Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

EXTRACTION OF DNA

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1 ISOLATION OF DNA

NOTES: Upon completion of analysis, DNA extracts from evidentiary items, excluding Casework Direct extracts used for screening purposes, will be dried down and returned to the submitting agency with the evidence. If greater than 50% of 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 dried and returned to the submitting agency with the evidence.

The return of DNA extracts from casework reference samples (buccal swabs, bloodstain cards, etc.) is optional unless greater than 50% of the casework reference sample was consumed.

The return of DNA extracts from unidentified remains not entered into CODIS is optional.

Wet DNA extracts may be stored at room temperature for approximately 24 hours while in use but shall be either refrigerated or frozen at all other times.

EXAMPLE: Manually extracted samples may be left at room temperature overnight for quantitation, amplification, and the 1.5mL tube transfer the next day but will be refrigerated or frozen once these processes are complete.

DNA may be extracted from bloodstains, sperm cells, buccal cells, hair, tissue, bone, and other samples. Slightly different extraction procedures are available for use and are outlined in this chapter.

For tracking purposes, the samples will be listed on the worksheets in the order in which they are processed/handled.

EXCEPTION: Reagent blanks, although interspersed within an extraction set when manually processed, may be moved to/listed in/loaded in empty wells, as needed, to accommodate spacing requirements when loaded for robotic processes.

All samples will be processed in accordance with all procedures and policies outlined in all Forensic Biology Section Procedure Manuals.

If more than one microcentrifuge tube was used to collect a stain for extraction, the entire sample should be condensed into one sample tube during the manual purification step or after the robotic purification step.

Special Precautions:

- At a minimum, a reagent blank will be processed with each set of samples extracted with the same procedure (procedure reagent blank) to check for the presence of contaminating DNA in the reagents. 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 with the respective case samples. For any set of samples extracted using a differential procedure, a separate reagent blank will be processed for each scaled volume of reagents used. For any set of samples extracted using a non-differential procedure, at least a single reagent blank using the highest volume used will be processed.
- The manual steps of the DNA extraction of evidence samples will be performed at a separate time or space from the manual steps of the DNA extraction of reference samples. This helps to prevent potential cross-contamination between evidence samples and reference samples.
- The manual steps of the DNA extraction of evidence samples will be performed prior to the manual steps of the DNA extraction of reference samples, if possible.
- When processed in the same batch of samples, the manual steps of the DNA extraction from samples expected to contain low levels of DNA (single hairs, trace DNA samples, etc.) will be performed prior to the DNA extraction from samples expected to contain high levels of DNA (for example, tissue) 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 tube. If liquid from the cap of the tube comes in contact with the disposable glove, the glove will be changed before proceeding to the next sample tube.
- All work surfaces will be thoroughly cleaned with a 10% solution of bleach or a solution that will remove/degrade the DNA. Subsequently, Isopropanol or a 70% solution of ethanol will be used to remove the residue left by the chemicals. Disposable bench paper will also be used to prevent the accumulation of human DNA on permanent work surfaces.
- 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.
- Different lot numbers of the same reagent will not be combined. If a small volume of one lot of a reagent remains, the extraction set may be separated into two smaller sets, each with its own reagent blank processed with all of the same lots as used on the samples.
- To avoid splashing and minimize aerosols, all liquid will be centrifuged to the bottom of the closed tube before the tube is opened.
- 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.

1.1 Equipment

- Heat block or incubator, 37 °C
- Heat block or incubator, 56 °C
- Heat block, 70 °C
- Microcentrifuge
- Microcentrifuge tube rack
- Vortex mixer
- Tweezers
- Microcentrifuge tube rack
- Pipettes – 2 µL, 10 µL, 100 µL, 200 µL, and 1000 µL
- Refrigerator/Freezer
- Biological Safety Hood (hood)
- Scalpel and blades
- Beaker, 50 mL
- Mortar and Pestle
- Chisel and hammer
- High Speed Electric Drill and drill bits
- Rotary shaft tool (tooth procedure)

