FORENSIC BIOLOGY SECTION
PROCEDURES MANUAL

SECTION IV

BIOMEK®2000 AUTOMATION WORKSTATION PROCEDURES MANUAL

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THE DNA IQ™ SYSTEM

Many procedures are available for the purification of DNA from evidentiary samples containing biological material. Commonly used methods are phenol/chloroform extraction, Chelex® extraction, and QIAGEN® column affinity purification. Phenol/chloroform extraction of samples requires the use of hazardous chemicals and may result in some loss of material. While rapid, the Chelex® extraction method does not remove amplification inhibitors and the DNA can degrade over time. Purification on silica matrices such as the QIAGEN® column has some advantages since the method does not require organic compounds, is easy to use and usually removes PCR inhibitors. However, it can be costly.

Promega Corporation’s DNA IQ™ System is also an affinity based purification system that utilizes the affinity of DNA for silica in a manner similar to the QIAGEN® column method. However, unlike the QIAGEN® columns where the silica is utilized as a filter, the DNA IQ™ System uses a silica coated paramagnetic resin. Since a paramagnetic resin is utilized with the system, no centrifugation steps are required and instead, cell debris, buffers and washes are removed when the paramagnetic resin/cell lysate suspension is placed into a magnetic stand or if used in conjunction with the BIOMEK®2000 robot, placed onto the magnetic plate. The paramagnetic resin is pulled to the side of the tube in proximity to the magnet and the buffer can be removed without disrupting the paramagnetic resin pellet. The paramagnetic particles used with the DNA IQ™ System are designed not to clump when coated with DNA, which is a problem with many other silica particles. One benefit to the use of the paramagnetic resin is its easy adaptation to “hands off” robotics since no centrifugation or filtration steps are required.

The DNA IQ™ System is designed to efficiently purify small quantities of DNA. The paramagnetic resin saturates at approximately 100 ng of bound DNA. Excess DNA is removed during the DNA purification process. The DNA IQ™ System becomes more efficient with samples containing less than 50 ng of DNA, which is frequently the case with evidentiary forensic samples.

THE BIOMEK®2000 AUTOMATION WORKSTATION

The BIOMEK®2000 Automation Workstation is manufactured by Beckman Instruments, Inc. The robot is used extensively in biotechnology research and private industry laboratories, as well as in pharmaceutical laboratories. The particular BIOMEK®2000 Automation Workstation pictured in Figure 1 has been modified by Promega Corporation for the purpose of utilizing the DNA IQ™ System for DNA extraction.

The BIOMEK®2000 Automation Workstation is a fairly simple robot. It picks up tips, 8 at a time, which constitutes a column on a 96 well plate. It also has a gripper tool for picking up and moving plates around. A Teleshake shaker for mixing samples has been attached to the right hand side of the robot. Also mounted on the right side, is an electronic heater for the elution of the DNA from the DNA IQ™ paramagnetic resin. Specially adapted aerosol resistant tips are used with the BIOMEK®2000 Automation Workstation. The tip boxes have enclosed wells so that the tips can be re-used wherever possible, but the used tips will not contaminate any neighboring tips.

The BIOMEK®2000 Automation Workstation has a limited number of sensors. For example, it is unable to detect a clogged tip. Therefore samples with large fragments of undigested material, such as what is frequently found in a tissue digest, should be centrifuged for several minutes to pellet the debris, prior to loading the lysate into a 96 well plate for robotic DNA extraction.

All of the labware holders (gray clamps) and tip box holders (black clamps) can be easily removed and repositioned on the deck. Likewise the tool stands (used to hold the Gripper, MP200, P200L, MP20, and P20 tools) lifts out and can be positioned elsewhere on the deck if another method requires it.

Software methods have been written by scientists at Promega Corporation for use with the DNA IQ™ System on the BIOMEK® 2000 Automation Workstation. The methods are employed for the extraction of mixed stains, tissue, bloodstains and buccal cells described in this manual. The methods are the same except for the number of samples extracted (16, 24, 32, 40, 48, 56, 64, 72, and 80 samples). The methods involve the use of deep well plates for the
initial loading of cell samples, automated resin addition and dispensation of purified DNA into strip tubes. Once the DNA has been quantitated, normalized and used to set up the PCR reactions, the DNA extracts are transferred to 1.5 mL tubes for long term storage.

**Figure 1.** The BioMek® 2000 Automation Workstation with modifications for DNA extraction using the DNA IQ™ System.

1 = Tools (position A1)
2 = Marsh deepwell 96 well plate for loading cell samples/waste plate (position B4)
3 = Magnetic plate – Magnabot (position B5)
4 = Thermal exchange unit (position A6)
5 = Teleshake Shaking unit
1 ISOLATING DNA USING THE DNA IQ™ ISOLATION SYSTEM

When the DNA examiner has completed his/her analysis, all remaining evidence samples, including extracted DNA which has been dried down using a DNA concentrator/evaporator (Refer to Appendix J, Procedure For Drying Down And Resolubilizing Extracted DNA, of the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section III, PowerPlex® 16 BIO System) or other suitable methods, will be returned to the submitting agency. However, amplified DNA samples WILL NOT be returned to the submitting agency, but instead will be discarded in the designated area to prevent possible transfer of amplified DNA to the remaining evidence samples. If the biological sample deposited on the evidence is consumed during the analysis then the extracted DNA sample and the cutting (in separate tubes) will be returned to the submitting agency with the evidence. Proper case file documentation and chain of custody documentation for these samples must be maintained.

DNA may be extracted from bloodstains, sperm cells, buccal cells, hair, tissue, bone, and other samples. Slightly different extraction procedures are required for each type of specimen and therefore are outlined in this chapter. It is important to handle all samples aseptically to prevent contamination by extraneous DNA. It is also important to prepare evidence samples at a separate time and or space from reference samples to prevent possible cross-contamination.

NOTES: For tracking purposes the samples will be listed on the worksheet in the order in which they were processed/handled. To the extent possible, the samples will be maintained in the same order throughout the rest of the analysis until the typing step. All samples will be processed in accordance with the procedures and policies outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section I, General Documentation and Evidence Handling Requirements, Chapter 3, Contamination Prevention and Detection Procedures, and Section VI, Quality Assurance Program DNA Typing of Biological Materials, Chapter 6, Dedicated PCR Facilities.

The portion size of the swab or bloodstain removed for DNA extraction should be judged based on a number of criteria, such as whether the stain appears dilute (for bloodstains) or if the sample may be heavily soiled or possibly degraded. Examine the bloodstain or buccal swab and remove a reasonable portion for DNA extraction. For example, a 3 mm² section may be all that is necessary to remove from a heavily bloodstained item.

Special Precautions:

- At a minimum, a reagent blank will be processed with each set of samples extracted with the same procedure (procedure reagent blank). For any cases in which more than 50% of an evidentiary stain has been consumed, a devoted reagent blank (case reagent blank) will be processed in situ with the respective case samples.
- The manual steps of the DNA extraction and PCR setup of evidence samples will be performed at a separate time or space from the DNA extraction and PCR setup of reference samples. This helps to prevent potential cross-contamination between evidence samples and reference samples.
- The manual steps of the DNA extraction from samples containing high levels of DNA (for example, tissue) will be performed separately from samples expected to contain low levels of DNA (single hairs, small bloodstains, etc.) to minimize the potential for sample-to-sample contamination.
- Disposable gloves will be used at all times. Gloves will be changed frequently to avoid sample-to-sample contamination with DNA and whenever moving between work areas. Gloves will be changed if suspected direct contamination has occurred from the sample DNA.
- To minimize transferring DNA to the disposable gloves a clean Kimwipe will be used to open each microcentrifuge/amplification tube. If the evidence (i.e., stained area or the liquid from the cap of the tube) comes in contact with the disposable glove, change gloves before proceeding to the next stained area, item of evidence, or sample tube.
- Scissors and tweezers will be thoroughly cleaned with a 10% solution of bleach or a solution that will remove/degrade the DNA after cutting each item/stain. Subsequently use isopropanol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces. A fresh scalpel blade may also be used to cut each item/stain.
1 Isolating DNA Using the DNA IQ™ Isolation System

- A clean cutting surface will be used for each piece of evidence.
- Disposable and/or plugged pipette tips and microcentrifuge tubes will be used.
- Pipette tips will be changed between samples.
- Reagents will be stored in small quantities to reduce the risk of possible contamination to the stock solution.
- To avoid splashing and minimize aerosols, all liquid will be centrifuged to the bottom of the closed tube before the tube is opened.
- Reagent blank controls will be included with each set of DNA extractions to check for the presence of contaminating DNA in the reagents.
- All work surfaces will be thoroughly cleaned with a 10% bleach solution or a solution that will remove/degrade the DNA. Subsequently use isopropanol to remove the residue left by the chemicals, using special care to remove all residue left on surfaces before setting up the DNA Extraction Work Area. Disposable bench paper or Kimwipes will be used to prevent the accumulation of human DNA on permanent work surfaces.
- The quantity of samples handled during a single analysis will be limited to a manageable number. This precaution reduces the risk of sample mix-up and the potential for sample-to-sample contamination.
- A dedicated lab coat will be worn for pre-amplification sample handling when working in the DNA Extraction Work Area.
- A dedicated lab coat will be worn when working with amplified DNA in the PCR Post Amplification Work Area.

1.1 Technical Notes

1.1.1 The DNA IQ™ Isolation System is designed to rapidly purify small quantities of DNA, approximately 100 ng or less, and becomes more efficient with samples containing less than 50 ng of DNA.

1.1.2 The DNA IQ™ Isolation System will isolate genomic DNA in general and is not human specific.

1.1.3 The DNA IQ™ Lysis buffer contains Guanidine Thiocyanate (GTC), which is a chaotropic agent used to attach the DNA to a silica-coated bead.

1.1.4 Proteinase K (Pro K) is a proteolytic enzyme that reduces proteins to their constituent amino acids. In particular, Pro K removes the histone groups that keep the DNA tightly bound within the cell. The enzymatic activity of Pro K lasts for approximately 2 hours, and eventually, it will self-digest.

1.1.5 A differential lysis is included in the procedure for the analysis of mixed stains containing spermatozoa and other biological fluid(s). It includes what is referred to as a sperm and a non-sperm fraction. The non-sperm fraction is contained in the aqueous portion remaining after a gentle lysis treatment of the stain. A more rigorous treatment is conducted for the pelleted material (generally sperm); this is referred to as the sperm fraction.

1.2 Equipment

- Heat block or incubator, 37 °C
- Heat block or incubator, 56 °C
- Microcentrifuge
- Vortex mixer
- Scissors
- Tweezers
- Microcentrifuge tube rack
- Pipettes - 10 µL, 20 µL, 100 µL, 200 µL, and 1000 µL
- Refrigerator/Freezer
- Scalpel and blades (for DNA extraction from swabs)
- Stereo microscope (for DNA extraction from hair)
- Chisel and hammer (for DNA extraction from bone)
- High Speed Electric Drill (bone procedure)
1 Isolating DNA Using the DNA IQ™ Isolation System

- Rotary shaft tool (tooth procedure)
- 1/8” or 9/64” drill bit (bone procedure)

1.3 Materials

- Microcentrifuge tubes, 1.5 mL
- Microcentrifuge tubes, 2.0 mL, Sarstedt Cat 72.689.001 or 2.0 mL tubes, LPS Cat#M850003
- Transfer pipettes
- Sterile ART tips for pipettes - 40 µL, 20 µL, 100 µL, 200 µL, and 1000 µL
- Microcentrifuge tube lids
- Kimwipes
- Spin-Ease basket (Optional)
- Gloves
- Conical tubes, 15 mL and 50 mL (for DNA extraction from bone)
- White/black paper (for DNA extraction from hair)
- Weigh boats (for DNA extraction from bone)
- Microscope slides (Optional)
- Plastic Ziploc baggies

1.4 Reagents

- Proteinase K - 20 mg/mL (Keep on ice.)
- TNE
- 20% Sarkosyl
- 0.39 M Dithiothreitol (DTT)
- Sterile Type I Water
- PCR Digestion Buffer
- 95% Ethanol
- CaCl₂
- DNA IQ™ Lysis Buffer
- DNA IQ™ Resin

1.5 DNA IQ™ Extraction Method for Buccal and Epithelial Cell Type Samples and Bloodstains.

1.5.1 Cut an appropriate portion of a blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube. If a stain is smeared over a large area of fabric, more than one microcentrifuge tube may be needed to extract the stain. If the sample is a buccal swab, remove a small portion of the swab and place into a labeled microcentrifuge tube.

1.5.1.1 If a sample contains a weak smear over a large surface area the sample should be placed in several 1.5 mL microcentrifuge tubes and the entire sample condensed into one sample tube after purification.

NOTE: Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.5.2 Add 250 to 375 µL of DNA IQ™ Lysis buffer, a sufficient volume should be added to saturate the sample. If the biological sample is dispersed over a large area of the substrate, up to 425 µL of DNA IQ™ Lysis buffer may be added to the microcentrifuge tube.

NOTE: A maximum volume of 375 µL may be loaded into the deepwell plate.

1.5.3 Vortex vigorously for 20-30 seconds, then pulse spin to force the cutting into liquid.
1.5.4 Place the microcentrifuge tube into a 56 °C heat block for a minimum of 30 minutes. If the sample is deposited on FTA paper, place the microcentrifuge tube into a 56 °C or 95 °C heat block for a minimum of 30 minutes.

1.5.5 If using a Spin-Ease basket, vortex vigorously for 20-30 seconds, then pulse spin the tube, remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 1.5mL tube and close the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

1.5.6 If using a recessed lid, vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

**NOTE:** Samples can be left at room temperature in DNA IQ™ Lysis buffer for up to 24 hours after heating and centrifugation before proceeding to the Biomek® 2000 Automation Workstation. If the samples are stored in a refrigerator before proceeding to Section 2, Preparation of the Biomek® 2000 Automation Workstation, place the samples in a 56 °C heat block for 5 minutes to resolubilize the samples.

It is not necessary to spin down cuttings made from known reference samples.

1.5.7 Proceed to Chapter 2, Preparation of the Biomek® 2000 Automation Workstation.

**1.6 DNA IQ™ Extraction Method for Mixed Body Fluid Stains When Spermatozoa Have Been Identified (Differential Procedure)**

1.6.1 Cut a sample of stain or cotton swab proportional to the number of sperm identified. Place the cutting into a labeled 1.5 mL microcentrifuge tube. If the stain is smeared over a large area on the fabric or the body fluids on a swab are weak and more than one swab must be used, more than one microcentrifuge tube may be needed to extract the stain.

1.6.1.1 If a sample contains a weak smear over a large surface area the sample should be placed in several 1.5 mL microcentrifuge tubes and the entire sample condensed into one sample tube after the purification step.

1.6.2 Add:

- 400 µL TNE
- 25 µL 20% Sarkosyl
- 75 µL Sterile Type I Water
- 5 µL Proteinase K

in proportional amounts to saturate the cutting.

1.6.3 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid.

1.6.4 Place the tube into a 37 °C incubator or heat block for a minimum of 2 hours.

1.6.5 If using a Spin-Ease basket, vortex vigorously for 20-30 seconds, then pulse-spin the tube, remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 1.5mL tube and close the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence. Proceed to 1.6.7.
1.6.6 If using a recessed lid, vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in
the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in
a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater
than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned
with the evidence.

1.6.7 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new 1.5 mL
labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The
supernatant removed from the pellet is the NON-SPERM FRACTION.

1.6.8 At this stage, set the non-sperm fraction tube aside and wait until the sperm fraction is ready, and then
proceed to Section 2, Preparation of the Biomek® 2000 Automation Workstation, with both the sperm and
non-sperm fractions.

NOTE: If necessary the sample(s) may be capped and left at room temperature overnight before
proceeding.

1.6.9 If using a recessed lid, remove and discard the old lid and cutting from the original tube containing the
pellet. (unless the cutting will be returned with the evidence). Place a new lid on the tube. This tube
contains the SPERM FRACTION.

1.6.10 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digest buffer by vortexing briefly.
Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a sterile pipette,
remove all but 50 µL of the supernatant and discard.

1.6.11 Repeat the wash in step 1.6.10 an additional 2 times. If a low sperm count has been determined, the
sperm pellet may be washed up to 5 times. After the final wash, remove all but 50 µL of the digest
buffer and discard. The sperm cells are not lysed at this point. The sperm cells are lysed during the
isolation step using the Biomek® 2000 Automation Workstation.

NOTE: If necessary the sample(s), once capped, can remain at room temperature overnight or be
stored in a refrigerator before proceeding to Chapter 2. If stored in a refrigerator, place the
samples in a 56 ºC heat block for 5 minutes to resolubilize the DNA before proceeding.

OPTION: If desired, a sperm search can be conducted by removing remove 3 µL of the resuspended
pellet and spotting the sample onto a glass microscope slide for examination.

1.6.12 Proceed to Chapter 2, Preparation of the Biomek® 2000 Automation Workstation, with both the sperm
and non-sperm fractions.

1.7 DNA IQ™ Extraction Method for Hair Highly Concentrated Bloodstains and Low Level Samples

NOTE: This procedure may also be used for samples that are believed to have a low concentration of
DNA (i.e., envelopes, stamps, cigarette butts) or highly concentrated bloodstain (i.e., dried
blood flakes).

1.7.1 Follow the steps outlined below for the extraction of hairs. Proceed to step 1.7.2 for all other sample
types:

1.7.1.1 Wash the hair to reduce surface dirt and contaminants by immersing the hair in sterile Type I
Water. If the hair contains a biological fluid that is important to the investigation DO NOT
wash the hair.

1.7.1.2 Use a clean scalpel blade to cut a 0.5 to 1 cm portion from the root end of the hair and then
place the hair root into a 1.5 mL microcentrifuge tube and proceed to step 1.7.3.
1.7.2 Cut an appropriately-sized blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube.

1.7.3 Add to the 1.5 mL microcentrifuge tube:

- 37.5 µL TNE
- 12.5 µL 20% Sarkosyl
- 10.0 µL 0.39M DTT
- 32.5 µL Sterile Type I Water
- 10.0 µL Proteinase K

Depending on the size of the sample, the volumes may be doubled. However, if it is necessary to increase the volume greater than approximately 200 µL to saturate the cutting, proceed to 1.9.3 and use the Large Volume extraction method. If using the 200 µL volume, refer to Chapter 2 for a modification to the loading process.

1.7.4 Mix by hand or lightly vortex, then pulse spin the microcentrifuge tube to force the sample into the liquid.

1.7.5 Place the tube into a 56 ºC incubator or heat block for minimum of 1 hour.

1.7.6 For hairs pulse spin the tube in a microcentrifuge for 10 seconds to force the condensate to the bottom of the tube.

1.7.7 For all other samples if using a Spin-Ease basket, vortex vigorously for 20-30 seconds, then pulse-spin the tube, remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 1.5mL tube and close the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence. Proceed to step 1.7.9.

1.7.8 For all other samples if using a recessed lid, vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

1.7.9 Proceed to Chapter 2, Preparation of the Biomek® 2000 Automation Workstation.

1.8 DNA IQ System Extraction Method for Tissue Samples

1.8.1 Cut a very thin slice of tissue (i.e. 1 mm thick slice of muscle) and place the sample into a labeled 1.5 mL microcentrifuge tube. If the tissue sample is degraded, a slightly larger portion of the sample may be used. Alternatively, cellular material may be collected from the tissue by swabbing the sample and placing a portion of the swab into a labeled 1.5 mL microcentrifuge tube. If swabbing was used to collect the sample from the tissue or the tissue is relatively fresh, the DNA IQ™ extraction method for buccal and epithelial cell type samples and bloodstains may be used in place of the CaCl2 buffer method.

1.8.2 Add 90 µL of 1X CaCl2 buffer and 10 µL of Proteinase K to saturate the sample. The volume may be increased proportionately in order to fully saturate or cover the sample.

1.8.3 Mix by hand or light vortexing and pulse spin to force the sample into the liquid.

1.8.4 Place the tube into a 56 ºC incubator or heat block for a minimum of 2 hours.

**NOTE:** If the sample has been preserved in Formaldehyde or Formalin, add a second 10 µL aliquot of Proteinase K after the 2 hour incubation, then place the tube into a 56 ºC incubator or heat block for an additional 2 hours.
1.8.5 Spin the 1.5 mL microcentrifuge tube at ~12,000 rpm for 5 minutes to pellet any undigested debris. If a swab was used the cutting may be removed as described previously (see 1.7.7).

1.8.6 Proceed to Chapter 2, Preparation of the Biomek® 2000 Automation Workstation

1.9 Large Volume (LV) Extraction Method (For Use with Low Level DNA Samples, Diffuse or Problematic Samples)

The LV method utilizes a 500 µL Proteinase K buffer digest and can provide a higher DNA yield, particularly for blood samples. It is designed to be used for samples which require a larger (i.e., 500 µL) Proteinase K digest buffer in order to produce optimal results. This method can also be used for differential extractions where the non-sperm fraction is suspected of having few or limited epithelial cells, with bone and teeth samples and diffuse samples requiring a large cutting. Caution must be employed when utilizing the LV method on the robot since samples that may provide a reasonably high yield (such as the non-sperm fraction of a vaginal swab) using the standard protocol, may provide a much higher yield with the LV method, thus potentially making the extraction more susceptible to carry over.

NOTE: If the sample is suspected to contain abundant DNA, then either a smaller portion should be removed for extraction or the standard protocol used instead of the LV method.

All reagents used are the same as for a standard 100 µL Proteinase K digest followed by DNA IQ™ DNA purification on the Biomek 2000.

1.9.1 Scale up the standard Proteinase K digest to approximately 500 µL for each tube with sample cutting.

187.5 µL TNE
62.5 µL 20% Sarkosyl
50 µL 0.39M DTT
187.5 µL Sterile Type I H₂O
25 µL Proteinase K (20 mg/mL)
512.5 µL Total volume

1.9.2 Add a maximum of 500 µL of Pro K buffer to each sample which has been cut and placed into either a labeled 1.5 or 2.0 mL microfuge tube. Ensure that cutting or swab is fully submerged or saturated by the buffer.

