COPYRIGHT © 2015

Department of Forensic Science

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

FORENSIC BIOLOGY SECTION PROCEDURES MANUAL

SECTION IX

BIOMEK®NX^{P} AUTOMATION WORKSTATION PROCEDURES MANUAL

UNCONTROLLED COPY

TABLE OF CONTENTS

Introduction

1 Isolating DNA Using the DNA IQ™ Isolation System

2 Preparation of the BIOMEK®NX^{P} Automation Workstation for DNA Isolation

3 Quality Assurance Measures for Isolating DNA Using the BIOMEK®NX^{P} Automation Workstation

4 DNA Quantitation

5 Normalization and PCR Set Up

6 Transfer of Extracted DNA for Permanent Storage

Appendix A Troubleshooting

Appendix B Reagents

Appendix C Calibration of the BIOMEK® NX^{P} Automation Workstation

Appendix D Performing the Stratagene Mx3005P™ Instrument Qualification Test

Appendix E Using the Biomek® NX^{P} DNA IQ™ Recovery Method

Appendix F Probe Alignment

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE
UNCONTROLLED
COPY

INTRODUCTION

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® NX^P 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® NX^P AUTOMATION WORKSTATION

The Biomek® NX^P Automation Workstation is manufactured by Beckman Coulter, Inc. The robot is used in biotechnology research and private industry laboratories. The particular Biomek® NX^P Automation Workstation pictured in Figure 1 has been modified for the purpose of utilizing the DNA IQ™ System for DNA extraction.

The Biomek® NX^P Automation Workstation relies on liquid displacement for pipetting. The deck has elevated labware positions and holders called ALPS. Each probe of the Span-8 tool can pick up a tip independently of the other probes and can pipette independently as well as pipette different volumes. This versatility makes the pipetting tool very rapid and increases functionality since it can fit P20, P50, P250 and P1000 tips. There is also has a gripper tool for picking up and moving plates around. An orbital shaker for mixing samples is defined as its own ALP and is located in the center back part of the deck. An electronic heater is located in an ALP position on the right hand side of the robot. Behind the heater unit on the deck, equipped with a pronged heat transfer plate, is the rapid shucker device. This allows for tips to be re-racked and re-used, but it can only accommodate P20, P50, P100, and P250 tips. Specially adapted aerosol resistant tips are used with the Biomek® NX^P Automation Workstation. The tip boxes have enclosed wells so re-used tips will not contaminate any neighboring tips.

The Biomek® NX^P Automation Workstation has liquid level sensing ability for the pipetting tool, however only the P1000 tips used for the robotic methods described in this manual have that capacity. The robot 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.

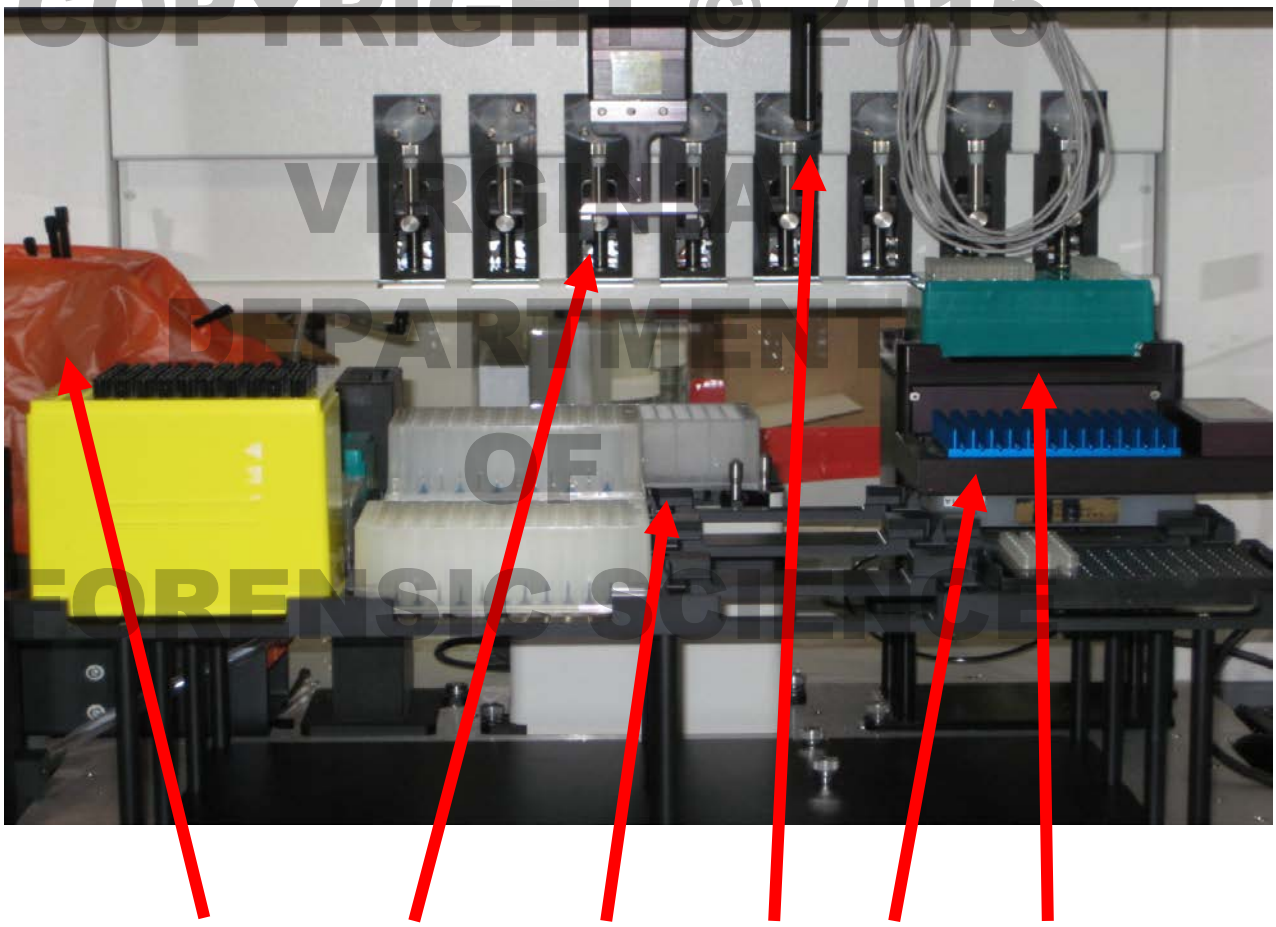
Software methods have been written by scientists at Promega Corporation for use with the DNA IQ™ System on the Biomek® NX^P Automation Workstation, with optimization performed by VDFS. A single method, the GIInterface method, is employed for the extraction of mixed stains, tissue, bloodstains, epithelial and buccal cells described in this manual. If samples are in the DNA IQ™ Lysis buffer, then the range of volumes the extraction method can accommodate ranges from 200-800 µL. If the samples are in a Proteinase K containing buffer (aqueous), the volume range it can accommodate is from 50-400 µL. 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

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

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® NX^{P} Automation Workstation with modifications for DNA extraction using the DNA IQ™ System.

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE



1 2 3 4 5 6

1 = Trash
2 = Gripper
3 = Orbital shaker (with plate)
4 = Span 8 pipetting tool
5 = Heater unit with heat transfer plate
6 = Rapid shucker (for re-using P20, P50, P100 and P250 tips)

UNCONTROLLED
COPY

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® 16BIO 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.

NOTE: For tracking purposes the samples will be listed on the worksheet in the order in which they were processed/handled. The samples will be maintained in the same order throughout the rest of the analysis until the DNA analysis 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.

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.
- 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.
- 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

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

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.

- A clean cutting surface will be used for each piece of evidence.
- Disposable 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 and/or with each case with one or more consumed samples in order 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 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 then 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 semen and other biological fluid. 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

1.2.1 Heat block or incubator, 37ºC

1.2.2 Heat block or incubator, 56ºC

1.2.3 Microcentrifuge

1.2.4 Vortex mixer

1.2.5 Scissors

1.2.6 Tweezers

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

1.2.7 Microcentrifuge tube rack

1.2.8 Pipettes - 10 µL, 100 µL, 200 µL, and 1000 µL

1.2.9 Refrigerator/Freezer

1.2.10 Scalpel and blades (for DNA extraction from swabs)

1.2.11 Stereo microscope (for DNA extraction from hair)

1.2.12 Beaker, 50 mL (for DNA extraction from hair)

1.2.13 Chisel and hammer (for DNA extraction from bone)

1.2.14 High Speed Electric Drill (bone procedure)

1.2.15 Rotary shaft tool (tooth procedure - maintained in the firearms section)

1.2.16 1/8” or 9/64” drill bit (bone procedure)

1.3 Materials

1.3.1 Microcentrifuge tubes, 1.5 mL

1.3.2 Microcentrifuge tubes, 2.0 mL, Sarstedt Cat 72.689.001 or 2.0 mL tubes, LPS Cat#M850003

1.3.3 Transfer pipettes

1.3.4 Sterile ART tips for pipettes - 10 µL, 100 µL, 200 µL, and 1000 µL

1.3.5 Microcentrifuge tube lids

1.3.6 Kimwipes

1.3.7 Spin-Ease basket (Optional)

1.3.8 Gloves

1.3.9 Conical tubes, 15 mL and 50 mL (for DNA extraction from bone)

1.3.10 White/black paper (for DNA extraction from hair)

1.3.11 Weigh boats (for DNA extraction from bone)

1.3.12 Microscope slides (Optional)

1.3.13 Plastic Ziploc baggies

1.4 Reagents

1.4.1 Proteinase K - 20 mg/mL (Keep on ice.)

1.4.2 TNE

1.4.3 20% Sarkosyl

1.4.4 0.39*M* Dithiothreitol (DTT)

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

1.4.5 Sterile Type I Water

1.4.6 PCR Digestion Buffer

1.4.7 95% Ethanol

COPYRIGHT © 2015

1.4.8 $CaCl_2$

1.4.9 DNA IQ™ Lysis Buffer

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

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

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, only a 3 mm^2 section may be all that is necessary to remove from a heavily bloodstained item.

NOTE: Process a reagent blank along with each set of samples. If the majority of the sample may be consumed during the typing process, then an additional reagent blank to be associated with that case needs to be processed. This is so that a reagent blank will be available for any additional testing of the sample.

1.5.1 Cut an approximate 3 mm^2 blood stain or other biological stain and place into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. 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.

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 from 200 to 800 uL of DNA IQ™ Lysis buffer, a sufficient volume should be added to saturate the sample.

NOTE: A maximum volume of 800 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 56ºC heat block for a minimum of 30 minutes. If the sample is deposited on FTA paper the sample maybe extracted at 56ºC or at 95° C for a minimum of 30 minutes.

UNCONTROLLED COPY

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® NX^P Automation Workstation. If the samples are stored in a refrigerator before proceeding to Section 2,

Preparation of the Biomek® NX^P Automation Workstation, place the samples in a 56ºC 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® NX^P Automation Workstation.

COPYRIGHT © 2015

1.6 DNA IQ™ Extraction Method for Mixed Body Fluid Stains (Differential Procedure)

1.6.1 Cut an approximate 3 mm^2 stain or a portion of a cotton swab proportional to the number of sperm identified. Place the stain into a labeled 1.5 mL microcentrifuge tube with a depression in the lid. 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.

VIRGINIA

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 during the purification step.

DEPARTMENT

NOTE: Process a reagent blank along with each set of samples.

1.6.2 Add:

OF

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

FORENSIC SCIENCE

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 37ºC 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.

UNCONTROLLED

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.

COPY

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® NX^P 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 Remove and discard the old lid (if holes were punched into the lid) and cutting from the original tube containing the pellet. (If the majority of the biological sample deposited on the evidence was consumed, the cutting will be returned with the evidence). Place a new colored 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 digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 µL of the supernatant and discard.

1.6.11 Repeat the wash in step 1.6.9 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 wash buffer and discard. The sperm cells are not lysed at this point. The sperm cells are lysed during the isolation step using the Biomek® NX^P 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 56ºC heat block for 5 minutes to resolubilize the DNA before proceeding.

OPTION: If a sperm search has not previously been conducted, remove 3 µL of the supernatant and spot the sample onto a glass microscope slide for examination.

1.6.12 Proceed to Chapter 2, Preparation of the Biomek® NX^P 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 Using clean tweezers, place the hair on a clean piece of white or black paper, whichever is appropriate, and examine under a stereo microscope for the presence of sheath material. Note the presence of any body fluids on the hair.

1.7.1.2 Wash the hair to reduce surface dirt and contaminants by immersing the hair in sterile Type I Water in a clean 50 mL beaker or other suitable container. If the hair contains a biological fluid that is important to the investigation DO NOT wash the hair.

1.7.1.3 Return the hair to the stereo microscope. 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.

NOTE: Process a reagent blank along with each set of samples.

1.7.2 Cut an approximate 3 mm^2 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:

160 µL DNA IQ Proteinase K Buffer
20.0 µL 0.39*M* DTT
20.0 µL Proteinase K

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

Depending on the size of the sample the volumes may scaled up. The maximum volume that can be loaded into the sample plate for robotic extraction is 400 µL

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

COPYRIGHT © 2015

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

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

1.7.7 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 1.7.9.

VIRGINIA DEPARTMENT OF

1.7.8 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® NX^P Automation Workstation.

FORENSIC SCIENCE

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.

1.8.2 Add 90 µL of 1X $CaCl_2$ buffer and 10 µL of Proteinase K to saturate the sample. The volumes can be scaled up to a maximum volume of 400 µL.

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 °C incubator or heat block for an additional 2 hours.

UNCONTROLLED

1.8.5 Spin the 1.5 mL microcentrifuge tube at ~12,000 rpm for 5 minutes to pellet any undigested debris.

1.8.6 Proceed to Chapter 2, Preparation of the Biomek® NX^P Automation Workstation.

COPY

2 PREPARATION OF THE BIOMEK® NX^P AUTOMATION WORKSTATION FOR DNA ISOLATION

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® NX^P Workstation deck:

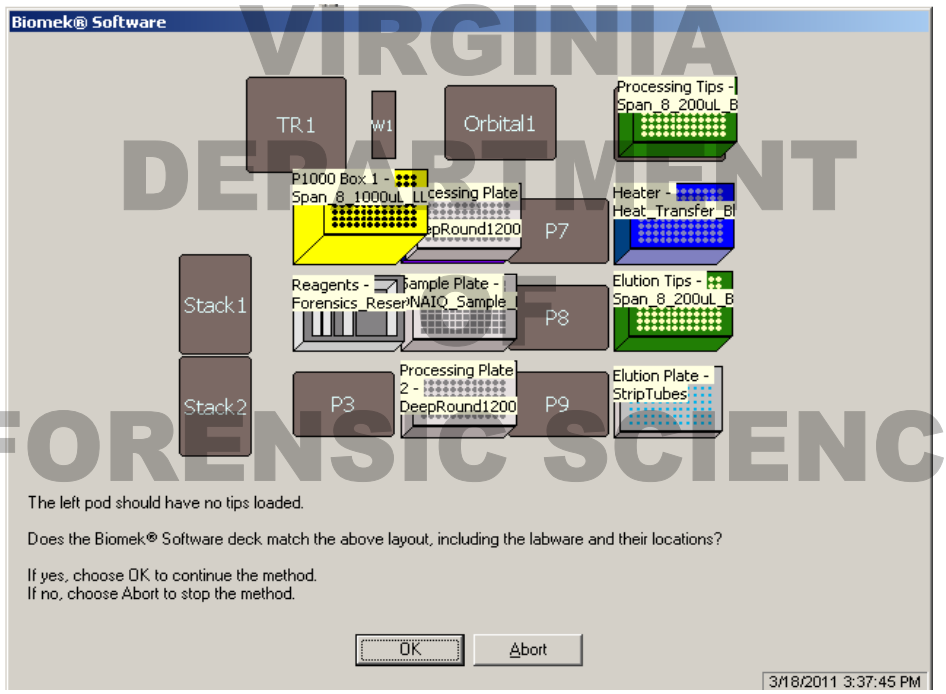


Figure 1. Schematic view of the Biomek® NX^P Workstation deck.

2.1.1 The electronic heating unit connected to the Biomek® NX^P Workstation is set at a temperature of 90°C 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 % 200mM NaCl. 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 (> 50ºC), the yield of DNA obtained will be lower than expected.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

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.

2.2 Equipment

2.2.1 Biomek® NX^P Automation Workstation

2.2.2 Magnabot with ¼ foam spacer – deep well prongs

2.2.3 Watlow Electronic Heating Unit

2.2.4 Heat transfer plate

2.2.5 Computer with Biomek® NX^P Software

2.2.6 Teleshake unit

2.2.7 Pipettes – 100 µL, 200 µL, and 1000 µL

2.2.8 96 well thermal cycler rack

2.2.9 4-Beckman 24 Microfuge tube holders – Beckman Catalog # 373661

2.2.10 Beckman white 1.5 mL tube inserts – Beckman Catalog # 373656

2.3 Materials

2.3.1 ABGene 96 deep round well plates (Do not substitute these plates with a different brand)

2.3.2 Marsh 96 deep square well plates (Do not substitute these plates with a different brand)

2.3.3 8-Reaction tube strips, 0.2 mL

2.3.4 8-Cap strips, 0.2 mL

2.3.5 P250 Tips – aerosol resistant – (Beckman catalog #379503)

2.3.6 P1000 Tips – aerosol resistant – (Beckman catalog #987925)

2.3.7 Quarter module reservoirs divided by length (Beckman catalog #372788)

2.3.8 Quarter module reservoirs (Beckman catalog #372790)

2.3.9 Half module reservoirs (Beckman catalog #372786)

2.3.10 Tape

2.3.11 Microscope slides (optional)

2.3.12 Sterile ART tips for pipettes - 100 µL, 200 µL, and 1000 µL

2.4 Reagents

2.4.1 DNA IQ™ System Reaction Kit

2.4.2 DNA IQ™ Lysis Buffer

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

2.4.3 DNA IQ™ Wash Buffer

2.4.4 DNA IQ™ Resin

2.4.5 DTT

2.4.6 DNA IQ™ Elution Buffer

COPYRIGHT © 2015

2.5 Starting the BIOMEK® NX^P Automation Workstation

2.5.1 Turn on the computer.

2.5.2 Turn on the Biomek® NX^P Automation Workstation using the power button located on the back right side of the unit under the power cord.

VIRGINIA

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

DEPARTMENT

2.5.4 Complete the Biomek® NX^P Automation Workstation loading sheet 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 (Figure 2).

OF

2.5.5 Place the 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 and STR setup procedure when the extracted DNA is prepared for permanent storage (the 1.5 mL transfer method).

FORENSIC SCIENCE

2.5.5.1 Place the pre-labeled PCR amplification strip 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 Biomek plate loading sheet.

NOTE: If known and unknown samples are loaded into the same 96 deep well plate, the known and unknown samples must be separated a column of empty wells or reagent blanks.

2.5.6 Depending on the sample type, pipette the following volume of sample into the appropriate well of a clean 96 deep square 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 800 µL of the lysate into the Marsh deep well plate.

UNCONTROLLED

2.5.6.2 For non-sperm fractions, pipette up to 400 µL of the lysate into the Marsh deep well plate.

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 prevent carry-over.

COPY

2.5.6.3 For sperm fractions, pipette the entire sample (this is approximately 30-50 uL 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 up to 400 µL of the lysate into the Marsh deep well plate.

2.5.6.5 For tissue samples, pipette up to 400 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 Loading evidence samples:

COPYRIGHT © 2015

2.5.7.1 Once a column of evidence samples has been loaded into the 96 deep well plate, place a piece of tape over the wells to prevent 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.

VIRGINIA

2.5.7.2 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 (including samples loaded diagonally to each other – i.e., if an evidence sample is loaded into well F7 (Figure 2) then the known samples can start at well H8). There will be one full column of blanks between evidence samples and known samples as indicated in Figure 2 column 8.

DEPARTMENT

NOTE: A set of evidence samples may also be separated by a column of blank wells from another examiner's set of samples (column 6 in Figure 2).

OF

2.5.7.3 If evidence samples are loaded on both sides of a column of known/reference samples (i.e., columns 5 and 9 in Figure 2), the evidence samples and the known/reference samples must be separated by a column of blanks (i.e., columns 6 and 8 shown below).

FORENSIC SCIENCE

Plate with Knowns and Unknowns

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

UNCONTROLLED COPY

Figure 2. Plate with knowns and unknowns. Both of the 11th and 12th columns must be unused (shown in red) since the Plexor HY controls will be in columns 11 and 12 on other tubes and plates.

2.5.8 Loading known/reference samples:

2.5.8.1 If known/reference samples are loaded into the evidence sample 96 deep well plate a column of blank wells or reagent blanks will be used to separate the known/reference samples from the evidence samples as demonstrated in the diagram above (i.e., wells A8 through H8).

COPYRIGHT © 2015

2.5.8.2 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.

2.5.8.3 Alternate known samples are loaded with the known/reference samples.

2.6 Biomek® NX^P Automation Workstation Operating Procedure

VIRGINIA

All reagents used for the DNA IQ™ ISOLATION SYSTEM must be properly prepared prior to use.

DEPARTMENT

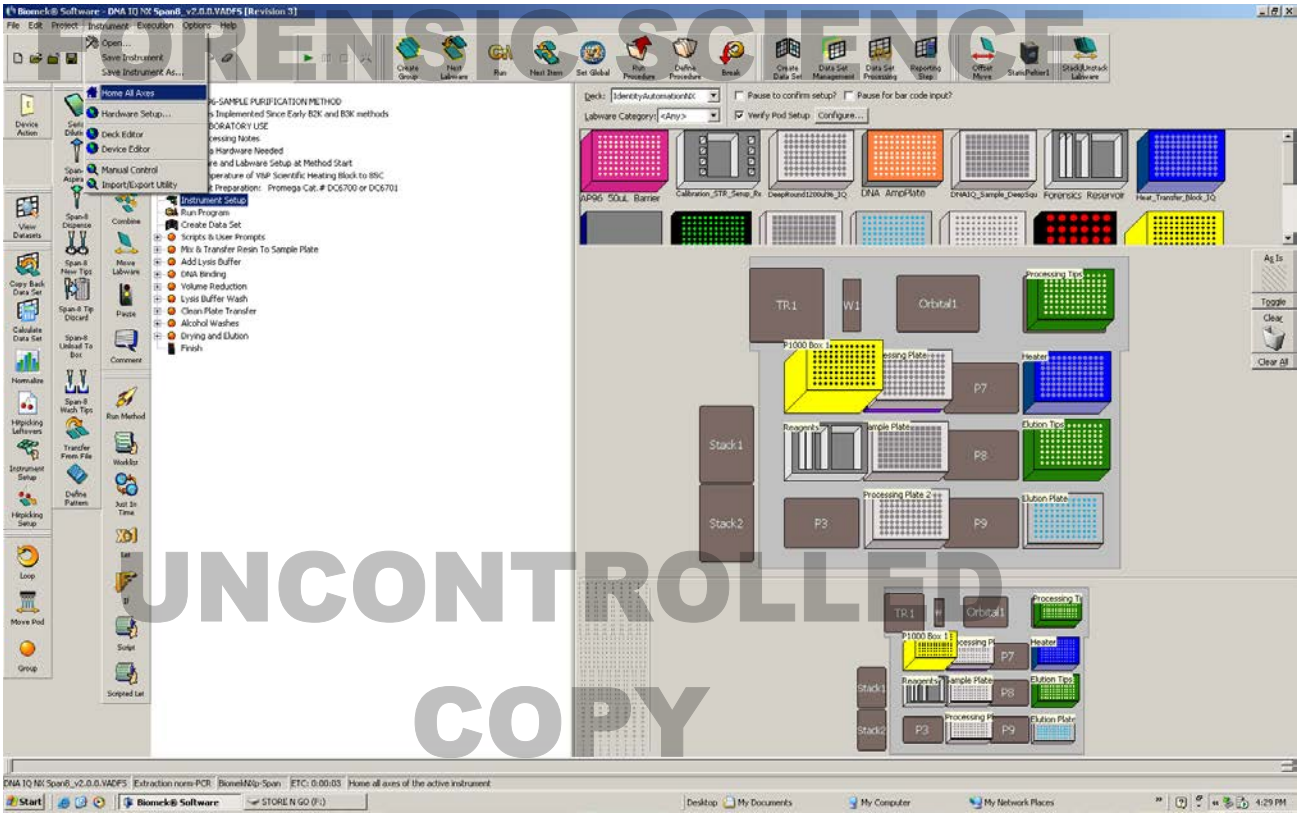
NOTE: Some of the reagents are sensitive to evaporation, **DO NOT** fill the reservoirs for the Biomek® method until just prior to initiating the robot run.

2.6.1 If the Biomek® NX^P Automation Workstation was not on, push in the power button and once fully booted up, click on the desktop shortcut for the NX software.

OF

2.6.2 When the NX software opens up the window shown in Figure 3 will appear.

FORENSIC SCIENCE



UNCONTROLLED
COPY

Figure 3. The drop-down window under the instrument heading is shown with Home All Axes highlighted.