1.2 Materials

- Microcentrifuge tubes, 1.5 mL
- Microcentrifuge tubes, 2.0 mL
- Transfer pipettes
- Sterile ART tips for pipettes – 10 µL, 20 µL, 100 µL, 200 µL, and 1000 µL
- Microcentrifuge tube lids
- Kimwipes
- Spin-Ease baskets
- CW spin baskets
- Gloves
- Conical tubes, 15 mL and 50 mL

- Weigh boats
- Microscope slides
- Plastic zip-closure bags
- Parafilm
- Diamond mini cutting disc

1.3 Reagents

- Stain Extraction Buffer
- Proteinase K - 20 mg/mL (frozen when not in use)
- TNE
- 20% Sarkosyl
- 0.39*M* Dithiothreitol (DTT)
- Sterile Type I Water
- PCR Digestion Buffer
- 10% bleach solution
- Reagent Grade Ethanol
- Isopropanol
- Liquid Nitrogen (may be maintained in/retrieved from another Section of the Laboratory)
- $CaCl_2$ buffer
- DNA IQ™ Lysis Buffer
- DNA IQ™ Proteinase K Buffer
- Casework Direct Reagent
- 1-Thioglycerol
- 5X AmpSolution™ Reagent
- Amplification grade water

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

NOTES: This procedure may also be used for most evidentiary type samples, including cellular material from tissue that was collected onto swabs, but not for hair.

The spinning down of cuttings, as referred to in 1.4.5 of this procedure, from casework reference samples (i.e., buccal swabs, bloodstain cards, etc.) is optional.

1.4.1 Add 2.5 µL of 0.39 *M* DTT per 100 µL of DNA IQ™ Lysis buffer to the DNA IQ™ Lysis buffer to be used if not added previously as stated in 1.4.1.1.

1.4.1.1 This addition may be made and the DNA IQ™ Lysis buffer with DTT stored and/or used for up to a month if capped tightly and marked with the date of preparation. If this tube is for personal use by one individual, it does not need to be recorded in the reagent log as an in-house made reagent.

1.4.2 Add a sufficient volume of DNA IQ™ Lysis buffer with DTT, ranging from 200 µL to 800 µL, to saturate the sample.

1.4.3 Vortex vigorously for 20-30 seconds, then pulse spin to force the cutting into liquid.

1.4.4 Place the microcentrifuge tube into a 56 °C heat block for a minimum of 30 minutes. If the sample is deposited on FTA paper the sample maybe extracted at 56 °C or at 95 °C for a minimum of 30 minutes.

1.4.5 Vortex vigorously for 20-30 seconds and pulse spin the tube. Remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 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.

1.4.6 Remove the Spin-Ease basket containing the cutting and discard (unless greater than 50% of the sample was consumed and the cutting must be retained).

1.4.7 Proceed to either 2.4.1 in Chapter 2, Manual Purification of DNA or Chapter 3, Loading Samples for Robotic Isolation/Purification of DNA.

NOTE: Samples may be left at room temperature or refrigerated for up to 24 hours after heating and centrifugation before proceeding to Chapter 2 or 3.

1.5 Organic/DNA IQ™ Extraction Method for Mixed Body Fluid Stains (Differential Procedure)

1.5.1 Add:

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

in proportional amounts to saturate the cutting.

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

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

1.5.4 Vortex vigorously for 20-30 seconds and pulse spin the tube. Remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 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.

1.5.5 Remove the Spin-Ease basket containing the cutting and discard (unless greater than 50% of the sample was consumed and the cutting must be retained).

1.5.6 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new labeled microcentrifuge 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. The pellet on the bottom of the tube will become the SPERM FRACTION.

1.5.7 At this stage, set the non-sperm fraction tube aside.

1.5.8 Wash the pellet as follows: Add 500 µL of PCR digestion buffer and resuspend the pellet by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Remove all but 50 µL of the supernatant and discard.

1.5.9 Repeat the wash in step 1.5.8 an additional 2 times. The sperm pellet may be washed up to 5 times if a low sperm cell count is suspected. After the final wash, remove all but 50 µL of the PCR digestion buffer and discard. Resuspend the pellet in the remaining 50 µL of PCR digestion buffer. This is the SPERM FRACTION.

OPTION: A 3 µL spot of the SPERM FRACTION onto a glass microscope slide may be created for examination/sperm search.