1.9.3 Place tubes into 56 ºC heat block for a minimum of 2 hours. Halfway through the incubation, vortex each tube, pulse spin, and return the tubes to the heat block.

1.9.4 If using a Spin-Ease basket, vortex vigorously for 20-30 seconds, then pulse-spin the tube, remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 1.5mL tube and close the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

1.9.5 If using a recessed lid, vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

1.9.6 Transfer a maximum of 500 µL of the lysate from the cutting into a labeled 2.0 mL microfuge tube if the sample is not already in a 2.0 mL microfuge tube.

NOTE: Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.9.7 Add 1 mL of DNA IQ Lysis buffer to the tube.
1.9.8 Vigorously vortex the bottle of DNA IQ™ Resin for 10-15 seconds prior to dispensing. Then add 8 µL of IQ resin to each tube containing lysate/IQ Lysis buffer mixture.

1.9.9 After adding the resin, vigorously vortex each tube for a full 30 seconds. Incubate at room temperature for 5 minutes.

1.9.10 Vigorously vortex the tube a second time for a full 30 seconds and then incubate at room temperature for 5 minutes.

1.9.11 Pulse spin to remove condensation from the lid of the tube.


1.10 Large Volume DNA IQ™ Extraction Method for Mixed Body Fluid Stains When Spermatozoa Have Been Identified (Differential Procedure)

If the non-sperm fraction is suspected of having few or limited epithelial cells, the LV Teleshake method may be used to enhance the DNA yield.

1.10.1 Cut a piece of stain or cotton swab proportional to the number of sperm identified. Place the cutting into a labeled 1.5 mL microcentrifuge tube. If the stain is smeared over a large area on the fabric or the body fluids on a swab are weak and more than one swab must be used, more than one microcentrifuge tube may be needed to extract the stain.

1.10.1.1 If a sample contains a weak smear over a large surface area the sample should be placed in several 1.5 mL microcentrifuge tubes and the entire sample condensed into one sample tube during the purification step.

1.10.2 Add:

- 400 µL TNE
- 25 µL 20% Sarkosyl
- 75 µL Sterile Type I Water
- 5 µL Proteinase K

in proportional amounts to saturate the cutting.

1.10.3 Mix by hand or light vortexing then pulse spin to force the cutting into the liquid.

1.10.4 Place the tube into a 37 °C incubator or heat block for a minimum of 2 hours.

1.10.5 If using a Spin-Ease basket, vortex vigorously for 20-30 seconds, then pulse spin the tube, remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 1.5mL tube and close the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

1.10.6 If using a recessed lid, vortex vigorously for 20-30 seconds, then pulse spin the tube, punch 2-3 holes in the lid of the tube, remove the cutting from the liquid and place in the lid. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to remove the excess liquid from the cutting. If greater than 50% of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence.

1.10.7 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new, labeled 2.0 mL tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet and placed into the 2.0 mL tube is the NON-SPERM FRACTION.
1.10.8 At this stage, set the non-sperm fraction tube aside and wait until the sperm fraction is ready, and then proceed to 1.10.11 with both the sperm and non-sperm fractions.

1.10.9 If using a recessed lid, remove and discard the old lid and cutting from the original tube containing the pellet (unless the cutting will be returned with the evidence). Place a new lid on the tube. This tube contains the SPERM FRACTION.

1.10.10 Wash the pellet as follows: Resuspend the pellet in 500 µL of PCR digest buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette, remove all but 50 µL of the supernatant and discard.

1.10.11 Repeat the wash in step 1.10.10 an additional 2 times. If a low sperm count has been determined, the sperm pellet may be washed up to 5 times. After the final wash, remove all but 50 µL of the digest buffer and discard. The sperm cells are not lysed at this point.

NOTES: If necessary, the sample(s) once capped can remain at room temperature up to 3 days or be stored in a refrigerator before proceeding to Chapter 2. If stored in a refrigerator place the samples in a 56 ºC heat block for 5 minutes to resolubilize the DNA before proceeding.

Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.10.12 Add 1 mL of DNA IQ Lysis buffer to both the sperm pellet and the non-sperm lysate.

1.10.13 Vigorously vortex the bottle of DNA IQ™ Resin for 10-15 seconds prior to dispensing. Then add 8 µL of IQ resin to each tube containing lysate/IQ Lysis buffer mixture.

1.10.14 After adding the resin vigorously vortex each tube for a full 30 seconds. Incubate at room temperature for 5 minutes.

1.10.15 Vigorously vortex the tube a second time for a full 30 seconds and then incubate at room temperature for 5 minutes.

1.10.16 Pulse spin to remove condensation from the lid of the tube.

OPTION: If desired, a sperm search can be conducted by removing 3 µL of the resuspended pellet and spotting the sample onto a glass microscope slide for examination.

1.10.17 Proceed to Chapter 2, Preparation of the Biomek 2000 Automation Workstation.

1.11 Large Volume DNA IQ™ Extraction Method for Bone Samples

1.11.1 Prepare the drilling setup prior to beginning to drill the bone for bone powder. The drill bit must be cleaned thoroughly with 10% bleach followed by 95% Ethanol or isopropyl alcohol prior to use and between bone samples. An incubator must be pre-set to 56 ºC.

1.11.2 Prepare the following cleaning solution for cleaning the outside of the bone.

\[
\begin{align*}
1.2 \text{ mL TNE} \\
75 \mu\text{L} 20\% \text{ Sarkosyl} \\
225 \mu\text{L Type I water}
\end{align*}
\]

Mix solution and pre-heat in heat block or incubator set to 56 ºC.

1.11.3 Fold a few Kimwipes to produce a pad capable of absorbing the cleaning solution.
1.11.4 Remove the cleaning solution from the heat block or incubator and add 15 µL Proteinase K (20 mg/mL) and mix well.

1.11.5 Apply the cleaning solution to a Kimwipe and apply to the bone surface.

1.11.6 Place the bone in a plastic Ziploc bag large enough to hold the bone sample and place into an incubator for 30 minutes.

1.11.7 Remove the bone sample from the plastic bag and remove the Kimwipe from the bone.

1.11.8 Clean the area on the bone that was digested with the Proteinase K solution with 95% Ethanol.

   1.11.8.1 Clean by applying 95% Ethanol to a clean Kimwipe and wipe the surface.

   1.11.8.2 Wipe one to two times enough to remove all active Proteinase K solution

1.11.9 Allow the bone to dry briefly inside of a hood.

1.11.10 Drill a small area (1-2 mm deep) out of the bone within the area that was cleaned with the cleaning solution.

1.11.11 To prevent the possibility of introducing exogenous DNA from the outside surface of the bone, dispose of the initial drilled material into a waste container.

1.11.12 Clean the drill bit with 10% bleach followed by 95% Ethanol or isopropanol.

1.11.13 Drill out a new portion of bone material from the same hole and collect the bone powder in weigh paper or weigh boat. Collect an amount that resembles the size of a pea.

1.11.14 Transfer the bone material to a 1.5 mL microcentrifuge tube.

1.11.15 Add 35 µL of 20 mg/mL Proteinase K to 500 µL of Bone Digest Buffer for each bone sample.

1.11.16 Incubate between 2 hours and overnight at 56 ºC.

1.11.17 Place the tube into a microcentrifuge and spin at ~10,000 rpm for 5 minutes to separate out the undigested bone material and transfer the supernatant to a 2.0 mL tube. Use caution not to transfer any debris.

**NOTE:** Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.11.18 Add 1 mL of DNA IQ Lysis buffer to the bone lysate.

1.11.19 Vigorously vortex the bottle of DNA IQ™ Resin for 10-15 seconds prior to dispensing. Then add 8 µL of IQ resin to each tube containing lysate/IQ Lysis buffer mixture.

1.11.20 After adding the resin vigorously vortex each tube for a full 30 seconds. Incubate at room temperature for 5 minutes.

1.11.21 Vigorously vortex the tube a second time for a full 30 seconds and then incubate at room temperature for 5 minutes.

1.11.22 Pulse spin to remove condensation from the lid of the tube.

1.11.23 Proceed to Chapter 2, Preparation of the Biomek 2000 Automation Workstation.
1.12 Large Volume DNA IQ™ Extraction Method for Teeth

**NOTE:** Molars are the tooth of choice for DNA recovery.

1.12.1 Clean the outer surface of the tooth with a Kimwipe and 10% bleach (avoid introducing the bleach solution into any cracks in the tooth). Subsequently remove the 10% bleach residue using a Kimwipe and sterile water followed by isopropanol.

1.12.2 While working in a hood, using a sterile diamond saw blade and a rotary shaft tool cleaned with bleach and isopropanol, cut/grind away the upper crown portion of the tooth until the pulp chamber becomes visible. In addition, make small nicks in the sides of the tooth to facilitate crushing the lower portion of the tooth. Refer to the diagrams below.

**NOTE:** It will take between 5 and 10 minutes to remove the crown of the tooth.

1.12.3 To gain access to the pulp, place the tooth minus the crown into a small sterile Ziploc plastic bag. Insert the Ziploc plastic bag into a second Ziploc bag. Ensure the Ziploc bags are sealed.

1.12.4 Lay the Ziploc plastic bag on a hard surface. Using a hammer, pulverize the tooth sample inside of the Ziploc plastic bag. Be careful not to puncture the Ziploc plastic bag.

1.12.5 While holding the plastic bag with the Ziploc portion of the plastic bag up, shake the sample to transfer the pulverized sample to the corner of the Ziploc plastic bag. Open the Ziploc plastic bags and transfer the pulverized/crushed tooth to a 1.5 mL microcentrifuge tube.

1.12.6 Add 10 µL of 20 mg/mL Proteinase K to the Bone Digest Buffer, 400 µL for each tooth sample:

1.12.7 Incubate overnight at 56 ºC.
1.12.8 Prepare DNA IQ Lysis buffer for use (add 2.5 µL 0.39 M DTT/100 µL DNA IQ™ Lysis buffer prior to use).

1.12.9 Spin out tooth material at ~10,000 rpm for 5 minutes and using a pipet, transfer the supernatant to a 2 mL microcentrifuge tube, taking care not to transfer any debris.

1.12.10 Place the tube into a microcentrifuge and spin at ~10,000 rpm for 5 minutes to separate out the undigested tooth material and transfer the supernatant to a 2.0 mL tube. Use caution not to transfer any debris.

**NOTE:** Prior to the addition of the DNA IQ™ Lysis buffer, DTT at the concentration described in the reagent preparation section (Appendix B) MUST be added.

1.12.11 Add 1 mL of DNA IQ Lysis buffer to the tooth lysate.

1.12.12 Vigorously vortex the bottle of DNA IQ™ Resin for 10-15 seconds prior to dispensing. Then add 8 µL of IQ resin to each tube containing lysate/IQ Lysis buffer mixture.

1.12.13 After adding the resin vigorously vortex each tube for a full 30 seconds. Incubate at room temperature for 5 minutes.

1.12.14 Vigorously vortex the tube a second time for a full 30 seconds and then incubate at room temperature for 5 minutes.

1.12.15 Pulse spin to remove condensation from the lid of the tube.

2 Preparation of the BIOMEK 2000 Automation Workstation for DNA Isolation

2 PREPARATION OF THE BIOMEK® 2000 AUTOMATION WORKSTATION FOR DNA ISOLATION – TELESHAKE AND LARGE VOLUME TELESHAKE SYSTEMS

2.1 Technical Notes

Unless specifically indicated, no substitution for the plates or other consumables can be made. Do not substitute another brand because small differences in the well formation or depth of the well can cause the tips to “bottom out”. When tips “bottom out” the tips are in contact with the bottom of the well which can form a vacuum in some tips when the tool aspirates liquid and affect the accuracy of pipetting.

Detailed view of the Biomek® 2000 Workstation deck:

- MP200 Gripper
- Strip tubes
- Tip box holder
- Tip box holder
- Empty or Labware holder and ABGene 1.2ml plate
- Labware holder
- Electronic Heater
- A6
- B1
- B2
- B3
- B4
- B5
- B6
- Reagents
- Marsh plate (Sample/ waste)
- Magnabot (ABGene 1.2ml plate goes here)
- Teleshake shaker

2.1.1 The electronic heating unit connected to the Biomek® 2000 Workstation is set at a temperature of 85 ºC and is used to heat the pronged heat transfer plate, used for eluting the DNA from the paramagnetic resin.

2.1.2 The DNA IQ™ Lysis buffer is a proprietary detergent mixture containing Guanidine Thiocyanate (GTC). The GTC is a chaotropic agent, necessary for the DNA to stick to the silica-coated, paramagnetic resin.

2.1.3 The DNA sticks to the resin through hydrophobic and possibly hydrogen bonding interactions, however the exact mechanism is unknown.

2.1.4 The DNA IQ™ Wash Buffer is a low salt buffer, which is 50% alcohol (50% isopropyl: 50% ethanol), and 50% NaAcetate. The alcohol in the wash keeps the DNA stuck to the resin and the low salt buffer helps to remove excess salt (Guanidine Thiocyanate) from the DNA bound to the resin. If the Guanidine Thiocyanate is not removed it can inhibit the PCR amplification.

2.1.5 The DNA IQ™ Elution Buffer is added in order to release the DNA from the resin. If the temperature at which the material is heated is not sufficient (>50 ºC), the yield of DNA obtained will be lower than expected.

2.1.6 When the DNA is initially eluted from the DNA IQ™ paramagnetic resin, some of it is single stranded and therefore cannot be quantitated on a Yield Gel.
2 Preparation of the BIOMEK 2000 Automation Workstation for DNA Isolation

2.2 Equipment

- Biomek®2000 Automation Workstation
- Magnabot with ¼ foam spacer – deep well prongs
- Watlow Electronic Heating Unit
- Heat transfer unit
- Computer with BioWorks Software
- Teleshake unit
- Pipettes – 100 µL, 200 µL, and 1000 µL
- 96 well thermal cycler rack
- 4-Beckman 24 Microfuge tube holders – Beckman Catalog # 373661
- Beckman white 1.5 mL tube inserts – Beckman Catalog # 373656

2.3 Materials

- ABGene 96 deep round well plates (Do not substitute these plates with a different brand.)
- Marsh 96 deep square well plates (Do not substitute these plates with a different brand.)
- 8-Reaction tube strips, 0.2 mL
- 8-Cap strips, 0.2 mL
- P250 Tips – aerosol resistant – Beckman Catalog # 140505
- P20 Tips – aerosol resistant – Beckman Catalog # 609043
- Quarter module reservoirs
- Tape
- Microscope slides (optional)
- Sterile ART tips for pipettes - 100 µL, 200 µL, and 1000 µL

2.4 Reagents

- DNA IQ™ System Reaction Kit
- DNA IQ™ Lysis Buffer
- DNA IQ™ Wash Buffer
- DNA IQ™ Resin
- DTT
- DNA IQ™ Elution Buffer

2.5 Starting the BIOMEK® 2000 Automation Workstation

2.5.1 Turn on the computer.

2.5.2 Turn on the Biomek® 2000 Automation Workstation using the power button located on the back left side of the unit under the power cord.

2.5.3 Turn on the Watlow electronic heating unit using the switch located on the front of the unit.

2.5.4 Complete the Biomek® 2000 Automation Workstation loading sheet (Document 210-F500, Worksheet 4) to reflect in which well each sample will be loaded. A column of empty wells will be used to separate the evidentiary samples from the reference samples.

2.5.5 Place pre-labeled 1.5 mL microcentrifuge tubes into the four Beckman 24 Microfuge tube holders on the work bench. The pre-labeled tubes will be placed into the Microfuge tube holders in the same order as the samples listed on the 96 deep well plate loading sheet. These tubes will be set aside and used after the DNA Normalization Wizard and PCR setup procedures when the extracted DNA is prepared for permanent storage.
2 Preparation of the BIOMEK 2000 Automation Workstation for DNA Isolation

2.5.1 If amplifying samples for PowerPlex® 16 BIO, place the pre-labeled attached cap PCR amplification tubes into a 96 well black PCR support base. The pre-labeled tubes will be placed into the support base in the same order as the samples listed on the 96 well plate loading sheet, except the tubes will be spaced into the support bases such that an empty column will be left between each column of tubes to accommodate the attached caps. Therefore, the tubes will be spaced over two 96 well PCR support bases. Refer to the figures 17A and 17B in Chapter 5 as a reference for the tube setup. If amplifying samples for PowerPlex® 16, a PCR plate or PCR amplification tubes may be used.

NOTE: If known and unknown samples are loaded into the same 96 deep well plate, the known and unknown samples must be separated by blank wells. Reagent blanks maybe placed in the blank wells.

2.5.6 Depending on the sample type, pipette the following volume of sample into the appropriate well of a clean 96 square deep well Marsh plate for the regular automated isolation method. If it is suspected that a high concentration of DNA may be present, it is acceptable to load proportionally less volume than what is specified below:

2.5.6.1 For buccal cell type samples and bloodstains in DNA IQ Lysis buffer, pipette up to 375 µL of the lysate into the Marsh deep well plate.

2.5.6.2 For non-sperm fractions, pipette 100 µL of the lysate into the Marsh deep well plate.

NOTE: If it is suspected that a low level of epithelial cells exists, the volume may be increased to 200 µL. However, 175 µL of DNA IQ Lysis buffer must be added to the lysate or to the sample well in the Marsh plate so that the entire volume is ~375 µL. This will ensure a 2:1 ratio of DNA IQ Lysis buffer to the Proteinase K buffer and provide the most efficient recovery of DNA.

NOTE: If it is suspected that there are a large number of epithelial cells in the non-sperm fraction and, hence, a potentially high DNA yield, less lysate can and should be loaded to reduce potential for carry-over.

2.5.6.3 For sperm fractions, pipette the entire sample (this is approximately 30-50 µL of the sperm cell suspension) into the Marsh deep well plate. Take care to transfer the entire sperm pellet. If desired, DNA IQ Lysis buffer (~100 µL) can be added to it prior to the transfer of the pellet or to rinse the tube out after the transfer of the pellet.

2.5.6.4 For hair samples (optional: envelopes, stamps, cigarette butts, flakes of blood, and other low level samples), pipette 100 µL of the lysate into the Marsh deep well plate.

NOTE: If it is suspected that a low level of epithelial cells exists, the volume may be increased to 200 µL. Follow the directions for increased volume under 2.5.6.2 if loading a 200 µL volume.

2.5.6.5 For tissue samples, pipette 100 µL of the lysate into the Marsh deep well plate. Caution must be used to prevent aspirating any of the pelleted debris and loading the debris into the Marsh deep well plate.

2.5.7 If the Large Volume (LV) automated isolation method will be used, the entire 1.5 mL of the lysate/resin/lysis buffer cocktail will be loaded into the 96 deep square well Marsh deep well plate.

2.5.8 Loading evidence samples:

2.5.8.1 Once a column of evidence samples has been loaded into the 96 deep well plate, place a piece of tape over the wells or take other appropriate measures to avoid other samples from being
pipetted into the same well. Alternatively, a glass microscope slide may be used to cover empty wells while loading the plate.

### Plate with Knowns and Unknowns

![Plate with Knowns and Unknowns](image_url)

**Figure 1.** Plate with knowns and unknowns. Both the 11th and 12th columns must be unused (shown in red), as they will be used for the Plexor™ HY Standards during DNA quantitation.

**NOTE:** A set of evidence samples may be separated by a column of blank wells from another examiner’s set of samples.

2.5.8.2 Knowns and unknowns may be loaded on the same 96 well plate or on separate plates.

2.5.9 Loading known/reference samples:

2.5.9.1 If known/reference samples are loaded into the sample 96 deep well plate, empty wells will be used to separate the known/reference samples from the evidence samples. An example of this is demonstrated in the diagram above (i.e., wells A8 through H8). Reagent blanks may be placed in the in the empty wells separating the two sample types.

2.5.9.2 Known/reference samples will be loaded sequentially (i.e., A9 – H9). A column of reference samples may be loaded adjacent to another column of reference samples.

2.5.10 Proceed to Section 2.6, Biomek® 2000 Automation Workstation Operating Procedure.

2.6 **BIOMEK® 2000 Automation Workstation Operating Procedure**

An **EMERGENCY STOP BUTTON** is located at the lower front area on the robot, just below the deck. If this button is pressed, the robot will abort the method it is currently running. The emergency stop only needs to be used when the robot cannot be stopped by software means or the
robot could be damaged by crashing into the deck or a tool could be damaged by crashing into something on the deck. If what is desired is simply to stop the method while it is running, simply click on the “Stop” button. The method will then pause and several options will be available. The button labeled “Continue” can be used to resume the method, the button labeled “Trace” can be used to advance to the next step (this function is useful when calibrating), the button labeled “Go Up” will move the pod up and the button labeled “Quit” will terminate the method.

When the BioWorks robot program is initiated it will prompt the user regarding the proper placement, number and type of pipette tips to use. A prompt will also ask about the temperature of the electronic heater. Each method has prompts that will ask if the correct volume of reagent has been placed into the modular reservoir at the appropriate position. A series of prompts will come up when a method is initiated asking whether or not the electronic heating unit is at temperature, the tip boxes are appropriately placed and if the proper volume of the various reagents has been placed into the modular reservoir at the appropriate position. Responding to the prompts will ensure the user has correctly set up the deck for the selected method. **All reagents used for the DNA IQ™ ISOLATION SYSTEM must be properly prepared prior to use.**

**NOTE:** Some of the reagents are sensitive to evaporation, **DO NOT** fill the reservoirs for the Biomek® method until just prior to initiating the robot run.

2.6.1 Prior to selecting a method to run, the Teleshake shaking unit must be connected or tested to ensure it is connected. This is performed by double clicking on the desktop shortcut labeled “shortcut to teleshaker set-up”.