2.6.2.1 Under the instrument heading drop-down window, select **Home All Axes**.

2.6.2.2 The robot will display the prompt shown in Figure 4.

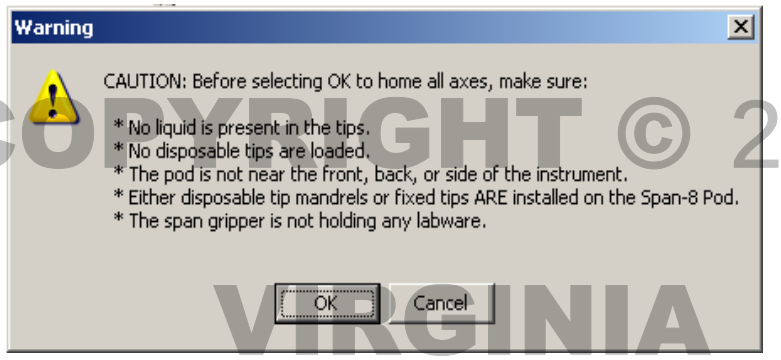


Figure 4. Home all axes prompt.

2.6.2.3 Ensure that all of the conditions are met and then click OK. If the pod is near the front, back or side of the instrument, gently slide the pod over by pushing the metal bars above the pod, not the pod itself.

2.6.2.4 The robot will home the axes and then pause when it gets to the step shown in Figure 5.

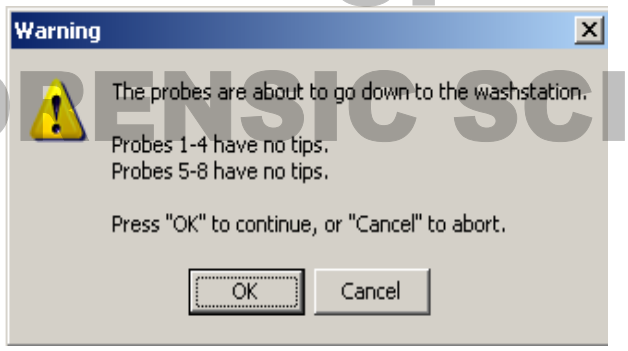


Figure 5. Prompt window prior to the step for purging any air from the lines.

2.6.2.5 If the probes have no tips on them, click OK and allow the plunges to push any air out of the lines.

2.6.2.6 After the plungers have pushed any bubbles out, which usually only takes a few iterations of the plungers, click on the OK in the box shown in Figure 6 to stop purging the lines.

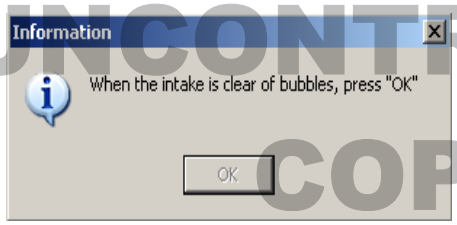


Figure 6. Prompt window to stop purging the lines.

2.6.3 The robot is now ready for running a method. Open the Extraction Norm-PCR project which contains the DNAIQ NX method.

2.6.3.1 Using either the folder icon or under the File drop-down menu, open the DNA IQ NX method.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

2.6.3.2 The window shown in Figure 7 will pop up.

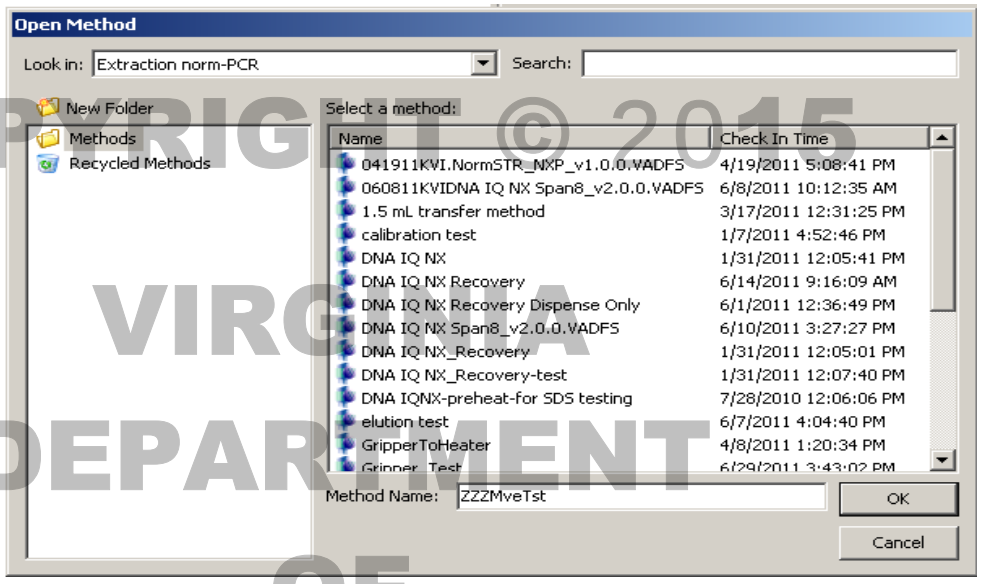


Figure 7. Open method window.

2.6.3.3 Select the DNA IQ NX method by double clicking or clicking on it and the OK button.

2.6.3.4 A series of prompts will appear, shown in Figure 8.

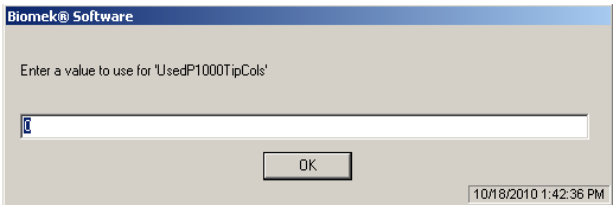


Figure 8. P1000 tip usage window.

2.6.3.5 In the window shown in Figure 8, enter the number of columns of tips, if any, that have already been used in the indicated P1000 tip box and click OK. A window identical to this except for the used P250 tips (the prompt says P200 which is the same) will open up, indicate the number of columns of used tips, if any for the P250 tips and click OK. This applies to both boxes of P250 tips.

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE
UNCONTROLLED
COPY

2.6.3.6 The user interface window shown in Figure 9 will open.

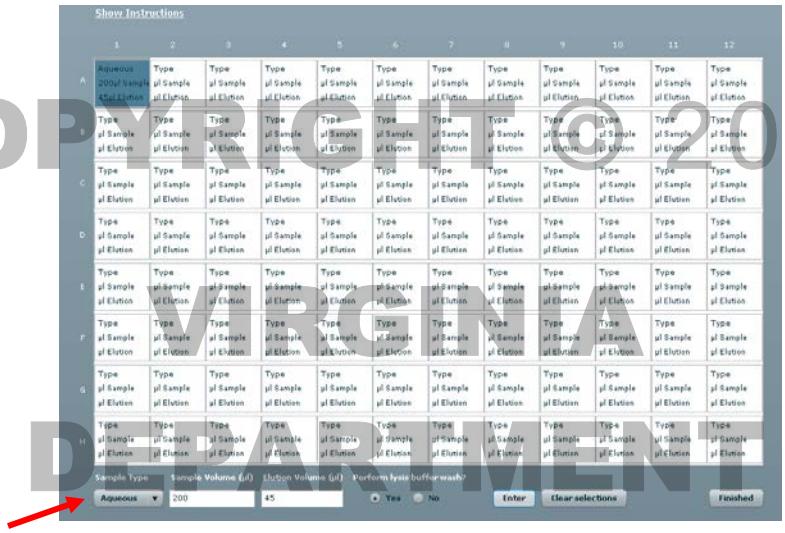


Figure 9. User interface window for entering sample information.

2.6.3.7 Highlight each well containing sample and indicate the nature of the buffer the sample is in from the drop-down menu indicated in Figure 9 with an arrow (aqueous [proteinase K buffer] or Lysis buffer), the volume of the lysate and the volume desired for the elution step. The elution volume will typically be set at 45 µL. Holding down on the Control and Alt keys will allow the user to cherry pick all of the wells that they wish to have the same settings. Once the wells have the information entered, click on Enter. This will need to be repeated for each sample type (for e.g., if another sample type uses 300 µL of Lysis buffer) and the Enter button clicked to enter that sample information for that well or wells. If the entire column will have the same information entered, highlight the well in the A row and while holding down the Control and Shift keys, highlight the well in the same column in the H row and this will result in the entire column of samples being highlighted. Enter the information and then click Enter. Once the information for all wells has been entered, click Finish.

NOTE: For optimal yields, do not underestimate the volume of the lysate. It is better to overestimate the volume and round up to the nearest 50 µL than to underestimate.

2.6.3.8 The following window, shown in Figure 10, will open up.

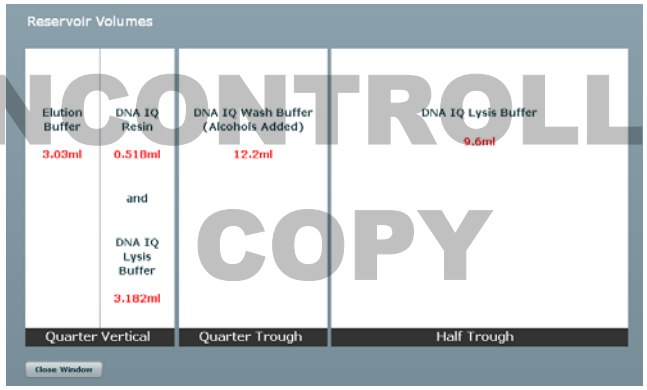


Figure 10. Estimated reagent volumes for a specific plate of samples.

2.6.3.9 Use the volumes indicated for each of the reagent troughs shown in Figure 10 to set up the reagents for the specific run. The values are calculated for each plate of samples from the

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

information entered in the screen shown in Figure 9. Click on the Close Window button when all of the reagent troughs have been filled with the appropriate reagents using the volumes indicated.

NOTE: The DNA IQ™ resin should be vortexed on high for approximately 15 seconds before pipetting into the second trough from the left. The DNA IQ™ Lysis buffer must have DTT added to it prior to use. The DNA IQ™ Wash buffer must be correctly prepared using the appropriate volumes of alcohol as indicated in the Appendix B.

COPYRIGHT © 2016

2.6.3.10 The method will then commence. Prompts regarding the tip boxes will appear as shown in Figure 11. Check to be certain that the tip box is placed on the deck in the appropriate position and click, OK.

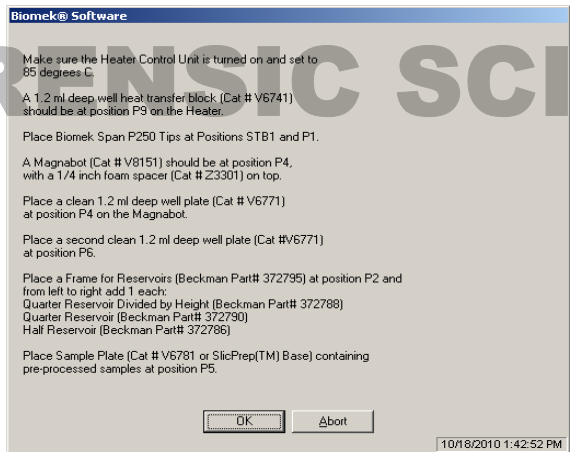
VIRGINIA

DEPARTMENT

Figure 11. First tip box prompt.

2.6.3.11 The window shown in Figure 12 will pop up. Check to make certain that all of the directions for placing labware on the deck are followed and click OK.

OF



FORENSIC SCIENCE

Figure 12. Labware directions.

UNCONTROLLED
COPY

2.6.3.12 A prompt showing the deck layout will appear as shown in Figure 13.

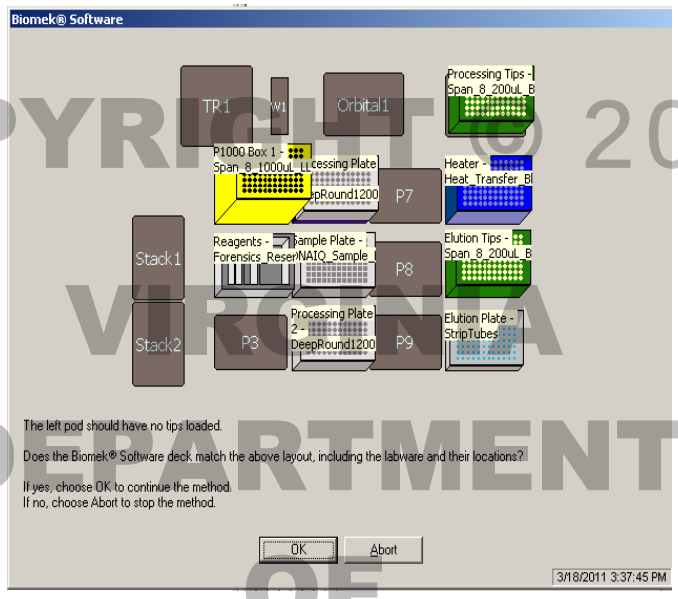


Figure 13. Deck layout for the DNA IQ™ extraction method.

NOTE: Once the DNA extraction is finished, the DNA will be eluted into ~35 µL of DNA IQ™ Elution buffer, then pipetted into the strip tubes at deck position P12 (lower right hand corner of Figure 12).

2.6.4 After the DNA isolation is complete, carry the samples forward for quantitation as outlined in the Section 4, DNA Quantitation.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

3 QUALITY ASSURANCE MEASURES FOR ISOLATING DNA USING THE BIOMEK® NX^P AUTOMATION WORKSTATION

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

3.1.1 If a signal is detected above the LOD at several loci in a reagent blank, all samples associated with the reagent blank (i.e., samples extracted in conjunction with a reagent blank) will be considered inconclusive, and the sample(s) will be re-extracted **and/or** re-amplified if possible. If a small peak is observed above the LOD 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.

COPYRIGHT © 2015
VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

4 DNA QUANTITATION

4.1 Plexor HY Human DNA Quantitation

4.1.1 Technical Notes

COPYRIGHT © 2015

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.

VIRGINIA DEPARTMENT OF

4.1.1.3 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.

4.1.1.4 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.

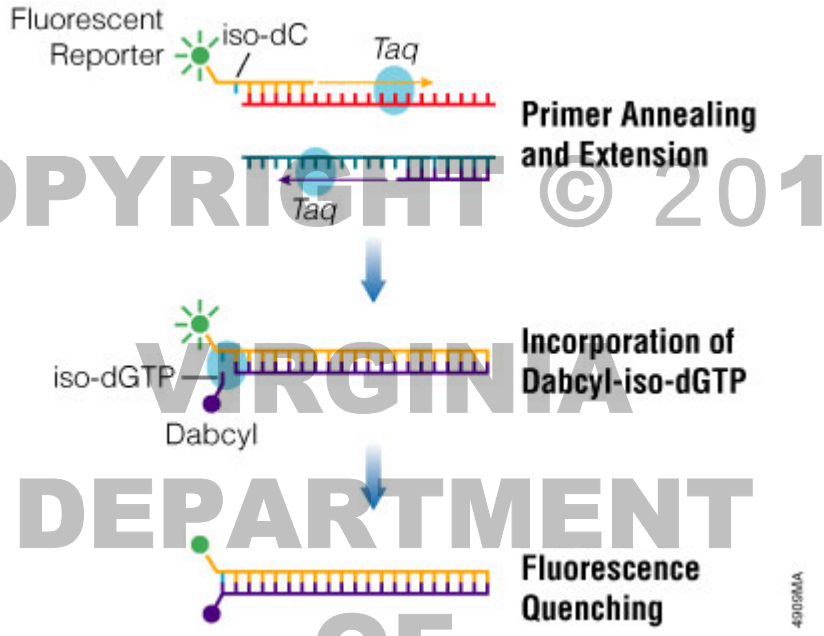
FORENSIC SCIENCE

4.1.1.5 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.

4.1.1.6 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 is 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.

UNCONTROLLED COPY

4.1.1.7 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.



COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

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.)

4.1.1.8 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

4.1.2.1 Stratagene Mx3005P™ Quantitative PCR instrument

4.1.2.2 Biomek® NX^{P} Automation Workstation

4.1.3 Materials

4.1.3.1 96-well qPCR Plate - Greiner P/N 652260

4.1.3.2 Optically Clear Strip Caps – Phenix Research P/N 101100-082 or Greiner P/N 373250

4.1.3.3 Optional: Optically clear film – VWR Cat#82050-994

4.1.3.4 Micro Amp strip tubes (in strips of 8 tubes)– ABI Cat# N801-0580

4.1.3.5 Black PCR support base (96 well) – ABI Cat# N801-0531

4.1.3.6 P20 Tips – aerosol resistant – Beckman Cat# 379506

4.1.3.7 P50 Tips – aerosol resistant – Beckman Cat# A21586

4.1.3.8 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:

COPYRIGHT © 2015

- Plexor® HY 2X Master Mix
- Plexor® HY 20X Primer/IPC Mix
- Nuclease free or Type I water

Type I water is considered nuclease free and may be used instead of the amplification grade water that comes with the Plexor® HY kit.

VIRGINIA

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

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

DEPARTMENT

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

OF

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.

FORENSIC SCIENCE

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® NX^P 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.

UNCONTROLLED

COPY

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.

Step	Temperature	Time	Number of Cycles
Initial denaturation:	95°C	2 minutes	1 cycle
Denaturation:	95°C	5 seconds	38 cycles
Annealing and extension:	60°C	40 seconds	
Melt temperature curve:	65°C initial temperature with 0.6°C increase each cycle, 40-second hold each cycle		48 cycles

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

Figure 2. Thermocycling parameters. (Figure obtained from the Plexor® HY System Technical Manual.)

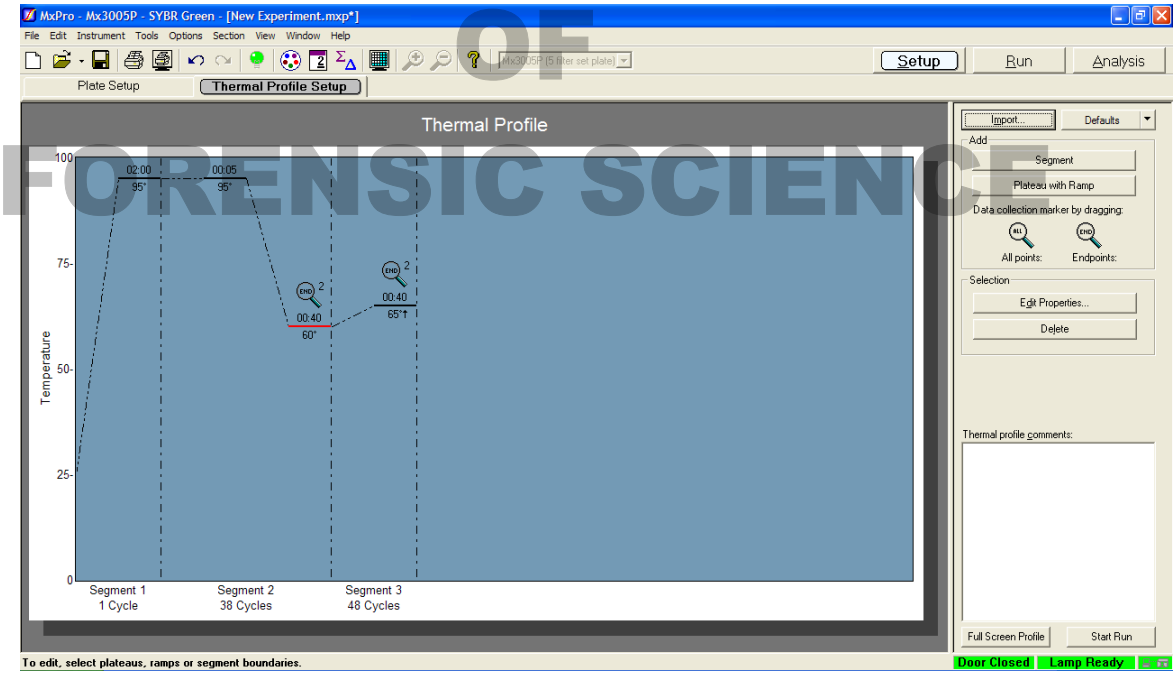


Figure 3. Thermal Profile Setup for the Plexor® HY System assay.

UNCONTROLLED COPY

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 or 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 NX^P Workstation

4.1.7.1 Turn on the computer.

4.1.7.1.1 Turn on the Biomek® NX^P Automation Workstation using the power button located on the back right side of the unit.

COPYRIGHT © 2015

4.2 Biomek® NX^P Automation Workstation Operating Procedure

4.2.1 If the Biomek® NX^P Automation Workstation was not on, push in the power button and once fully booted up, click on the desktop shortcut for the NX software.

VIRGINIA

4.2.2 When the NX software opens up the window shown in Figure 4 will appear.

DEPARTMENT OF FORENSIC SCIENCE

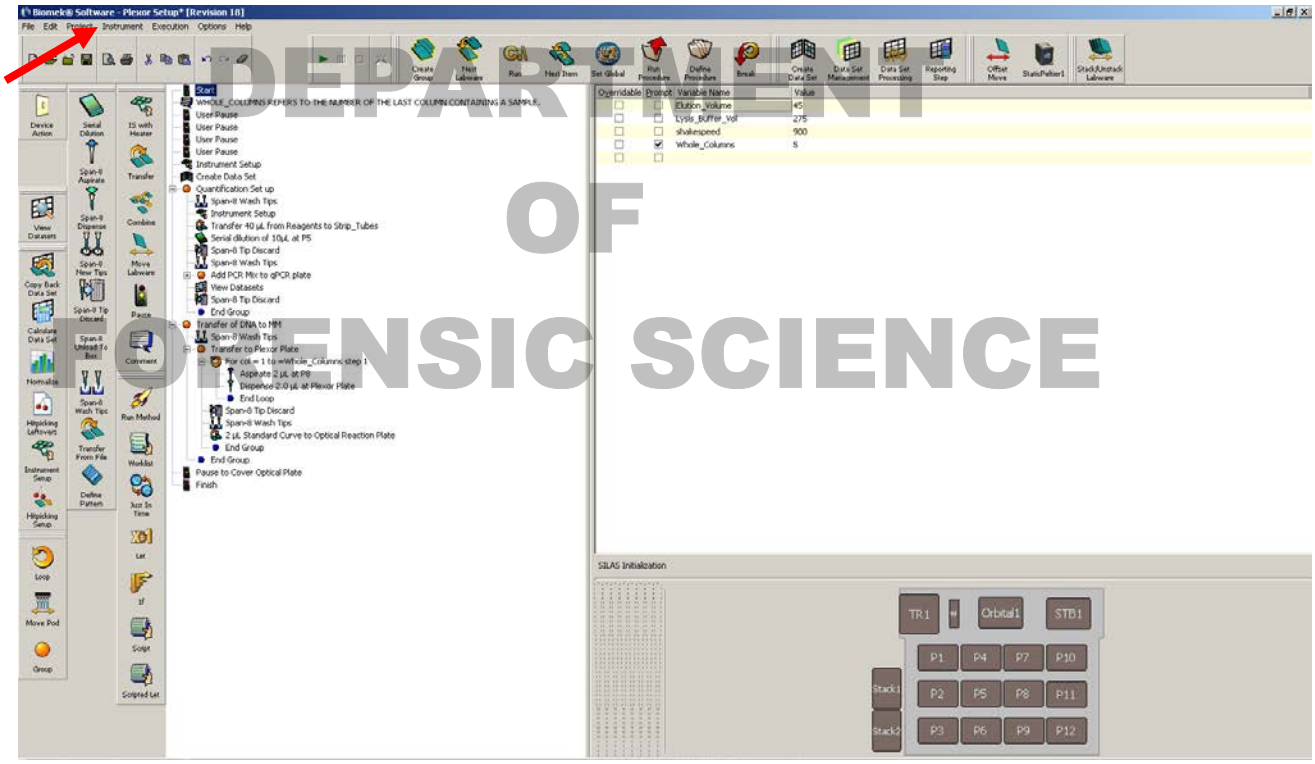


Figure 4. Window of the NX software showing the Plexor setup method. Red arrow points to the instrument drop-down window.

4.2.2.1 Under the instrument heading drop-down window (shown by arrow), select Home All Axes.

UNCONTROLLED COPY

4.2.2.2 The robot will display the prompt shown in Figure 5.

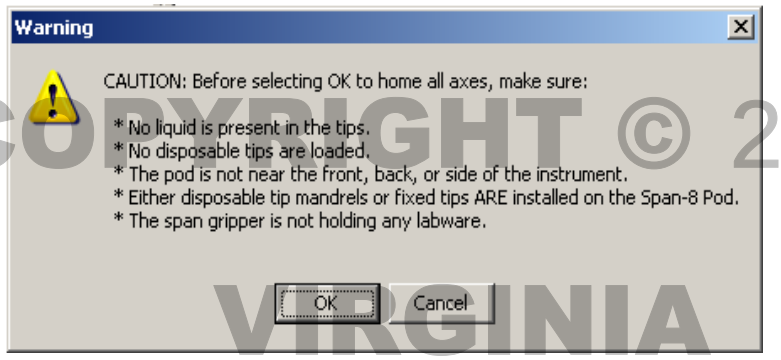


Figure 5. Home all axes prompt.