- If the slide is examined and no spermatozoa are observed, the slide may be discarded.
- If the slide is either not examined (spotted for possible future examination only) OR if at least one spermatozoon is identified on the slide, it must be returned with the evidence.

1.5.10 Proceed to either Chapter 3, Loading Samples for Robotic Isolation/Purification of DNA with both the non-sperm and sperm fractions OR continue to 1.5.11 with the sperm fraction for organic procedure or Chapter 2, Manual Purification of DNA (Section 2.4) for manual DNA IQ™ procedure.

NOTES: If proceeding directly to Chapter 3, Loading Samples for Robotic Isolation/Purification of DNA, the sperm cells will by lysed during the robotic isolation/purification steps.

The sperm and non-sperm fractions may be capped and left at room temperature overnight or refrigerated before proceeding.

1.5.11 Add to each sperm fraction:

150 µL TNE
50 µL 20% Sarkosyl
40 µL 0.39*M* DTT
150 µL Sterile Type I Water
10 µL Proteinase K

1.5.12 Mix by hand or light vortexing and place the tube into a 56 °C incubator or heat block for a minimum of 2 hours.

1.5.13 Pulse spin the tube to force the condensate to the bottom of the tube.

1.5.14 Proceed with both the non-sperm fraction and sperm fraction to Chapter 2, Manual Purification of DNA (Section 2.5).

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

NOTE: This procedure may be used for hair, cellular material from tissue that was collected on a swab, samples that are believed to have a low concentration of DNA (i.e., envelopes, stamps, cigarette butts) or highly concentrated bloodstains (i.e., dried blood flakes).

1.6.1 Add:

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

in proportional amounts to saturate the sample.

A final volume of up to 400 µL may be used if proceeding with robotic isolation/purification in Chapter 3.

1.6.2 Mix by hand or lightly vortex and pulse spin the microcentrifuge tube to force the sample into the liquid.

1.6.3 Place the tube into a 56 °C incubator or heat block for a minimum of 1 hour.

1.6.4 Pulse spin the tube in a microcentrifuge to force the condensate to the bottom of the tube.

1.6.5 Vortex vigorously for 20-30 seconds and pulse spin the tube. For samples other than hair, remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 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.

1.6.6 Remove the Spin-Ease basket containing the cutting and discard (unless greater than 50% of the sample was consumed and the cutting must be retained).

1.6.7 Proceed to either 2.4.1 in Chapter 2, Manual Purification of DNA or to Chapter 3, Loading Samples for Robotic Isolation/Purification.

1.7 DNA IQ™ System Extraction Method for Tissue Samples

1.7.1 Add:

90 µL of 1X $CaCl_2$ buffer
10 µL of Proteinase K

in proportional amounts to saturate the sample.
A final volume of up to 400 µL may be used if proceeding with robotic isolation/purification in Chapter 3.

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

1.7.3 Place the tube into a 56 °C 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 and place the tube into a 56 °C incubator or heat block for an additional 2 hours.

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

1.7.5 Proceed to either 2.4.1 in Chapter 2, Manual Purification of DNA or to Chapter 3, Loading Samples for Robotic Isolation/Purification.

1.8 Organic Extraction Method for Buccal Cell Type Samples, Bloodstains and Tissue Samples

NOTES: This procedure may also be used for other evidentiary type samples, including cellular material collected from tissue that was collected on a swab. It should not be used for hair, bones, teeth or samples to be extracted differentially.

The spinning down of cuttings, as referred to in 1.8.6 and 1.8.7 of this procedure, from casework reference samples is optional.

1.8.1 Add:

400 µL of stain extraction buffer
10 µL of Proteinase K

in proportional amounts to saturate the sample.

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

1.8.3 Place the tube into a 56 °C 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 and place the tube into a 56 °C incubator or heat block for an additional 2 hours.

1.8.4 If extracting tissue, spin the microcentrifuge tube at ~12,000 rpm for 5 minutes to pellet any undigested debris and proceed to 2.5, Microcon® Purification Procedure.

1.8.5 For samples other than tissue, vortex vigorously for 20-30 seconds and pulse spin the tube. Remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 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.

1.8.6 Remove the Spin-Ease basket containing the cutting and discard (unless greater than 50% of the sample was consumed and the cutting must be retained).