2.6.2 The following box (Figure 2) will open:

![Teleshake Test Program](image)

**Figure 2.** Teleshake Test Program
2 Preparation of the BIOMEK 2000 Automation Workstation for DNA Isolation

2.6.3 Under “Options”, select “Interface”. The following box (Figure 3) will appear:

![Interface Figure](image)

**Figure 3. Interface**

2.6.4 Select “Com 2” and click “OK”.

2.6.5 Click on “File”, and then click on “Connect”. The “Device No 1” line should now populate with the Teleshaker settings as pictured below, Figure 4. Do not change these settings for any reason. The Start button DOES NOT need to be clicked to ensure it is connected. The lighter panels confirm that the connection has been made.

![Teleshake Test Settings](image)

**Figure 4. Teleshake Test Settings**

2.6.6 Methods for running specific programs on the Biomek 2000 Automation Workstation are located in the BioWorks folder. Click on the BioWorks folder located on the desktop.

2.6.7 Double click on the Lab Book Manager icon in the Bioworks folder and verify that the selected lab book is the desired one. To select a lab book, highlight the line of the desired lab book and click on “select as current lab book”, then click on the close button to close the window.
2.6.8 To select a method to run, double click on the Edit icon in the Bioworks folder. Then, either using the method drag-down window or using the open folder icon, select Open. The following window, Figure 5, will open and a list of all methods will be displayed (i.e., 56 sample method). Double click on the method you wish to open or highlight the method and click on the Open button.

![Open Method window](image)

**Figure 5. List of available methods**

**NOTE:** Before utilizing the Biomek® 2000 Automation Workstation for DNA extraction, a test of the shaker MUST be performed. This needs to be performed once a day when starting up the computer, the Biomek® 2000 Automation Workstation, and the shaker, unless there is reason to believe that the shaker has gone “off-line” with the workstation and computer. A method, the Teleshake/Grip Test, was created to test whether or not the shaker is “on line” with the workstation and computer. During this test method, the robot will precisely pick up a round well deep well plate block from the Magnabot, move it to the teleshaker unit and initiate shaking according to the computer command. When trying to execute the Teleshake/Grip Test method, if an error message box comes up stating that the computer is unable to communicate with the shaker, check the interface connection into Com Port 2. The test must then be repeated. Allow the Teleshake/Grip Test to run to completion as explained in section 2.6.9.

2.6.9 Run the Teleshake/Grip Test once a day when starting up the computer, BioMek® 2000 Automation Workstation, and shaker as follows:

2.6.9.1 Using the Edit function, open the Open Method window.

2.6.9.2 Scroll to the bottom of the window where the Teleshake/Grip Test method is visible. Double click on the Teleshake/Grip Test or highlight it then click “Open”. The method will open with an inset window showing the deck layout. Verify that a round deep well plate is at deck position B5 on top of the pronged heat transfer plate atop the Magnabot. Verify that the Gripper tool is in the tool rack at deck position A1.

2.6.9.3 Click on the running man icon on the tool bar to initiate the method. A window will appear showing a stop light with a red light. It will state that the light will turn green once communication has been established. It will take a minute or less for the computer to establish communication with the robot. Once this occurs, the light will turn green and the window will disappear. An inset window method will ask if you accept the deck layout, click accept, then the method will initiate.
2 Preparation of the BIOMEK 2000 Automation Workstation for DNA Isolation

NOTE: The gripper will move a 96 deep round well ABGene plate from position B5 to position B6 and begin shaking. Allow the Teleshake/Grip test to run to completion. Now the robot is ready for executing a DNA extraction method.

2.6.10 Double click on the DNA IQ™ extraction method to run the appropriate program depending on the number of samples.

NOTE: The prompts and messages for the method will appear in green.

2.6.11 Following the directions provided by the program in green type, set up the deck components (i.e., tips, plates, quarter module reservoirs, and buffers). Descriptions of all the reservoirs and their contents are in green type. The deck layout will also come up as represented in Figure 6. This will allow the user to set up all the deck components appropriately for the selected method prior to its execution.

![Figure 6. Deck layout for sample method](image)

NOTE: Unless specifically indicated, no substitution for the plates can be made. Do not substitute another brand because small differences in the well formation or depth of the well can cause the tips to “bottom out”. When tips “bottom out” the tips are in contact with the bottom of the well and when the tool attempts to aspirate liquid, a vacuum can form in some tips, which could affect the accuracy of pipetting and also cause filter wetting.

2.6.11.1 Place a clean, unused 96 deep round well ABGene plate onto the deep pronged magnet with spacer located at deck position B5. If running a two plate method, a clean 96 deep well round well ABGene plate should be placed onto a gray Labware holder at position A4.
2.6.11.2 Place the appropriate number and type of clean Beckman tip boxes in the designated position in row A. Make sure that the tip boxes are fitting squarely into the black clamps. It is necessary to push back on the spring manually to get the tip box into the holder.

2.6.11.3 Place the quarter module reservoirs in the holders at position B3 of the deck.

2.6.11.4 In deck position B1, place a black 96 well PCR support base. Starting from left to right, place the appropriate number of strip tubes depending on the number of samples that are being extracted (i.e., for the 40 sample method, 5 columns consisting of 8 strip tubes each. **ENSURE THAT THE TUBES ARE FULLY SEATED IN THE 96 PCR SUPPORT BASE.**

**NOTE: DO NOT** pre-mix the DNA IQ™ Lysis Buffer containing DTT with the resin. The Biomek® 2000 Automation Workstation will mix these two solutions. DNA IQ™ Resin should be vortexed vigorously for 10-15 seconds prior to dispensing.

2.6.11.5 Once all samples have been loaded into the Marsh plate, place the 96 deep well plate onto the Biomek® 2000 Automation Workstation deck at position B4. Ensure that all tape or other covering has been removed from the wells. This plate will also serve as the waste collection plate later in the method.

2.6.11.6 Click on the button showing an image of a running man on the menu bar. This will initiate the method.

2.6.11.7 Once a method has been initiated the following window, Figure 7 will open showing the deck layout appropriate for the selected method. Click on Accept All.

![Figure 7](image)

*Figure 7. Deck layout for the selected method*

2.6.11.8 The robot will initiate the method and will take the user through a series of prompts beginning with a prompt asking whether the electronic heating unit is at 85 ºC. If the heating unit is not yet to 85 ºC, proceed with the method anyway. The unit typically gets to temperature within 5-10 minutes, so it will be ready by the time it is used.
2.6.11.9 The following prompts will ask about the tip boxes, the 96 deep well plates, the strip tubes and the reagents in the reservoirs. Click on OK for each prompt after verifying that the step has been completed. Once all the steps/prompts have been completed, the robot will start the DNA extraction procedure.

**NOTE:** Once the DNA extraction is finished, the DNA will be eluted into ~40 µL of DNA IQ™ Elution buffer, then pipette into the strip tubes at deck position B1.

2.6.12 After the DNA isolation is complete, carry the samples forward for quantitation as outlined in the Section 4, DNA Quantitation.
3 QUALITY ASSURANCE MEASURES FOR ISOLATING DNA USING THE BIOMEK® 2000 AUTOMATION WORKSTATION

3.1 For a typing result to be reported the controls must work appropriately.

3.1.1 If an allele(s) peak/band is detected at several loci in a reagent blank, all samples associated with the reagent blank (i.e., case samples extracted in conjunction with a case reagent blank or non-consumed samples extracted in conjunction with a procedure reagent blank) will be considered inconclusive, and the sample(s) will be re-amplified and/or re-extracted if possible. If a peak/faint band is observed at one or two loci, those loci may be deemed inconclusive for the associated samples, but the remainder of the loci will be reported for the evidentiary samples. Alternatively, these samples may be re-amplified and/or re-extracted.

3.1.2 Evidence samples will be loaded into the 96 deep well Marsh plate in a format such that the evidence samples will not be directly adjacent to any known samples. There will be blanks between evidence samples and known samples as indicated in Figure 1.

![Plate with Knowns and Unknowns](image)

Figure 1. Plate with knowns and unknowns.

3.1.3 When known/reference samples and evidence samples are loaded into the same 96 deep well plate, empty wells will be used to separate the known/reference samples from the evidence samples. An example of this is demonstrated in the diagram above (i.e., wells A8 through H8). Reagent blanks may be placed in the empty wells separating the two sample types.
4 DNA QUANTITATION

4.1 Plexor HY Human DNA Quantitation

4.1.1 Technical Notes

4.1.1.1 The Plexor® HY System is a real-time quantitative PCR assay for the simultaneous quantitation of total autosomal and male DNA.

4.1.1.2 Unless specifically indicated, no substitution for the plates or other consumables can be made. Do not substitute another brand because small differences in the well formation or depth of the well can cause the tips to “bottom out”. When tips “bottom out” the tips are in contact with the bottom of the well which can form a vacuum in some tips when the tool aspirates liquid and affect the accuracy of pipetting and potentially cause filter wetting.

4.1.1.3 The black labware holders are specifically designed to hold tip boxes. The gray labware holders are for plates and other items.

4.1.1.4 The Plexor® HY System assay utilizes two primers – one with a modified base (iso-dC) and a fluorescent label at the 5’ end. The second PCR primer is unlabeled. During amplification, the modified base, iso-dC, base pairs specifically with iso-dGTP, included in the reaction mixture, as it is incorporated into the complementary strand. The iso-dGTP is modified to include a quencher, dabcyl. Thus, upon extension and incorporation of the iso-dG base, quenching of the fluorescent signal is observed.

4.1.1.5 The Plexor® HY System can accurately quantitate human DNA sample in the range of ~8 pg/µL to 50 ng/µL. Quantitation values <8 pg/µL should be considered as indistinguishable from background, although values below 8 pg/µL have yielded partial profiles.

4.1.1.6 The Plexor® HY System uses primers specific to a 99 bp multi-copy target on chromosome 17 to quantitate the total amount of human DNA in a sample. A second pair of primers specific to a 133 bp multi-copy target on the Y-chromosome is used to quantitate the total amount of male DNA in a sample.

4.1.1.7 The Plexor® HY System also simultaneously amplifies an internal positive control (IPC) which can be used to assess the qPCR reaction. A synthetic DNA template is added to the PCR master mix and, thus, is present in every reaction at approximately the same quantity. As a result, a similar C_T value (± 1 C_T) is expected for the IPC in each well. If one of the samples is not within 1 C_T value of that exhibited by the standards, then that may be suggestive of the presence of a PCR inhibitor in the sample. However, lack of an indication of a PCR inhibitor by the IPC does not rule out the possibility of inhibition in subsequent amplifications or the possibility that the inhibitor is present in a concentration that is undetected by the qPCR assay, but still affects the STR amplification.

4.1.1.8 The Plexor® HY System relies upon the specific interaction between the two modified bases for quantitation. The two modified bases, isoguanine (iso-dG) and 5’-methylisocytosine (iso-dC), base pair, whereas iso-dG and the unmodified cytosine or, alternatively, iso-dC and the unmodified guanine, do not base pair. During amplification, the fluorescently-labeled primer anneals and extends, becoming template for subsequent cycles. During subsequent cycles of amplification, the iso-dC base on the fluorescently-labeled primer is paired with the iso-dG (modified with an attached quencher) in the reaction mixture. The proximity of the fluorescent label and quencher result in a decrease in fluorescent signal detected by the instrument. This process is illustrated in Figure 1.
Figure 1. PCR amplification of a target sequence using the Plexor® technology. The first step involves the primer annealing and extension. Note that one primer contains a 5’ modified base (iso-dC) and fluorescent label. Upon extension of the DNA in subsequent cycles, a second modified base (dabcyl-iso-dG) is incorporated. The proximity of the fluorescent label and quencher (dabcyl) results in a decrease in fluorescent signal, indicating accumulation of product. (Figure reproduced from the Plexor® HY System Technical Manual.)

4.1.1.9 Gloves must be worn at all times when performing the Plexor® HY System reactions because the introduction of nucleases that could occur from un-gloved handling would interfere with the reaction.

4.1.2 Equipment

- Stratagene Mx3005P™ Quantitative PCR instrument
- Biomek® 2000 Automation Workstation

4.1.3 Materials

- 96-well qPCR Plate - Greiner P/N 652260
- Optically Clear Strip Caps – Phenix Research P/N 101100-082 or Greiner P/N 373250
- Optional: Optically clear film – VWR Cat#82050-994
- Micro Amp strip tubes (in strips of 8 tubes) – ABI Cat# N801-0580
- Black PCR support base (96 well) – ABI Cat# N801-0531
- P20 Tips – aerosol resistant – Beckman Catalog # 609043
- Quarter module reservoir – Beckman Cat# 372788

4.1.4 Reagents

4.1.4.1 Plexor® HY System kit (Promega Cat# DC1000 = 800 determinations or Promega Cat# DC1001 = 200 determinations) (stored at -20 °C) which contains:

- Plexor® HY 2X Master Mix
- Plexor® HY 20X Primer/IPC Mix
- Nuclease free or Type I water
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Type I water is considered nuclease free and may be used instead of the amplification grade water that comes with the Plexor® HY kit.

4.1.4.2 Plexor® HY Genomic DNA Standard (50 ng/µL) – stored at 4 ºC after initial thawing.

NOTE: The genomic standard must be stored at 4 ºC after thawing. Freezing and thawing can affect accuracy of the standard.

4.1.5 Starting the Stratagene Mx3005P Quantitative PCR instrument by using the power button located on the back left side of the unit.

4.1.5.1 Turn on the Stratagene Mx3005P™ Quantitative PCR instrument by using the power button located on the back left side of the unit.

4.1.5.2 Turn on the computer.

4.1.5.3 Open the Mx3005P™ software “MxPro” and select “SYBR Green (with Dissociation Curve)” from the New Experiment Options window. In the same window, check the box “Turn lamp on for warm-up?” Select “OK”. The lamp requires 20 minutes to warm up before running the assay. The software can be configured and the plate set up on the BioMek® 2000 Automation Workstation while the lamp is warming up.

4.1.5.4 The software will ask “Do you wish to use SYBR Green Plate Setup from the active set “plexor”? Click “yes” to load the Plexor® HY assay default plate setup.

4.1.5.5 The software will ask “Do you wish to use SYBR Green Thermal Profile Setup from the active set “plexor”? Click “yes” to load the Plexor® HY assay default thermal profile.

4.1.6 Programming the Stratagene Mx3005P for the Thermocycling

4.1.6.1 Select the Plate Setup tab.

4.1.6.2 Select the wells that will NOT be used and choose “blank” as the well type.

4.1.6.3 Select the Thermal Profile Setup tab. The default thermocycling conditions should be programmed for the Plexor® HY assay as shown below in Figures 2 and 3.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation:</td>
<td>95°C</td>
<td>2 min</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation:</td>
<td>95°C</td>
<td>5 sec</td>
<td>35 cycles</td>
</tr>
<tr>
<td>Annealing and extension:</td>
<td>60°C</td>
<td>40 sec</td>
<td></td>
</tr>
<tr>
<td>Melt temperature curve:</td>
<td>65°C Initial temperature with 0.6°C increase each cycle, 30-sec hold each cycle</td>
<td></td>
<td>48 cycles</td>
</tr>
</tbody>
</table>

**Figure 2.** Thermocycling parameters. (Figure obtained from the Plexor® HY System Technical Manual.)
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Figure 3  Thermal Profile Setup for the Plexor® HY System assay.

4.1.6.4 If you plan to use film to seal the plate, select “Instrument” and “Set Filter Gain Settings”. In the dialog box that opens, the filter settings should be changed to:

1X for each dye except FAM, which should be 2X. If optically clear caps will be used, then use the default filter gain settings.

NOTE: The default filter gain settings are configured for the caps (1X for each dye except 2X for CO560 and 4X for FAM)

4.1.6.5 The instrument is now ready for the running the Plexor® HY System assay once the reactions are prepared in the qPCR amplification plate.

4.1.7 Starting the BIOMEK 2000 Workstation

4.1.7.1 Turn on the computer.

4.1.7.2 Turn on the BioMek® 2000 Automation Workstation using the power button located on the back left side of the unit.

4.1.8 BIOMEK® 2000 Workstation Operating Procedure

An EMERGENCY STOP BUTTON is located at the lower front area on the robot, just below the deck. If this button is pressed, the robot will abort the method it is currently running. The emergency stop only needs to be used when the robot cannot be stopped by software means or the robot could be damaged by crashing into the deck or a tool could be damaged by crashing into something on the deck. If what is desired is simply to stop the method while it is running, simply click on the “Stop” button. The method will then pause and several options will be available. The button labeled “Continue” can be used to resume the method, the button labeled “Trace” can be used to advance to the next step (this function is useful when calibrating), the button labeled “Go Up” will move the pod up and the button labeled “Quit” will terminate the method.
The Plexor® HY System setup methods for the Biomek® 2000 Automation Workstation are accessed in the same manner as the DNA IQ™ methods. The BioWorks folder is opened on the desktop by double clicking on the folder. The Plexor methods can be found in the Quantitative PCR lab book. Once the BioWorks folder is opened, the Edit icon is double-clicked to display all of the robot methods. Reagents used for the Plexor® HY System come prepared for use. It is critical for optimal performance that the reagents thaw completely and are well mixed prior to use. Unused reagents may be re-frozen at -20 ºC, except the genomic DNA standard, which MUST be stored at 4 ºC between uses.

4.1.8.1 Remove the Plexor® HY System reagents from the -20 ºC freezer and allow them to thaw prior to use. All reagents should be thawed and well mixed prior to use.

4.1.8.2 Specific methods for the Biomek® 2000 Automation Workstation are located in the BioWorks folder. Click on the BioWorks folder located on the desktop. The open folder contains a number of software program icons. To select a method, double click on the “Edit” icon. Then, either using the method drag-down window or using the open folder icon, select Open. A window will open (Figure 4) and a list of methods will be displayed (i.e., Plexor Setup, Plexor Setup 56, etc.). To setup the 96-well qPCR plate for 56 samples or fewer (not including the DNA standards), double click on the method entitled “Plexor Setup.” To setup the 96-well qPCR plate for greater than 56 samples (not including the DNA standards), double click on the method entitled “Plexor Setup 56”.

NOTE: A maximum of 80 unknown samples at a time in a 96 well thermocycle plate can be quantitated with the Plexor assay since two columns must be reserved for the standards.

Figure 4. Open Method window.

4.1.8.3 After a Plexor® method is opened, the selected method and a deck lay out window will appear. Use the deck lay out window (Figure 5) to properly place the robot tools, tip boxes and the reagent holder onto the deck.

NOTE: With the Plexor Setup method, only 1 box of P20 barrier tips is needed unless the samples (not including standard) are in more than seven columns.
NOTE: The tool holder stand is different from the one used for the DNA IQ™ methods since the Gripper tool is not needed. Instead the tool stand has slots only for pipetting tools. The tool stand and the pipetting tools are labeled indicating the “Front” orientation. The tools need to be placed in the tool stand according the deck layout window. Place the tools in the positions in the tool stand according the deck layout window. The MP20 and P20 pipetting tools are used with the Plexor® methods.

4.1.8.4 Prepare the Plexor master mix in a 1.5 mL tube as described below:

NOTE: If using the Plexor setup 56 method, it will be necessary to prepare the master mix in a 2.0 mL tube and subsequently split it into two 1.5 mL tubes with six excess reactions for each 1.5 mL tube, as described below.

- Plexor 2X Master Mix – 10 µL per reaction
- Nuclease-free (or Type I) H₂O – 7 µL per reaction
- 20X Primer/IPC Mix – 1 µL per reaction

Prepare sufficient reaction mix for the desired number of reactions, making certain that the two columns of standards (16) are included as well as an appropriate number of excess reactions (approx. 6).

4.1.8.5 Place the Plexor master mix in the 1.5mL tube into position A1 of the 24 sample Microfuge rack at robot deck position B3. If using the Plexor setup 56 method, it will be necessary to prepare the master mix in a 2.0 mL tube and split it (after preparation) into two 1.5 mL tubes as follows: Place sufficient master mix (plus the six reactions) for columns 1-6 in the 1.5mL tube in position A1 of the microfuge rack. To calculate the volume (in µL) of master mix to be placed in tube 1, add up the number of samples in the first six columns of the Biomek sheet, add six for the excess reactions, and multiply that number by 18. Place the remaining master mix for columns 7-12 (plus the six excess reactions) in the 1.5 mL tube in position A2 of the microfuge rack.

NOTE: The volume of master mix in each tube will be automatically calculated and indicated on the populatable worksheet

4.1.8.6 Type 1 H₂O is placed into a quarter module reservoir at B4 as specified in the screen prompt.

4.1.8.7 Set up the remaining deck components (i.e., tips, plates, samples, and the DNA standard) by following the directions provided by the program in green type (Figure 5).

NOTE: The DNA standard should be added to a strip tube in column 12 of the plate at deck position B1. This DNA should be placed in position A12, as indicated in the green text.

CAUTION: When manually pipetting the genomic DNA into the strip tube and master mix into the 1.5 mL tube, it is imperative to visually inspect the tubes to ensure that no bubbles were introduced. If bubbles were introduced, they must be manually removed.

4.1.8.8 Keep the PCR support base containing the extracted DNA sample strip tubes at deck position B1 for the Plexor® methods. DO NOT re-arrange the strip tubes in the PCR support base from the orientation established during the DNA IQ isolation process.

4.1.8.9 If manually extracted samples need to be quantitated using the Plexor® method, the examiner may pipette either the entire DNA sample (if sample is to be carried through Normalization Wizard and robotic PCR amplification setup) or a 10 µL portion of the DNA sample (if the sample is for quantitation only) of the DNA sample into a clean strip tube. Alternatively, a 2µL portion of the DNA sample may be added directly to the qPCR plate. GREAT CARE
4 DNA Quantitation

MUST BE TAKEN TO ENSURE THAT BUBBLES ARE NOT INTRODUCED WHEN TRANSFERRING THE SMALL ALIQUOTS OF DNA TO THE STRIP TUBES. If any bubbles are introduced, they must be manually removed or removed using a centrifuge capable of spinning the strip tubes or qPCR plate. The strip of 8 tubes should remain connected since the entire strip of tubes will be loaded into an empty column in the PCR support base along with the Biomek/DNA IQ extracted samples. The DNA samples and the positions in the strip tubes in the 96 well PCR support base will be written or typed in the appropriate positions on the Biomek® 2000 Automation Workstation/Plexor® HY System loading sheet (Excel spreadsheet, form 210-F500).