4.2.2.3 Ensure that all of the conditions are met and then click OK. If the pod is near the front, back or side of the instrument, gently slide the pod over by pushing the metal bars above the pod, not the pod itself.

4.2.2.4 The robot will home the axes and then pause when it gets to the step shown in Figure 6.

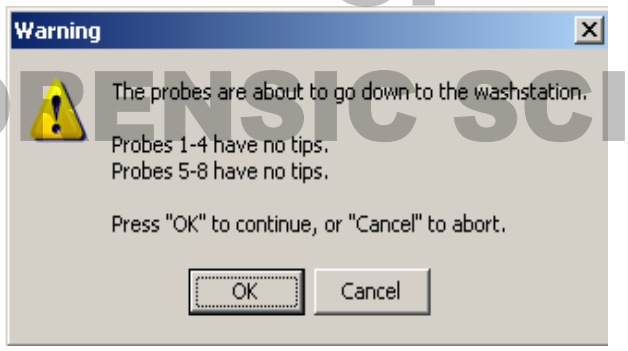


Figure 6. Prompt window prior to the step for purging any air from the lines.

4.2.2.5 If the probes have no tips on them, click OK and allow the plunges to push any air out of the lines.

4.2.2.6 After the plungers have pushed any bubbles out, which usually only takes a few iterations of the plungers, click on the OK in the box shown in Figure 7 to stop purging the lines.

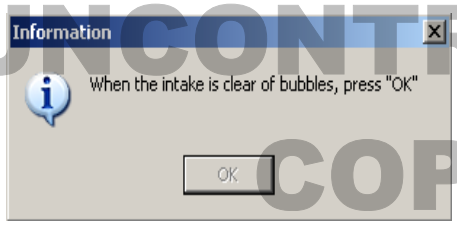


Figure 7. Prompt window to stop purging the lines.

4.2.3 The robot is now ready for running a method. Open the Plexor project folder which contains the Plexor Setup method.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

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 -20ºC, except the genomic DNA standard, which **MUST** be stored at 4ºC between uses

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

COPYRIGHT © 2015

4.2.3.2 Prior to starting the Plexor method, ensure that the sample information is entered into the first worksheet of the populatable worksheets and click on the NX button (Figure 8).

4.2.3.3 A text file will be created which is a worklist, called Run Sheet, to be imported into the Plexor setup method. This file should be created at another workstation and saved and transported to the Biomek® NX^P robot computer. Then Run Sheet file then needs to be saved to the desktop of the computer. When prompted that another file named Run Sheet is there and would you like to replace it with the current Run Sheet file, click Yes.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

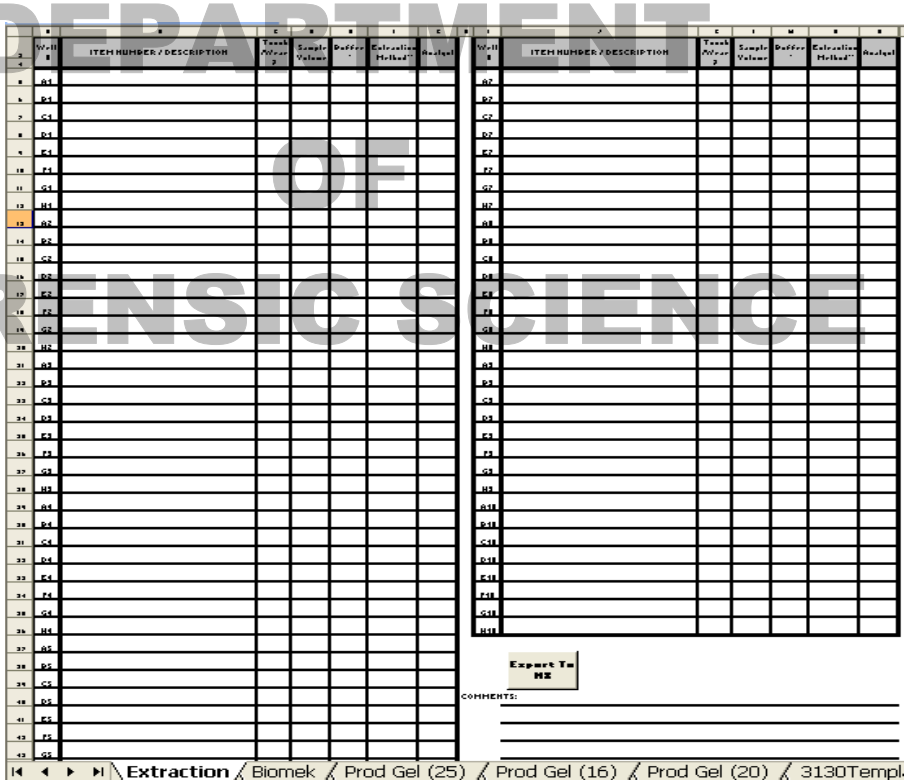


Figure 8. Populatable worksheets with export to NX^P function.

UNCONTROLLED COPY

4.2.3.4 To ensure that the proper Run Sheet will be imported into the Plexor setup method, do the following:

4.2.3.4.1 Click on the Finish line of the method (shown in Figure 9, red arrow). Clicking on the Finish line of the method causes the method to be auto-checked and will also import the Run Sheet from the desktop.

4.2.3.4.2 Click on the View Datasets line (shown in Figure 9, black arrow). To view the appropriate dataset, click on the Plexor Plate to highlight (if it has not been already) and select View One Dataset. If not already designated in the drop-down window, select SAMPLE. Check to ensure that the Run Sheet is populated with the appropriate samples in the correct pattern.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

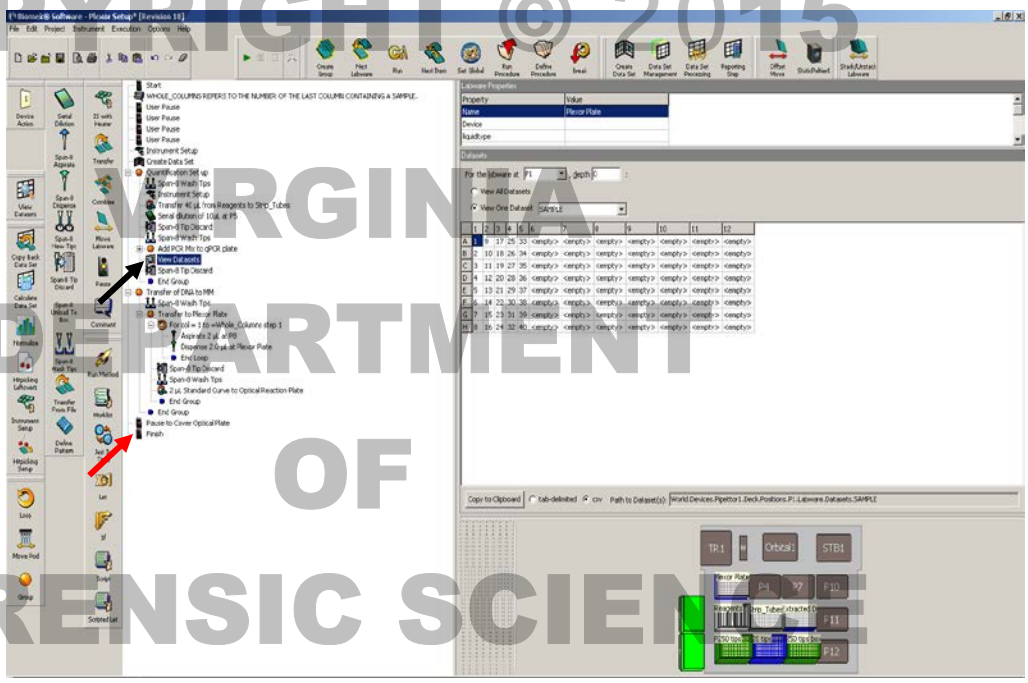
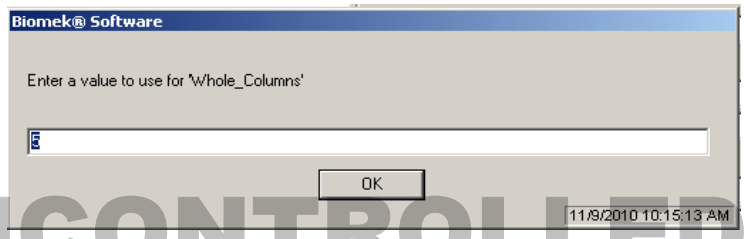


Figure 9. Checking the Plexor setup method to ensure that the plate specific Run Sheet has been imported. The red arrow points to the line of method that states "Finish". The black arrow points to the View Datasets line. The Dataset is shown on the right part of the screen.

4.2.3.5 Click on the green arrow to initiate the method. The following window will open and a box with a prompt will open (Figure 10).



UNCONTROLLED COPY

Figure 10. Open Plexor setup method window with first prompt**.**

4.2.3.6 The first prompts the user to enter a value for Whole Columns and click OK. This refers to the last column containing DNA samples and does not refer to the standards. For example, if you extracted 45 samples that were loaded in columns 1-8, then the value for Whole Columns would be 8. A prompt will pop up immediately afterwards explaining what Whole Columns means. Click OK.

NOTE: If samples need to be manually added, then the column(s) of strip tubes containing the manually pipetted samples must be included in the Whole Columns number unless the column(s) of samples can be inserted into an empty column. The manually added samples should be typed into the populatable

worksheets to ensure that sufficient qPCR master mix is created and pipetted to the appropriate sample wells.

4.2.3.7 The next prompt will be as shown in Figure 11. Follow the direction and click OK.

Figure 11. Prompt for directing the number of P250 tip boxes to place on the deck.

4.2.3.8 After responding to the prompt in Figure 11, the prompt in Figure 12 will pop up directing the user as to where to place the Plexor® HY System mastermix and the Type I water.

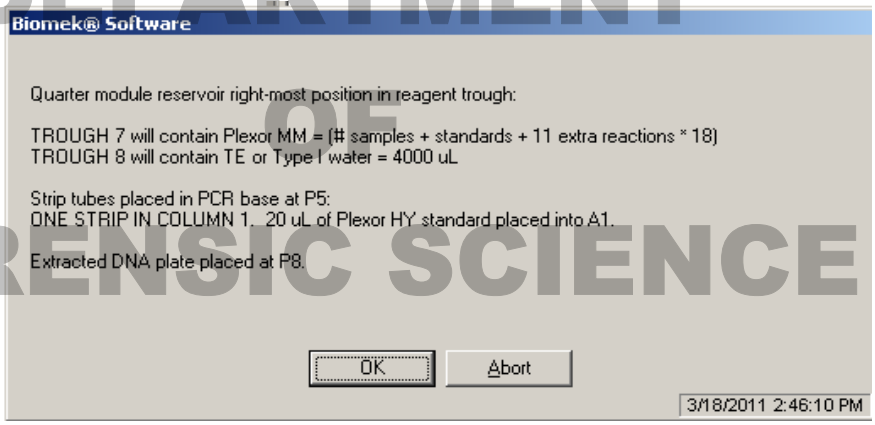


Figure 12. Placing Plexor® HY mastermix and Type I water into the appropriate troughs and the Plexor HY standard into the strip tubes.

4.2.3.9 Prepare the Plexor® HY System master mix in a 1.5 or 2.0 mL tube as described below:

- Plexor® 2X Master Mix – 10 µL per reaction
- Nuclease-free (or Type I) H_2O – 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 11 excess reactions (vortex well).

4.2.3.10 Place the Plexor HY master mix in quarter module reservoir divided by length, in the trough on the left (#7). Make certain to dispense the mastermix so that it is evenly distributed along the bottom of the reservoir. Place the appropriate volume or greater of Type I water or TE-4 into the trough on the right side (#8) and place the reservoir into the reservoir holder in the rightmost slot.

4.2.3.11 Continuing to follow the directions in Figure 13, remove the Plexor HY standard from the 4ºC refrigerator and vortex on high briefly before dispensing 20 µL of the standard into one tube of a strip of amplification tubes. It must be dispensed into an end tube with no bubbles in the bottom of the tube and the strip placed into a black PCR support base in the first column with the tube containing the standard in the A (top) row. Once this is completed, as well as the previous step, click OK for the prompt shown in Figure 12.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

4.2.3.12 Another prompt will come up asking if you verified that the Run Sheet has been properly imported into the method, shown in Figure 13.

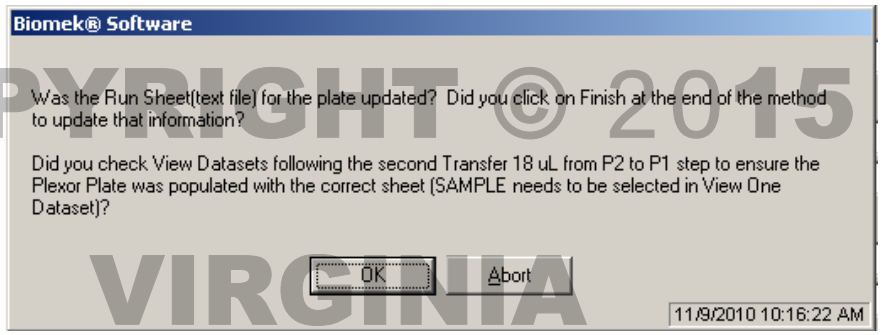


Figure 13. Check the View Datasets to ensure that the run specific Run Sheet was imported into the method.

4.2.3.13 If Finish has not been clicked to update the Plexor setup method prior to initiating the run, then follow the directions on the prompt and abort the method and follow the directions in 4.2.3.4. This is only necessary for checking to make certain that the plate specific worklist has been generated and saved to the Biomek NX computer's desktop since the method will import the Run Sheet whenever the method is initiated.

4.2.3.14 Allow the method to complete. Remove the qPCR plate at deck position P1. Carefully seal the plate with optically clear strip caps or film. If using film, try to center the film over the plate and press down carefully all around the plate to ensure that the seal is tight.

4.2.3.15 Spin the plate briefly in a table top centrifuge to drive any bubbles in the bottoms of the wells to the top.

4.2.3.16 The plate should be immediately taken to the qPCR instrument 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.

4.2.3.17 Cover the strip tubes containing the isolated DNA with a piece of parafilm or something similar and a weight to prevent evaporation of the samples.

NOTE: These samples will be uncovered and used during the Normalization and PCR Setup method (section 5.0).

4.2.4 Stratagene Mx3005P™ Operating Procedure

4.2.4.1 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.

4.2.4.2 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.

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

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

4.2.5 Data Analysis

4.2.5.1 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.

4.2.5.2 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 14. 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 14. Import parameters. The instrument and data collection should be selected as shown for the Plexor® HY system amplification.

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

4.2.5.3 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.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

4.2.5.4 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 15), 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 15. 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.

4.2.5.5 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”.

4.2.5.6 The defaults are used for all settings, as shown in Figure 16, except the expected melt temperature. The approximate T_m values should be: A = 81.23°C, Y = 82.59°C, and IPC = 82°C. These melt temperatures are imported when you select the Plexor® analysis template. Click “OK”. The T_m value can be adjusted slightly manually by dragging the vertical bar to the left or right to center the T_m (Figure 17, red arrow).

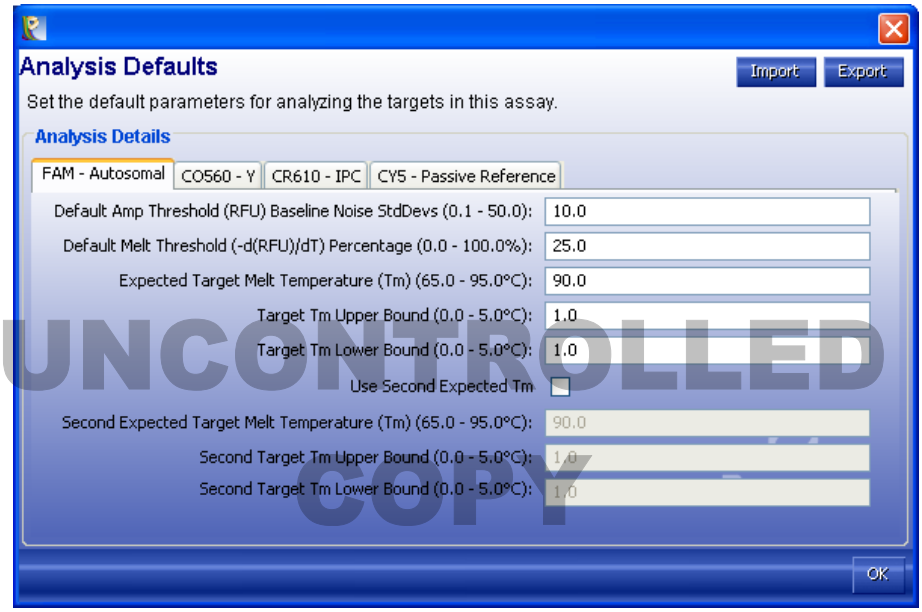


Figure 16. Analysis Template Window**.** After importing the analysis template, the expected melt temperature for the autosomal amplicon should be 81.23°C.

4.2.5.7 Click “Finish” to see the analyzed data.

COPYRIGHT © 2015 KANSAS DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

4.2.5.8 The sample IDs can now be imported from the Biomek populatable worksheet. Open the Populatable Worksheet and select the “Raw Data” Tab. Copy the sample names from the “sample names” grid (the bottom-most 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>.

COPYRIGHT © 2015

4.2.5.9 The current version of the software may not import the standards properly from the template, so 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 17). Click on the standard series definition button, indicated by the arrow in Figure 17 and the inset window shown in Figure 17 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”.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

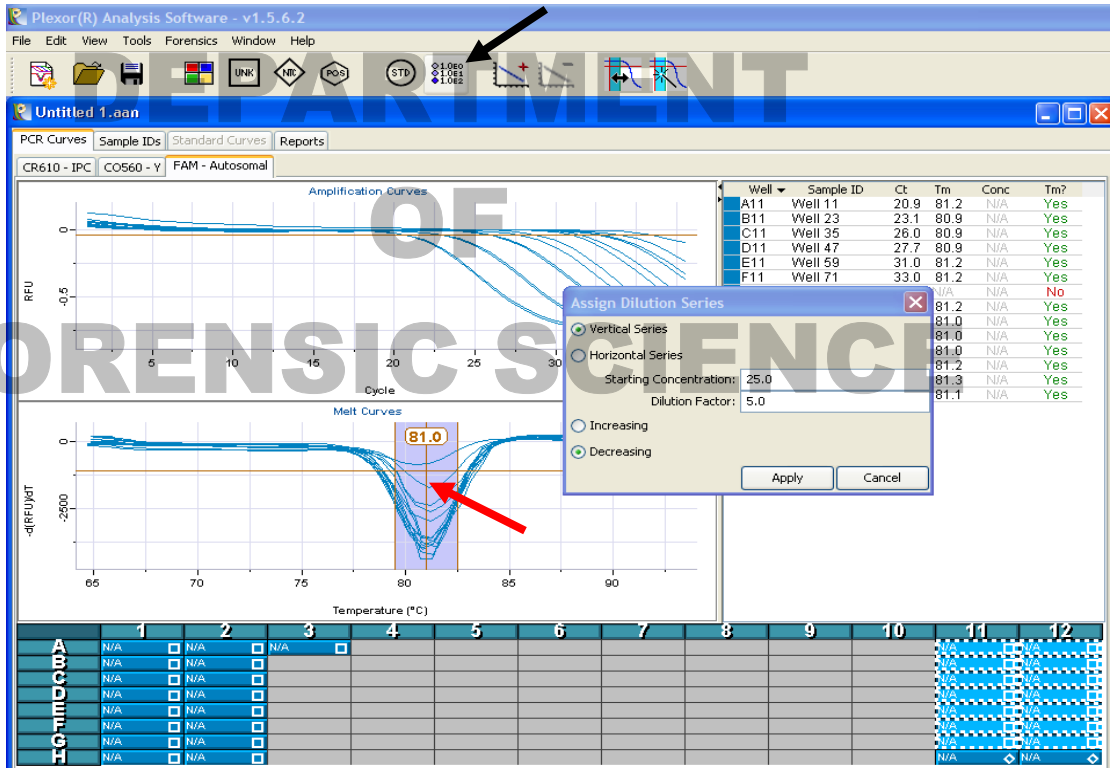


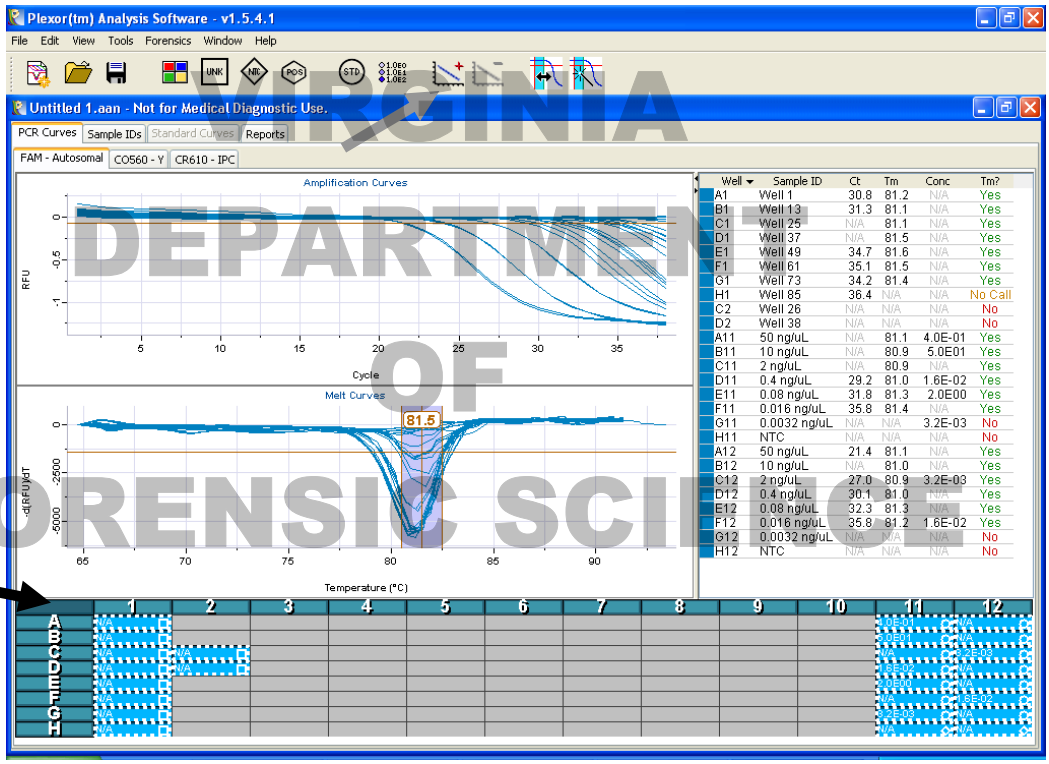
Figure 17. Defining the standard dilution series. The black arrow points to the button for assigning the dilution series. The red arrow points to the melt curve T_m.

UNCONTROLLED COPY

4.2.5.10 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 18. This creates a standard curve for the dye selected and quantitates the unknowns for that dye as well. Click on the tab for CO560 – Y and repeat to create a standard curve for the Y amplicon.

COPYRIGHT © 2015

NOTE: No standard curve is necessary for the IPC.



VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

Figure 18. 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.

4.2.5.11 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 column of standard for a total of 6 data points when necessary to produce a linear standard curve.

UNCONTROLLED COPY

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.

COPYRIGHT © 2015

4.2.5.12 Generation of the Forensics Report and Sample Details Report.

NOTE: If the Forensics Report and Samples Details Report will not be used, skip to 4.2.5.13 to generate the quantitation report (Form 210-F504).

4.2.5.12.1 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-STR dye is CO560. The IPC dye is CR610 and the default to flag an improper (inhibited) amplification for the IPC is 2 C_Ts.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

4.2.5.12.1.1 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.

4.2.5.12.1.2 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.

4.2.5.12.1.3 The Forensics report indicates the sample concentrations (in ng/uL) 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.

UNCONTROLLED COPY

4.2.5.12.2 Generation of the Sample Details Report.

4.2.5.12.2.1 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.