1.8.7 Proceed to 2.5, Microcon® Purification Procedure.

1.9 Organic Extraction Method for Hair

1.9.1 Add:

400 µL of stain extraction buffer
10 µL of Proteinase K

1.9.2 Mix by hand or lightly vortex and pulse spin the microcentrifuge tube to force the hair sample into the liquid.

1.9.3 Place the tube into a 56 °C incubator or heat block overnight (18-24 hours).

1.9.4 Pulse spin the tube in a microcentrifuge to force the condensate to the bottom of the tube.

1.9.5 Proceed to 2.5, Microcon® Purification Procedure.

1.10 Organic Extraction Method for Teeth

1.10.1 Add:

400 µL of stain extraction buffer
10 µL of Proteinase K

in proportional amounts to saturate the sample.

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

1.10.3 Place the tube into a 56 °C incubator or heat block for a minimum of 2 hours.

1.10.4 Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to force the pulverized tooth sample to the bottom of the tube.

1.10.5 Proceed to 2.5, Microcon® Purification Procedure.

1.11 Organic Extraction Method for Bone

1.11.1 Add:

800 uL of stain extraction buffer
28 µL of Proteinase K

in proportional amounts to saturate the bone sample.

If necessary, the extraction may be performed in a 15 mL conical tube to accommodate a larger volume.

1.11.2 Place the tube into a 56 °C incubator or heat block. After one hour of incubation, mix thoroughly and re-secure the cap of the tube. Continue to incubate the bone sample at 56 °C overnight.

1.11.3 Proceed to 2.5, Microcon® Purification Procedure.

1.12 Organic/DNA IQ™ Extraction Method for Isolating DNA from Heat Fixed and Permounted Slides/Smears (Differential Procedure)

NOTE: If the slide/smear has not been heat fixed and/or permounted, skip to step 1.12.5

1.12.1 Place the stained, fixed/permounted slide/smear into a clean glass Petri dish with a cover.

1.12.2 Pour a sufficient volume of xylene over the slide/smear until completely submerged.

1.12.3 Incubate the slide/smear overnight at room temperature. If the coverslip does not float off easily, continue to soak the slide/smear in xylene until the coverslip can be removed easily.

NOTE: Slides/smears that have been mounted in permount may need to be incubated in xylene for several days.

1.12.4 Once the coverslip floats off, remove the slide/smear from the Petri dish and air dry for a minimum of 5 minutes. DO NOT DISCARD THE COVERSLIP.

1.12.5 Scrape the cellular material off the slide/smear with a clean, unused scalpel or razor blade and place it into a labeled microcentrifuge tube.

1.12.6 Remove half the cotton from a sterile cotton tipped swab. Wet the remaining portion of the swab attached to the stick slightly with sterile water and use the stick and swab to scrape the slide/smear to remove any remaining cellular material.

1.12.7 Remove the cotton from the stick or break off the portion of the stick and swab used to collect the remaining cellular material and add it to the scrapings in the microcentrifuge tube.

1.12.8 Retain the slide/smear along with the coverslip until it is certain that DNA has successfully been obtained. Slide/smears submitted by external agencies must be returned to the submitting agency along with the associated coverslips.

1.12.9 Add:

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

in proportional amounts to saturate the material in the tube.

1.12.10 Mix by hand or light vortexing and pulse spin to force the material into the liquid.

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

1.12.12 Vortex vigorously for 20-30 seconds and pulse spin the tube. Remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the 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.

1.12.13 Remove the Spin-Ease basket containing the cutting and retain in a separate tube.

1.12.14 Using a pipette, carefully transfer all but approximately 50 µL of the supernatant into a new labeled microcentrifuge 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. The pellet on the bottom of the tube will become the SPERM FRACTION.

1.12.15 At this stage, set the non-sperm fraction tube aside.

1.12.16 Wash the pellet as follows: Add 500 µL of PCR digetstion buffer and resuspend the pellet by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Remove all but 50 µL of the supernatant and discard.

1.12.17 Repeat the wash in step 1.12.16 an additional 2 times. The sperm pellet may be washed up to 5 times if a low sperm cell count is suspected. After the final wash, remove all but 50 µL of the PCR digestion buffer and discard. Resuspend the pellet in the remaining 50 µL of PCR digestion buffer. This is the SPERM FRACTION.