4.1.8.10 Two lines in the method (three lines in the “Plexor Setup 56” method), as indicated in Figures 5 and 6, must be edited to indicate the location of samples in the strip tubes/96-well qPCR plate. A detailed description of the changes that need to be made follows.

Figure 5. “Plexor Setup” window indicating which lines in the method must be edited to select the appropriate wells of the 96-well plate to which qPCR Master Mix and DNA should be dispensed. The black (top, darker) arrow indicates the line in the method to program to which wells master mix will be dispensed. The gray (bottom, lighter) arrow points to the line in the method where one will select the columns containing DNA and those to which DNA will be dispensed.
Figure 6  “Plexor Setup 56” Window indicating which lines in the method must be edited to select the appropriate wells of the 96-well plate to which qPCR Master Mix and DNA should be dispensed. The black (top, darker) arrows indicate the lines in the method to program to which wells master mix will be dispensed. The gray (bottom, lighter) arrow points to the line in the method where one will select the columns containing DNA and those to which DNA will be dispensed.
4.1.8.11 The wells to which qPCR master mix should be dispensed must be selected. Double click on the line “Pipette 18.00 µL from B3 to B2 using P20 – P20 Barrier”, as shown in Figure 7. Only select wells which correspond to either a DNA sample or reagent blank using the plate map or Biomek loading sheet as a guide and click “OK”. The last two columns (11 and 12) are used for the DNA standard and are selected on a separate line in the method. **DO NOT** add columns 11 and 12 to this line of the method.

![Figure 7. Window to designate the wells to which qPCR master mix should be dispensed. In the example shown here, Plexor® Master Mix will be dispensed to the first two columns as well as wells A3 and B3 in the qPCR plate.](image)

4.1.8.12 The columns to which extracted sample (DNA, reagent blanks, etc) should be dispensed must be selected. Double click on the line “Pipette 2 µL from B1 to B2 using P20 – P20 Barrier”, as shown in Figure 8. Only select columns which contain either a DNA sample or reagent blank using the plate map or Biomek loading sheet as a guide and click “OK”. The last two columns (11 and 12) are used for the DNA standard and are selected on a separate line in the method. **DO NOT** add columns 11 and 12 to this line of the method.

4.1.8.13 Click on the running man icon located on the tool bar to initiate the method. A window will appear showing a stop light with a red light. The light will turn green once communication has been established. If communication is not already established between the robot and the computer, it will take approximately a minute to establish the link. Once this occurs, the light will turn green and the window will disappear. An inset window method will ask if you accept the deck layout, click accept and the method will initiate.

4.1.8.14 Once the Plexor® method has been initiated, prompts will appear to verify that the deck layout as well as the dispensation of reagents have been properly set up. Click “OK” for each of
the prompts once the double check of the reagents has been performed. A prompt will also ask if the wells for dispensing the master mix have been selected. Click “OK” if this has been done. If not, exit the program and restart after selecting the appropriate wells, as described above. The method will run for approximately 15 minutes. Once the method is complete, the robot will emit a faint beeping sound and a window will appear with the following instructions:

- Seal the qPCR plate in position B2 with optically clear strip caps (or optically clear film).
- Place the 96 well plate in the Stratagene Mx3005P thermocycler and start run.

4.1.8.15 Remove the qPCR plate at deck position B2. Visually examine the wells to ensure that no bubbles are present. If a bubble is observed, dislodge it with a sterile pipette tip or using a plate centrifuge and seal the plate with optically clear strip caps or film.

4.1.8.16 If using the optically clear film, ensure that a secure seal is made with the plate such that no evaporation of liquid will occur. In addition, if you are using optically clear film, be sure that the filter gain settings have been adjusted in the MxPro data collection software as described in 4.1.6.4.

4.1.8.17 Cover the strip tubes containing the isolated DNA to minimize evaporation.

**NOTE:** These samples will be uncovered and used during the DNA Normalization step outlined in Chapter 5, Normalization Wizard and Amplification Set Up.

4.1.8.18 Click OK and the method will end.

4.1.8.19 The plate should be immediately taken to the thermocycler to initiate the run. If the run cannot be started immediately, the plate should be stored at room temperature away from light (such as in a drawer), to prevent potential deleterious effects on the reactions. The plate should not be stored (prior to running) for longer than two hours. Short wait times (less than two hours, at room temperature) are permissible, as the reactions utilize a hot-start polymerase. The reaction plate should be stored out of the light to prevent potential photobleaching that could negatively affect the sensitivity of the assay.

4.1.9 Stratagene Mx3005P™ Operating Procedure

4.1.9.1 Place the 96 well qPCR plate in the Stratagene Mx3005P thermocycler. Note that the plate holder in the thermocycler opens like a waffle maker. The plate should be under the black top.

4.1.9.2 The run can be initiated by selecting “Start Run”. The run should be launched immediately after the qPCR plate is placed into the instrument. The computer will prompt the user to designate a filename in which the data will be saved.

4.1.9.3 Once the run is over, remove the qPCR plate containing the Plexor® reactions from the thermocycler. It should be discarded into the waste.

4.1.10 Data Analysis

4.1.10.1 To analyze the data prior to import into the Plexor software, in the Stratagene data collection program, click “File” and “Export Instrument Data”. The export function provides a drop-down window. From that window, select “Export Instrument Data to text file” and “Type 3 – Grouped by wells”. A dialog box will appear asking for a filename to save the file. Choose the same name as was used for the Stratagene file, which is typically the date followed by the operator’s initials. This will not overwrite the Stratagene data collection file (raw data) as this will be a *.txt file.
4.1.10.2 Open the Plexor® Analysis Desktop program. Choose “File”, “Import New Run”. Type “Plexor HY” in the Assay Name box. The parameters should be as shown below in Figure 9. Click “Next>”.

**NOTE:** If the setup does not look like that shown below, click “Import” in the upper right hand corner of this box and browse to the assay setup template called “plexor.atp”.

![Figure 9. Import parameters. The instrument and data collection should be selected as shown for the Plexor® HY system amplification.](image)

**NOTE:** The order of the dyes shown in the “Data Collection” box is not important, although the boxes selected for each dye are important.

4.1.10.3 At the “Run Info” dialog box, fill in the Experiment Title with the robot run name. Also fill in the operator name. The date should be set correctly, if the computer’s date is incorrect. Notebook ID may be left blank. Notebook ID and Reagent ID fields may be left blank. Click “Next”.
4.1.10.4 At the File Import screen, click “Browse” and find the *.txt file that was generated in which the raw data was saved. Click “Run Template”. In the “Run Template” dialog box (Figure 10), click on the “Plate Setup” tab and choose “Import” and select “Plexor_run template.rtp”. Click “OK” in the bottom right hand corner of the dialog box.

Figure 10. Run Template screen for analysis of Plexor® HY System data. The standard curves and no template controls (columns 11 and 12) are indicated by the circles and diamonds, respectively.

4.1.10.5 The Plexor® Analysis settings can be imported from the file “Plexor_analysistemplate.ntp” At the File Import screen, click “Analysis Template”. In the “Analysis Template” dialog box, choose “Import” and select “Plexor_analysistemplate.ntp”. Click “Open”.

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4.1.10.6 The defaults are used for all settings, as shown in Figure 11, except the expected melt temperature. For autosomal it is approximately $T_m = 81.23 \, ^\circ C$, for Y it is approximately $T_m = 82.59 \, ^\circ C$, and for the IPC, the $T_m = 82 \, ^\circ C$ (The $T_m$ can be adjusted manually to be centered in the melt curve trough). These melt temperatures are imported when you select the Plexor® analysis template. Click “OK”.

![Figure 11. Analysis Template Window.](image_url)

After importing the analysis template, the expected melt temperature for the autosomal amplicon should be approximately 81.23 °C.

4.1.10.7 Click “Finish” to see the analyzed data.

4.1.10.8 The sample IDs can now be imported from the Biomek populatable worksheet (Excel spreadsheet, Form 210-F500). Open the Populatable Worksheet and select the “Sample Names” Tab. Copy the sample names from the “sample names” grid by selecting the appropriate wells and pressing <Ctrl-C>. Do not copy column or row headers (i.e. A, B, C...or 1, 2, 3...). In the Plexor Analysis software, click on the Sample IDs tab, and paste the sample IDs from Template by pressing <Ctrl-T>.
4.1.10.9 The current version of the software may not import the standards properly from the template. If they are not imported properly, they must be defined. Define the standard curves by highlighting the wells of the two columns of standards, but not the No Template Control wells (Figure 12). Click on the standard series definition button, indicated by the arrow in 12 and the inset window shown in Figure 12 will appear. Be certain that the most concentrated standard is defined as 25 ng/μL and that the series is a vertical series, decreasing by a factor of five. Click “Apply”.

Figure 12. Defining the standard dilution series.
4.1.10.10 In the “PCR curves” tab, click on the box in the upper left hand corner of the plate map. This will highlight the entire plate. Be sure you are in the FAM tab, for the autosomal quantitation data. Choose “Edit”, “Add Standard Curve” or click on the corresponding icon in the toolbar, as indicated in Figure 13. This creates a standard curve for the dye selected and quantitates the unknowns for that dye selection as well. Click on the tab for CO560 – Y and repeat to create a standard curve for the Y amplicon.

NOTE: No standard curve is necessary for the IPC.

Figure 13. Making the Standard Curves. After selecting the entire plate of samples by clicking in the box indicated by the gray arrow, a standard curve can be created by clicking the appropriate icon as indicated by the second (black) arrow.

4.1.10.11 Evaluate the standard curves by selecting the Standard Curves tab. Assess the curves for the autosomal and Y DNA quantitation separately by selecting each of the tabs separately (FAM – Autosomal and CO560 – Y). The linearity ($r^2$) and efficiency (eff) of both curves should be assessed. Each standard curve should have an $r^2$ value of 0.98 or above to be acceptable. Evaluate the curve generated to determine if any outlier data points need to be de-selected to improve the quality of the extrapolated curve generated. Up to 3 data points can be de-selected per curve when necessary to produce a linear standard curve.

NOTE: This includes data points in the standard curves (A11-G11 and A12-G12) that are not detected. The software does not include any undetected data points when constructing the standard curve. The de-selection of data points from either standard curve must be indicated on the report. See Appendix A for a detailed description of Plexor troubleshooting methods, including how to de-select data points from a standard curve.

The efficiency is usually 100% ± 15%.
NOTE: The baseline regions are computed and selected automatically. Should the baseline of any specific amplification plot be improperly estimated, it can be adjusted manually. Each well can be highlighted individually to inspect the amplification plot and adjust the baseline. Refer to Appendix A for direction on baseline adjustment: when it is needed and how to perform it.

4.1.10.12 A copy of either the “Forensics Report” (prepared in 4.1.10.13.1) and the “Sample Details Report” (as described in 4.1.10.13.2) or the quantitation report (Form 210-F504, prepared and printed in 4.1.10.14) should be printed for each examiner with samples in the Plexor® run. If the report does not indicate the $r^2$ value for the two standard curves, this should be added by hand and the notation initialed.

NOTE: There may be apparent discrepancies between the quantitation reports and the normalization wizard. These apparent discrepancies will be minor (usually 0.01 ng/μL or less) and originate from the rounding and truncating that each software program performs.

4.1.10.13 Generation of the Forensics Report and Sample Details Report.

4.1.10.13.1 Generation of the Forensics Report. To generate a report (“Forensics Report”) of the quantitation data, click “Forensics” and “Set Normalization and IPC parameters”. A check mark should appear near the top of the dialog box so that the report is limited to the concentration data and $C_T$ only. The default parameters are correct and should read that the autosomal dye is FAM and the Y dye is CO560. The IPC dye is CR610 and the default to flag an improper (inhibited) amplification for the IPC is 2 $C_T$s.

NOTE: If the Forensics Report and Samples Details Report will not be used, skip to 4.1.10.14 to generate the quantitation report (Form 210-F504).

4.1.10.13.1.1 The forensics report should contain the following columns: Sample Name, Location, Sample Type, [Auto], [Y], and [Auto]/[Y]. To change the columns shown, right-click on the table (not on the column headings) and choose “Change columns shown” from the drop-down menu. The columns shown are arranged by dye in the subsequent window. To rearrange the order of the columns shown on the Sample Details report, click and drag the column header horizontally to the desired location.

4.1.10.13.1.2 To print this report, select the entire table and click on the printer icon in the upper right hand corner of the window. Printing from the “File” drop down menu will only print a screenshot. If the Forensics Report does not indicate the $r^2$ value for the two standard curves, this should be added by hand and the notation initialed.

4.1.10.13.1.3 The Forensics report indicates the sample concentrations (in ng/μL) as well as the ratio of autosomal to Y DNA in a sample. This ratio of autosomal to Y DNA may be utilized by an examiner, along with other pertinent case information, to recommend Y STR typing.

NOTE: A result of “N/A” in the concentration column indicates that no DNA (either autosomal, Y, or both, depending on the location of the N/A result) was detected. “N/A” may be reported for
a sample’s ratio of autosomal/male DNA concentration if no Y DNA is detected in the sample.

4.1.10.13.2 Generation of the Sample Details Report.

4.1.10.13.2.1 In the Plexor Analysis software, navigate to the Sample Details tab. Make sure that only the correct columns are showing and in the correct order (Location, Sample ID, CR610 Ct, CO560 Conc, CO560 NS Call, FAM Conc, FAM NS Call). To change the columns shown, right-click on the table (not on the column headings) and choose “Change columns shown” from the drop-down menu. The columns shown are arranged by dye in the subsequent window. To rearrange the order of the columns shown on the Sample Details report, click and drag the column header horizontally to the desired location.

4.1.10.13.2.2 Sort the samples into column order (if desired) by clicking on the top of the “Location” column.

4.1.10.13.2.3 Highlight all of the samples and standards. Click on the printer icon in the upper right corner to print the report.

4.1.10.14 Generation of the Quantitation Report (Form 210-F504)

4.1.10.14.1 If the quantitation report (Form 210-F504) will not be printed (see 4.1.10.12), skip to step 4.1.10.15.

4.1.10.14.2 In the Plexor Analysis software, navigate to the Sample Details tab. Make sure that only the correct columns are showing and in the correct order (Location, Sample ID, CR610 Ct, CO560 Conc, CO560 NS Call, FAM Conc, FAM NS Call). To change the columns shown, right-click on the table (not on the column headings) and choose “Change columns shown” from the drop-down menu. The columns shown are arranged by dye in the subsequent window. To rearrange the order of the columns shown on the Sample Details report, click and drag the column header horizontally to the desired location.

4.1.10.14.3 Sort the samples into column order (if desired) by clicking on the top of the “Location” column.
4.1.10.14.4 Click on the icon in the upper right corner that is called the “Export Selected” icon as indicated in Figure 14.

**Figure 14.** Sample details tab of the Plexor HY reports. The arrow points to the “Export Selected” icon for exporting Plexor HY data to a tab-delimited format for preparing the report for the casefile.

4.1.10.14.5 Save the file when prompted.

4.1.10.14.6 Open Microsoft Excel. Choose File>Open and browse to the file saved in a designated folder to import the tab-delimited data into Excel.

**NOTE:** It may be necessary to change the “Files of Type:” to “All Files” for the exported data file to be observed.
4.1.10.14.7 At the prompt, choose “Finish” as shown in Figure 15.

![Figure 15](image)

Figure 15: Import of Plexor HY data into a Microsoft Excel spreadsheet. The arrow points to the “Finish” button to complete the data import.

4.1.10.14.8 On the spreadsheet, click <CTRL-p> to generate the Plexor HY report for the casefile.

4.1.10.14.9 The report may be printed via File>Print. The print settings may be altered using File>Page Setup, if desired.

4.1.10.15 The “CR610, Ct” column is a flag for whether inhibition of a sample may be observed via the IPC, as determined by whether the cycle at which the IPC crossed the threshold was within the expected result. The IPC should cross the threshold at a similar cycle number in each sample with a degree of tolerance between samples. If a given sample’s IPC Ct is not within 2 cycles of those observed in the standard curve, this may be an indication of inhibition by the sample/sample matrix. This range is typically 21.5 ± 2 cycles. Routinely, these samples are amplified for autosomal STRs regardless of whether inhibition is indicated. However, given this information, the examiner may choose to amplify a dilution of the sample to overcome the potential inhibition. In addition, if a sample is believed to contain PCR inhibitors, a Microcon® 100 may be utilized to improve typing results.

4.1.10.16 The “NS Call” column is an indication of whether non-specific amplification is potentially observed in the melt curve for the sample. Any melt observed outside of the expected melt window (81.23 ± 1.5°C for autosomal and 82.59 ± 1.5°C for Y) and of sufficient magnitude to cross the threshold will result in a “YES” in the “NS Call” column. Routinely, these samples are amplified for autosomal STRs regardless of whether non-specific amplification is indicated. If a “YES” is observed in several samples (greater than 5 that have concentrations of greater than 50 pg/μL), this could be an indication of poor reagents or thermocycling conditions. If this occurs, QC measures should be taken to ensure the instrument and reagents are functioning properly.

4.1.10.17 Saving the Plexor Analysis File. Under File, select Save As and give the Plexor® Analysis file the same filename as used for the qPCR setup and *.txt export. This will not overwrite the previous files, as it has a *.aan extension. This file should be saved to a designated folder in the computer for future reference or use. If the Normalization Wizard will be used, the quantitation data can be exported to a file (as described in 4.1.10.18) that can be imported into the Normalization Wizard.
4.1.10.18 Export of quantitation data to a file for import into the Normalization Wizard.

4.1.10.18.1 Under the “Forensics” drop-down window, select “IPC and Normalization Wizard Parameters” and make certain that the correct dyes are selected for each of the targets (e.g. FAM for autosomal) and then click OK. In the Plexor Analysis Software, choose “Forensics” and “Export Concentrations in 96 Well Plate Format” and “Export FAM – Autosomal Concentrations”. (If amplification setup based on the Y chromosome quantitation is desired, choose “Export CO560 – Y Chromosome Concentrations”). A dialog box will appear asking for a filename. Save this file with an appropriate filename on the network or if the Plexor computer is directly linked to the Biomek computer, to the appropriate designated folder on the C-drive of the Biomek robot. This data can then be accessed from the Biomek Workstation computers for the Normalization Wizard.

4.1.10.18.2 Once the data has been exported to a format recognizable by the Normalization Wizard, proceed to step 5.5 in Chapter 5 which directs the user how to import the quantitation data file into the Normalization Wizard.

4.1.10.19 While still in the Stratagene data collection software, verify that the lamp has been turned off by looking at the indicator in the bottom right hand corner of the screen. Close out the Plexor Analysis Software and Stratagene data collection software. If prompted to save the changes, click “YES”. The instrument can then be turned off.

4.1.10.20 Proceed to 4.1.11 once the quantitation data are returned to the examiners for additional testing for Y-STR typing.

4.1.11 Using Plexor® Quantitation Data for Y-STR Typing Decisions

4.1.11.1 If no male reference samples were submitted with the case in question, no Y-STR analysis will be performed. The case may be re-submitted for Y-STR testing when male reference samples are obtained.

4.1.11.2 Examine the Plexor® HY Quantitation System data during or after PowerPlex® 16 BIO or PowerPlex® 16 typing of casework samples.
4.1.11.3 Use the Decision Tree, shown in Figure 16, to determine if Y-STR typing should be performed.

**Plexor HY System DNA Quantitation Data**

- (optional)
  - Samples from known vasectomized males or samples with extreme female:male mixtures

- All samples setup for PowerPlex 16 or PowerPlex 16 BIO amplification using the Biomek Automation Workstation

- All samples run on Product Gel (if necessary) STR Typing gel or capillary electrophoresis

**Samples typed for Y-STRs**

- Probative male profile generated

- Some alleles observed but no probative male profile generated or very weak partial profile or case description indicated the possibility of a male contributor.

**No Probative male profile**

- Examined Plexor quantitation data to determine if sufficient male DNA was detected.

**No Y-STR typing**

- No autosomal and no Y-DNA detected by Plexor. **No Y-STR testing performed.**
  - Typing process may be repeated if inhibition or inaccurate quantitation is suspected or if sample is re-extracted.

**Samples typed for Y-STRs**

*Figure 16. Decision Tree for Y-STR Typing*
5 NORMALIZATION WIZARD AND AMPLIFICATION SET UP

The Normalization Wizard is designed to utilize previously generated DNA quantitation data (ng/µL) to create a customized Biomek® 2000 Automation Workstation method for the dilution of those samples to a concentration specified by the user. An Excel table containing quantitated DNA (ng/µL) data can be imported into the Normalization Wizard as part of the procedure for the generation of a customized robotic method for diluting the samples on the Biomek® 2000 Automation Workstation. An aliquot of the diluted DNA samples, programmed in by the user, is then added to PowerPlex® 16/PowerPlex® 16 BIO amplification master mix, which is aliquoted by the robot into a PCR amplification plate or PCR amplification tubes. When the entire process is complete, a set of PCR reactions will be prepared in a customized fashion and ready for amplification. Afterwards, the DNA extracts are pipetted into 1.5 mL tubes, using the 1.5 mL Transfer Method, for long term storage.