COPYRIGHT © 2015

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

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

VIRGINIA

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

4.2.5.13.1 If the quantitation report (Form 210-F504) will not be printed, skip to step 4.2.5.15.

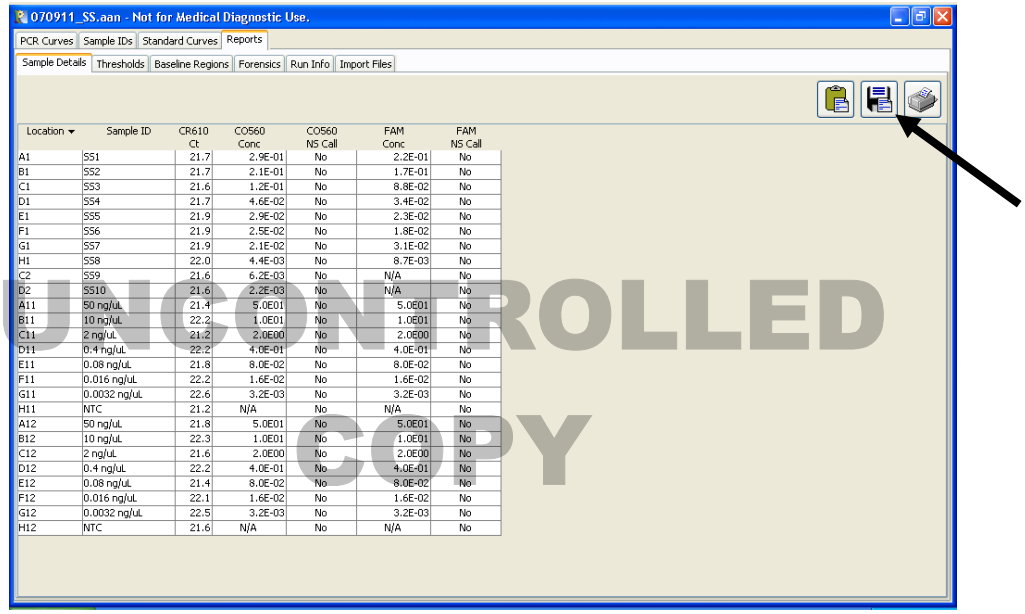
DEPARTMENT

4.2.5.13.2 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.

OF
FORENSIC SCIENCE

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

4.2.5.13.4 Click on the icon in the upper right corner that is called the “Export Selected” icon as indicated in Figure 19.



Location	Sample ID	CR610 Ct	CO560 Conc	CO560 NS Call	FAM Conc	FAM NS Call
A1	SS1	21.7	2.9E-01	No	2.2E-01	No
B1	SS2	21.7	2.1E-01	No	1.7E-01	No
C1	SS3	21.6	1.2E-01	No	8.8E-02	No
D1	SS4	21.7	4.6E-02	No	3.4E-02	No
E1	SS5	21.9	2.9E-02	No	2.3E-02	No
F1	SS6	21.9	2.5E-02	No	1.8E-02	No
G1	SS7	21.9	2.1E-02	No	3.1E-02	No
H1	SS8	22.0	4.4E-03	No	8.7E-03	No
C2	SS9	21.6	6.2E-03	No	N/A	No
D2	SS10	21.6	2.2E-03	No	N/A	No
A11	50 ng/uL	21.4	5.0E01	No	5.0E01	No
B11	10 ng/uL	22.2	1.0E01	No	1.0E01	No
C11	2 ng/uL	21.2	2.0E00	No	2.0E00	No
D11	0.4 ng/uL	22.2	4.0E-01	No	4.0E-01	No
E11	0.08 ng/uL	21.8	8.0E-02	No	8.0E-02	No
F11	0.016 ng/uL	22.2	1.6E-02	No	1.6E-02	No
G11	0.0032 ng/uL	22.6	3.2E-03	No	3.2E-03	No
H11	NTC	21.2	N/A	No	N/A	No
A12	50 ng/uL	21.8	5.0E01	No	5.0E01	No
B12	10 ng/uL	22.3	1.0E01	No	1.0E01	No
C12	2 ng/uL	21.6	2.0E00	No	2.0E00	No
D12	0.4 ng/uL	22.2	4.0E-01	No	4.0E-01	No
E12	0.08 ng/uL	21.4	8.0E-02	No	8.0E-02	No
F12	0.016 ng/uL	22.1	1.6E-02	No	1.6E-02	No
G12	0.0032 ng/uL	22.5	3.2E-03	No	3.2E-03	No
H12	NTC	21.6	N/A	No	N/A	No

UNCONTROLLED
COPY

Figure 19. 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.

4.2.5.14 A copy of either the "Forensics Report" (prepared in 4.2.5.12.1) and the "Sample Details Report" (as described in 4.2.5.12.2) *or* the quantitation report (Form 210-F504, prepared and printed in 4.2.5.13) 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.

COPYRIGHT © 2015

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.

4.2.5.14.1 Save the file when prompted.

VIRGINIA

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

DEPARTMENT

NOTE: It may be necessary to change the "Files of Type:" to "All Files" for the exported data file to be observed.

4.2.5.14.3 At the prompt, choose "Finish" as shown in Figure 20.

OF

FORENSIC SCIENCE

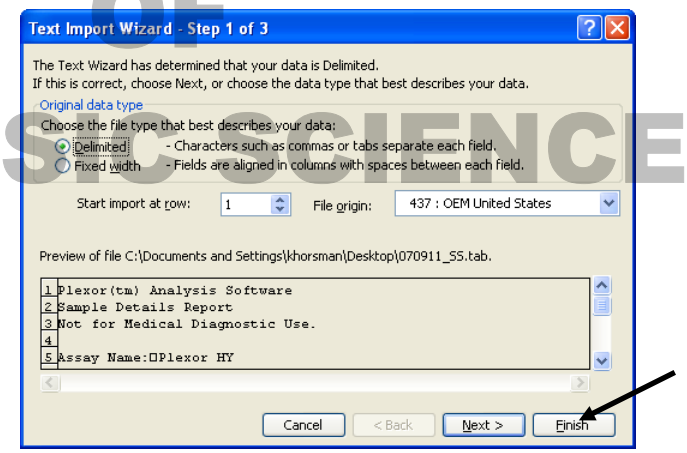


Figure 20. Import of Plexor HY data into a Microsoft Excel spreadsheet. The arrow points to the "Finish" button to complete the data import.

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

UNCONTROLLED

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

COPY

4.2.5.15 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 C_T is not within 2 cycles of those observed in the standard curve, this may be an indication of inhibition by the sample/sample matrix. 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.

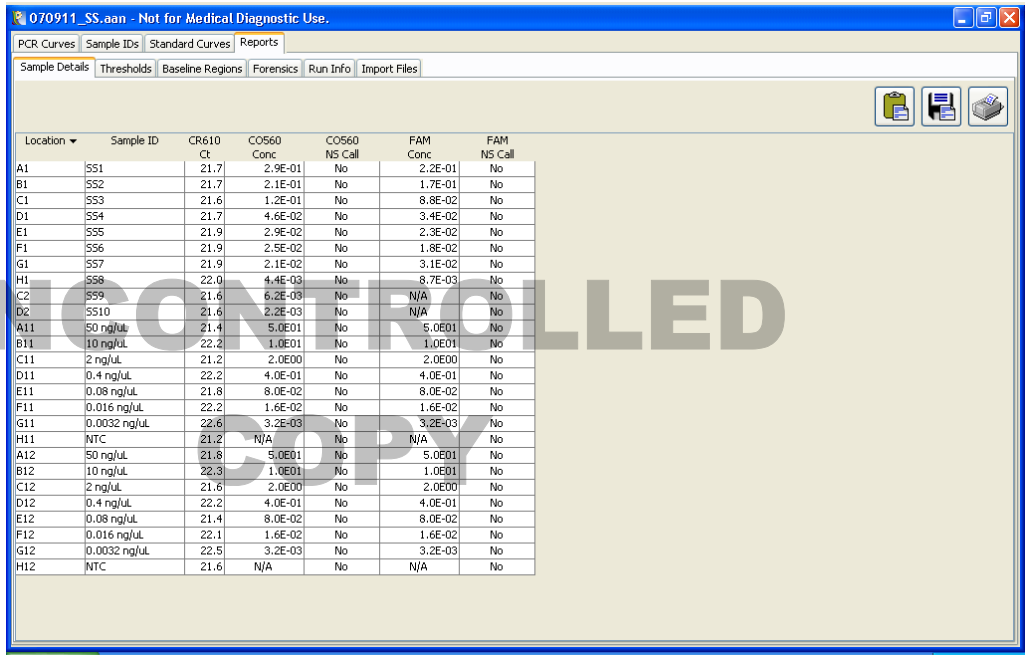
4.2.5.16 The “NS Call” column is an indication of whether non-specific amplification is potentially observed in the melt curve for the sample. Any melt observed outside of the expected melt window (generally 79-81 ± 1.5°C for autosomal and 81-83 ± 1.5°C for Y) and of sufficient magnitude to cross the 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.

4.2.5.17 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.

4.2.5.18 Export of quantitation data to a file for import into the Normalization Wizard.

4.2.5.18.1 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 computer, 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.

4.2.5.18.2 Open the .aan Plexor run file for the particular run of samples for which the normalization and PCR setup method will be used (shown in Figure 21).



Location	Sample ID	CR610 Ct	CO560 Conc	CO560 NS Call	FAM Conc	FAM NS Call
A1	SS1	21.7	2.9E-01	No	2.2E-01	No
B1	SS2	21.7	2.1E-01	No	1.7E-01	No
C1	SS3	21.6	1.2E-01	No	8.8E-02	No
D1	SS4	21.7	4.6E-02	No	3.4E-02	No
E1	SS5	21.9	2.9E-02	No	2.3E-02	No
F1	SS6	21.9	2.5E-02	No	1.8E-02	No
G1	SS7	21.9	2.1E-02	No	3.1E-02	No
H1	SS8	22.0	4.4E-03	No	8.7E-03	No
C2	SS9	21.6	6.2E-03	No	N/A	No
D2	SS10	21.6	2.2E-03	No	N/A	No
A11	50 ng/uL	21.4	5.0E01	No	5.0E01	No
B11	10 ng/uL	22.2	1.0E01	No	1.0E01	No
C11	2 ng/uL	21.2	2.0E00	No	2.0E00	No
D11	0.4 ng/uL	22.2	4.0E-01	No	4.0E-01	No
E11	0.08 ng/uL	21.8	8.0E-02	No	8.0E-02	No
F11	0.016 ng/uL	22.2	1.6E-02	No	1.6E-02	No
G11	0.0032 ng/uL	22.6	3.2E-03	No	3.2E-03	No
H11	NTC	21.2	N/A	No	N/A	No
A12	50 ng/uL	21.8	5.0E01	No	5.0E01	No
B12	10 ng/uL	22.3	1.0E01	No	1.0E01	No
C12	2 ng/uL	21.6	2.0E00	No	2.0E00	No
D12	0.4 ng/uL	22.2	4.0E-01	No	4.0E-01	No
E12	0.08 ng/uL	21.4	8.0E-02	No	8.0E-02	No
F12	0.016 ng/uL	22.1	1.6E-02	No	1.6E-02	No
G12	0.0032 ng/uL	22.5	3.2E-03	No	3.2E-03	No
H12	NTC	21.6	N/A	No	N/A	No

Figure 21. Plexor HY run file (.aan file).

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

4.2.5.18.3 Select the Forensics tab. With that window open, right click and select the "change columns shown" option (Figure 22). The Sample Name, Location, Sample Type, Auto, Y and Auto/Y will already be checked. Scroll down and also check (if not already checked) the IPC Expect Ct, IPC Status, and Curves Status and then click OK.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

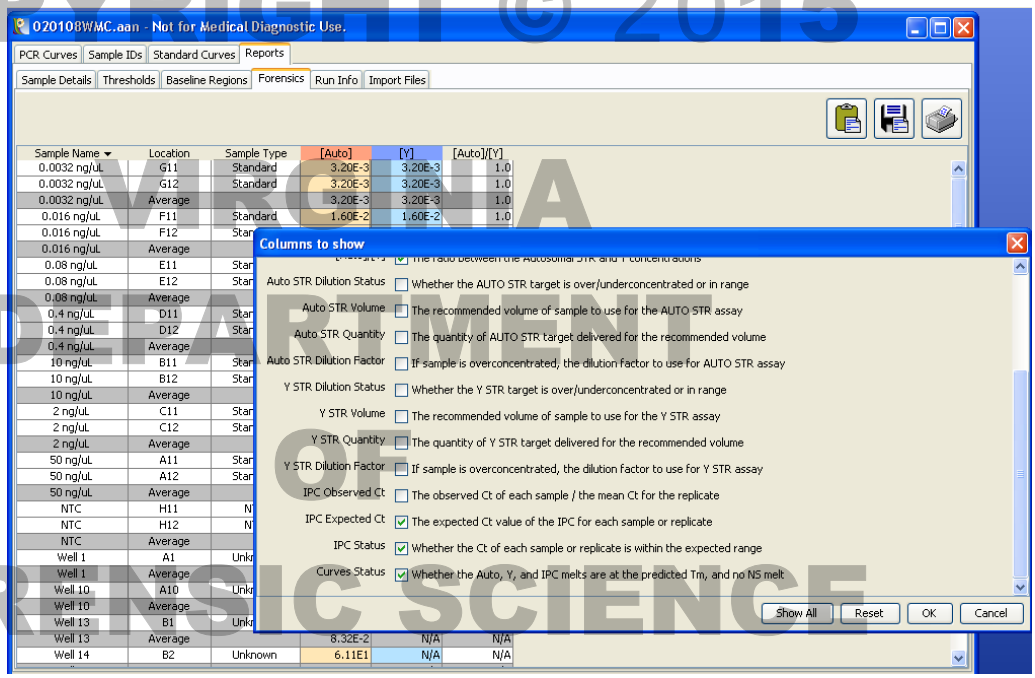


Figure 22. Changing the columns in the Forensic Report.

4.2.5.18.4 Select all of the rows and click on the "export selected icon", shown highlighted in the upper right corner of Figure 23.

UNCONTROLLED COPY

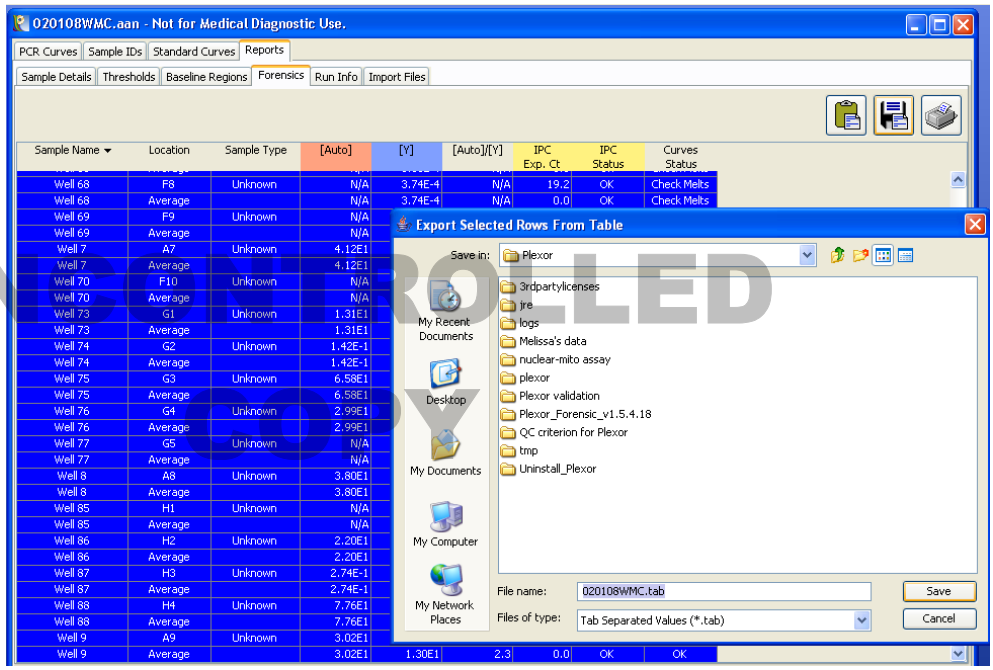


Figure 23. Biomek® Plate Import Prompt

4.2.5.18.5 Save the quantitation data as a tab file in the desired location, such as the desktop or to a flash drive. This data file will need to be physically transferred to the Biomek® NX^P workstation in order to for the normalization and PCR setup method to import it.

COPYRIGHT © 2015

4.2.5.19 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.

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

VIRGINIA

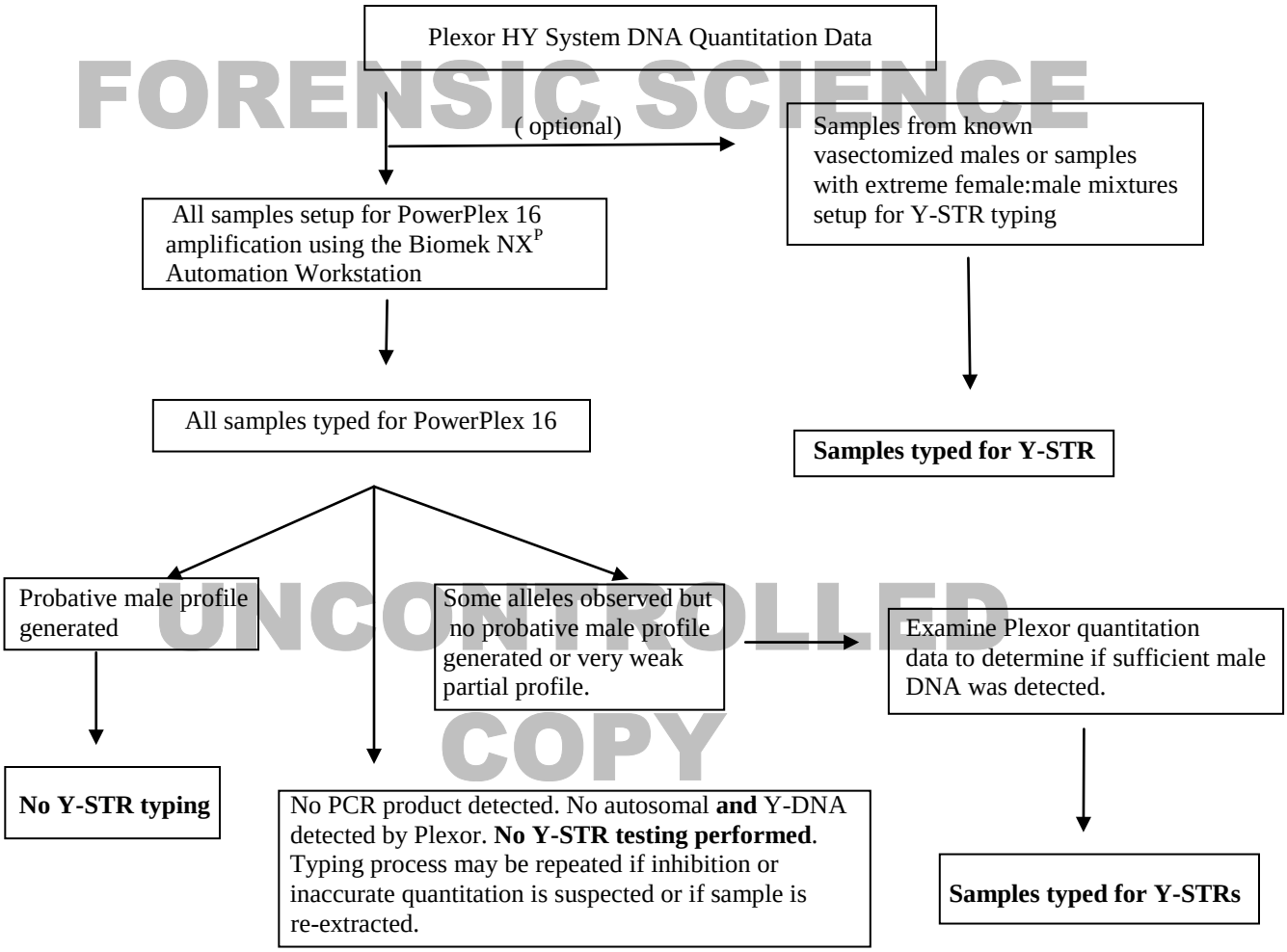
4.2.6.1 **If no male reference samples were submitted with the case in question, no Y-STR analysis will be performed**. The case may be re-submitted for Y-STR testing when male reference samples are obtained.

DEPARTMENT

4.2.6.2 Examine the Plexor® HY Quantitation System data during or after PowerPlex® 16 typing of casework samples.

4.2.6.3 Use the Decision Tree, shown in Figure 21, to determine if Y-STR typing should be performed.

OF
FORENSIC SCIENCE



UNCONTROLLED
COPY

Figure 21. Decision Tree for Y-STR Typing

5 NORMALIZATION AND PCR SET UP

The Normalization and PCR Setup method is designed to import DNA quantitation data (ng/µL) and utilize that information to determine the method of DNA normalization. The method, created by Promega Corp. using the Beckman software and Visual Basic scripting, directs the Biomek® NX^P perform a DNA normalization procedure that will accommodate that specific sample. It can perform a direct transfer of DNA samples to the STR mastermix (undiluted), a small dilution in the pipette tip itself, it can use a single plate for a dilution or it can perform a serial dilution using two plates. To further increase the one-plate and two-plate dilution factors, the robot can pair either one or two plate dilutions with an in-tip dilution. When the entire process is complete, a set of PCR reactions will be prepared, with the DNA input quantity normalized to the target unless it fails to meet the target concentration, in which case the robot will add the maximum volume (5 µL) to the STR amplification reaction.

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE
UNCONTROLLED
COPY

5.1 Equipment

5.1.1 Biomek® NX^P Automation Workstation

5.1.2 Biomek® Span-8 Tool

5.2 Materials

5.2.1 Black PCR support base (96 well) – ABI Cat# N801-0531

5.2.2 MicroAmp® tubes (attached caps) – USA Scientific # 1402-8100 (clear) or #1402-8108 (multi-colored)

5.2.3 Modular reservoir half module 150 mL - Beckman Catalog #372786

5.2.4 96 well normalization plate – Innovative Microplate Catalog # S30026

5.2.5 P250 Tips–aerosol barrier - Beckman Catalog # 379503

5.2.6 P50 tips – aerosol resistant - Beckman Catalog # A21586

5.2.7 P20 tips – aerosol resistant - Beckman Catalog # 379506

5.2.8 Microcentrifuge tubes, 1.5 mL with unattached or attached lids

5.2.9 Thermocycle plate

5.3 Reagents

5.3.1 Type I Sterile Water

5.3.2 AmpliTaq™ Gold DNA polymerase

5.3.3 PowerPlex® 16 BIO System amplification kit

5.4 Preparation for Using the Normalization Wizard

NOTE: The tool and the Biomek® NX^P robot deck must be wiped off with a solution that will remove/degrade the DNA prior to running the Normalization Wizard method.

5.4.1 Open the project folder entitled, “Extraction Norm-PCR”. If the Biomek® NX^P was off, follow the steps in Chapter 2 for homing all axes prior to initiating a method.

5.4.2 Select the Norm STR_NX method and click on the green arrow to initiate the method. Prompts will pop up asking the user to enter the first tip that can be used for the P250 tips (Figure 1). Enter the value and click OK. Another prompt will pop up asking the user to enter the first tip that can be used for the P50 tips. Enter the value and click OK.

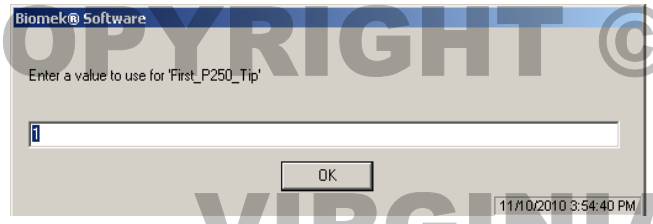


Figure 1. First tip for use for the P250 tips.

5.4.3 A new prompt will pop up asking the user to enter a value for manual master mix preparation. The default is False, as shown in Figure 2. The "False" value means that the method will create the STR mastermix on the deck using tubes of Taq polymerase, buffer and primer. If a True value is entered, this indicates to the method that the STR mastermix will be created off-deck and a single tube placed into the rack for dispensing into the amplification tubes.

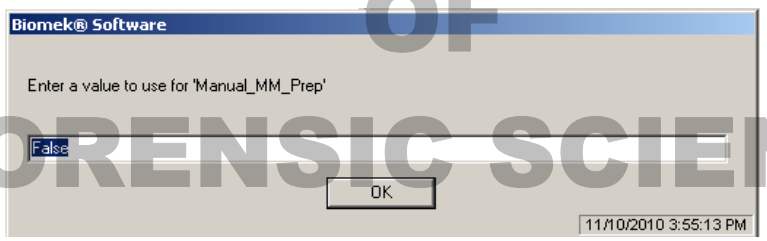


Figure 2. Prompt for manual versus robotic mastermix preparation.

5.4.4 The next window to pop open will be for entry of plate specific information into the STR Normalization Manager interface, shown in Figure 3.

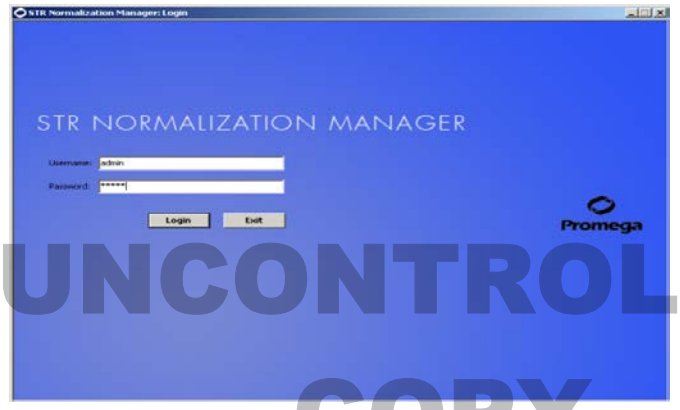


Figure 3. The STR Normalization Manager

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

5.4.5 Enter either admin for the username and password if a project coordinator (qualified examiner) or user for both if an FLS. The following window, shown in Figure 4, will pop up and require the user to enter the run or analysis name, which will typically be the date and the operator's initials. Select the "PP16 half rxn use flagged samples" template from the drop-down window for analysis templates.

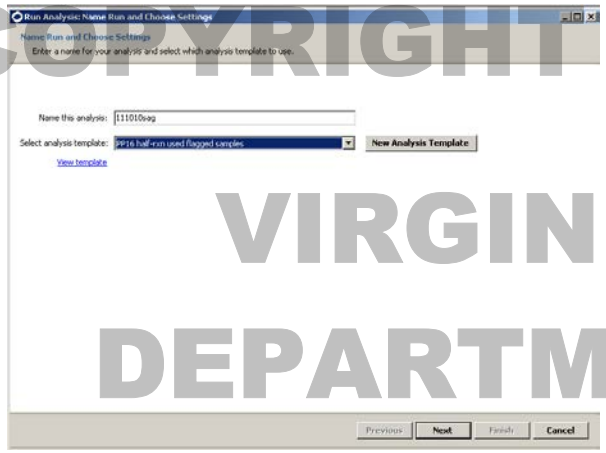
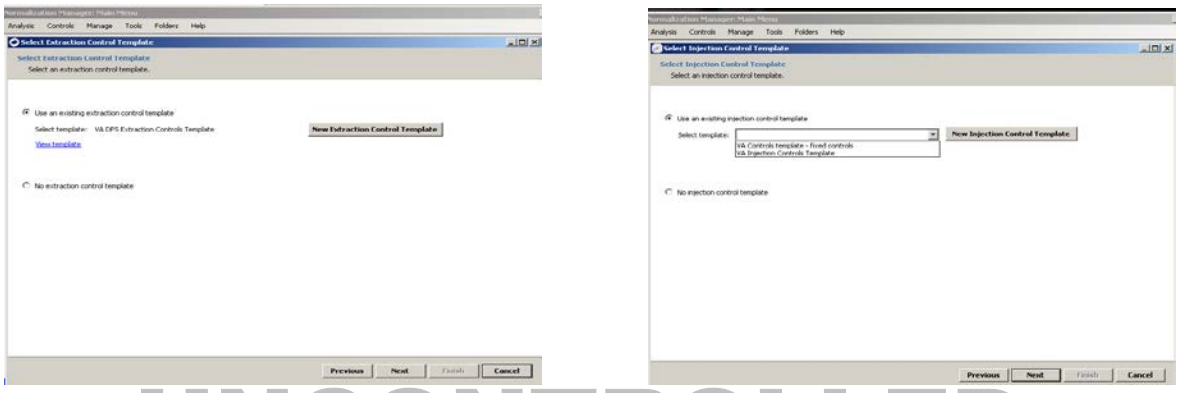


Figure 4. Entering the analysis name and selecting the analysis template.

5.4.6 Two windows will pop up asking the user to first select an extraction control template and then select an injection control template, as shown in Figure 5. The injection controls template contains two choices. The template that has "fixed controls" in its name will automatically place a single positive and a single negative control into the plate map in column 12. If additional positive or negative controls are desired, then choose the other injection control template. Click next after selecting the control template.



A B

Figure 5. Setting extraction (panel A) and injection control templates (panel B).

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

5.4.7 The next window to pop up will instruct the user to import the tab delineated file created from the Plexor HY data for the run. Use the browse function to locate the file. Click on the Next button as shown in Figure 6.

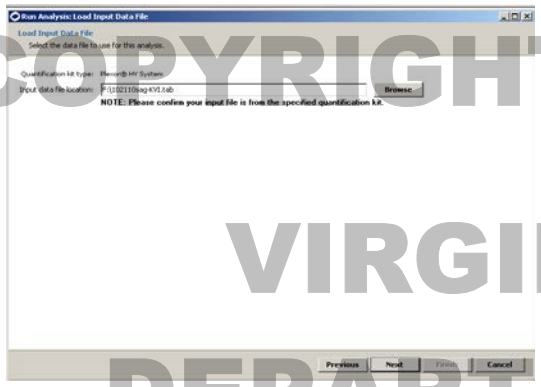


Figure 6. Importing the quantitation data.

5.4.8 The next window to open up will have a summary of the number of samples included in that run (Figure 7). Click on the Review Extraction Plates in the upper right in order to view detailed information about the plate of samples.

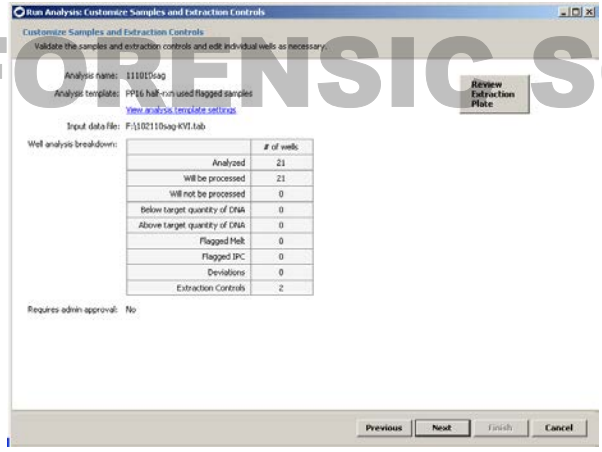


Figure 7. Summary of the sample information for the plate of samples to be amplified.

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE
UNCONTROLLED
COPY

5.4.9 The Plexor HY quantitation data can be reviewed for each well individually by clicking on the well. The detailed information shows up on the left-hand side of the screen as shown in Figure 8. It is at this step that manually added samples are included (such as those samples that were manually extracted and quantitated). See Figure 9 for additional instruction on manually adding samples to a plate. Samples can also be excluded from normalization and STR setup at this point by highlighting the sample and selecting "No" where it says Amplify (red arrow in Figure 8). Click Save. If no samples need to be added to the plate, then click "Done", otherwise go to step 5.4.10 and see Figure 9 for instruction on adding samples to the run.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

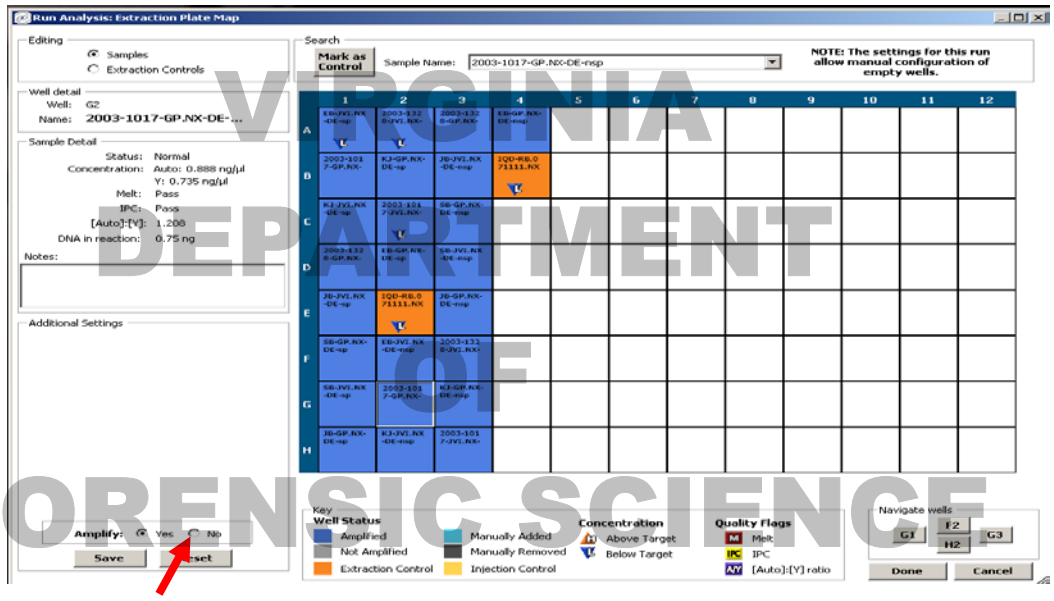


Figure 8. Extraction plate detailed information.

5.4.10 Click on the well where the sample has been manually added. The well detail box (red arrow) will now open a line for entering in the sample name (see the left side of Figure 9). The sample detail box will now allow for concentration information also to be entered. The dilution factor will be automatically calculated unless it is specified by the user. Once the information has been entered for a sample, click on save and move onto the next well for manual addition. Once all of the information for the manual samples has been entered and saved, click Done.

UNCONTROLLED COPY

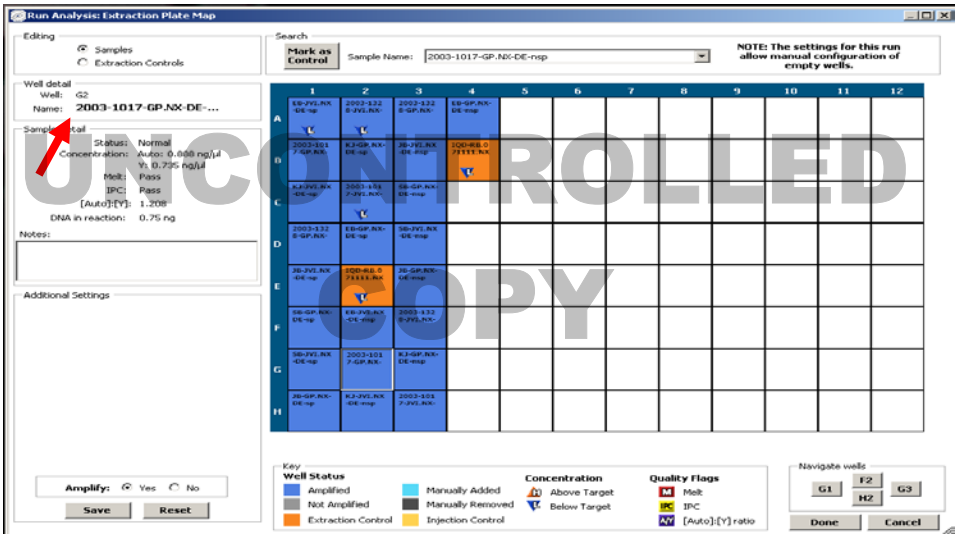


Figure 9. Manually adding samples to a plate of samples

5.4.11 The next window that will pop up will concern the amplification and injection controls (Figure 10). Only the positive and negative controls will be utilized for this step. The positive, negative and allelic ladder controls require a minimum of one. To place the positive, negative and allelic ladder controls click on the Review Amplification Plate button.

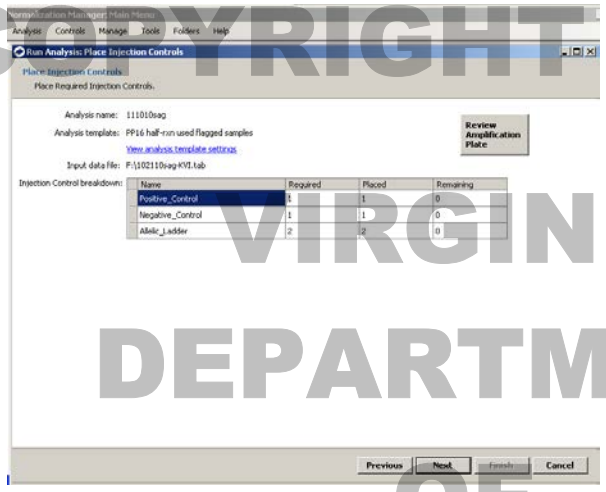


Figure 10. Placing the injection controls.

5.4.12 If the control template was chosen that did not have fixed controls, to add the positive, negative and allelic ladder controls, highlight the control in the box in Figure 11 (black arrow) and click on the well where the control is to be placed and then click Save. Click Done when finished.

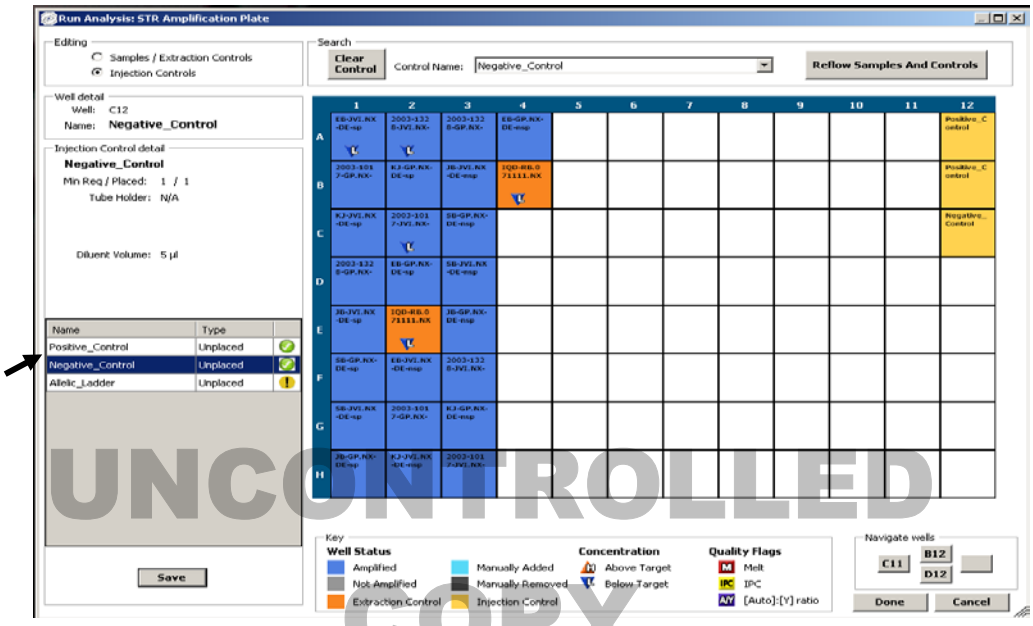


Figure 11. Adding Controls to a plate of amplified samples.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

5.4.13 If the fixed controls were chosen, the plate setup Figure 12 will be shown. Click Done when finished reviewing the plate.

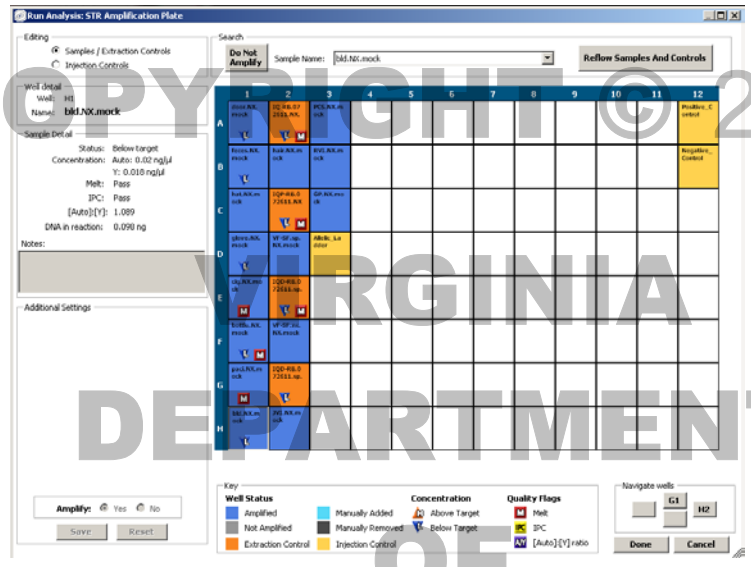


Figure 12. Injection plate showing fixed controls.

5.4.14 The next window will require entry of the login and password again (to confirm the credentials) as shown in Figure 13. Simply enter in the login and password used at the beginning of the Normalization Manager information entry process and then click on Finish.

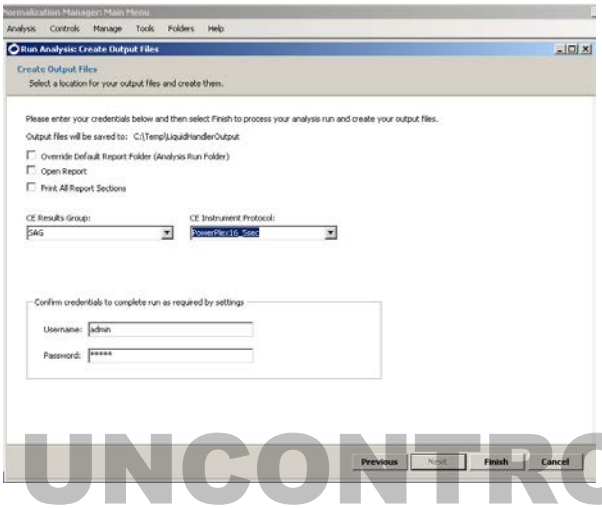


Figure 13. Confirmation of credentials window.

5.4.15 The final window of the Normalization Manger will indicate that the output files have been generated and that the window will close in a number of seconds. The user can either click on the Close Now button or let it close automatically. The method will now begin using the information entered into the Normalization Manager for the method.

5.4.16 After the Normalization Manager closes, there will be a prompt instructing the user to place labware on the deck and the volume of diluent (Type I water) needed, as shown in Figure 14. It will indicate whether for that particular plate of samples (with its range of DNA concentrations) one or two dilution plates will be needed. Click OK once the plates and diluent are placed on the deck in the

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

appropriate positions. The volume indicated is the minimum volume of diluent required, so pipetting a larger volume is acceptable.

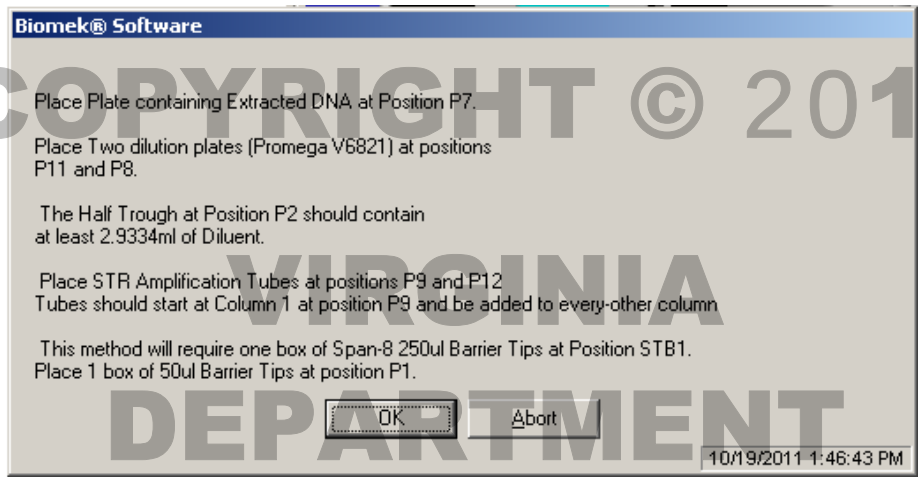


Figure 14. Prompt for plate placement on the deck, diluent volume and for the number of dilution plates needed.

5.4.17 The next prompt, shown in Figure 15, will indicate where the boxes of tips need to be placed and if one or two boxes of P50 tips will be needed. Click OK once the tips are placed on the deck.

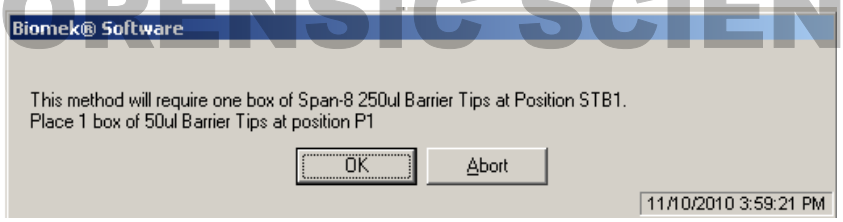


Figure 15. Tip box placement on the deck.

5.4.18 The next prompt will indicate in what positions in the left side of the tube holder the reagent tubes belong as shown in Figure 16. Once the reagent tubes and empty tube are loaded into the rack, click OK. Ensure that each of the reagent tubes contains at least the volume indicated in the prompt, which will vary depending on the number of samples for that specific plate of samples. If manually preparing the mastermix this prompt will indicate in what position the tube of mastermix belongs. Position 1 is the rearmost position and Position 4 is the most forward position.

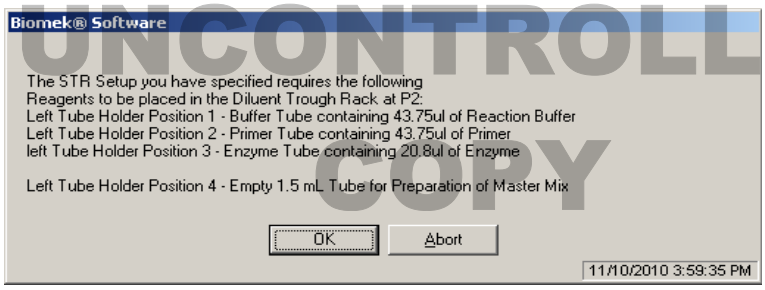


Figure 16. Tube and reagent positions in the tube rack.

5.4.19 The next prompt will indicate in which position on the right side of the tube holder rack the positive control belongs (Figure 17). Prepare a dilution of the positive control so that the concentration is 0.15

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

ng/µL unless it is necessary to utilize a different concentration. If that is necessary, then the positive control template in the Normalization Manager may need to be modified or a new one created.

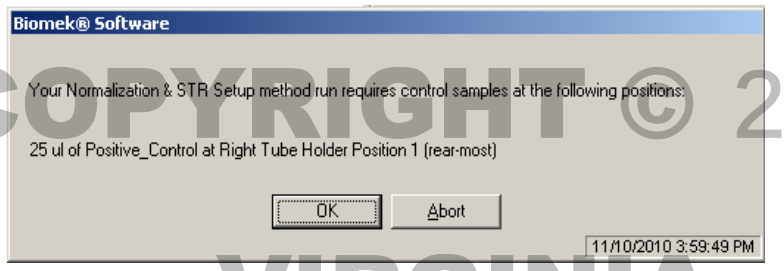


Figure 17. Placement of the positive control in the tube holder.

5.4.20 The final prompt, shown in Figure 18, is the deck setup and the placement of labware and tips. The deck setup needs to be confirmed prior to clicking OK to continue the method.

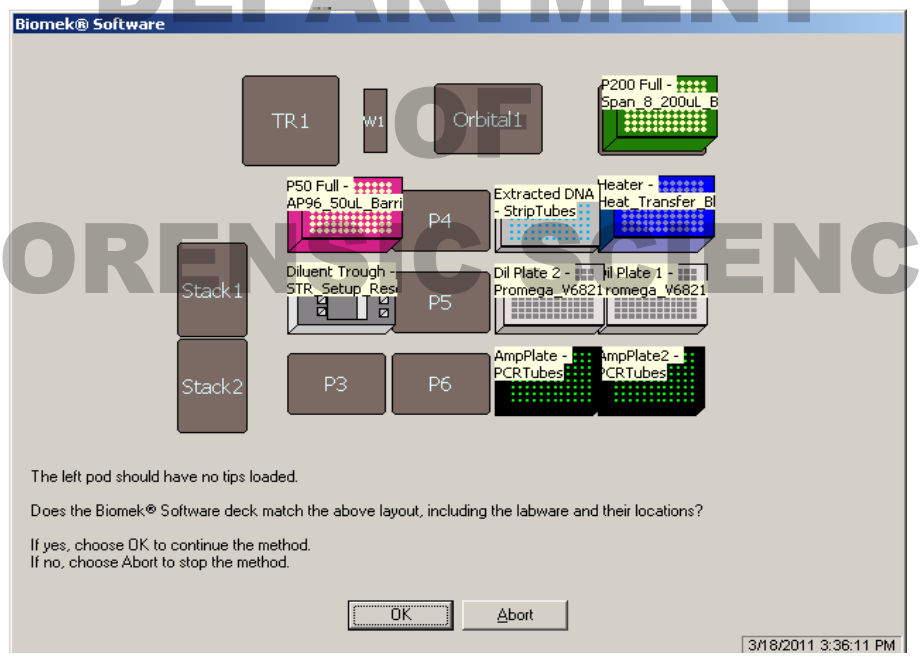


Figure 18. Deck setup for the Normalization and PCR setup method.

5.4.21 The method will take approximately 30 minutes for a large plate of samples

5.4.22 Depending on whether the normalization and PCR setup method designed to pipette into a PCR plate or into tubes was selected, once the method is complete, the plate or tubes needs to be covered with a foil seal or capped and placed into the thermocycler for PowerPlex® 16 amplification. If using a plate with a foil seal, then make certain the foil cover is well sealed and place a pad on top of the plate to reduce evaporation prior to initiating the PCR process.