5.1 Equipment

- Biomek® 2000 Automation Workstation
- Biomek® MP200 Pipette Tool
- Biomek® MP20 Pipette Tool
- Biomek® P200L Pipette Tool
- Biomek® P20 Pipette Tool

5.2 Materials

- 2 - Black PCR support bases (96 well) – ABI Cat# N801-0531
- MicroAmp® tubes (attached caps) – USA Scientific # 1402-8100 (clear) or #1402-8108 (multi-colored)
- 96-well PCR amplification plate (TempPlate® III, half-skirted – USA Scientific #1402-9700)
- Modular reservoir full module 150 mL - Beckman Catalog # 372784
- 96 well normalization plate – Innovative Microplate Catalog # S30026
- 4 - Beckman 24 Microfuge tube holders- Beckman Catalog # 373661
- Beckman white 1.5 mL tube inserts- Beckman Catalog # 373656
- P250 Tips– no aerosol barrier - Beckman Catalog # 372655
- P250 tips – aerosol resistant - Beckman Catalog # 140505
- P20 tips – aerosol resistant - Beckman Catalog # 609043
- Microcentrifuge tubes, 1.5 mL with unattached or attached lids

5.3 Reagents

- Type I Sterile Water
- AmpliTaq™ Gold DNA polymerase
- PowerPlex® 16 System amplification kit
- PowerPlex® 16 BIO System amplification kit

5.4 Preparation for Using the Normalization Wizard

NOTE: The tools and the Biomek® robot must be wiped off with 10% bleach, followed by isopropanol prior to running the Normalization Wizard method.

5.4.1 Open the Plexor® HY data expressed in ng/µL and in an Excel format for the plate of DNA extracts of interest that will be prepared for amplification set up.

5.4.2 Open the BioWorks folder on the desktop. Click on the Lab Book Manager icon.
5 Normalization Wizard and Amplification Set Up

5.4.3 A list of the Lab Books will be displayed as shown in Figure 1. Highlight the appropriate Wizard Lab Book and click the “Set as Current Lab Book” button. Close the Lab Book Manager window.

![Lab Book Manager](image)

**Figure 1.** Lab Book Manager

5.5 Creating a Normalization Wizard Method

5.5.1 In the BioWorks folder, double click on the Normalization GI Wizard icon to initiate the program. The first box is the introduction, select “Next” to begin generating the normalization plate protocol.
5 Normalization Wizard and Amplification Set Up

5.5.2 The next dialog box to appear will be the Import Plates screen (Figure 2). Click on the Import Plates button and select the file containing the quantitation data to be imported. The Import Plates box will have “Biomek®” listed under the Plate Name, the Plexor® HY run filename will not be displayed. The lowest and the highest sample concentrations present in the opened file will be shown; select “Next” to proceed.

NOTE: Based on the Sample Details Report/Quantitation Report, if a sample has a concentration greater than 14 ng/µL (if targeting 0.625 ng total DNA in the amplification cocktail), 17.0 ng/µL (if targeting 0.75 ng), 23 ng/µL (if targeting 1.0 ng), 28 ng/µL (if targeting 1.25 ng), 34 ng/µL (if targeting 1.5 ng) or 45.3 ng/µL (if targeting 2.0 ng), that sample should be manually diluted to meet the amplification target.

![Figure 2](image.png)

**Figure 2.** Importing plates into the Normalization Wizard.
5.5.3 Change the Final Concentration and the Sample Volume to a target of 0.125 to 0.4 ng/µL for the concentration field and 8 to 10 µL for the volume field as shown in the boxes below on the Configure Options dialog screen (Figure 3); select “Next” to proceed.

5.5.4 Verify that the parameters are set as appears in the boxes below on the Configure Transfer screen (Figure 4):
NOTE: Settings may need minor adjustments to accommodate minor differences in robot workstations.

5.5.5 Uncheck the “Tip Touch” box on the Configure Transfer screen. Check the “Mix” option box and change the settings to the values shown in the Mix Values screen below (Figure 5). Select OK, then next on the Configure Transfer screen to proceed.

5.5.6 The View Plates dialog screen (Figure 6) allows the user to exclude samples or empty wells that will not be used in the procedure. Highlight the wells that need to be excluded and click on the “Exclude Well” button. The sample volume and diluent volume used can also be viewed by clicking on the circle next to the Sample Volume or Diluent Volume designation; select “Next” to proceed.

NOTE: Samples that fall below the specified range (0.125 to 0.40 ng/µL) will be shown in red.

Figure 5. Setting mix values.

Figure 6. View Plates.
5.5.7 If the desired wells have been excluded from the procedure then answer “Yes” to the next prompt (Figure 7), if changes need to be made select “No” and return to the View Plates box (Figure 6).

![Warning dialog box](image)

**Figure 7.** Exclude errors prompt

5.5.8 Verify that the strip tubes, containing the samples to be analyzed, have been uncovered, then select “OK” from the Warning dialog box (Figure 8).

![Warning dialog box](image)

**Figure 8.** Warning dialog box.
5.5.9 The Generate Method dialog screen will appear (Figure 9). The Method name will have the date and time that the normalization procedure was created (i.e., Norm04/19/2004 15:48). **DO NOT CHANGE THE METHOD NAME.** Click on the ‘Generate Method’ button to create the new normalization method to be created. This creates the customized dilution method which can be accessed in the Lab Book that is currently open. Periodically delete the specific normalization method generated after completion, using the delete function under Edit in the Bioworks folder.

![Figure 9. Generate Method](image-url)
5.5.10 Click on the “Next” button to proceed.

5.5.11 Place a check in the “Open in Excel” box on the Summary dialog screen (Figure 10), then click on the “Save Plate Maps” button. Select “Finish” to proceed.

**Figure 10.** Summary dialog box
5.5.12 If the Excel spreadsheet containing the Plate Map information doesn’t immediately come up, it can be accessed using the Start Menu. Scroll up to Documents and when the side window listing the Recent Documents opens, select the file of interest. The file will be named with the current date as shown in Figure 9. The date and operator’s initials will be written on the top header of the Summary Plate Map sheet (Figure 11).

![Summary plate map](image)

**Figure 11.** Summary plate map
5 Normalization Wizard and Amplification Set Up

5.5.13 Print the Plate Map sheet, then exit the Normalization Wizard program. Double click on the Edit button in the BioWorks folder to open the method list under the edit function. Locate and select the Normalization method generated (e.g., Norm04/19/2004 15:58) as represented in the Figure 12.

Figure 12. Normalization method

5.5.14 Use the deck layout (e.g., in Figure 12) as a guide to place the labware onto the robot deck.

5.5.14.1 Verify that the tools located in deck position A1 are in the correct order. Additionally, the P20 tool is used during the Normalization Wizard and the PCR amplification set up methods.

5.5.15 Place Beckman P20, Filtered tips in the deck position A2.

NOTE: The deck layout will have a second box of Beckman P20, Filtered tips in deck position A3. The second box of tips is used in the PCR amplification set up. It will also have a box of Beckman P250, Filtered tips at deck position A5 also used in the PCR amplification setup.

5.5.16 Place a box of Beckman P250 tips with NO barrier in deck position A4. These tips are utilized for pipetting the diluent (sterile water) only and lack of a filter barrier allows the tool to pipette larger volumes of the diluent.

NOTE: If a clean tip is not placed in the A row of the first column, the robot will automatically search the next row for an available tip.

5.5.17 Place a Full Modular Reservoir with up to 20 mL of Type 1 sterile water in deck position B3.

5.5.18 Place a 96-well normalization plate (1.1 mL Innovative Microplate) with well A1 in the upper left corner at deck position B4. This plate has a set maximum volume of 1.145 mL, which is less than the actual volume of the plate wells, but allows for displacement during mix steps.

5.5.19 In deck position B5, place the black PCR support base containing the strip tubes of extracted DNA samples. The tubes must be in the same orientation and position as indicated on the Sample Set Up For Biomek Robot / DNA Quantitation / Normalization Wizard sheet.
5.5.20 When all the tools and labware are in the proper position, click on the running man button on the menu bar to initiate the method.

5.6 Setting Up the PCR Amplification Reaction

5.6.1 Once the Normalization procedure is completed, close the Edit window and open Lab Book Manager. Change the current lab book to “PCR SETUP/TRANSFER”.

5.6.2 Exit the Lab Book Manager, double click on the Edit icon in the Bioworks folder and open the ‘PCR SETUP’ (or ‘PCR SETUP (plate)’, if amplifying in a plate) method (Figure 13 A and B). The PCR SETUP and PCR SETUP (plate) methods require some customization based on the plate of samples that are to be amplified.

Figure 13A.

Figure 13B.

Figure 13. PCR Amp Setup method. A. PCR SETUP (plate) method for use with a thermocycler plate. B. PCR SETUP method using tubes with attached caps.
5.6.3 The PCR Setup method requires an adjustment in the aspiration height for the transfer of the Master Mix to the samples in position B2 and this is dependent on the number of samples to be amplified. A screen prompt will appear once the method has been initiated, reminding the operator of this step (Figure 14).

![Figure 14](image1)

**Figure 14.** Modifications to the aspiration height for the transfer of the Master Mix.

5.6.4 This modification to the aspiration height must be made prior to initiating the method and needs to be customized for each set of samples. The line in the method which may need this modification is directly under the green line “Begin Master Mix Transfer From B1 to B2” seen in Figures 13 and 15.

5.6.5 If amplifying with PowerPlex® 16 BIO for analysis using the Hitachi FMBIO gel imaging system (or amplifying with PowerPlex® 16 in tubes), follow steps 5.6.5.1 – 5.6.5.22. If amplifying PowerPlex® 16 in a PCR amplification plate, go to 5.6.6.

![Figure 15](image2)

**Figure 15.** Editing the aspiration height for the master mix addition. An arrow points to the value which may need to be modified in order to obtain the most accurate pipetting.
5.6.5.1 Make the appropriate adjustment to the aspiration height according to how many tubes will be setup for amplification. Only the first line, highlighted in Figure 15, corresponding to the aspiration height for the PCR Master Mix into amplification tubes located in the first 96 well rack at position B2, needs to be modified.

5.6.5.2 Use the deck layout in Figure 13B as a guide to set up the labware. Remove the used box of Beckman P20, filtered tips at deck position A2 and the barrier free P250 tips at deck position A4. The box of barrier free P250 tips does not need to be discarded. Since only one tip is used for each Normalization Wizard setup, that tip can be removed manually and a clean, unused tip moved into the vacant position in the top left of the tip box, A1.

**NOTE:** If a clean tip is not placed in the A row of the first column, the robot will automatically search the next row for an available tip.

5.6.5.3 At deck positions B1 and B5 place microfuge tube racks that can hold 24-1.5 mL pre-labeled microcentrifuge tubes.

5.6.5.4 If amplifying more than 72 samples, the robot will pause and beep indicating that the used box of Beckman P20, filtered tips need to be discarded and replaced with a new, unused box of Beckman P20, filtered tips.

5.6.5.5 Verify that a box of Beckman P250, filtered tips has been placed in deck position A5.

5.6.5.6 Prepare the PCR master mix just prior to dispensation into the PCR amplification tubes. This can be done while the robot is mixing the diluted DNA samples in the Normalization plate.

5.6.5.6.1 If prepared prior to initiating the method, vigorously mix the PCR master mix just prior to the robot pipetting the master mix into the PCR amplification tubes.

5.6.5.6.2 Count the number of samples that need to be amplified and add an approximately 15 additional if greater than 56 samples will be amplified. Add approximately 9 additional if less than 56 samples will be amplified. Include master mix volume for the positive and negative controls. Only wells containing samples, controls, or reagent blanks should be included in the sample count since PCR master mix will be added to these wells.

5.6.5.6.3 Multiples of the positive and negative controls may be prepared for each set of samples to be amplified. At a minimum, 1 positive and 1 negative control must be run.
5.6.5.7 Double click on the “Mix 60 µL at B4 . . . .” line to open the window shown in Figure 16. On the Mix at Location screen, highlight the number of columns that need to be mixed for the diluted DNA samples in the Normalization plate. Since the MP200 tool is used, all wells in those columns will be mixed, including the empty ones. Click OK when finished.

Figure 16. Designating columns of diluted DNA samples to mix.

5.6.5.8 For the PCR master mix dispensing steps, the 0.2 mL attached cap amplification tubes must be pre-labeled. Computer generated labels can be placed on all 0.2 mL amplification tubes by the reporting examiner or their designee prior to the PCR amplification set up.
5.6.5.9 Since 0.2 mL PCR tubes with attached caps are used, the pattern of samples will be extended so that each column of PCR tubes is separated by a blank column in order to accommodate the caps (Figure 17A, represents samples 1 - 48 and Figure 17B, represents samples 49 – 88, plus positive and negative amplification controls or just the positive and negative amplification controls if 48 or less samples have been amplified). The pattern of the samples will be maintained. The 11th column in the second PCR plate, will be designated for the setup of the positive and negative controls.

**NOTE:** PCR tubes SHOULD NOT be placed into wells that DO NOT contain a sample or reagent blank.

**NOTE:** The uncapped PCR tubes should be labeled ahead of time and placed into the black PCR support base. The uncapped tubes should be covered to prevent anything from falling into the tubes while stored in the support base off of the deck.

![Figure 17A. Window for pipetting from B1 to B2](image-url)
5 Normalization Wizard and Amplification Set Up

5.6.5.10 Double click on the black line “Pipette 7.5 µL from B1 to B2 using P20 repeat – P20 barrier” to open the window shown in Figure 17A. This line is just below the green line stating “Begin Master Mix Transfer”. Double click on the “Pipette 7.5 µL from B1 to B3....” to open the window shown in Figure 17B.

5.6.5.11 The Pipette Transfer windows will open as shown in Figures 17A and 17B. The window displays the settings for the aspiration from the 1.5 mL microfuge tube containing the PCR master mix shown in plate position A1 on the left side of the window, known as the “Source Labware”. The window also displays the dispense settings of the master mix into the PCR strip tubes for the amplification on the right side of the window, known as the “Destination Labware”.

NOTE: Aspiration and dispense heights may need to be adjusted for different Biomek robots.

5.6.5.12 Using the Biomek and Plexor (for manually extracted samples) loading worksheet as a guide for the samples and reagent blanks, highlight the wells on the right, lower portion of the Pipette Transfer window (i.e., 96 well grid) which contain samples and reagent blanks. In addition, highlight the wells in the 11th column of the second plate where the positive and negative controls are to be pipetted. The Biomek robot will skip the non-highlighted tubes/wells. Click OK for the settings in the windows shown in Figures 17A and 17B when finished.

NOTE: This specific well designation needs only to be performed at the PCR master mix transfer step and when the specific tubes are designated for dispensing positive and negative controls. A single channel pipette tool will be used to dispense the master mix into the tubes/wells that have been designated. A multi-channel pipette tool is
used to transfer the diluted DNA sample to the PCR tube. Only air will be transferred to wells that contain no sample or reagent blank.

5.6.5.13 Place a 24 well Microfuge holder in deck position B1 and B5 containing white inserts that will hold 1.5 mL tubes firmly in place. Place the capped 1.5 mL centrifuge tube containing master mix in deck position A1 of the Microfuge holder at deck position B1. Place a 1.5 mL tube containing the appropriately diluted positive control (9947A) into deck position A1 of the Microfuge holder at deck position B5. Place extra diluted positive control (approximately 10 additional µLs) into the tube to ensure there is a sufficient quantity. Place a 1.5 mL tube containing approximately 50 µL of the negative control (sterile Type I water) into deck position A3 of the Microfuge holder at deck position B5. The operator will be given a prompt when to uncap the lids.

5.6.5.14 As shown in Figures 18A and 18B, double click on the method line stating “Pipette 5 µL from B4 to B2 using MP20-P20 Barrier” that appears just below the line in green stating “Begin DNA transfer from B4 to B2”. The columns of DNA to be transferred must be selected under Source labware and using the Pipette Transfer window that pops up. Under the Destination labware, select the alternating columns of PCR tubes to which the DNA needs to be transferred. The software is capable of directing the robot to function in sequential order (e.g., transferring the 2nd column of normalized DNA in the Normalization plate to the 3rd column containing the PCR tubes). Normalized DNA columns 1-6 may be transferred to the first 96 well PCR support base containing 6 alternating columns of amplification tubes. Click OK when finished.

5.6.5.15 Double click on the line just below the line described above “Pipette 5 µL from B4 to B3 using MP20-P20 Barrier” to transfer the remaining columns of normalized DNA also using the alternating columns of amp tubes in the Destination labware setting. Do not select the positive and negative control column. Click OK when finished. If amplifying fewer than 7 columns of samples this step requires that you select a transfer of an empty column in the wizard plate to an empty column in the PCR rack.

Figure 18A. Transferring the normalized DNA samples to the amplification tubes.
5 Normalization Wizard and Amplification Set Up

Figure 18B.
5.6.5.16 In order to instruct the robot into which tubes to pipette the positive control, double click on the line “Pipette 5 µL from B5 to B3 using MP20-P20 Barrier” that appears below the green line “Transfer positive and negative controls” (Figure 19). This step will only need to be customized for the Destination Labware to accommodate the number of tubes to amplify positive control. Highlight those tubes into which the positive control will be pipetted. Click OK when finished.

Figure 19. Transferring the positive control.
5.6.17 Double click on the second line that reads, “Pipette 5.00 µL from B5 to B3 using P20 1-P20 Barrier” in order to define the transfer of the negative controls (Figure 20). This step will only need to be customized for the Destination Labware to accommodate the number of tubes to amplify negative control. Highlight those tubes into which the negative control will be pipetted. Click OK when finished.

Figure 20. Transferring the negative control.

5.6.18 Once the labware is in place, click the running man button on the menu bar to initiate the method.

5.6.19 The program will initially mix all the normalized DNA samples, then once completed, the operator will be prompted to uncap the tubes containing the master mix (in position A1 of the 24 Microfuge holder at deck position B1), the positive control (in position A1 of the 24 Microfuge holder at deck position B5) and the negative control (in position A3 of the 24 Microfuge holder at deck position B5). Additionally, the operator will be prompted to check for bubbles in the normalization plate. The operator will also be prompted to place the black PCR support base onto the deck, which contains the uncapped PCR tubes that were pre-labeled by the examiner or the designee. The black PCR support bases will now serve as a thermal cycler rack for carrying the amplification tubes into the post-amplification room. Select ‘OK’ when this has been completed.

NOTE: Remove the cover from labeled PCR tubes before placing the support base(s) onto the robot deck.

5.6.20 The master mix will be aliquoted to the individual PCR amplification tubes by the robot and then DNA sample added to the PCR tubes containing the master mix. The process takes approximately 30 minutes for 80 samples.
5 Normalization Wizard and Amplification Set Up

5.6.5.21 Once the procedure has completed, close the attached caps of the PCR tubes containing samples and the positive and negative controls.

5.6.5.22 The entire rack of PCR-prepared samples is ready for transfer to the post-amplification room and for placement into the thermal cycler.

5.6.6 If amplifying with PowerPlex® 16 and in a thermocycler plate, use the PCR SETUP (plate) method.

5.6.6.1 Use the deck layout in Figure 13A as a guide to set up the labware. Remove the used box of Beckman P20, filtered tips at deck position A2 and the barrier free P250 tips at deck position A4. The box of barrier free P250 tips does not need to be discarded. Since only one tip is used for each Normalization Wizard setup, that tip can be removed manually and a clean, unused tip moved into the vacant position in the top left of the tip box, A1.

**NOTE:** If a clean tip is not placed in the A row of the first column, the robot will automatically search the next row for an available tip.

5.6.6.2 At deck positions B1 and B5 place microfuge tube racks that can hold 24-1.5 mL pre-labeled microcentrifuge tubes.

5.6.6.3 If amplifying more than 72 samples, the robot will pause and beep indicating that the used box of Beckman P20, filtered tips need to be discarded and replaced with a new, unused box of Beckman P20, filtered tips.

5.6.6.4 Verify that a box of Beckman P250, filtered tips has been placed in deck position A5.

5.6.6.5 Prepare the PCR master mix just prior to dispensation into the PCR amplification plate. This can be done while the robot is mixing the diluted DNA samples in the Normalization plate.

5.6.6.5.1 If prepared prior to initiating the method, vigorously mix the PCR master mix just prior to the robot pipetting the master mix into the PCR amplification plate.

5.6.6.5.2 Count the number of samples that need to be amplified and add approximately 15 additional if greater than 56 samples will be amplified. Add approximately 9 additional if less than 56 samples will be amplified. Include master mix volume for the positive and negative controls. Blank wells should NOT be included in the sample count since these wells will be excluded from the addition of PCR master mix.

5.6.6.5.3 Multiples of the positive and negative controls may be prepared for each set of samples to be amplified. At a minimum, 1 positive and 1 negative must be prepared with each run.
Double click on the “Mix 60 µL at B4 . . .” line to open the window shown in Figure 21. On the Mix at Location screen, highlight the number of columns that need to be mixed for the diluted DNA samples in the Normalization plate. Since the MP200 tool is used, all wells in those columns will be mixed, including the empty ones. Click OK when finished.

Figure 21. Designating columns of diluted DNA samples to mix.

For the PCR master mix dispensing steps, the PCR amplification plate must be pre-labeled. This can be accomplished using two computer generated labels (corresponding to the plate name which is typically the date followed by the examiners initials included in the run) which will be placed on the amplification plate by the reporting examiner or their designee prior to the PCR amplification set up. Alternatively, the plate name may be hand written using a permanent marker on the plate.

The labeled PCR amplification plate should be placed into deck position B2.

**NOTE:** Aspiration and dispense heights may need to be adjusted for each individual Biomek and adjustments may need to be made after service or repair of the instrument.
Double click on the black line “Pipette 7.5 µL from B1 to B2 using P20 repeat – P20 barrier” to open the window shown in Figure 22. This line is just below the green line stating “Begin Master Mix Transfer”.

The Pipette Transfer windows will open as shown in Figure 22. The window displays the settings for the aspiration from the 1.5 mL microfuge tube containing the PCR master mix shown in plate position A1 on the left side of the window, known as the “Source Labware”. The window also displays the dispense settings of the master mix into the PCR plate for the amplification on the right side of the window, known as the “Destination Labware”.

NOTE: Aspiration and dispense heights may need to be adjusted for different Biomek robots.

Using the Biomek/Plexor loading worksheet as a guide for the samples and reagent blanks, highlight the wells on the right, lower portion of the Pipette Transfer window (i.e., 96 well grid) which contain samples and reagent blanks. In addition, highlight the wells in the 12th column of the plate where the positive and negative controls are to be pipetted. The Biomek robot will skip the non-highlighted wells. Click OK for the settings in the windows shown in Figure 22 when finished.