5.4.23 For the case file records, print out Section 4, Sample Dilution Tracking, of the automatically stored version of that particular analysis (stored as a pdf file C:\Documents and Settings\All Users\Application Data\Promega Corporation\STR Normalization Manager\Analysis Run Performed). The printout shall include the amplification controls.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

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, the Plexor HY quantitation and the PCR setup), that order and separation between one examiner's set of samples and another's is maintained for the transfer method. Each Beckman 24 Microfuge tube holder holds 24-1.5 mL tubes, so in order to accommodate a plate of 80 DNA extracts, four Beckman 24 Microfuge tube holders are placed onto the deck and the pattern of sample loading maintained.

COPYRIGHT © 2015

6.1 Equipment

6.1.1 Biomek® NX^P Automation Workstation

VIRGINIA

6.2 Materials

6.2.1 Microcentrifuge tubes, 1.5 mL

DEPARTMENT

6.2.2 4 - Beckman 24 Microfuge tube holders- Beckman Catalog # 373661

6.2.3 1.5 mL eppendorf tubes

OF

6.2.4 Beckman white 1.5 mL tube inserts- Beckman Catalog # 373656

6.2.5 Black PCR support base (96 well) – ABI Cat# N801-0531

FORENSIC SCIENCE

6.2.6 P250 Tips–aerosol barrier - Beckman Catalog # 379503

6.2.7 Gloves

6.2.8 Tough tag labels for 1.5 mL tubes

6.3 Starting the Biomek® NX^P Workstation

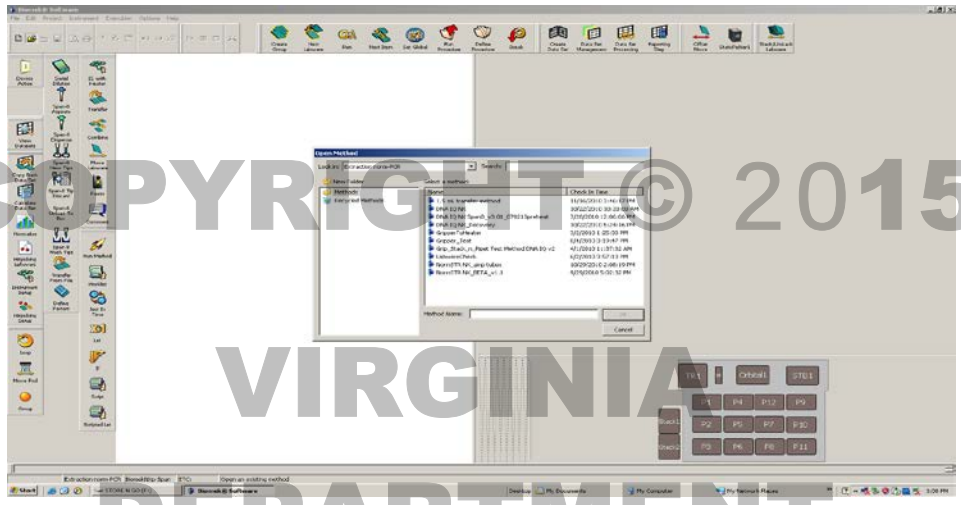
6.3.1 If the power is not already on for the robot, then turn on the power switch on the back, right-hand side of the robot. Follow the directions in section 2.6.1 – 2.6.2.6 for in order to home all axes and prepare the robot for running a method.

NOTE: The 1.5 mL tubes need to be pre-labeled prior to placing into the 24 well microfuge racks using the same pattern as the samples were loaded into the sample plate for extraction.

6.4 Once the Biomek® NX^P has been homed, using either the File drop-down window or the folder icon, open the Open Method window.

UNCONTROLLED

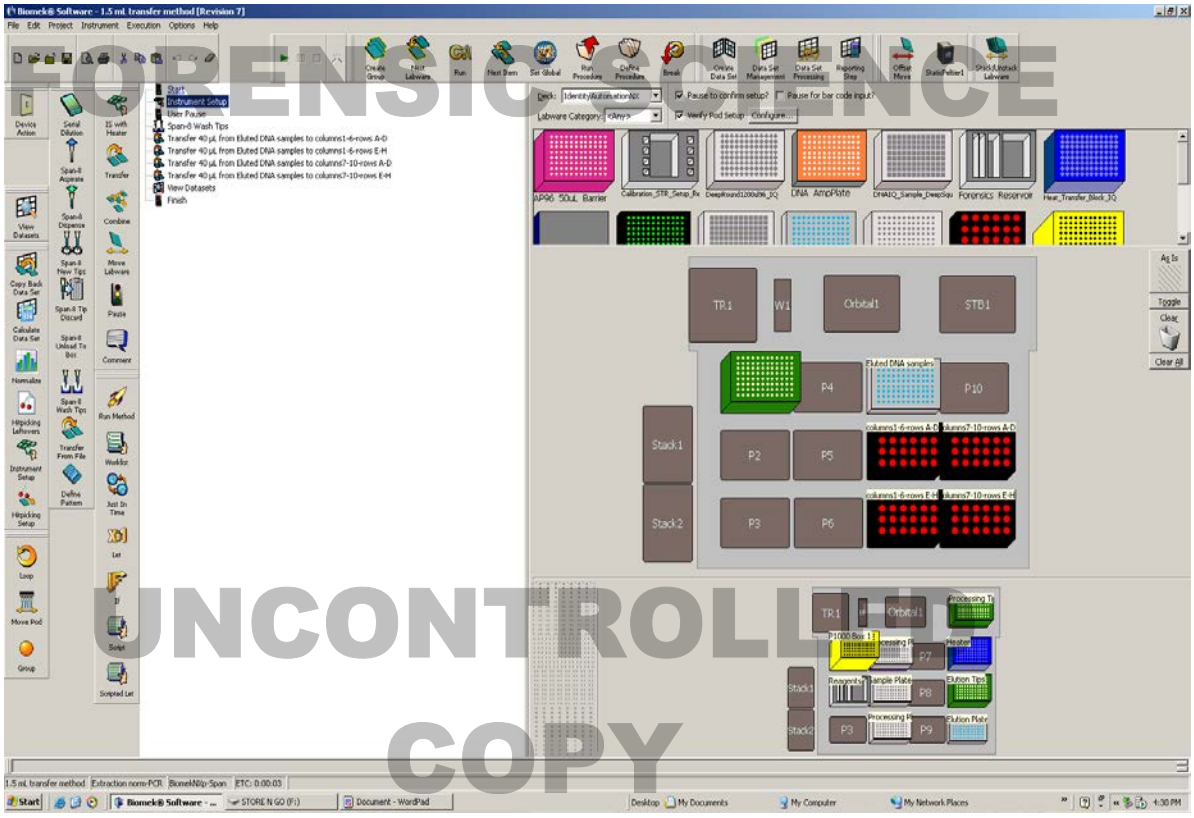
COPY



COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

Figure 1. From the Project folder window, open the 1.5 mL transfer method.

6.4.1 Select the 1.5 mL transfer method. With the method open, click on the green arrow to start the method. The method will begin and a prompt window will open showing the deck layout for the method (Figure 2). Place the labware in the designated positions and click OK.



UNCONTROLLED COPY

Figure 2. Deck layout for the 1.5 mL transfer method.

6.4.2 The next window to pop up is shown in Figure 3. Follow the directions. Step 6.4.4 and Figure 4 will provide direction on disabling the lines of the method, if necessary. If it is necessary to disable lines of method, then the method must be aborted, the lines disabled and the method re-started.

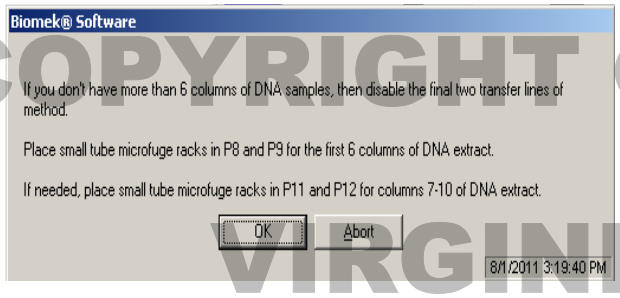


Figure 3. Placing tubes and tips on the deck.

6.4.3 If six columns or fewer of DNA samples will be transferred, then disable the last two transfer steps, which transfer samples in columns 7-10, rows A-D and columns 7-10, rows E-H. As shown in Figure 4, highlight the line in the method and right click. Select Disable. The line will appear grayed out and with a red X once is has been disabled.

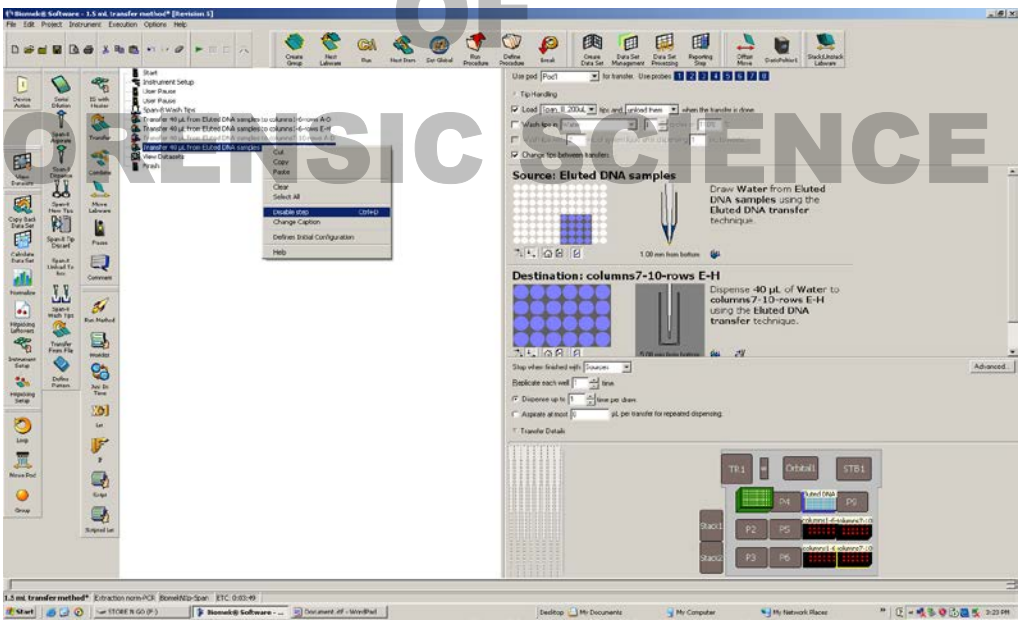


Figure 4. Disabling transfer steps in the 1.5 mL transfer method.

6.4.4 The method will now run until completion. Once it has finished and the 1.5 mL method is being closed, the user will be asked if he/she would like to save the changes (that will occur if any of the transfer steps were disabled). Click "No" so that the disabled steps will not be disabled the next time the method is opened.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

APPENDIX A – TROUBLESHOOTING

1. General Biomek® NX^P automation workstation operation

   1.1 Span-8 pipettor tool

   In order to obtain the most accurate pipetting, air bubbles need to be purged from the lines. This will be performed every time the Home All Axes function is performed. Perform a visual check to ensure that no large air bubbles are present in the plungers. Make certain that the carboy containing the system fluid doesn't run out of fluid, so the carboy should be at least ¼ full. The system fluid consists of tap deionized water.

2. What to do when the Biomek NXP has a malfunction and must be aborted during the DNA IQ NX method. The Restore method (Appendix F) should be run in the event of an unplanned method abortion. The Restore method will allow the user to pick up where the method left off and to complete the extraction.

3. Plexor Data Analysis

   3.1 Removing outlier data points from a standard curve

   3.1.1 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 column of standards can be deselected when necessary to produce a linear standard curve. 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.

   3.1.2 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.

   3.1.3 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.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

3.1.4 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 1. Note that this selection is specific to the dye selected via the tab above the data.

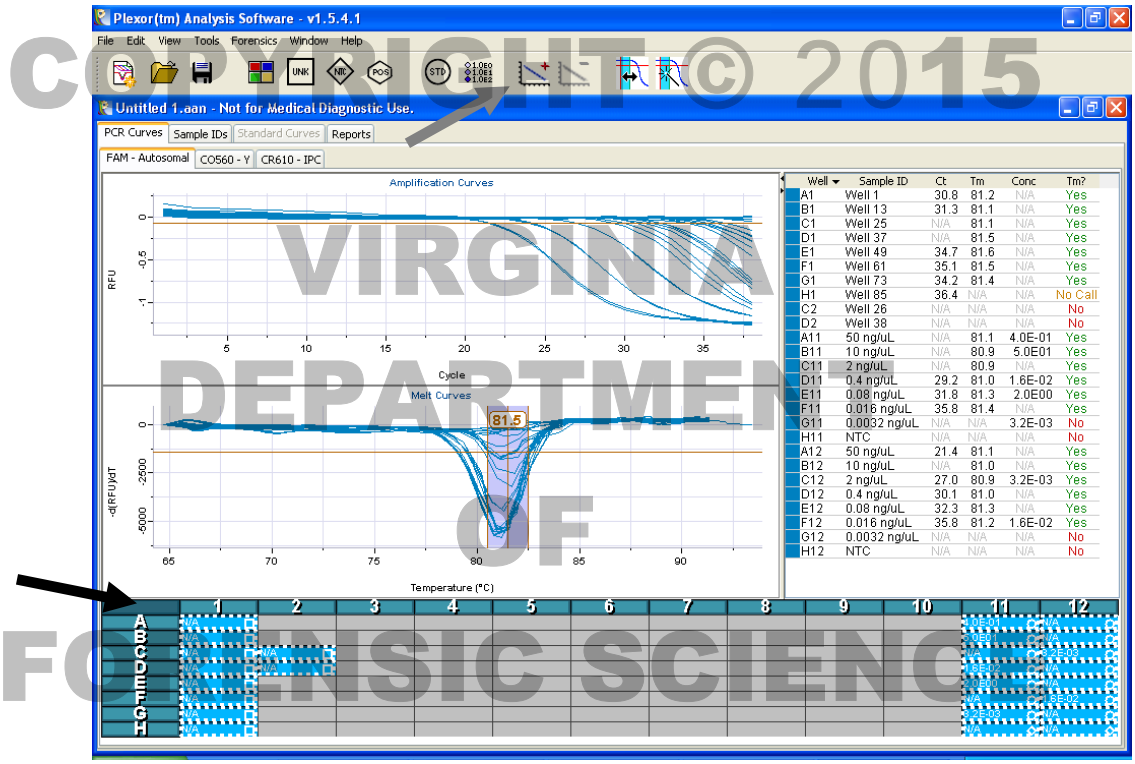


Figure 1. Making the Standard Curves**.** After selecting the entire plate of samples by clicking in the box indicated by the black arrow, a standard curve can be created by clicking the appropriate icon as indicated by the second (gray) arrow.

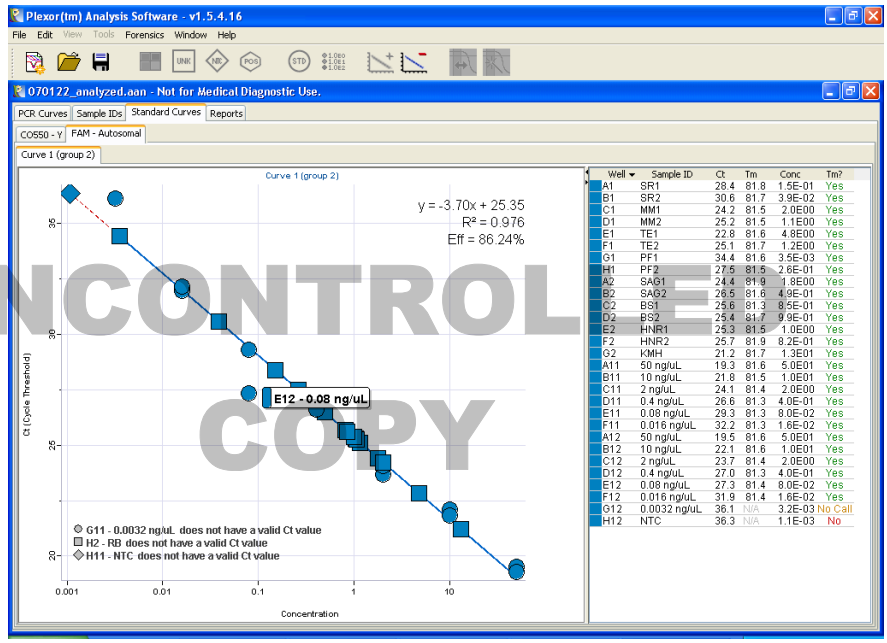


Figure 2. Standard Curve**.** The well location of the outlier data point is displayed (as shown) by placing the mouse (arrow) over the data point in question.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

3.1.5 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 2. This or these data point(s) are the points which should be de-selected to improve the standard curve.

COPYRIGHT © 2015

3.1.6 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.

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

VIRGINIA

DEPARTMENT

3.1.8 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”.

OF

3.1.9 If de-selection of data points on the other standard curve is necessary, be sure to select the other tab (as shown in Figure 1) and repeat steps 3.1.3 – 3.1.7, above.

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

FORENSIC SCIENCE

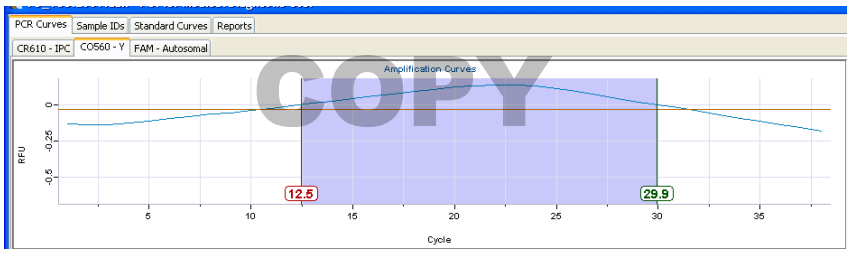
3.2 Adjusting a Sample’s Baseline to More Optimal Values

3.2.1 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.

3.2.2 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.

3.2.3 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 3.

UNCONTROLLED



COPY

Figure 3. Improperly-set baseline region for an individual sample.

3.2.4 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 4 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.

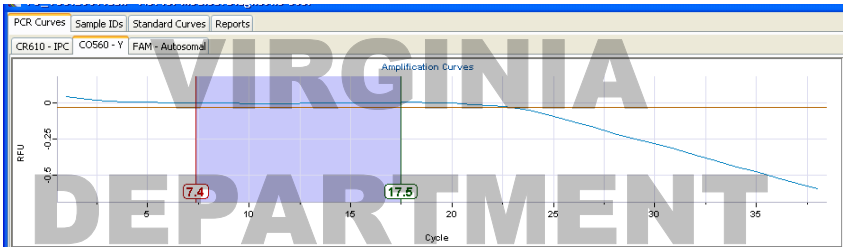


Figure 4. Amplification plot from same sample as in Figure 3, but showing a properly-selected baseline region.

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE
UNCONTROLLED
COPY

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 other wise 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 Forensic 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.

CALCUM CHLORIDE, 1M

Expiration date: Twelve months from the date of preparation

	MW	100 mL
$CaCl_2$	110.9	11.09 g
Type I Water		90 mL

Dissolve the appropriate amount of $CaCl_2$ into Type I Water. Bring up to final volume

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

$CaCl_2$ - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number C1016, 100 g bottle.

$CaCl_2$ BUFFER 1X

Expiration date: Twelve months from the date of preparation

COPYRIGHT © 2015

100 mL

5 mL of 1M Tris (8.0)
1 mL of $CaCl_2$ (1M)
94 mL of Sterile Type I water

VIRGINIA

Mix reagents together and filter sterilize.

DITHIOTHREITOL, 0.39 M (DTT)

DEPARTMENT

Expiration date: Twelve months from date of preparation

	MW	25 mL
DTT	154.2	1.50 g
Type I Water		15 mL

OF

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 C.

FORENSIC SCIENCE

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

UNCONTROLLED

  Add 2.5 µL of 0.39 M DTT for every 100 µL of DNAIQ™ 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.

COPY

- 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.

DNA IQ-PROTEINASE K BUFFER

Expiration date: Twelve months from date of preparation

	50 mL	100 mL	500 mL
TNE	18.75 mL	37.5 mL	187.5 mL
20% Sarkosyl	5.0 mL	10.0 mL	50.0 mL
Type I Water	18.75 mL	37.5 mL	187.5 mL

Pipette all solutions and mix well. Dispense into aliquots.

ETHYLENEDIAMINETETRAACETIC ACID (EDTA), 0.5 M

Expiration date: Twelve months from date of preparation

	MW	500 mL	1L	2 L
EDTA	372.2	93.05 g	186.1 g	372.2 g
Type I Water		375 mL	750 mL	1.5 L

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.

HYDROCHLORIC ACID (250 mM)

Expiration date: AluQuant™ Human Quantitation System kit expiration data

Dilute the HCl reagent from 250 mM to 200 mM prior to use. After vortexing or swirling to mix the HCl reagent, remove 12 mL of HCl reagent using a pipette and transfer to a clean 15 mL conical tube. Add 3 mL of sterile Type I water and mix by inverting several times. Label the tube “200 mM HCl”, date and initial it. It can be stored at room temperature at the robot workstation.

The HCl Acid (250 mM) Solution is supplied as a component in the AluQuant™ Human Quantitation System kit

PCR DIGESTION BUFFER, 1.0%

Expiration date: Twelve months from date of preparation

Stock	100 mL
1 M Tris	1 mL
0.5 M EDTA	2 mL
NaCl	0.29 g

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

20% SDS	5 mL
Type I Water	91mL

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.

COPYRIGHT © 2015

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).

VIRGINIA

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

DEPARTMENT

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/ □L
    - Diluted DNA standard should be freshly prepared each day of use.
- H_2O, AMPLIFICATION GRADE

OF

PROTEINASE K ENZYME (20 mg/mL)

FORENSIC SCIENCE

Expiration date: Twelve months from date of preparation

Stock	5 mL	15 mL	25 mL
Proteinase K	100 mg	300 mg	500 mg
Sterile Type I Water	5 mL	15 mL	25 mL

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 °C. 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 °C.
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

UNCONTROLLED

	MW	250 mL	500 mL	2 L
N-Lauroylsarcisine	293.4	50 g	100 g	400 g
Type I Water		200 mL	400 mL	1600 mL

COPY

Add the appropriate amount of N-Lauroylsarcisine 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-Lauroylsarcisine, Sodium Salt - Purchased from Sigma Chemical Co., St, Louis, MO., Catalog number L 5125, 500 g bottle.

SODIUM CHLORIDE (NaCl), 5 M

Expiration date: Dispose of when necessary

	MW	500 mL	1 L	4 L	16 L
NaCl	58.44	146.1 g	292.2 g	1168.8 g	4675.2 g
Type I Water		500 mL	1 L	4 L	16 L

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

	MW	500 mL	1 L	2 L
Tris 1 M	121.1	60.55 g	121.10 g	242.2 g
Tris 2 M	121.1	121.10 g	242.20 g	484.4 g
Type I Water		300 mL	600 mL	1200 mL

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.

For HCl see this list.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

UNCONTROLLED COPY

APPENDIX C – CALIBRATION OF THE BIOMEK®NX^P AUTOMATION WORKSTATION

Calibration tests should be performed on a routine basis for the Biomek®NX^P Automation Workstation. Home All Axes should be performed each day prior to use or whenever the robot has been turned off. The deck, including the Orbital Shaker and the STB1 ALPs should be framed once every three months or when needed. The deck framing provides coordinates to the software for each of the deck positions. The Accuframe tool is used to help perform this function.

COPYRIGHT © 2015

1. Homing All Axes

1.1 If the robot was turned off and then turned on and a Home All Axes function is not performed, the software will prompt the user that it needs to be performed and will not allow the user to initiate a method.

VIRGINIA

1.2 Section 2.6 provides the procedure for Homing All Axes. Follow the directions from 2.6.1 to 2.6.2.6.

2. Framing the Deck

DEPARTMENT

2.1 The deck should be framed once every three months or whenever needed.

2.2 Using the Instrument drop-down window, shown in Figure 1, select the Deck Editor.

OF
FORENSIC SCIENCE

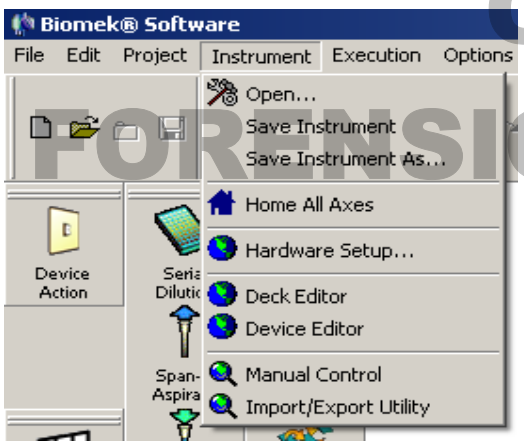


Figure 1. Selecting the Deck Editor from the Instrument drop-down window.

2.3 The Deck Editor window will open up showing deck all of the deck positions (Figure 2). Positions P3, P12 and P10 will be framed for the 4X3 ALP in the center of the deck.

UNCONTROLLED
COPY

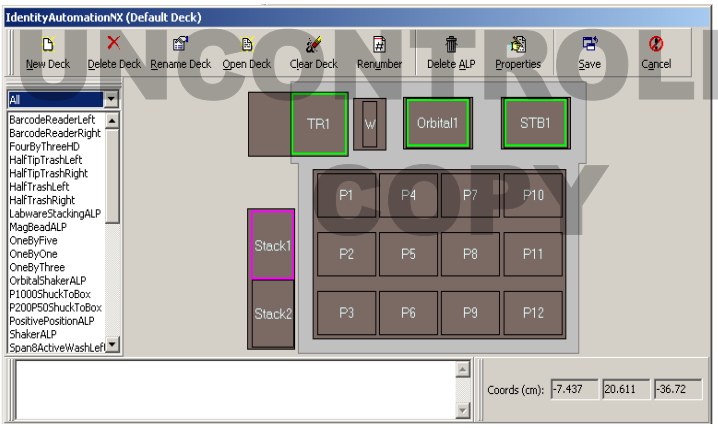


Figure 2. Deck positions.

2.4 Double-click on position P3 and the framing window will open (Figure 3). Depending on the position selected (e.g., P3 versus P10), the Auto Teach button will indicate which probe is to be used. The probe numbers begin at #1 in the back and end at #8 in the front-most position.

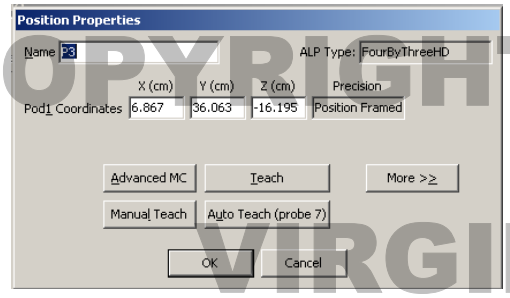


Figure 3. Position properties for framing.

2.5 To prepare the probe for framing, unscrew the black mandrel surrounding the #7 probe (2nd probe from the front) and remove it. Next unscrew the silver probe, disconnect it from the system fluid tubing and remove it. Push the tubing up so that it is flush with end of the piece from which it protrudes. Next screw on the silver framing piece. It is asymmetrical, so it will only screw on in one orientation.

2.6 Place the framing tool into the ALP position (#3), making sure that the Beckman Coulter and Accuframe logos are facing the front and the framing device is sitting flush in the ALP position.

2.7 Click on the Auto Teach button (shown in Figure 3).

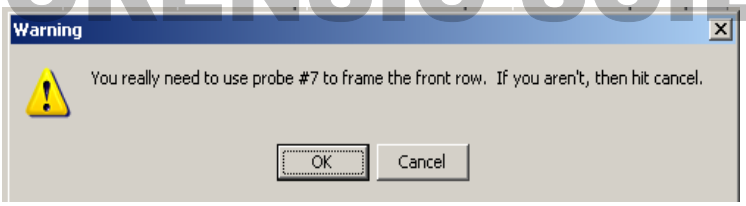


Figure 4. Warning about the proper probe to use.

2.8 A window will pop up warning the user to use probe #7 for the deck framing at this position (Figure 4). After checking that probe #7 is being used, click OK.

2.9 Another warning will pop up, shown in Figure 5. After confirming that the distance moved will be tolerated, click OK and the framing will be performed.

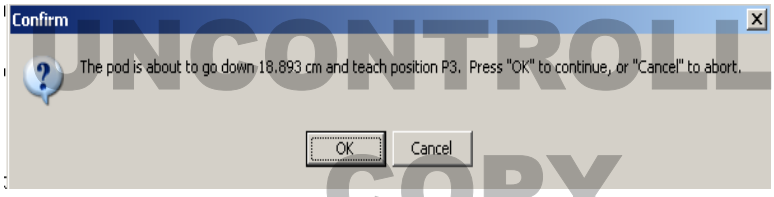


Figure 5. Confirm that the pod can move down in order to teach position P3.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

2.10 After the framing is performed, another window will open up which provides the measured coordinates for the new framing as compared to the current coordinates (an example is shown in Figure 6).

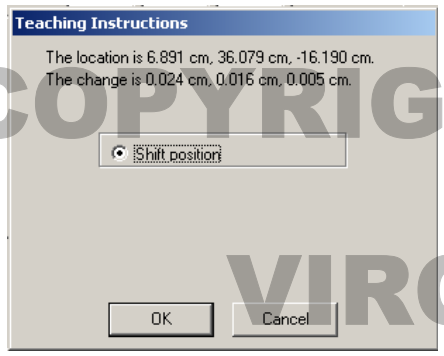


Figure 6. New framing coordinates.

2.11 Framing coordinates are listed in the x, y and z-axes. **Cancel the changes unless one of the axes has a change greater than 0.025 cm.** If one of the coordinates displays a change greater than 0.025 cm, click OK.

2.12 Move the framing tool to the P12 position and repeat the process.

2.13 For framing P10, the Orbital Shaker and the STB1, a different probe will be used. Remove the silver framing tool from the #7 probe position. Push the tubing back down so it protrudes and feed it onto the silver probe. Screw the probe into place and then screw the black mandrel back into position covering the probe. Remove the black mandrel from probe #1. Remove the silver probe and push the tubing back into the opening from which it protrudes. Screw the framing tool into the probe #1 position.

2.14 Repeat the process for framing but select the P10 position. Remove any device or piece of labware that might be placed into that ALP position prior to framing it. The window shown in Figure 7 will pop up. The Auto Teach button will now indicate that probe #1 should be used.

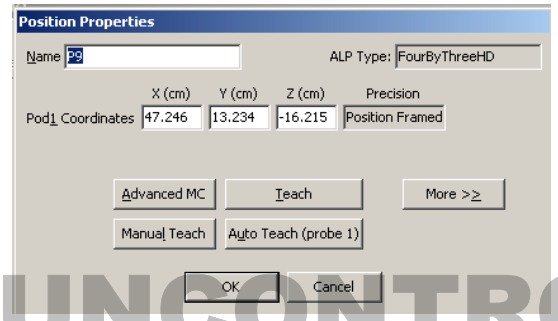


Figure 7. Position properties for framing.

2.15 Click on the Auto Teach button and follow the prompts as done previously for P3 and P12.

2.16 Click on Start. As directed in step 2.11 and in Figure 6, if the resulting values after framing are equal to or less than 0.025 at the x, y and z coordinates, then click Cancel. If one is greater than 0.025, then click OK.

2.17 Repeat the process for the STB1, going back to the deck screen shown in Figure 2 in order to double click on the STB1 ALP position.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

2.18 Go through all of the same steps to frame the STB1 position. When the new coordinates have been measured, a window will pop up with the new coordinates, as shown in Figure 8.

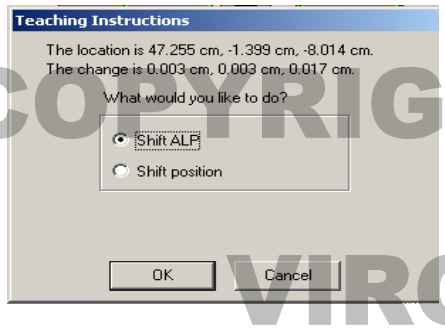


Figure 8. New framing coordinates for the STB1.

2.19 Again, unless the new coordinates are greater than 0.025 cm, then click cancel. If greater than 0.025, then either the Shift ALP or the Shift position can be checked before clicking OK.

2.20 Once the user returns to the deck layout, shown in Figures 2 and 9, the user will need to exit this prior to framing the orbital shaker. If any changes were made to the P3, P12, P10 or STB1 coordinates, then the user will need to click on the Save button otherwise the changes will not be saved. If no changes adopted for those deck positions, then click Cancel.

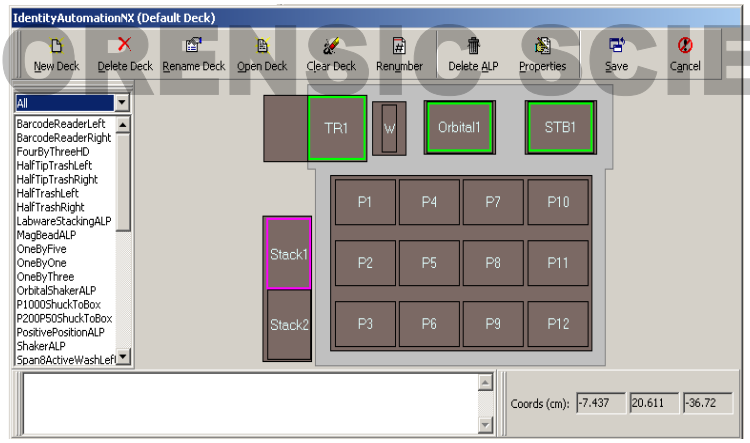


Figure 9. Deck layout.

2.21 The Orbital Shaker ALP should only be framed on an as needed basis (for e.g., if the Gripper is having trouble moving plates into it and this can't be remedied by adjusting the offsets). Move the Accuframe framing device to the Orbital Shaker ALP.

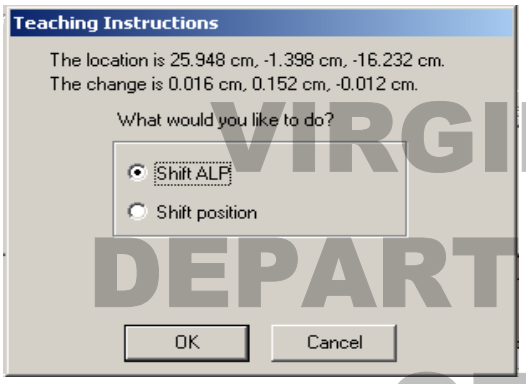
2.22 Under the Instrument drop-down window, select Deck Editor.

2.23 Double click on the Orbital Shaker ALP and follow the prompts as done previously for P3, P12, P10 and the STB1.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

2.24 Frame the Orbital Shaker ALP. Once the window appears which provides the new coordinates, an example of which is shown in Figure 10, decide whether or not the new coordinates should be adopted. In the example shown in Figure 10, the y-coordinate is greater than 0.025 cm which would normally merit accepting the new coordinates, however, the y-axis coordinate was manually adjusted instead of adjusting an offset in order to accommodate plate movement onto the Orbital Shaker. Before accepting new coordinates for the Orbital Shaker, be certain that no manual adjustments to the framing coordinates were made.

COPYRIGHT © 2015



VIRGINIA DEPARTMENT OF

Figure 10. New coordinates for the Orbital Shaker ALP.

2.25 Click either OK to accept the new coordinates or Cancel. If new coordinates were accepted, then be certain to click Save when exiting the Deck Editor or the changes will be lost.

FORENSIC SCIENCE

NOTE: A test of plate movement and pipettor function must be performed if the Orbital Shaker is framed and changes to any of the axes are retained. Adjustments to the x, y or z offsets for a specific piece of labware may need to be made in order to move the plate properly and to pipette from the plate when sitting on the Orbital Shaker.

2.26 Remove the silver framing tool from the probe #1 position, push the tubing back out, re-thread the silver probe #1 into the tubing and screw back into place. Follow that by screwing the mandrel back onto the probe.

UNCONTROLLED COPY

APPENDIX D – PERFORMING THE STRATAGENE MX3005P™ 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 Mx3005P™ 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 icon "Mx3005P Qualification Test" on the desktop of the computer controlling the Stratagene Mx3005P™. Click "Next" (located in the bottom right corner of screen).

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

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

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).

COPYRIGHT © 2015

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.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

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.

UNCONTROLLED COPY

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

APPENDIX E - USING THE BIOMEK® NX^P DNA IQ™ RECOVERY METHOD

The DNA IQ NX Recovery method is designed to be used in the event of a crash or malfunction while running the normal Biomek NX^P DNA IQ extraction method. If the extraction method cannot be continued after the crash or malfunction and it must be aborted, then the DNA extraction can be completed using the DNA IQ NX Recovery method. For the recovery of the DNA samples to be successful, the status of the extraction must be carefully noted at the time of the crash (for e.g., the crash occurred in column 2 during the volume reduction step. The samples from column 2 have been aspirated from the sample plate, but the liquid (with resin) still remained in the tips when the crash occurred). If critical liquid (liquid containing sample) still remains in the pipette tips from the crash, then the DNA IQ NX Recovery Dispense Only method must be used prior to the DNA IQ NX Recovery method.

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE

1. Reset pod 1 to prepare for the recovery method.

NOTE: The recovery dispense only and tip shuck methods need to be run by a project coordinator or other qualified individuals with administrator privileges on the Biomek® NX^P Software.

NOTE: If the liquid in the pipette tips does not need to be retained (i.e., there is no resin with DNA attached in the tips) or the pipettor has tips mounted with nothing in them, **run the A-tip Shuck method to remove the tips** (see 1.2 for instruction on running the A-tip Shuck method). It is not necessary to run the DNA IQ NX Recovery Dispense Only method prior to the Recovery method.

1.1 Dispense liquid in the tips (if necessary), otherwise proceed to step 1.2.

1.1.1 Open the DNA IQ NX Recovery Dispense Only method.

1.1.1.1 Open the Extraction Norm-PCR project folder which contains the DNA IQ NX Recovery Dispense Only method.

1.1.1.2 Using either the folder icon or the under the File drop-down menu, open the DNA IQ NX Recovery Dispense Only method.

1.1.1.3 Select the DNA IQ NX Recovery Dispense Only method by double clicking or clicking on it and the OK button.

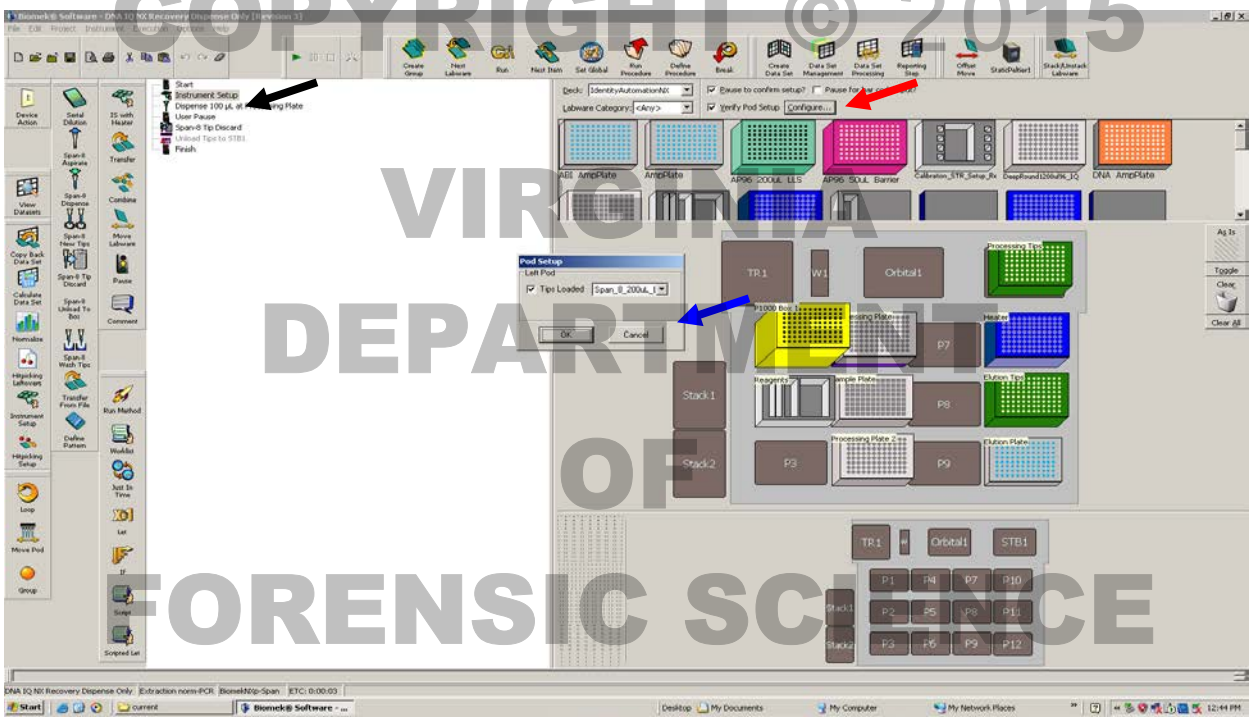
UNCONTROLLED
COPY

1.1.2 Select the tips (containing the liquid) that are loaded onto pod 1.

1.1.2.1 Click on the “Instrument Setup” line of the method (shown in Figure 1, black arrow).

1.1.2.2 Press the “Configure” button (shown in Figure 1, red arrow).

COPYRIGHT © 2015



VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

Figure 1. Shows the steps involved in selecting the tip type for the DNA IQ NX Recovery Dispense Only method.

1.1.2.3 Select the appropriate tip type from the pull down menu (shown in Figure 1, blue arrow).

1.1.2.4 Press “Ok” in the pod setup box to save the tip type.

UNCONTROLLED COPY

1.1.3 Select the conditions for the dispense step.

1.1.3.1 Click on the “Dispense” line of the method (shown in Figure 2, black arrow).

1.1.3.2 Select the labware that the liquid is going to be dispensed into by clicking on it on the deck layout (shown in Figure 2, red arrow). Verify that the labware type and position are correct.

1.1.3.3 Check to make sure the “Empty Tips” box is checked (shown in Figure 2, green arrow) to dispense all of the liquid in the tips.

1.1.3.4 Designate the wells where the liquid will be dispensed by selecting them on the plate diagram (shown in Figure 2, blue arrow).

COPYRIGHT © 2015
VIRGINIA
DEPARTMENT
OF
FORENSIC SCIENCE

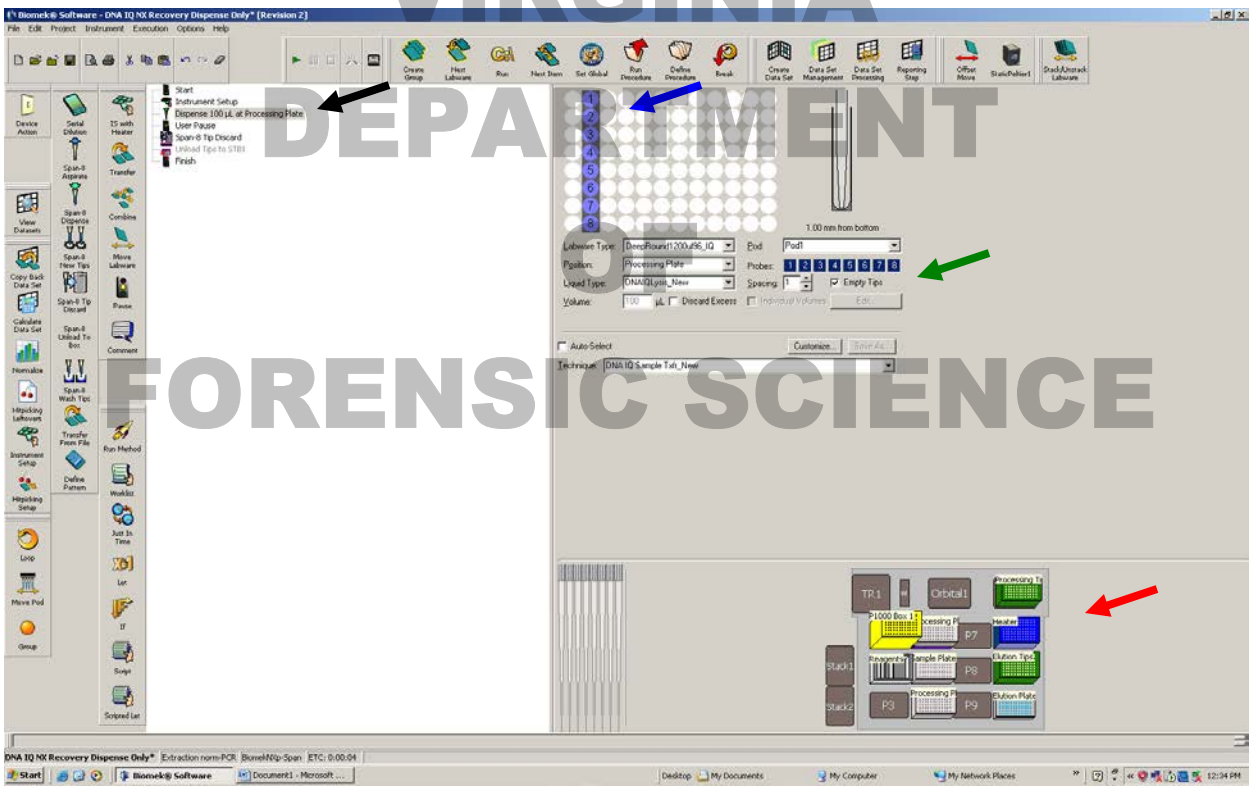


Figure 2. Shows where to designate the location, labware, and volume for the dispense step.

UNCONTROLLED
COPY

1.1.4 Click “Finish” in the method, and then run the DNA IQ NX Recovery Dispense Only method.

1.1.4.1 A prompt showing the deck layout will appear (shown in Figure 3).

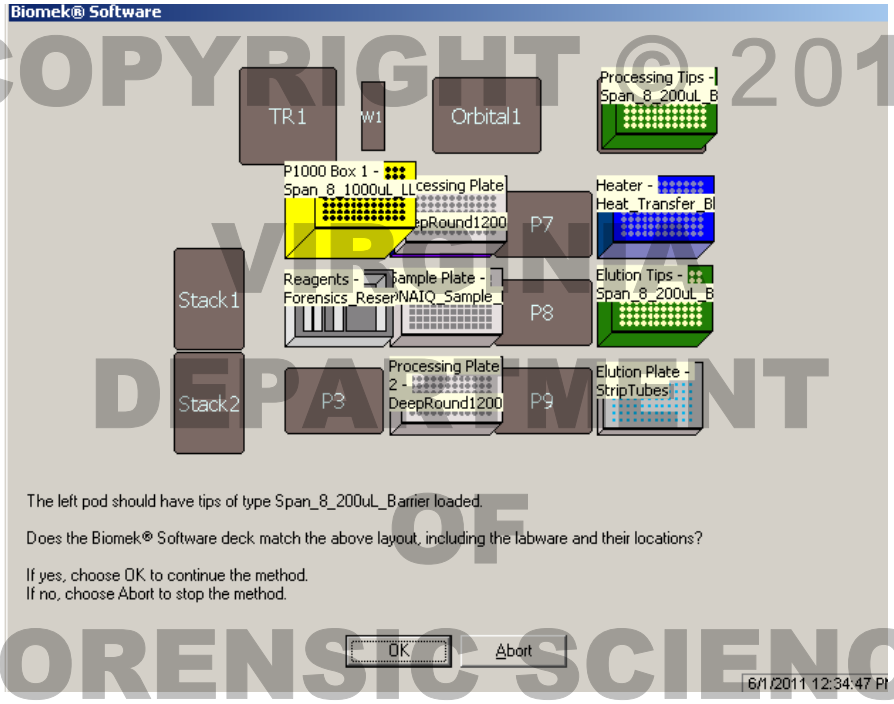


Figure 3. Deck layout for the DNA IQ NX Recovery Dispense Only method.

1.1.4.2 After the dispense step the instrument will pause and display the prompt shown in Figure 4.

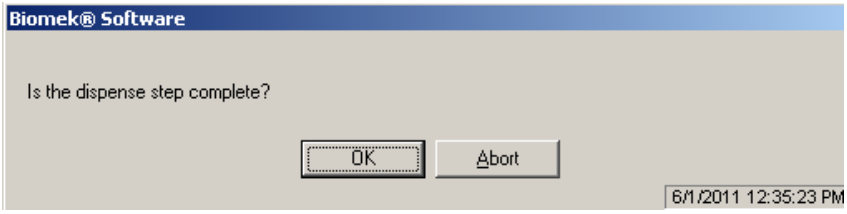


Figure 4. Dispense complete prompt.

1.1.4.3 Verify that the liquid has been dispensed from the tips.

1.1.4.4 The tips will be discarded in the trash after the dispense step has completed.

1.1.5 Proceed to step 1.3.

1.2 If there are tips on pod 1 that need to be discarded, but no liquid needs to be dispensed then run the tip shuck method, otherwise proceed to step 1.3.