NOTE: This specific well designation needs only to be performed at the PCR master mix transfer step and when the specific wells are designated for dispensing positive and negative controls. A single channel pipette tool will be used to dispense the master mix into the wells that have been designated. A multi-channel pipette tool is used to transfer the diluted DNA sample to the PCR plate. Only air will be transferred to wells that contain no sample/reagent blank/plate blank.

Place a 24 well Microfuge holder in deck position B1 and B5 containing white inserts that will hold 1.5 mL tubes firmly in place. Place the capped 1.5 mL centrifuge tube containing master mix in position A1 of the Microfuge holder at deck position B1. Place a 1.5 mL tube containing the appropriately diluted positive control (9947A) into position A1 of the Microfuge holder at deck position B5. Place extra diluted positive control (approximately 10
additional µLs) into the tube to insure there is a sufficient quantity. Place a 1.5 mL tube containing approximately 50 µL of the negative control (sterile Type I water) into position A3 of the Microfuge holder at deck position B5. The operator will be given a prompt when to uncap the lids.

5.6.6.13 As shown in Figure 23, double click on the method line stating “Pipette 5 µL from B4 to B2 using MP20-P20 Barrier” that appears just below the line in green stating “Begin DNA transfer from B4 to B2”. The columns of DNA to be transferred must be selected under Source labware and using the Pipette Transfer window that pops up. Under the Destination labware, select the columns of the wells to which the DNA needs to be transferred. All columns containing sample DNA are indicated at this step. Click OK when finished. Do not select the positive and negative control column.

Figure 23. Transferring the normalized DNA samples to the amplification plate.
5.6.6.14 In order to instruct the robot into which wells to pipette the positive control, double click on the line “Pipette 5 µL from B5 to B2 using P20 1 - P20 Barrier” that appears below the green line “Transfer positive and negative controls” (Figure 24). This step will only need to be customized for the Destination Labware to accommodate the number of wells to amplify positive control. Highlight those wells into which the positive control will be pipetted. Click OK when finished.

Figure 24. Transferring the positive control.
5 Normalization Wizard and Amplification Set Up

5.6.15 Double click on the second line that reads, “Pipette 5.00 µL from B5 to B2 using P20 1- P20 Barrier” in order to define the transfer of the negative controls (Figure 25). This step will only need to be customized for the Destination Labware to accommodate the number of wells to amplify negative control. Highlight those wells into which the negative control will be pipetted. Click OK when finished.

![Image of a computer interface with a diagram of a robot deck and DNA samples]

**Figure 25.** Transferring the negative control.

5.6.16 Once the labware is in place, click the running man button on the menu bar to initiate the method.

5.6.17 The program will initially mix all the normalized DNA samples, then, once completed, the operator will be prompted to uncap the tubes containing the master mix (in position A1 of the 24 Microfuge holder at deck position B1), the positive control (in position A1 of the 24 Microfuge holder at deck position B5) and the negative control (in position A3 of the 24 Microfuge holder at deck position B5). The operator will also be prompted to place the black PCR support base onto the deck, which contains the uncovered PCR plate that was pre-labeled by the examiner or the designee. Select ‘OK’ when this has been completed.

**NOTE:** Remove the cover from labeled PCR plate before placing the support base onto the robot deck.

5.6.18 The master mix will be aliquoted to the individual PCR amplification wells by the robot and then DNA sample added to the PCR wells containing the master mix. The process takes approximately 30 minutes for 80 samples.
5.6.6.19 Once the procedure has completed, place a foil sealing film on the plate. Ensure that the film is fully sealed in order to prevent evaporation of the PCR amplification reaction components during thermal cycling.

5.6.6.20 The samples are ready for transfer to the post-amplification room and for placement into the thermal cycler. A compression pad (ABI P/N 4312639 or similar) must be added on top of the sealing foil prior to placing the thermocycler lid over the samples and tightening.
6 TRANSFER OF EXTRACTED DNA FOR PERMANENT STORAGE

This is a transfer method designed to transfer DNA extracts from the PCR strip tubes into pre-labeled 1.5 mL tubes. Since sample order (e.g. sample #44 loaded into well A3) is maintained throughout the process (loading into the Biomek deep well plate for extraction, DNA quantitation and the Normalization Wizard), that order and separation between one examiner’s set of samples and another’s is maintained for the transfer method. Each 24 Microfuge tube holder holds 24-1.5 mL tubes (no lids), so in order to accommodate a plate of 80 DNA extracts, four Microfuge tube holders are placed onto the deck and the pattern of sample loading maintained.

6.1 Equipment

- Biomek® 2000 Automation Workstation

6.2 Materials

- Microcentrifuge tubes, 1.5 mL
- Black PCR support base (96 well) – ABI Cat# N801-0531
- P250 Tips (aerosol resistant) – Beckman Cat# 140505
- Gloves
- Tough tag labels for 1.5 mL tubes

6.3 Starting the BIOMEK® 2000 Workstation

6.3.13 Turn on the computer

6.3.14 Turn on the Biomek® 2000 Automation Workstation using the power button located on the back left side of the unit.

6.3.15 If the robot has been previous use and is still on, simply open the BioWorks folder on the desktop by double-clicking on the icon.

6.4 BIOMEK® 2000 Workstation Operating Procedure

An EMERGENCY STOP BUTTON is located at the lower front area on the robot, just below the deck. If this button is pressed, the robot will abort the method it is currently running. The emergency stop only needs to be used when the robot could be damaged by crashing into the deck or a tool could be damaged by crashing into something on the deck. If what is desired is simply to stop the method while it is running, simply click on the “Stop” button. The method will then pause and several options will be available. The button labeled “Continue” can be used to resume the method, the button labeled “Trace” can be used to advance to the next step (this function is useful when calibrating), the button labeled “Go Up” will move the pod up and the button labeled “Quit” will terminate the method.
6.4.13  Specific methods for the Biomek® 2000 Automation Workstation are located in the BioWorks folder. Click on the BioWorks folder located on the desktop. The open folder contains a number of software program icons. To select a method, double click on the “Edit” icon. Then, either using the method drag-down window or using the open folder icon, select Open. The following window will open (Figure 1) and a list of methods will be displayed (i.e., 1.5 mL transfer method). Double click on the “Transfer to 1.5 mL Tubes” method to open or highlight the method and then click on the Open button.

Figure 1. Open Method window
6.4.14 Once the 1.5 mL method is open, the deck configuration will be visible (Figure 2). The robot method will transfer, one at a time using the P200L tool, each DNA extract located in the strip tubes at deck position B2. The 1.5 mL tubes must be labeled prior to placement into the 24 Microfuge tube rack and they must be in the same order as the original Biomek loading sheet. The method is designed to transfer 80 samples every time. If, for example, a 40 sample DNA IQ method was used to generate the DNA extracts; then 1.5 mL tubes need to be placed only into the 40 wells of the 24 Microfuge racks associated with the samples to collect the extracted DNA (Figure 3).

6.4.15 Once the deck has been setup properly, click on the running man icon to initiate the method.

![Deck configuration for the 1.5 mL transfer method.](image)
6.4.16 Once the method has finished (approximately 25 minutes), caps must be placed onto the 1.5 mL tubes.

**Figure 3.** Placement of 1.5 mL tubes in the 24 Microfuge racks for transfer of DNA extracts for a 40 sample DNA IQ™ extraction.
Appendix A – Troubleshooting

1 GENERAL BIOMEK® 2000 AUTOMATION WORKSTATION

1.1 Biomek® 2000 Base Unit

An emergency stop button is located at the lower front area on the robot, just below the deck. If this button is pressed, the robot will abort the method it is currently running. The emergency stop only needs to be used when the robot could be damaged by crashing into the deck, a tool could be damaged by crashing into something on the deck or if you are unable to stop the robot. If the emergency stop button is used, the robot must be re-booted. If what is desired is simply to stop the method while it is running, simply click on the “Stop” button. The method will then pause and several options will be available. The button labeled “Continue” can be clicked to resume the method, the button labeled “Trace” can be clicked to advance to the next step (this function is useful when calibrating), the button labeled “Go Up” will move the pod up and the button labeled “Quit” will terminate the method.

1.2 Shaker

While in most instances, the Teleshake shaker is hardwired into Com Port 2 of the computer and no further action is needed to insure that the shaker will perform upon computer command, the connection of the Teleshake shaker to the computer should always be checked prior to use and will need to be reconnected whenever the computer is turned off. Refer to step 2.6.1 of the BIOMEK® 2000 AUTOMATION WORKSTATION Procedures Manual for proper connection instructions.

1.3 Pipette Tools

The seal between the pipette tool and the corresponding tips may not be perfect and this can result in inconsistent pipetting, which is most noticeable with the multi-channel pipette tool. Often, the pipettor probes simply need to be wiped off with a KimWipe and 95% Ethanol or Isopropyl alcohol. If no improvement in the pipetting consistency is observed, then the pipette tool may need to be serviced as this can be an indication that the quad rings need to be changed.

2 Plexor Data Analysis

2.1 Deselecting outlier data points from a standard curve

2.1.1 Evaluate the standard curves by selecting the Standard Curves tab. You will need to assess the curves for the autosomal and Y DNA quantitation separately by selecting each of the tabs (FAM – Autosomal and CO560 – Y). Each standard curve should have an $r^2$ value of 0.98 or above to be acceptable. Evaluate the curve generated to determine if any outlier data points need to be removed to improve the quality of the extrapolated curve generated. Up to 3 data points for each standard dilution series can be deselected when necessary to produce a linear standard curve. This total includes those data points that may be automatically deselected by the software which can occur when no CT value is detected for the standard. The de-selection of data points from either standard curve must be indicated on the report. Only a Project Coordinator may approve the de-selection of data points.

2.1.2 De-selection of data points from the standard curve does not result in deletion of the data. The data for those wells are still reported on the printed reports. De-selection of the wells simply results in not using that data for the generation of the standard curve, from which concentrations of the unknowns are estimated.

2.1.3 Standard Samples can be selected/de-selected for the generation of each standard curve, the autosomal and the Y, independently. That is, if a data point is an obvious outlier in the
standard curve for the autosomal target, this well may be de-selected for the generation of the autosomal standard curve. If the sample is not an outlier for the Y standard curve, it may be included in the generation of the Y standard curve.

2.1.4 In the generation of a standard curve, all samples and standards are selected by clicking on the box indicated by the black arrow, as shown in Figure 3. Note that this selection is specific to the dye selected via the tab above the data.

![Figure 3](image-url)

**Figure 3.** Making the Standard Curves. After selecting the entire plate of samples by clicking in the box indicated by the black arrow, a standard curve can be created by clicking the appropriate icon as indicated by the second (gray) arrow.
APPENDIX A – Troubleshooting

2.1.5 If after reviewing the standard curve and the $r^2$ value is lower than is acceptable, an examination of the curve may reveal what or which data points significantly deviate from the extrapolated curve. These data points can be identified by moving the mouse (arrow) over the outlier data point, which then displays the well location of the data point, as shown in Figure 4. This or these data point(s) are the points which should be de-selected to improve the standard curve.

![Figure 4. Standard Curve. The well location of the outlier data point is displayed (as shown) by placing the mouse (arrow) over the data point in question.](image)

2.1.6 Samples are deselected by holding down the <Ctrl> key on the keyboard while using the mouse to click on the sample of interest. This sample is then no longer highlighted.

2.1.7 The standard curve can then be generated in the usual manner, by clicking the icon shown below:

![Image](image)

2.1.8 If a standard curve was already generated for that dye, the software will prompt the user that the generation of a new standard curve will replace the existing standard curve. If this is desired, click “OK”.

2.1.9 If de-selection of data points on the other standard curve is necessary, be sure to select the other tab (as shown in Figure 3) and repeat the above steps.

2.1.10 The de-selection of data points must be indicated on the Plexor report(s) and initialed.

2.2 Adjusting a Sample’s Baseline to More Optimal Values

2.2.1 The baseline regions are computed and selected automatically by the Plexor Analysis Software. Should the baseline of any specific amplification plot be improperly estimated, it can be adjusted manually. Each well can be highlighted individually to inspect the
amplification plot and adjust the baseline. The need to manually adjust the baseline for a sample or standard should arise rarely if ever.

2.2.2 The baseline of individual samples should be adjusted, if needed. First, view the amplification plots for the entire plate, be sure you have selected the “PCR Curves” tab, then click on the empty box at the upper left hand corner of the sample plate. Choose “Edit”, “Display and manually adjust baseline regions” and click “OK” in the dialog box that appears.

2.2.3 The baseline should be set in a flat region of the amplification curve before the decrease in signal, which is indicative of accumulation of PCR product. Manual adjustment of the baseline region for each sample is possible, although rarely necessary. You can alternate between each dye to view the various amplification plots for each well. An improperly set baseline region can result in a skewed amplification plot, such as that shown in Figure 5.

![Figure 5. Improperly-set baseline region for an individual sample.](image1)

2.2.4 To correct the baseline region of a sample, if needed, each well can be highlighted individually. Choose “Edit”, “Display and manually adjust baseline regions” and click “OK” in the dialog box that appears. The baseline can then be adjusted by dragging the lower and upper limits. The upper limit should be approximately 5 cycles before the decrease in fluorescent signal in an area where the signal is flat. The lower limit is usually set to a region that creates a flat baseline, resulting in a baseline region of approximately 6 cycles or more. Figure 6 shows an amplification plot with an appropriately-selected baseline region. Note that the software automatically recalculates the estimated DNA concentrations and standard curves affected by the altered baseline region.

![Figure 6. Amplification plot from same sample as in Figure 5, but showing a properly-selected baseline region.](image2)
Appendix B - Reagents

1. This appendix describes the preparation of reagents necessary for the DNA analysis. For each reagent listed, the company and catalog number is included. As a reagent is prepared, it will be labeled to include the following information:

   Identity
   Concentration
   Lot number
   Date of preparation
   Initials of preparer
   Date of expiration

   and if appropriate:

   Date of autoclaving
   Storage requirements

2. All reagents will be prepared with Type I water, unless otherwise stated.

3. All chemicals and reagents will be stored according to the manufacturers' specifications. All chemicals containing biologicals will be disposed of in biohazard bags. Waste organic reagents will be placed in designated waste containers in a hood and removed from the laboratory by an appropriate means of disposal. All other reagents can be disposed of in the laboratory sink.

4. Any changes in chemical supply companies will be carefully checked by the Section Supervisor to ensure the chemical being provided meets the specifications necessary for the reagent. Any changes in chemical supply companies will be brought to the Biology Program Manager’s attention so the list can be updated as necessary.

5. Concentrations preceding reagent components reflect the final concentrations of that specific component in the resulting mixture.

BONE DIGEST BUFFER [50 mM Tris (7.5), 100 mM NaCl, 250 mM EDTA (8.0), 2% Sarkosyl]

Expiration date: Twelve months from the date of preparation

100 mL

5 mL of 1 M Tris (7.5)
2 mL of 5 M NaCl
50 mL of 0.5 M EDTA (8.0)
10 mL of 20% Sarkosyl
33 mL Sterile Type I water

Mix reagents together and sterilize using a filter apparatus.

CALCIUM CHLORIDE, 1M

Expiration date: Twelve months from the date of preparation

MW          110.9          100 mL
CaCl₂
Type I Water  11.09 g   90 mL

Dissolve the appropriate amount of CaCl₂ into Type I Water. Bring up to final volume
CaCl₂ - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number C1016, 100 g bottle.

CaCl₂ BUFFER 1X

Expiration date: Twelve months from the date of preparation

100 mL
5 mL of 1M Tris (8.0)
1 mL of CaCl₂ (1M)
94 mL of Sterile Type I water

Mix reagents together and filter sterilize.

DITHIOTHREITOL, 0.39 M (DTT)

Expiration date: Twelve months from date of preparation

MW  25 mL

DTT  154.2  1.50 g
Type I Water  15 mL

Add DTT to Sterile Type I Water and mix well. When DTT is completely dissolved, bring up to final volume with Sterile Type I Water. Filter sterilize and divide into 500 µL aliquots in sterile microcentrifuge tubes and store at -20 ºC.

DTT (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number D0632, 25 g bottle.

DNA IQ™ SYSTEM

Expiration date: Manufacturer's expiration date

Purchased from Promega Corporation, Madison, WI., Catalog Number DC6700 for 400 reaction kit.

Store all DNA IQ™ Isolation System reagents at room temperature.

Kit components included (expiration date same as kit unless specified):

- DNA IQ™ LYSIS BUFFER
  
  Expiration date: One month from the date when DTT is added
  
  Add 2.5 µL of 0.39 M DTT for every 100 µL of DNA IQ™ Lysis Buffer that is prepared. Mark and date label to record the addition of DTT. This solution can be stored at room temperature for up to a month if capped tightly.

- DNA IQ™ ELUTION BUFFER
- DNA IQ™ WASH BUFFER

Add 35 mL of 95% ethanol and 35 mL of Isopropyl Alcohol to 2X Wash Buffer. Be certain to accurately measure the alcohol volumes since this could negatively impact the performance of the robotic extraction. Mix contents and store at room temperature in a tightly capped container.
ETHYLENEDIAMINETETRAACETIC ACID (EDTA), 0.5 M

Expiration date: Twelve months from date of preparation

<table>
<thead>
<tr>
<th>EW</th>
<th>500 mL</th>
<th>1L</th>
<th>2 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>372.2</td>
<td>93.05 g</td>
<td>186.1 g</td>
</tr>
<tr>
<td>Type I Water</td>
<td>375 mL</td>
<td>750 mL</td>
<td>1.5 L</td>
</tr>
</tbody>
</table>

Add EDTA to Type I Water. Mix well and pH to 8.0 with 10 N NaOH (EDTA will not go into solution unless pH = 8.0). When totally dissolved, bring up to final volume with Type I Water and recheck pH. Dispense into appropriate container and autoclave at 215°F at 20 lb for 20 minutes.

EDTA (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number E 5134, 500 g bottle.

EDTA (Disodium Salt) purchased from Invitrogen, Carlsbad, CA, Catalog number 15575-028, 500 g bottle.

ETHANOL, 95%

Expiration date: Dispose of when necessary

Purchased as Reagent Alcohol (190 Proof) from VWR, Bridgeport, NJ, Catalog number 6590-1, 4 L bottle.

PCR DIGESTION BUFFER, 1.0%

Expiration date: Twelve months from date of preparation

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>100 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 M Tris</td>
<td>1 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 M EDTA</td>
<td>2 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>0.29 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% SDS</td>
<td>5 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I Water</td>
<td>91 mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix all reagents together and pH to 7.5 using dilute HCL. Adjust to final volume with Type I Water and store at room temperature.

PLEXOR® HY SYSTEM

Expiration date: Manufacturer’s expiration date.

Purchased from Promega Corporation, Madison, WI., Catalog Number DC1001 for 200 reaction kit (or DC1000 for 800 reaction kit).

Store all Plexor® HY System reagents at -20 °C upon receipt. After first use, the DNA standard must be stored at 4 °C, and all other kit components stored at -20 °C.

Kit components included (expiration date same as kit unless specified):

- PLEXOR® HY 2X MASTER MIX
- PLEXOR® HY 20X PRIMER/IPC MIX
- PLEXOR® HY MALE GENOMIC DNA STANDARD, 50 ng/µL
  - Diluted DNA standard should be freshly prepared each day of use.
- H₂O, AMPLIFICATION GRADE
APPENDIX B – Reagents

PROTEINASE K ENZYME (20 mg/mL)

Expiration date: Twelve months from date of preparation

<table>
<thead>
<tr>
<th>Stock</th>
<th>5 mL</th>
<th>15 mL</th>
<th>25 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase K</td>
<td>100 mg</td>
<td>300 mg</td>
<td>500 mg</td>
</tr>
<tr>
<td>Sterile Type I Water</td>
<td>5 mL</td>
<td>15 mL</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Add lyophilized Proteinase K to appropriate amounts of sterile Type I Water. When completely reconstituted, divide into 250 μL aliquots in microcentrifuge tubes and store at -20 ºC. Thaw at room temperature prior to use and keep on ice once thawed.

Purchased from Invitrogen, Carlsbad, CA., Catalog number 25530-031, 1.0 g bottle. Store dry at 2-5 ºC. or Purchased from Sigma Chemical CO., St. Louis, MO, Catalog number P 2308, 100 mg bottle.

SARKOSYL, 20%

Expiration date: Twelve months from date of preparation

<table>
<thead>
<tr>
<th>MW</th>
<th>250 mL</th>
<th>500 mL</th>
<th>2 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Lauroylsarcine</td>
<td>293.4</td>
<td>50 g</td>
<td>100 g</td>
</tr>
<tr>
<td>Type I Water</td>
<td>200 mL</td>
<td>400 mL</td>
<td>1600 mL</td>
</tr>
</tbody>
</table>

Add the appropriate amount of N-Lauroylsarcine to Type I Water and mix until completely dissolved and the solution is clear. Bring up to volume with Type I Water, filter sterilize and store in sterile bottles at room temperature.

N-Lauroylsarcine, Sodium Salt - Purchased from Sigma Chemical Co., St, Louis, MO., Catalog number L 5125, 500 g bottle.

SODIUM CHLORIDE (NaCl), 5 M

Expiration date: Twelve months from date of preparation

<table>
<thead>
<tr>
<th>MW</th>
<th>500 mL</th>
<th>1 L</th>
<th>4 L</th>
<th>16 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>58.44</td>
<td>146.1 g</td>
<td>292.2 g</td>
<td>1168.8 g</td>
</tr>
<tr>
<td>Type I Water</td>
<td>500 mL</td>
<td>1 L</td>
<td>4 L</td>
<td>16 L</td>
</tr>
</tbody>
</table>

Begin with 60-75% of the total volume of Type I Water in a beaker on a stir plate and add the NaCl slowly. Next add almost all of the required volume of Type I Water because it will be needed in order for the NaCl to go into solution. When the NaCl is dissolved, bring up to final volume by adding Type I Water. Larger volumes (4 L) that are to be used for denaturation solutions need not be autoclaved. NaCl for all other uses must be dispensed into appropriate containers and autoclaved at 215 F for 20 minutes at 20 lb.