1.2.1 Open the Tip Shuck method.

1.2.1.1 Open the Extraction Norm-PCR project which contains the A_Tip_Shuck method.

1.2.1.2 Using either the folder icon or the under the File drop-down menu, open the A_Tip_Shuck method.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

1.2.1.3 Select the A_Tip_Shuck method by double clicking or clicking on it and the OK button.

1.2.2 Select the tips that are loaded onto pod 1.

1.2.2.1 Click on the “Instrument Setup” line of the method (shown in Figure 5, black arrow).

COPYRIGHT © 2015

1.2.2.2 Press the “Configure” button (shown in Figure 5, red arrow).

1.2.2.3 Select the appropriate tip type from the pull down menu (shown in Figure 5, blue arrow).

1.2.2.4 Press “Ok” in the pod setup box to save the tip type.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

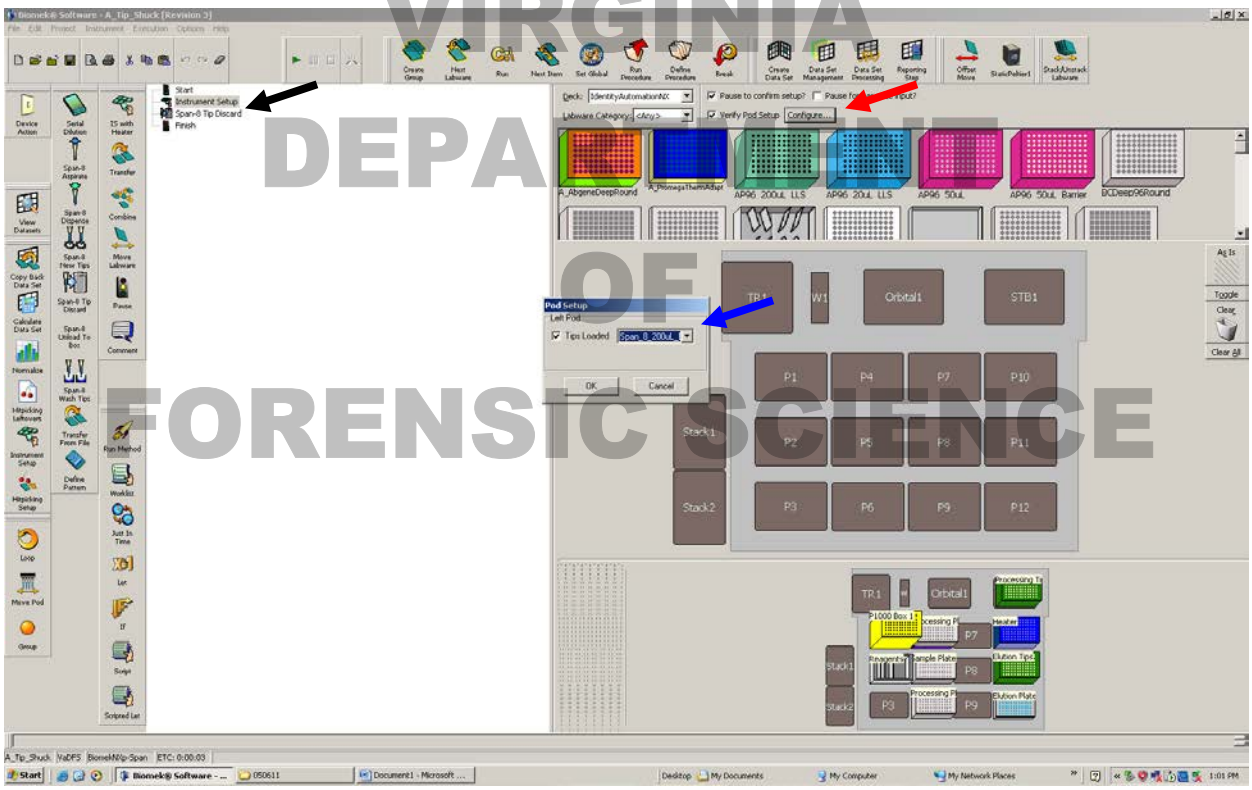


Figure 5. Shows the steps involved in selecting the tip type for the A_Tip_Shuck method.

1.3 Clean the deck and tools of the robot as necessary before going forward with the recovery or other method.

2. The robot is now ready for running the recovery method.

UNCONTROLLED COPY

2.1 Perform the Home All Axes function following the protocol in steps 2.6.2.1 - 2.6.2.6 of section 2 Preparation of the Biomek® NX^P Automation Workstation for DNA Isolation.

2.2 Open the Extraction Norm-PCR project which contains the DNAIQ NX Recovery method.

2.2.1 Using either the folder icon or the under the File drop-down menu, open the DNA IQ NX Recovery method.

2.2.2 Select the DNA IQ NX recovery method by double clicking or clicking on it and the OK button and start the run.

2.3 The initial deck layout will be the same as the starting layout for a standard extraction run regardless of the starting step.

2.3.1 Place clean tip boxes on the deck (they can be partial).

2.3.2 Replace additional labware and/or reagents as necessary.

NOTE: Any reagents that were partially consumed by the original run that will be used in the recovery run need to be replenished to their starting volume.

COPYRIGHT © 2015

2.3.3 The conditions for the recovery run now need to be set.

2.3.3.1 In addition to the regular series of prompts that appear in the standard extraction method (see steps 2.6.3.4 – 2.6.3.6 and 2.6.3.11 – 2.6.3.12 of section 2 Preparation of the Biomek® NX^P Automation Workstation for DNA Isolation), the GI Interface prompt (shown in Figure 6) will also appear.

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

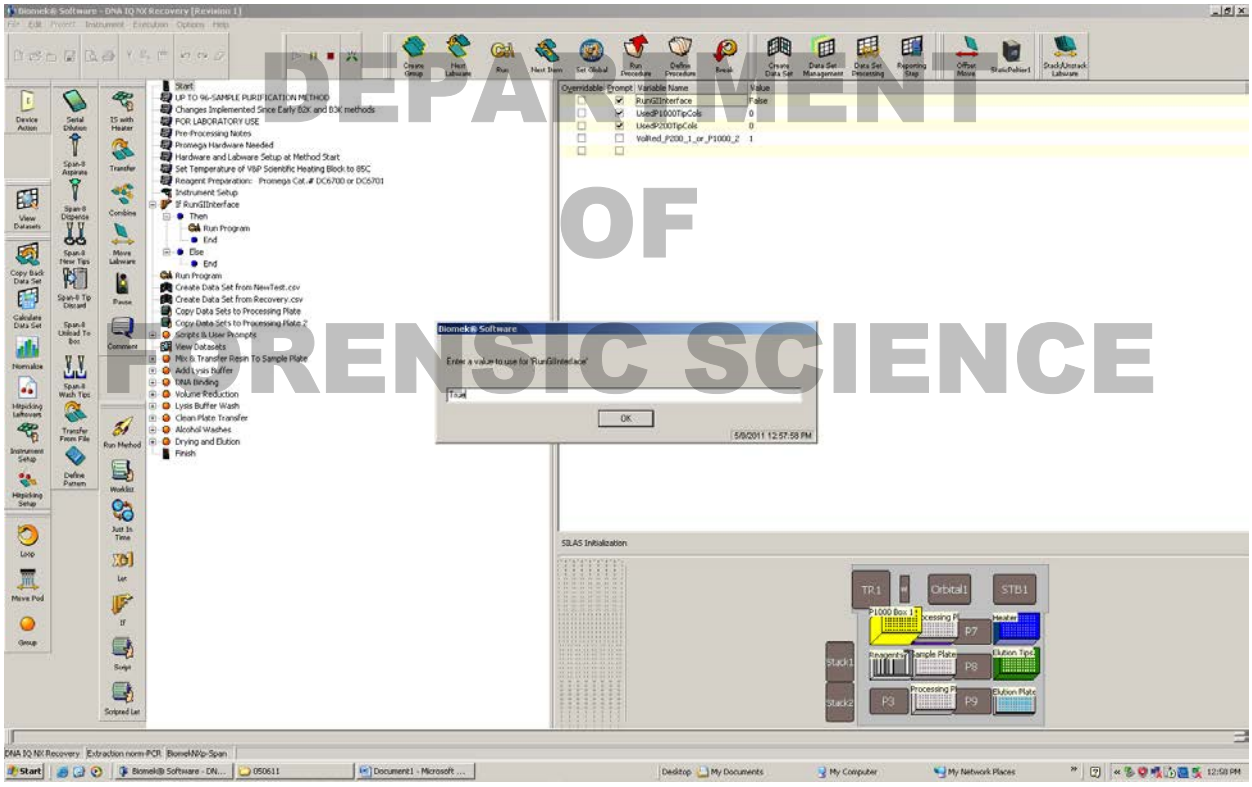


Figure 6. Prompt for running the GI Interface in the recovery method.

UNCONTROLLED COPY

2.3.3.1.1 If the "new Test.csv" file (created during a regular run) is incorrect, has been altered since the failed run, or volumes for the reagents are needed, enter "True" and the GI Interface will run and the information for the run must be entered in the same way it was entered for a standard run (see steps 2.6.3.7 – 2.6.3.10 of section 2 Preparation of the Biomek® NX^P Automation Workstation for DNA Isolation).

2.3.3.1.2 If nothing has changed with the "new Test.csv" file and the GI Interface does not need to be run leave the entry as "False".

2.3.3.2 The “Recovery.xls” file (shown in Figure 7) will be opened next by the software.

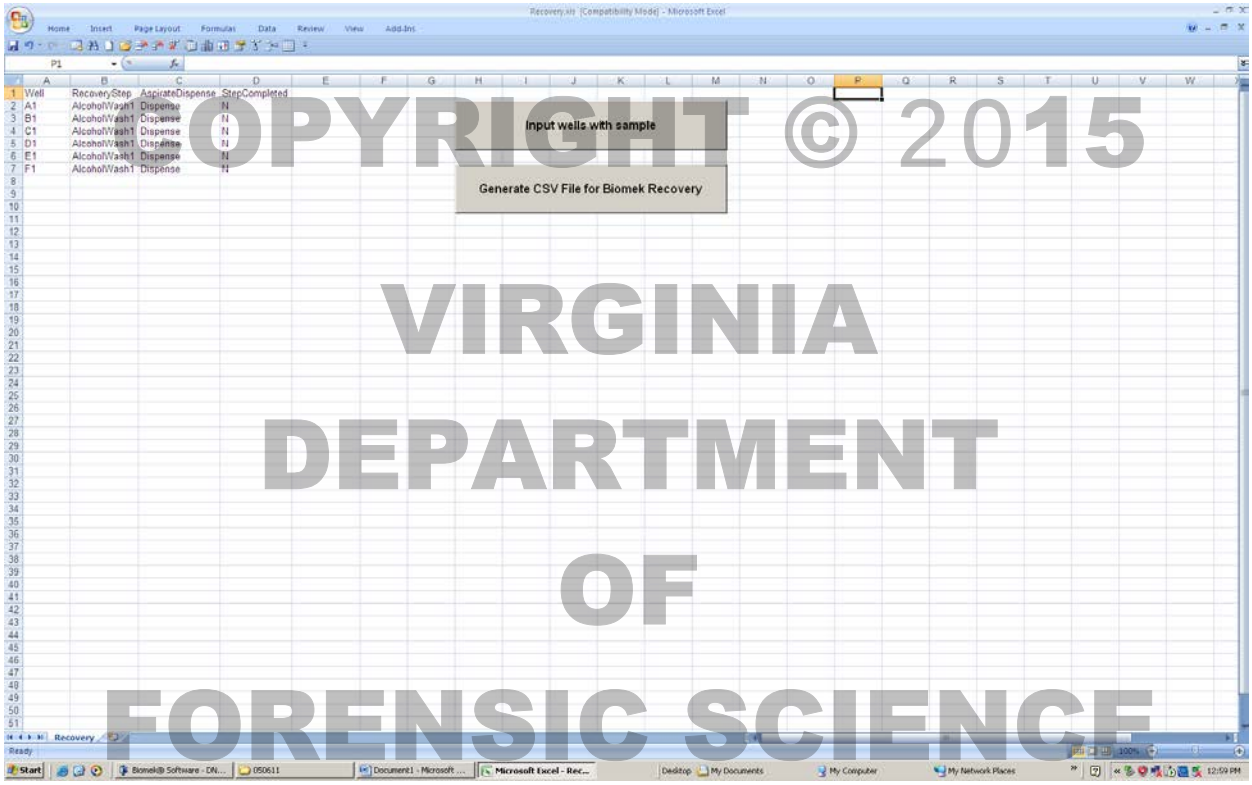


Figure 7. Recovery.xls worksheet for entering the conditions for the recovery run.

2.3.3.2.1 Click on the “Input wells with sample” button to bring in the well numbers from the new Test.csv file for the wells with sample in them.

2.3.3.2.2 Choose the “RecoveryStep”, “AspirateDispense”, and “StepCompleted” conditions (shown as column headers in Figure 7) to set the starting point for the recovery method for **EACH** well with sample (See Table 1).

2.3.3.2.3 Make sure that all of these fields are filled in for every well with sample.

2.3.3.2.4 Click on the “Generate CSV File for Biomek Recovery” button and click “Ok” when prompted if the worksheet has been completed to generate the Recovery.csv file for the run and close the worksheet.

OPYRIGHT © 2015
VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

Table 1. Failure conditions for the recovery run. Depending on which step the initial run failed, shown in the leftmost column, the information to be entered for the RecoveryStep, AspirateDispense and StepCompleted in the "Recovery.xls" file, are indicated in the respectively labeled columns.

Point at which run failed	RecoveryStep	AspirateDispense	StepCompleted
Lysis buffer with resin dispense (dispense has not occurred yet)	ResinDispense	Dispense	N
Lysis buffer with resin dispense (dispense completed)	ResinDispense	Dispense	Y
Initial lysis buffer dispense (dispense has not occurred yet)	LysisDispense	Dispense	N
Initial lysis buffer dispense (dispense completed)	LysisDispense	Dispense	Y
Volume reduction	VolumeReduction	Dispense or Aspirate	Y or N
Lysis buffer wash dispense (dispense has not occurred yet)	LysisWash	Dispense	N
Lysis buffer wash dispense (dispense completed)	LysisWash	Dispense	Y
Removal of lysis buffer wash	LysisWash	Aspirate	Y or N
Wash buffer dispense #1 (dispense has not occurred yet)	AlcoholWash1	Dispense	N
Wash buffer dispense #1 (dispense completed)	AlcoholWash1	Dispense	Y
Clean plate transfer (from Processing Plate to Processing Plate 2) and removal of wash buffer #1	CleanTransfer	Dispense	Y or N
Wash buffer dispense #2 (dispense has not occurred yet)	AlcoholWash2	Dispense	N
Wash buffer dispense #2 (dispense completed)	AlcoholWash2	Dispense	Y
Removal of wash buffer #2	AlcoholWash2	Aspirate	Y or N
Wash buffer dispense #3 (dispense has not occurred yet)	AlcoholWash3	Dispense	N
Wash buffer dispense #3 (dispense completed)	AlcoholWash3	Dispense	Y
Removal of wash buffer #3	AlcoholWash3	Aspirate	N
Dry resin prior to elution	AlcoholWash3	Aspirate	Y
Elution buffer dispense (dispense has not yet occurred)	Elution	Dispense	N
Elution buffer dispense (dispense completed)	Elution	Dispense	Y
Elution buffer transfer to strip tubes (elution on heatblock complete)	Elution	Aspirate	Y or N

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

NOTE: For conditions where there are two choices listed either choice will work and so it is not necessary to indicate whether it is a dispense or aspirate step or if it's been completed or not (Y or N).

3. After the DNA isolation is complete, carry the samples forward for quantitation as outlined in the Section 4, DNA Quantitation.

APPENDIX F – PROBE ALIGNMENT

If it is observed that one or more of the probes appears not to be aligned with the other probes in the z-axis, a probe alignment may be performed to better align the z-axis positioning. A probe may be out of z-axis alignment if it is observed that liquid within a particular row in a plate fails to be transferred repeatedly from one or more of the wells (or from tubes). This may be due to the z-axis being out of alignment which could cause the pipette tip to bottom out in the well or to be above the meniscus of the liquid, particularly if the volume of liquid is small.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY

1 Open Probe Alignment method

1.1 If the robot was turned off and then turned on and a Home All Axes function is not performed, the software will prompt the user that it needs to be performed and will not allow the user to initiate a method.

1.2 Section 2.6 provides the procedure for Homing All Axes. Follow the directions from 2.6.1 to 2.6.2.6.

1.3 The tips mounted on the Span-8 tool must come down onto a flat surface in order to evaluate whether or not the tips are contacting at the same z-axis position and if not, adjustments will be made using this method.

1.3.1 Invert the Magnabot and place it in the P3 position on the Biomek® NX^{P} deck. Make certain the surface facing up is horizontally flat.

1.4 Prior to starting the Probe Alignment Script method, P20 tips need to be loaded onto all probes of the Span-8 tool.

1.5 Open the Service project folder.

1.6 Open the P20 Tip Load method. The method will load one column of P20 tips onto the probes and then stop. **Do not discard the tips!**

1.7 Close P20 Tip Load method, but stay in the same project folder. Open the Probe Alignment Script method as shown in Figure 1 and click OK.

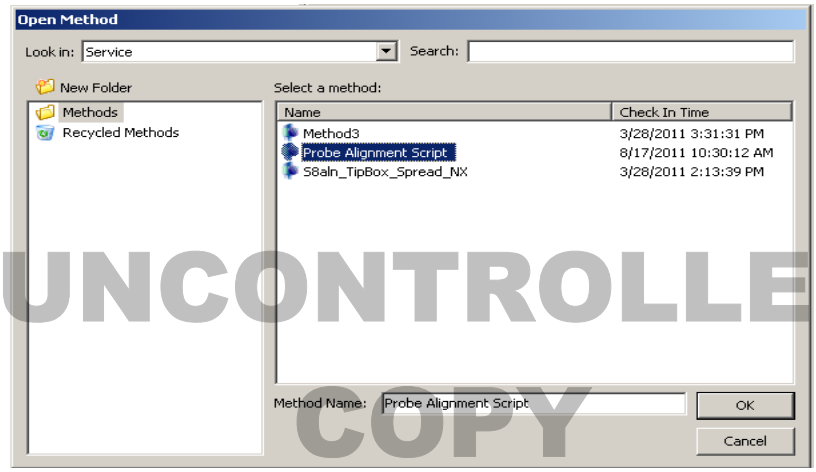


Figure 1. Opening Probe Alignment Script method.

1.8 The inverted Magnabot in the P3 position will provide a flat surface with which you will evaluate the probe z-axis alignment. You will use a Post-It note to determine if the tip is close enough to the flat surface. There should sufficient space between the Post-It note and the inverted Magnabot to insert the Post-It note, however, there should be a slight tug of the tip contacting the Post-It note when you move the paper under the tip.

1.9 Initiate the method by clicking on the green arrow, shown in Figure 2.

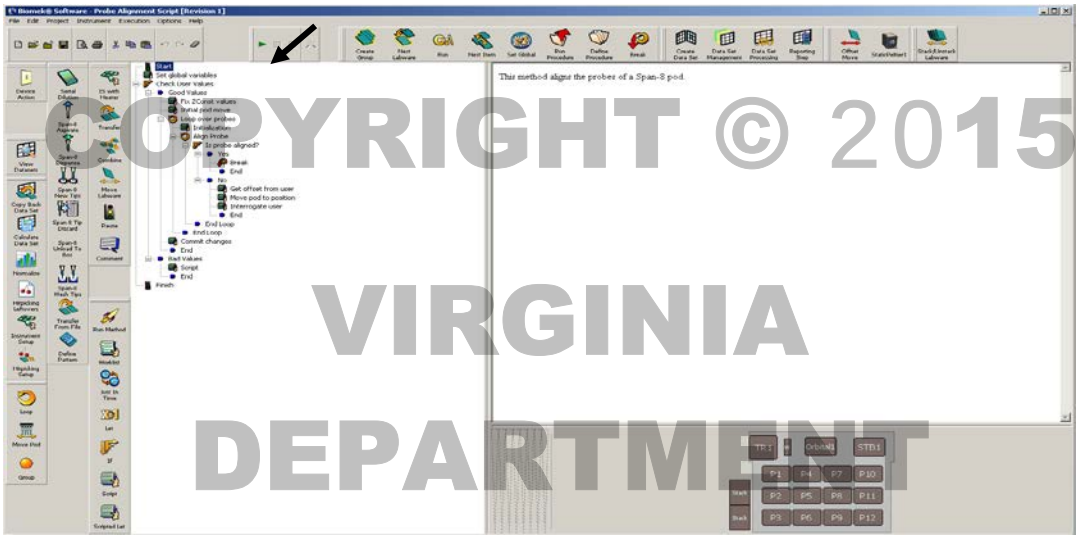


Figure 2. Initiating the Probe Alignment Script method. Arrow points to green arrove for starting a method.

1.10 The following prompt will pop up.

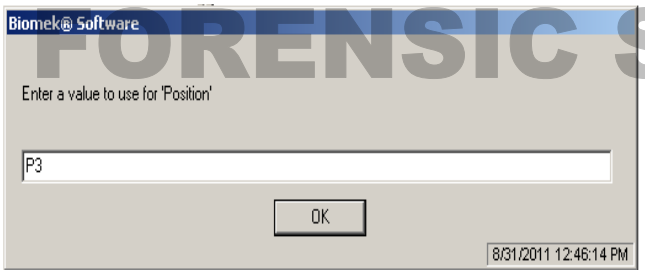


Figure 3. Positioning Pod 1 over the Magnabot.

1.11 Type in P3 and click OK.

1.12 The next prompt to pop is shown in Figure 4. The default is Pod1, so just click OK.

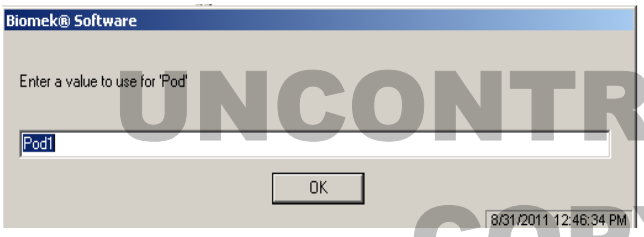


Figure 4. Selecting the Pod.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
UNCONTROLLED COPY

1.13 The next prompt is asking by how much the user should change the Pod1 z-axis position (Figure 5). The default is 3 and it is in cm. Do not change this value and just click OK.

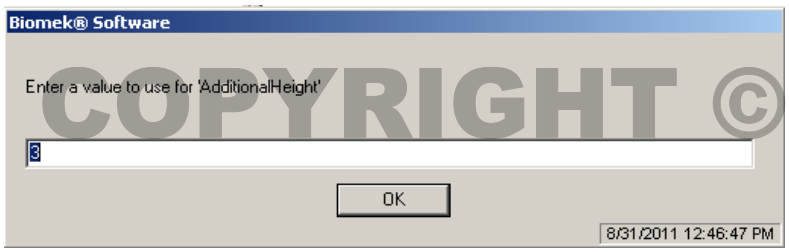


Figure 5. Pod 1 z-axis position.

1.14 The next prompt that opens up (Figure 6) will allow the user to adjust the probe positioning with respect to the Magnabot surface (in mm), one probe at a time. Adjusts should be made carefully, making every effort not to overestimate the distance and cause the tips to crash onto the Magnabot surface. To move the tips down, enter in negative values, e.g., -2, whereas to move the tips up, enter in positive values. This may need to be repeated several times before the tip (it does one probe at the time, although all of the probes move together) is aligned. To determine if the probe (probe #1 in this example) is properly aligned in its z-axis, insert a Post-It note between the P20 tip loaded onto probe #1 and the flat surface of the inverted Magnabot. A slight tug on the Post-It note should be felt as you try to move it out from between the P20 tip and the Magnabot. Click OK when the amount the tips should be moved is entered.

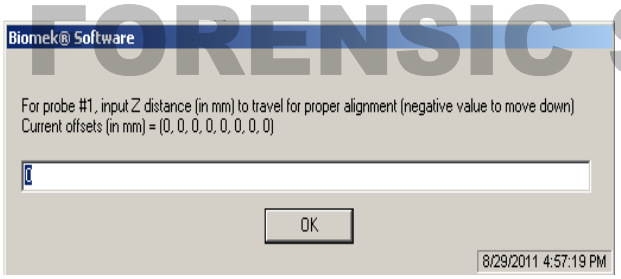


Figure 6. Moving the probe (in mm) up or down for proper alignment.

1.15 The next prompt window will open as shown in Figure 7.

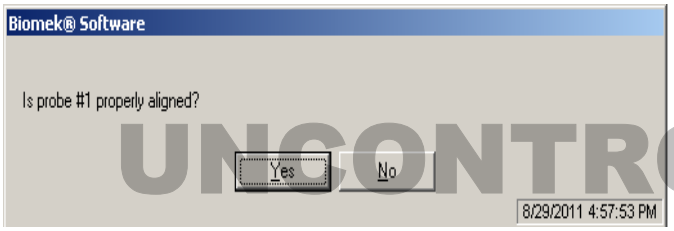


Figure 7. Determining if the probe is properly aligned.

1.16 If the probe is properly aligned with respect to the Magnabot as described in 1.14, then click Yes, otherwise click No and the process will be repeated.

1.17 The method will walk the user through the alignment of each of the probes, one at a time, with respect to the Magnabot surface. The method will finish once probe #8 is aligned. The method can be stopped at any point in the process by clicking on the stop icon.

COPYRIGHT © 2015 VIRGINIA DEPARTMENT OF FORENSIC SCIENCE UNCONTROLLED COPY