NaCl - Purchased from VWR, Bridgeport, NJ., Catalog number 7532, 12 kg box.

NaCl, 5 M - Purchased from Invitrogen, Carlsbad, CA., Catalog number 4740UB, 10 liter bottle.

TRIS, 1 M, pH 7.5 or pH 8.0

Expiration date: Twelve months from the date of preparation
Dissolve the appropriate amount of Tris base into Type I Water. Mix well and adjust to pH 7.5 for the pH 7.5 solution and adjust to pH 8.0 for the pH 8.0 solution with 37.1% HCl. Bring up to final volume and recheck pH. Dispense into bottles and autoclave at 215°F for 20 minutes. Store at room temperature.

Tris - Purchased from VWR, Bridgeport, NJ., Catalog number 7732, 2.5 kg box.
Appendix C – Calibration of the BIOMEK®2000 Automation Workstation

Calibration tests should be performed on a routine basis for the Biomek®2000 Automation Workstation. The Position Calibration MUST be performed weekly and the Base Module, Left Side Module, Right Side Module and the B2K Alignment MUST be performed monthly, or as needed.

1 Position Calibration

1.1 Open the BioWorks folder on the desktop. Icons for different programs will be visible.

1.2 Double click on the Diagnostics icon or highlight the Diagnostics icon and under “File” in the drop down menu, select “Open”.

1.3 A window will open showing a menu with the following choices: Align, Diagnose, View, Download and Help.

1.4 Select Align and a drop down menu will appear with the following choices: Work surface, Stacker Carousel, Position Calibrate, Gripper and Exit.

1.5 Select Position Calibrate and the robot will automatically perform the test. The robot will indicate whether or not the calibration is done (a box will appear stating that the position calibration is done. Click OK) or whether the robot failed the test (a box will appear stating that the robot failed the test). If the robot failed the test, repeat it. If the robot fails three times in a row, a service technician must be called to repair the robot.

2 Base Module Alignment

2.1 Click on the Align function in the Diagnostic program and the drop down window will appear. Select Work Surface and another drop down menu with appear giving the following selections: Base Module, Left Side Module and Right Side Module. Choose the Base Module. A window will open (shown below, Figure 1).

![Base Module Alignment](image)

**Figure 1.**
2.2 Click on Start. A window will open asking the user to place a LabWare Holder (gray clamp for this test) at position A2. After doing so, click OK. A message will come up asking the user to place the Alignment probe on the head. The Alignment probe is red in color and must be manually placed onto the head.

2.3 Depress the black button on the front of the head and align the pins of the Alignment probe so that they fit into the robot head. Pay attention the “Front” label and arrow pointing to the front direction on the probe to ensure that it is placed on the head correctly.

2.4 Place a single Beckman P20 tip onto the probe, then click OK and the program will initiate. The probe with tip will align over the target on a gray clamp first at position A2.

2.5 A window will appear asking if it is safe to move down 3 mm. If it is, click “Yes”, otherwise click “No”.

2.6 A window will appear saying “Move to the alignment point, click on ‘Advance’ when in place”, click OK.

2.7 The tip should be perfectly centered over the target and only a Post-It-Note should be able to be slipped between the tip on the probe and the target. It should be a tight fit, with some resistance when pulling out the Post-It-Note from underneath the tip.

2.8 If adjustment is necessary at the X, Y or Z axes, then click on the appropriate arrow to move the probe in the correct direction. The increment value ranges from 10 to 100, with 100 being the default. For fine adjustments, the 10 value should be used.

2.9 Once the tip has been successfully aligned, then click “Advance” and the Alignment probe will continue on to targets at B2, A5 and B5.

2.10 Once all alignments have been performed, click OK and those values will be saved.

2.11 A window will appear requesting that the Alignment probe be removed from the head. Click OK and the alignment will be finished.

3 Left Side Module Alignment

3.1 Click on the Align function in the Diagnostics program and the drop down window will appear. Select Worksurface and another drop down menu will appear giving the following selections: Base Module, Left Side Module and Right Side Module. Choose the Left Side Module. A window will open (similar to the window shown above for the Base Module Alignment).

3.2 Click on Start. A window will open asking the user to place a LabWare Holder (gray clamp for this test) at position A1. After doing so, click OK. A message will come up asking the user to place the Alignment probe on the head. The Alignment probe is red in color and must be manually placed onto the head.

3.3 Depress the black button on the front of the head and align the pins of the Alignment probe so that they fit into the robot head. Pay attention the “Front” label and arrow are pointing to the front direction on the probe to ensure that it is placed on the head correctly.

3.4 Place a single Beckman P20 tip onto the probe, then click OK and the program will initiate. The probe with tip will align over the target on a gray clamp first at position A1.

3.5 A window will appear asking if it is safe to move down 3 mm. If it is, click “Yes”, otherwise click “No”.

3.6 A window will appear saying “Move to the alignment point, click on ‘Advance’ when in place”, click OK.
APPENDIX C - Calibration of the BIOMEK®2000 Automation Workstation

3.7 The tip should be perfectly centered over the target and only a Post-It-Note should be able to be slipped between the tip on the probe and the target. It should be a tight fit, with some resistance when pulling out the Post-It-Note from underneath the tip.

3.8 If adjustment is necessary at the X, Y or Z axes, then click on the appropriate arrow to move the probe in the correct direction. The increment value ranges from 10 to 100, with 100 being the default. For fine adjustments, the 10 value should be used.

3.9 Once the tip has been successfully aligned, then click “Advance” and the Alignment probe will continue on to another point at A1. There is no target at the second point, so just position the Z axis, then click “Advance”.

3.10 A window will come up asking the user to a LabWare Holder (gray clamp) at position B1. After doing so click OK and continue with the alignment over the target at position B1.

3.11 Once all alignments have been performed, click OK and those values will be saved.

3.12 A window will appear requesting that the Alignment probe be removed from the head. Click OK and the alignment will be finished.

4 Right Side Module Alignment (Only for Newer Systems not Previously Equipped with Shaking Platform)

4.1 Click on the Align function in the Diagnostics program and the drop down window will appear. Select Work surface and another drop down menu will appear giving the following selections: Base Module, Left Side Module and Right Side Module. Choose the Right Side Module. A window will open (similar to the window shown above for the Base Module Alignment).

4.2 Click on Start. A window will open asking the user to place a LabWare Holder (gray clamp for this test) at position A6. After doing so, click OK. A message will come up asking the user to place the Alignment probe on the head. The Alignment probe is red in color and must be manually placed onto the head.

4.3 Depress the black button on the front of the head and align the pins of the Alignment probe so that they fit into the robot head. Pay attention that the “Front” label and arrow are pointing to the front direction on the probe to ensure that it is placed on the head correctly.

4.4 Place a single Beckman P20 tip onto the probe, then click OK and the program will initiate. The probe with tip will align over the target on a gray clamp first at position A6.

4.5 A window will appear asking if it is safe to move down 3 mm. If it is, click “Yes”, otherwise click “No”.

4.6 A window will appear saying “Move to the alignment point, click on ‘Advance’ when in place”, click OK.

4.7 The tip should be perfectly centered over the target and only a Post-It-Note should be able to be slipped between the tip on the probe and the target. It should be a tight fit, with some resistance when pulling out the Post-It-Note from underneath the tip.

4.8 If adjustment is necessary at the X, Y or Z axes, then click on the appropriate arrow to move the probe in the correct direction. The increment value ranges from 10 to 100, with 100 being the default. For fine adjustments, the 10 value should be used.

4.9 Once the tip has been successfully aligned, then click “Advance” and the Alignment probe will continue on to another point at A6. There is no target at the second point, so just position the Z axis, then click “Advance”.
4.10 A window will come up asking the user to a LabWare Holder (gray clamp) at position B6. After doing so click OK and continue with the alignment over the target at position B6.

4.11 Once all alignments have been performed, click OK and those values will be saved.

4.12 A window will appear requesting that the Alignment probe be removed from the head. Click OK and the alignment will be finished.

5 B2K Alignment/Calibration of LabWare and Devices

5.1 This alignment program is not found in the Diagnostics folder, but is instead a method written specifically for alignment of the labware on the shaker. In the BioWorks folder, click on the Lab book Manager Icon.

5.2 All of the lab books will be listed (Figure 2). Select the IQ TELESHAKE as the lab book by clicking on the ‘Set As Current Lab Book’ button. Once set, close the window.

Figure 2.

5.3 Double click on the Edit Icon. Once in the Edit function, either use the drop-down window under Method or click on the open folder icon.

5.4 Once the Open Method window is open, highlight B2K Alignment and double click or click on open.
5.5 The method will open in the edit mode as well as an inset window with showing the Biomek deck. The MP200 tool and Gripper at deck position will be placed at A1, a P165 tip box at position A2, strip tubes (column 6 only for this method) in a black support rack at deck position B1, gray labware holder in position B3, and a Marsh plate at deck position B4. A round well, 1.2 mL plate is needed at deck positions B2, B5 (on the Magnabot), B6 on the Teleshaker, and A6 on the thermal exchange unit. See below (Figure 3).

Figure 3.

5.6 Click on the running man icon to initiate the method.

5.7 A window will open showing the Biomek worksurface. Click on the Accept All button. The method will then begin.

5.8 As soon as the robot arm picks up a column of tips and is moving over to the strip tubes, click on the stop button which appears in the method window. The robot will not immediately stop, but will finish that step. The robot arm will end up with the tips positioned just above the strip tubes.

5.9 Verify that the tips won’t crash into the top of the strip tubes, but will instead go into the wells before continuing to the next step. If it appears as if the tips will hit the top of the plate, then click the Quit button and go directly to 5.12 – 5.14 in order to adjust the labware definitions enough so that the tips can go in the wells instead of crashing on top of the plate.

5.10 Click on the Trace button twice. The tips will then go down into the strip tube wells and stop. Check the tip alignment in the wells from the front (X-axis) and the side (Y-axis). Tips should be centered in the wells. The Z-axis is assessed by pulling up on the plate to evaluate the “wiggle room”. The plate should be able to be moved up from the base of the labware holder by about 1mm.

5.11 This procedure is called “tracing through” the method. Trace through each piece of labware on the deck (positions B1, B2, B4). If the tips need to be adjusted for any of the axes for any of the labware, then
click on the Quit button and go to step 5.13. Be sure to select the appropriate labware for editing. Otherwise, click on Continue and proceed to 5.12.

5.12 The labware settings must be checked and corrected for deck positions B1, B2, and B4 prior to moving on in the method. If a correction is needed, click on the Edit function of the method after it has been terminated, or from the bar at the bottom, click on the method in the Edit mode.

5.13 The method will appear in the Edit mode. Go under the Edit drag down window and click on Labware.

5.14 For Z axis adjustments, adjust the well depth setting of the labware accordingly. If the tips are too high in the wells, adjust the well depth number up slightly. **Only the Z axis can be adjusted for the labware definitions.** If the tips are too low in the wells and are jammed in the bottom of the well, then adjust the number down slightly. Once the changes have been made, click OK. The window will close and the Edit device window will still be open. Click Close. If corrections are made to the labware definition(s), proceed back to 5.5 and re-start the method.

5.15 **IMPORTANT** Repeat steps 5.6 through 5.14 until the labware settings (deck positions B1, B2 and B4) are exact, then proceed to 5.16.

5.16 Once the labware settings are correct, allow the method to continue. Trace through the rest of the method in a similar fashion as before with the labware. The robot is now checking the device settings for the Magnabot with ¼ spacer, VP heater, and Teleshake.

5.17 For the VP Heater only, disregard the amount of play in the Z axis. It should be set appropriately during the initial setup of the Teleshake System upgrade. This setting is deliberately set very high (about 4 mm above bottom). Do not change the Z setting. The X and Y axes can be adjusted if necessary.

5.18 If the tips need to be adjusted for any of the axes of the devices (i.e Magnabot ¼ spacer, VP heater, and Teleshake), click on the Quit button and go to step 5.19. Otherwise, click on Continue and proceed to 5.22.

5.19 If a correction is needed, click on the Edit function of the method after it has been terminated, or from the bar at the bottom, click on the method in the Edit mode.

5.20 The method will appear in the Edit mode. Go under the Edit drag down window and click on Devices.

5.21 In the Labware Position Difference box, there will be an X offset, a Y offset and a Z offset. Decreasing the X offset will move the tips to the left, decreasing the Y offset (numbers can be reduced to become increasingly more negative) will move the tips back, away from the front of the deck, and reducing the Z offset will bring the tips lower. It is best to make small increments (± 0.4) until it can be evaluated just how much the tip alignment needs to be adjusted.

5.22 Once the changes have been made, click OK. The window will close and the Edit device window will still be open. Click Close.

5.23 **IMPORTANT** Repeat steps 5.16 through 5.22 until the device settings are exact, then proceed to 5.24.

5.24 Once the labware and device settings are all correct, allow the method to continue. The robot will then pick up the Gripper and check alignment of the round well block and Marsh plate in different deck positions. This step is just to confirm that all the labware position offsets are correct and also that the gripper can correctly sense the labware it is moving.

5.25 Once the gripper is done moving plates around on the deck, the method will finish.
Appendix D - BIOMEK®2000 Tool Calibration

1.1 Technical Notes

1.1.1 To calibrate the Biomek® 2000 tools, each tool will go through two low and high volume measurements to achieve an average of the tools performance. Before any calibration measurements are made and after any adjustments have been made to the slope and offset of a tool, the user will first run the low volume measurement for the tool twice with water and unlabeled tubes. This helps to adjust the plunger movement of the tool.

1.1.1.1 Each set of measurements will be used to generate a corrected slope value (corrected slope from set 1 and corrected slope from set 2) and a corrected offset value (corrected offset from set 1 and corrected offset from set 2) value. The average of set 1 and 2 for the corrected slope and the average of set 1 and set 2 for the corrected offset will be used.

1.1.2 In a method, a specific tool may have several names, each name reflecting different tool parameters (i.e. P20, P20 calib, P20 PCR, etc.). A method can contain several different tool names but will only use the tool definition of the tool name selected for a particular pipetting process.

1.1.2.1 For the calibration methods the tool names used are P20 calib, MP20 calib, P200L calib, and MP200 calib. The ‘calib’ tool names must be used for calibration of the tools since all of the methods utilize the same tool definitions, thus a change in the tool definition will affect all methods within a lab book... If a tool requires an adjustment to the slope and offset, the new settings will be transferred to the regular (live) tool name (i.e. P20, P20 PCR, MP20, P200L, MP200) in all of the lab books once the settings have been validated.

1.1.3 Single-channel and eight-channel tools are used interchangeably to accommodate a varying range of liquid volume transfers allowing the utilization of a variety of labware.

1.1.4 BioWorks, the Windows-based software interface for the Biomek® 2000, contains pipetting specifications for all the liquid handling tools, which can be modified to customize and optimize a tool for special liquid transfer functions.

1.1.5 Calibration is a plot of the millimeters the tool plunger moves versus the actual volume dispensed in microliters. Tool calibration will be conducted annually, after tool repair, or when a new and unique liquid is being used in a method.

1.1.6 One milligram of water is equal to one microliter of volume after multiplication with the Temperature Compensation Factor.

1.2 Equipment

- Biomek® 2000 Automation Workstation
- Biomek® MP20 and MP200 Pipette tools
- Biomek® P20 and P200L Pipette tools
- P10 Pipetman
- Balance (must be able to accurately weigh milligrams)
- Thermometer (Celsius)

1.3 Materials

- P200 Beckman Barrier tips - Beckman Catalog# 140505
- P20 Beckman Barrier tips - Beckman Catalog# 609043
- 1-10µl tips for P10 pipetman
- Black PCR support base (96well) – ABI Catalog# N801-0531
- Micro Amp strip tubes (in strips of 8 tubes) – ABI Catalog# N801-0580
• Micro Amp strip tube caps (in strips of 8 caps) – ABI Catalog# N801-0535
• MicroAmp® tubes (attached caps) – USA Scientific Catalog# 1402-8100 (clear) or Cat # 1402-8108 (multi-colored)
• Clear plastic reservoir holder – Beckman Catalog# P2-11-15
• Quarter module reservoir divided by length – Beckman Catalog# 372788

1.4 Reagents

• Sterile Type I water

1.5 Starting the BIOMEK® 2000 Workstation

1.5.1 Turn on the computer

1.5.2 Turn on the Biomek® 2000 Automation Workstation using the power button located on the back left side of the unit.

1.5.3 If the robot has been in previous use and is still on, then simply open the BioWorks folder on the desktop by double-clicking on the icon.

1.6 BIOMEK® 2000 Workstation Operating Procedure

NOTE: Tool calibration will be conducted annually, after tool repair, or when a new and unique liquid is being used in a method. The calibration will be conducted following the procedure outlined below. All tool calibrations will be recorded using the worksheets (Documents 210-F502 and 210-F503). If a tool requires repair by an authorized vendor, record this service on the “Equipment Calibration and Maintenance Form” (210-F600) or comparable log form.

An EMERGENCY STOP BUTTON is located at the lower front area on the robot, just below the deck. If the button is pressed, the robot will abort the method it is currently running. The emergency stop button only needs to be used when the robot could be damaged by crashing into the deck or a tool could be damaged by crashing into something on the deck. If what is desired is simply to stop the method while it is running, simply click on the “Stop” button. The method will then pause and several options will be available. The button labeled “Continue” can be used to resume the method, the button labeled “Trace” can be used to advance to the next step (this function is useful when calibrating), the button labeled “Go Up” will move the pod up and the button labeled “Quit” will terminate the method.

1.6.1 Pre-label tubes to be used in the calibration of the tool(s) tested (P20, MP20, P200L, MP200). See below for the appropriate tubes to use for each tool.

1.6.1.1 P20: lightly cap and label 8 Micro Amp tubes with attached caps 1 to 8.

1.6.1.2 MP20: label 8 columns of strip tubes 1 to 8. Cap the tubes lightly with strip caps and label the caps 1 to 8 (corresponding to tube #).

1.6.1.3 P200L: lightly cap and label 8 Micro Amp tubes with attached caps 1 to 8.

1.6.1.4 MP200: label 8 columns of strip tubes 1 to 8. Cap the tubes lightly with strip caps and label the caps 1 to 8 (corresponding to tube #).

1.6.1.5 The tubes used to calibrate the MP20 tool can be reused for the calibration of the MP200 tool for the low volume measurement.

1.6.2 Use the Calibration Worksheet appropriate for the tool being calibrated to record all relevant data needed to properly calibrate the tool. (calibration worksheet for the MP20 and MP200 tools and calibration worksheet for the P20 and P200L tools are located in Document 210-F502). Fill in worksheet while measuring weights.
1.6.3 Weigh the capped strip tubes used for the calibration of the MP20 and MP200 tools. Record the initial weight in milligrams (mg; i.e.: 1.295, 1.306, etc.) for each, as it corresponds to the number on the tube, in the Tare Wgt column of the calibration worksheet (Figure 1).

1.6.3.1 Tubes used for the low volume measurement for the P20 and P200L tools do not have to be weighed as the initial (low) volume will be checked using a P10 Pipetman.

![Figure 1: Calibration Worksheet for MP20 and MP200 tools](image)

1.6.4 All methods for calibrating the Biomek® 2000 Workstation tools can be found in the Calibration Lab Book. Open the BioWorks folder located on the desk top. Double click on the Lab Book Manager Icon. A list of Lab Books will be displayed. Highlight the Calibration Lab Book and click on the ‘Set As Current Lab Book’ button and close out the Lab Book Manager window.

1.6.5 To select a method from BioWorks, double click on the Edit icon located in the BioWorks folder. Then, either using the method drag-down window or using the open folder icon, select Open. A list of methods will be displayed. There are several methods associated with the calibration of the tools.

1.6.6 Calibration of the Biomek® 2000 P20 tool

1.6.6.1 Record the current slope and offset for P20-calib on the calibration worksheet. To retrieve the current slope and offset for the specified tool name, see 1.6.10.

1.6.6.2 Open the ‘Calibration P20’ method in order to perform the low volume (2µl) measurement for the P20 tool.

**NOTE:** The low volume method has to be performed before the high volume method as a pipetman is used to measure the volume dispensed.

Use the deck layout as a guide to place the labware on the deck.

1.6.6.3 Place the tool rack at deck position A1 with the P20 tool in the first position (far left).

1.6.6.4 Place P20 barrier tips at deck position A2.
1.6.6.5 Place a reservoir holder in deck position B1 with a quarter module, divided by length, situated in the far right position of the reservoir holder (see Figure 3). Place 8ml of water in the left reservoir of the quarter module.

1.6.6.6 Place a black PCR support base, with eight labeled Micro Amp tubes in column six, at deck position B2.

1.6.6.6.1 Run the low volume method twice using water and unlabeled tubes before taking any calibration measurements (see Technical notes 1.1.1). Discard the test run tubes after use.

1.6.6.7 Verify that the dispense volume is set at 2µl. This can be verified by looking at the pipette command line in the method (Figure 2). If the value is incorrect, change it by double clicking on the “Pipette _µl from B1 to B2 using P20 calib – P20 Barrier” command. The dialog box containing the parameters for the pipette transfer step will appear (Figure 3). Change the Dispense Volume to 2µl per tip. Select ‘Okay’ and save the change for the method.

Figure 2: Verifying tool name and pipette volume.
Figure 3: Changing the Dispense Volume

1.6.6.8 Uncap the tubes and begin the method, by clicking on the running man.

**NOTE:** Cap each tube immediately after water has been pipetted into them.
1.6.6.9 Once completed, use a P10 Pipetman to check the volume in the tubes. Using your Pipetman, adjust it so that the actual measured volume is as accurate as possible. Record the volume, in microliters (µl; i.e.: 2.0, 1.8, 2.1, etc), as it corresponds to the number on the tube, in the Low Sample Vol column of the calibration worksheet (Figure 4).

![Calibration Worksheet for P20 and P200L tools](image)

**Figure 4: Calibration Worksheet for P20 and P200L tools**

1.6.6.9.1 The tubes will be reused for the high volume measurement of the P20 tool. Cap and weigh each tube and record the weight (in milligrams), as it corresponds to the number on the tube, in the Tare Wgt column of the calibration worksheet.

1.6.6.10 Next, perform the high volume (20µl) measurement for the P20 tool. Change the dispense volume in the ‘Calibration P20’ method to 20µl by following the instructions in 1.6.6.7.

1.6.6.11 The deck layout remains the same as the low volume (2µl) Calibration method.

**NOTE:** It is not necessary to use a new box of tips in A2. If following the low volume calibration of the P20 tool then the robot will use the next unused tip as it remembers the last tip used.

It is not necessary to place more water in the reservoir if the procedure follows the low volume measurement for the P20 tool.

1.6.6.12 Uncap, and place the tubes in column six of a black PCR support base at deck position B2. Begin the method by clicking on the running man.

**NOTE:** Cap each tube immediately after water has been pipetted into them.

1.6.6.13 Once the method is completed, weigh each tube and record the weight (in milligrams), as it corresponds to the number on the tube, in the High Vol Wgt column of the calibration worksheet (see Figure 4).

1.6.6.14 Obtain a second set of measurements for the P20 tool by repeating steps 1.6.6.1 to 1.6.6.13.

1.6.6.15 Proceed to 1.6.11 for Tool calibration calculations.
1.6.7 Calibration of the Biomek® 2000 MP20 tool

1.6.7.1 Record the current slope and offset for MP20-calib on the calibration worksheet. To retrieve the current slope and offset for the specified tool name, see 1.6.10.

1.6.7.2 Open the ‘Calibration MP20’ method in order to perform the low volume (2µl) measurement for the MP20 tool. Use the deck layout as a guide to place the labware on the deck.

1.6.7.3 Place the tool rack at deck position A1 with the MP20 tool in the first position (far left).

1.6.7.4 Place P20 barrier tips at deck position A2 and A3.

NOTE: It is not necessary to use a new box of tips in A2. If following the calibration of the P20 tool then the robot will use the next unused tip as it “remembers” the last tip used.

1.6.7.5 Place a reservoir holder in deck position B1 with a quarter module situated in the far right position of the reservoir holder. Place 8ml of water in the left reservoir of the quarter module.

1.6.7.6 At deck position B2, place a black PCR support base with the weighed strip tubes labeled 1 to 4 in columns 3, 5, 7 and 9. At deck position B3 place a black PCR support base with the weighed strip tubes labeled 5 to 8 in columns 4, 6, 8, and 10.

1.6.7.6.1 Run the low volume method twice using water and unlabeled tubes before taking any calibration measurements (see Technical notes 1.1.1). Discard the test run tubes after use.

1.6.7.7 Verify that the dispense volume is set at 2µl. This can be verified by looking at the Pipette command in the method (Figure 5). If the value is incorrect, change it by double clicking on the “Pipette µl from B1 to B2 using MP20 calib – P20 barrier” command. The dialog box containing the parameters for the Pipette transfer step will appear (similar to Figure 3). Change the Dispense Volume to 2µL per tip. Select Okay. Make sure that both Pipette commands are changed. Save the changes for the method.

1.6.7.8 Remove the strip caps and begin the method.

Figure 5: MP20 Calibration Method
NOTE: Lightly cap each column of strip tubes with its correspondingly labeled strip caps immediately after water has been pipetted into each column of tubes.

1.6.7.9 Once completed, weigh each capped column of strip tubes. Record the weight as it corresponds to the number and previously recorded tare weight in the Low Vol Wgt column of the calibration worksheet (see Figure 1).

NOTE: The new weight (Low Vol Wgt) for each capped strip tube will serve as the Tare weight for the high volume measurement.

1.6.7.10 Next, perform the high volume (20µl) measurement for the MP20 tool. Change the dispense volume in the Calibrate MP20 method to 20µl by following the instructions in 1.6.7.7. Make sure that both pipette commands are changed.

1.6.7.11 The deck layout remains the same as the low volume (2µl) calibration method.

NOTE: It is not necessary to use a new box of tips in A2 or A3. If following the low volume calibration of the MP20 tool then the robot will use the next unused tip as it remembers the last tip used.

It is not necessary to place more water in the reservoir if the procedure follows the low volume measurement for the MP20 tool.

1.6.7.12 Use the same strip tubes and caps used for the low volume measurement of the MP20 tool. At deck position B2 place a black PCR support base with the strip tubes labeled 1 to 4 in columns 3, 5, 7 and 9. At deck position B3 place a black PCR support base with the strip tubes labeled 5 to 8 in columns 4, 6, 8 and 10.

1.6.7.13 Remove the strip caps and begin the method by clicking on the running man.

NOTE: Lightly cap each column of strip tubes with its correspondingly labeled strip caps immediately after water has been pipetted into each column of tubes. See 1.6.7.7.

1.6.7.14 Once the method is completed, weigh each column of capped strip tubes. Record the weight (in milligrams) as it corresponds to the number and previously recorded tare weight (in 1.6.7.9) in the High Vol Wgt column of the calibration worksheet (Figure 1).

1.6.7.15 Obtain a second set of measurements for the MP20 tool by repeating steps 1.6.8.1 to 1.6.8.14.

1.6.7.16 Proceed to 1.6.11 for Tool calibration calculations

1.6.8 Calibration of the Biomek® 2000 P200L tool

1.6.8.1 Record the current slope and offset for P200L-calib on the calibration worksheet. To retrieve the current slope and offset for the specified tool name, see 1.6.10.

1.6.8.2 Open the ‘Calibration P200L’ method in order to perform the low volume (5µL) measurement for the P200L tool.

NOTE: The low volume method has to be performed before the high volume method as a pipetman is used to measure the volume dispensed.

Use the deck layout as a guide to place the labware on the deck.

1.6.8.3 Place the tool rack at deck position A1 with the P200L tool in the first position (far left).

1.6.8.4 Place P250 barrier tips at deck position A2.
1.6.8.5 Place a reservoir holder in deck position B1 with a quarter module situated in the last position (far right) of the reservoir holder. Place 8ml of water in the left reservoir of the quarter module.

1.6.8.6 Place a black PCR support base, with eight labeled Micro Amp tubes in column six, at deck position B2.

1.6.8.6.1 Run the low volume method twice using water and unlabeled tubes before taking any calibration measurements (see Technical notes 1.1.1). Discard the test run tubes after use.

1.6.8.7 Uncap the tubes and begin the method by clicking on the running man.

**NOTE:** Cap each tube immediately after water has been pipetted into them.

1.6.8.8 Once completed, use a P10 Pipetman to check the volume in the tubes. Using your Pipetman, adjust it so that the actual measured volume is as accurate as possible. Record the volume, in microliters (µl; i.e.: 5.0, 4.9, 5.1, etc), as it corresponds to the number on the tube, in the Low Sample Vol column of the calibration worksheet (see Figure 4).

1.6.8.8.1 The tubes will be reused for the high volume measurement of the P200L tool. Cap and weigh each tube and record the weight (in milligrams), as it corresponds to the number on the tube, in the Tare Wgt column of the calibration worksheet (see Figure 4).

1.6.8.9 Next, perform the high volume (125µl) measurement for the P200L tool. Open the ‘Calibration P200L125’ method.

1.6.8.10 The deck layout remains the same as the low volume (5µl) calibration method.

**NOTE:** It is not necessary to use a new box of tips in A2. If following the low volume calibration of the P200L tool then the robot will use the next unused tip as it remembers the last tip used.

It is not necessary to place more water in the reservoir if the procedure follows the low volume measurement for the P200L tool.

1.6.8.11 Uncap and place the tubes in column six of a black PCR support base at deck position B2. Begin the method by clicking the running man.

**NOTE:** Cap each tube immediately after water has been pipetted into them.

1.6.8.12 Once the method is completed, weigh each tube and record the weight (in milligrams), as it corresponds to the number on the tube, in the High Vol Wgt column of the calibration worksheet (Figure 4).

1.6.8.13 Obtain a second set of measurements for the P200L tool by repeating steps 1.6.9.1 to 1.6.9.12.

1.6.8.14 Proceed to 1.6.11 for Tool calibration calculations.

1.6.9 Calibration of the Biomek® 2000 MP200 tool

1.6.9.1 Record the current slope and offset for MP200-calib on the calibration worksheet. To retrieve the current slope and offset for the specified tool name, see 1.6.10.

1.6.9.2 Open the ‘Calibration MP200’ method in order to perform the low volume (5µl) measurement for the MP200 tool. Use the deck layout as a guide to place the labware on the deck.
1.6.9.3 Place the tool rack at deck position A1 with the MP200 tool in the first position (far left).

1.6.9.4 Place P250 barrier tips at deck position A2 and A3.

**NOTE:** It is not necessary to use a new box of tips in A2. If following the calibration of the P200L tool then the robot will use the next unused tip as it remembers the last tip used.

1.6.9.5 Place a reservoir holder in deck position B1 with a quarter module situated in the far right position of the reservoir holder. Place 10ml of water in the left reservoir of the quarter module.

1.6.9.6 At deck position B2 place a black PCR support base with the weighed strip tubes labeled 1 to 4 in columns 3, 5, 7 and 9. At deck position B3 place a black PCR support base with the weighed strip tubes labeled 5 to 8 in columns 4, 6, 8 and 10.

1.6.9.6.1 Run the low volume method twice using water and unlabeled tubes before taking any calibration measurements (see Technical notes 1.1.1). Discard the test run tubes after use.

1.6.9.7 Remove the strip caps and begin the method.

**NOTE:** Cap each column of strip tubes with its correspondingly labeled strip caps immediately after water has been pipetted into each column of tubes.

1.6.9.8 Once completed, weigh each capped column of strip tubes. Record the weight as it corresponds to the number and previously recorded tare weight in the Low Vol Wgt column of the calibration worksheet (see Figure 1).

**NOTE:** The new weight (Low Vol Wgt) for each capped strip tube will serve as the Tare weight for the high volume calibration measurement.

1.6.9.9 Next, perform the high volume (125µl) measurement for the MP200 tool. Open the “CalibrationMP200-125” method.

1.6.9.10 The deck layout remains the same as the low volume (5µl) calibration method.

**NOTE:** It is not necessary to use a new box of tips in A2 or A3. If following the low volume calibration of the MP200 tool then the robot will use the next unused tip as it “remembers” the last tip used.

It is not necessary to place more water in the reservoir if the procedure follows the low volume measurement for the MP200 tool.

1.6.9.11 Use the same strip tubes and caps used for the low volume measurement of the MP200 tool. At deck position B2 place a black PCR support base with the strip tubes labeled 1 to 4 in columns 3, 5, 7 and 9. At deck position B3 place a black PCR support base with the strip tubes labeled 5 to 8 in columns 4, 6, 8 and 10.

1.6.9.12 Remove the strip caps and begin the method.

**NOTE:** Lightly cap each column of strip tubes with its correspondingly labeled strip caps immediately after water has been pipetted into each column of tubes.

1.6.9.13 Once the method is completed, weigh each column of capped strip tubes. Record the weight (in milligrams) as it corresponds to the number and previously recorded tare weight (in 1.6.10.7) in the High Vol Wgt column of the calibration worksheet (Figure 1).
1.6.9.14  Obtain a second set of measurements for the MP200 tool by repeating steps 1.6.10.1 to 1.6.10.13.

1.6.9.15  Proceed to 1.6.11 for Tool calibration calculations.

1.6.10  Retrieving Current Slope and Offset values

1.6.10.1  In the BioWorks Edit window of the Tester Lab Book, select Edit from the toolbar and then highlight Tools in the drag down window. The Edit Tools window appears (Figure 6):

![Edit Tools Window](image)

**Figure 6: Edit Tools Window**
1.6.10.2 From the list of tool names, select the tool that is used in the calibration method, as it will contain the parameters tested. Highlight the tool name then double click or select the Edit button. The following window appears (Figure 7).

![Figure 7: Tool configurations](image)

1.6.10.3 Record the current calibration slope and offset for the tool selected in the appropriate calibration worksheet, click Cancel and then close the Edit Tools window.

1.6.11 Tool calibration calculations.

1.6.11.1 Calculations are performed using an Excel spreadsheet for each tool. The low volume and high volume measurements, Temperature Compensation Factor, and current calibration slope and offset are plugged into designated cells (Figure 8). The calibration calculation worksheets for each tool can be found in the Tool Calibration folder.

1.6.11.1.1 For the single channel tools (P20 and P200L) the low volume measurements (µl), acquired from the pipetman, will be entered into the Sample Volume column of the calibration calculation worksheets. The Tare Wgt and High Vol Wgt values will be entered into the Tare and Full columns, respectively, of the high volume measurement table (Figure 8).

1.6.11.1.2 For the multi-channel tools (MP20 and MP200), the Tare Wgt and Low Vol Wgt values will be entered into the Tare and Full columns, respectively, of the low volume measurement table. The Low Vol Wgt and the High Vol Wgt values will be entered into the Tare and Full column, respectively, of the high volume measurement table (Figure 9).
To obtain the Temperature Compensation Factor value, the room temperature must be measured. Then, select the Temp Comp tab at the bottom of the calculation worksheet (Figure 8 and 9). The following table appears (Figure 10).
Select and copy the Compensation Factor that correlates to the current room temperature and paste it in to the space provided in the calculation spreadsheet.

![Temperature Compensation Factor Table](image)

**Figure 10:** Temperature Compensation Factor Table.

1.6.11.2 Once all relevant values have been entered, the Average, Standard Deviation (SD) and Percent Coefficient of Variation (%CV) for the low and high measurements will be available. Use this information to determine the performance of a tool.

**NOTE:** Calculations must be performed for both sets of measurements. Two sets of measurements were taken, each containing low and high measurements for the tool tested. A Corrected Slope and Offset will be calculated for each set of measurements, resulting in a corrected slope value for set 1 and set 2 and a corrected offset value for set 1 and 2.

1.6.11.3 To determine if the current slope and offset for a tool will be corrected, use the following guidelines:

1.6.11.3.1 The Average Low Volume and the Average High Volume values should be close to the desired amount for the low and high volume measurement values for the tool being evaluated, with a %CV lower or equal to 5% (for all tools). If the %CV is greater than 5% then the slope and offset must be corrected.

1.6.11.3.2 It is important to note the individual measurements for each volume measured. For instance, the average may be close to the expected volume but there may be significant variability between the individual measurements (poor precision). If this is observed the measurement will have to be repeated. If, variability continues to exist then this may be an indication that the tool will need to be serviced, cleaned and/or the quad ring(s) replaced. Contact a project coordinator or Beckman service representative.

1.6.11.3.3 A tool may exhibit very good precision, resulting in a low SD and %CV, however, the average may be several microliters above or below the expected volume, i.e. poor accuracy. This is an indication that the slope and offset for the tool must be changed. If a corrected slope and offset were used to obtain the...
measurements then the tool must be reevaluated using the original slope and offset. Repeat the low and high volume measurements for the tool. Occasionally the measurements will have to be repeated several times (in duplicate) to produce the desired results. If a desired measurement can not be reached then the tool may need to be serviced.

1.6.11.4 Proceed to 1.6.12 if an adjustment has to be made to the current slope and offset of a tool.

1.6.12 Adjustments to Tool Slope and Offset

1.6.12.1 If it has been determined that an adjustment needs to be made to the current slope and offset of a tool, then the average of the two corrected slope values (from set 1 and set 2) and the average of the two corrected offset values (from set 1 and set 2) must be calculated. These values will be entered as the new/current slope and offset values.

1.6.12.2 In the BioWorks Edit window of the Tester Lab Book, select Edit from the toolbar and then highlight, Tools, in the drag down window. The Edit Tools window appears (Figure 6).

1.6.12.2.1 The Edit tools window can be accessed similarly in all the Lab Books.

1.6.12.3 From the list of tool names, highlight the tool name to which adjustments will be made, then double click or select the Edit button. The tool parameters for the tool selected appears (Figure 7).

1.6.12.4 Change the current Slope and Offset to the corrected slope and offset derived from the calibration calculations. The corrected slope and offset values are the averages of the corrected slope and offset values from the two separate calibration calculations of the tool (see 1.6.12.1). Click OK and Close the Edit Tools window.

IMPORTANT: When a change is made to a tool configuration, it will affect all methods where the tool, with the specific tool name, is used. Thus, the need for all of these measurements and modifications to be made using the P20-calib tool, etc. when testing a new slope or offset it is crucial that only the tool used in the calibration methods is changed (e.g. P20-calib) until the tool modifications have been properly validated.

1.6.12.5 Proceed to 1.6.1 to begin the recalibration/validation of the new tool settings.

1.6.12.6 Two sets of measurements must be performed with the new tool settings. Then proceed to 1.6.11 to perform the calibration calculations to determine if further adjustments will need to be made.

1.6.13 Once the corrected slope and offset of a particular tool are satisfactory as determined by the Robot Coordinator, the settings for slope and offset must be changed in all the Lab Books for the live tools (i.e., modifications made and tested for P20-calib will now be transferred to the P20 tool in all Lab books). For each Lab Book follow the steps in 1.6.12.1 to 1.6.12.4 to enter the corrected slope and offset for the specific tool name. A project coordinator is required to verify that the appropriate values have been entered into the appropriate live tool definitions.
Appendix E – Performing the Stratagene Mx3005PTM Instrument Qualification Test

1.1 Technical Notes

1.1.1 These instructions are adapted from that provided by the manufacturer in the Setup and User’s Guide to the Stratagene Mx3005PTM QPCR System.

1.1.2 This test is a quality assurance measure to verify that the performance of the instrument is within factory specifications. The run takes approximately 2.5 hours to complete.

1.1.3 This test uses a preconfigured plate setup and thermal profile setup. No additional software setup steps are required. Do not modify the qualification test experiment or analyze data during the Qualification Test run.

1.1.4 Always wear gloves when handling the Qualification test plate, so as to ensure that no fingertip oils or other debris is transferred to the optical seal.

1.2 Procedure for Performing the Stratagene Mx3005P™ Instrument Qualification Test

1.2.1 The qualification test plate can be obtained from Stratagene.

1.2.2 Prepare the qualification test plate just prior to starting the run. Thaw the plate at room temperature for 15-30 minutes, leaving the plate sealed in the foil wrapper while thawing.

1.2.3 After thawing, remove the foil wrapper and mix the contents of the wells by repeating this action five times:

1.2.3.1 Invert the plate. Use a sharp, swift, downward motion to move the liquid to the seal on top of each well. Observe the liquid to verify that the liquid has moved from the bottoms to the tops of the tubes.

1.2.3.2 Re-invert the plate, and use the same motion to return the liquid to the bottom of each well, again verifying liquid movement before proceeding.

1.2.4 After completing five mixing cycles, the liquid should be at the bottom of the wells. Briefly centrifuge the plate at 1000 rpm, if a plate centrifuge is available, to dislodge any bubbles and ensure all liquid is at the bottom of the wells. If no centrifuge is available, shake the liquid down to the bottom of the wells using the same motion described above. It is important to shake down any noticeable liquid on the tube seal. Small amounts of liquid spray retained on the tube sides should not affect test results.

1.2.5 Prior to loading the plate into the thermocycler, ensure that no bubbles are present at the bottoms of the wells. Small bubbles near the liquid meniscus should not affect the test results, although bubbles at the bottom of the wells will interfere with the fluorescence readings.

1.2.6 Turn on the instrument and computer. Double click on the Mx3005P software icon “MxPro” on the desktop of the computer controlling the Stratagene Mx3005PTM. If more than one instrument is connected to the computer, select the instrument to be qualified and click “OK”. The “New Options” window will appear. Click “Cancel”. Under the “Instrument” drop-down menu, choose “Qualification Test…”

1.2.7 Verify that the black “Perfect Fit” frame shipped with the instrument has been removed from the thermal block area. Load the plate in the thermal block, verifying the correct orientation (well A1 in the top, left corner).
NOTE: This opens much like a waffle iron – you should slide the black bar toward you and lift up. The plate should fit inside. The black top can then be placed over top of the plate and closed and latched to secure the plate.

1.2.8 Close the instrument door and click Start Run (located in the bottom right corner of the screen).

1.2.9 If the pre-run lamp warm-up dialog box appears, select Run Now. The system will begin the Instrument Qualification Test run.

NOTE: Do not open the instrument door during the run.

1.2.10 When the run is complete, the Instrument Qualification Test – Run complete dialog box will appear. Select Analyze. The software will open the Microsoft Excel software Instrument Qualification Validation template and will automatically export the Text Report data from the Instrument Qualification Test run into the Microsoft Excel software template, which has been preconfigured for data analysis.

NOTE: If a “Server Busy” error dialog box appears, click Retry until the data export succeeds.

1.2.10.1 If any Norton anti-virus dialog boxes appear, select “Notify Again in 1 Day”, if this option is available, and close the dialog box.

1.2.11 The Microsoft Excel software Validation template will open to the FAM analysis tab. First, verify that the data count cell reports the result “Good”, ensuring that all of the data has been correctly imported into Microsoft Excel. Next, verify that the Result cell reports a Pass result. A Pass result indicated that the instrument is working properly. In this case, it is not necessary to email the data to Stratagene Technical Services. If the test reports a Check result, it is important to call Stratagene Technical Services (1-800-894-1304) and send the three data files, including Customer Information.txt, into Stratagene technical Services by email for troubleshooting guidance.

1.2.12 After checking the results displayed on the Microsoft Excel software Validation template, go back into the MxPro software.

1.2.13 For a test report Check result only, the “Instrument Qualification Test – Email Experiment” dialog box will appear and present the option to automatically email the data or save the data to a disk to later email to Stratagene Technical Services (QPCR@stratagene.com). Include a contact name, Virginia Department of Forensic Science, and the instrument serial number when sending the data to Technical services.

1.2.14 The “Instrument Qualification Test – Complete” dialog box appears, indicating that the Instrument Qualification Test is complete. Click Finish to conclude the test.

1.2.15 Record the results of the test in the appropriate QC log book.