OPTION: A 3 µL spot of the SPERM FRACTION onto a glass microscope slide may be created for examination/sperm search.

- If the slide is examined and no spermatozoa are observed, the slide may be discarded.
- If the slide is either not examined (spotted for possible future examination only) OR if at least one spermatozoon is identified on the slide, it must be returned with the evidence.

1.12.18 Proceed to either Chapter 3, Loading of Samples for Robotic Isolation/Purification of DNA with both the non-sperm and sperm fractions or continue to 1.12.19 with the sperm fraction.

NOTES: If proceeding directly to Chapter 3, Loading of Samples for Robotic Isolation/Purification of DNA, the sperm cells will by lysed during the robotic extraction/purification steps.

The sperm and non-sperm fractions may be capped and left at room temperature overnight or refrigerated before proceeding.

1.12.19 Add to each sperm fraction:

150 µL TNE
50 µL 20% Sarkosyl
40 µL 0.39*M* DTT
150 µL Sterile Type I Water
10 µL Proteinase K

1.12.20 Mix by hand or light vortexing and place the tube into a 56 °C incubator or heat block for a minimum of 2 hours.

1.12.21 Pulse spin the tube to force the condensate to the bottom of the tube.

1.12.22 Proceed with both the non-sperm fraction and sperm fraction to Chapter 2, Manual Purification of DNA (either the DNA IQ™ Manual Purification Procedure or the Microcon® Purification Procedure may be used).

1.13 Casework Direct (CD) Extraction Method for Screening for Male DNA

1.13.1 Cut an appropriate amount of sample (e.g., approximately 1/8 of sample present, approximately 1/8 of each swab, etc.) and place in either a CW Spin Basket housed in a CW Microfuge tube (assembly tube) or in a 1.5 mL microcentrifuge tube.

1.13.2 Add 50 µL of 1-Thioglycerol to 450 µL of amplification grade water and vortex for 10-15 seconds, if not already diluted as stated in the note below.

NOTES: 1-Thioglycerol is viscous. To facilitate accurate pipetting, warm to room temperature, pipette slowly and avoid pipetting small volumes.

This dilution may be made and the diluted 1-Thioglycerol stored at 4 °C and used for up to 6 months if marked with the date of preparation. If this dilution is for personal use by one individual, it does not need to be recorded in the reagent log as an in-house made reagent.

1.13.3 Add the diluted 1-Thioglycerol (see 1.13.2) to Casework Direct Reagent in the following proportions to make enough Casework Direct Solution for the number of samples to be extracted (100 µL Casework Direct Solution per sample).

200 µL Casework Direct Reagent
1 µL diluted 1-Thioglycerol

1.13.4 Add 100 µL of Casework Direct Solution to each CW Spin Basket or 1.5 mL microcentrifuge tube, close the lid, and vortex for 5-10 seconds.

1.13.5 Place the CD assembly or tube in a 70 °C heat block for 30 minutes.

1.13.6 Vortex the CD assembly or tube for 5-10 seconds.

1.13.7 If using the CD assembly, centrifuge at room temperature for 5 minutes at maximum speed.

1.13.7.1 Ensure that the liquid has passed through the CW Spin Basket and into the assembly tube.

1.13.7.1.1 If all the liquid has passed through the CW Spin Basket and into the assembly tube, remove and discard the basket and cutting(s). Proceed to 1.13.9.

1.13.7.1.2 If liquid remains in the CW Spin Basket, transfer the cutting(s) and any liquid to a Spin-Ease basket and place the Spin-Ease basket into the assembly tube. Discard the CW Spin Basket OR using clean forceps, punch holes in the bottom of the CW Spin Basket to facilitate the passing of the liquid to the assembly tube and place the basket back into the tube.

1.13.7.1.3 Centrifuge the tube at room temperature for 5 minutes at maximum speed.

1.13.8 If using 1.5 mL microcentrifuge tube:

1.13.8.1 Pulse spin the tube.

1.13.8.2 Remove the cutting from the liquid and place into a new unused Spin-Ease basket. Place the basket in the tube and close the lid. Centrifuge the tube at room temperature for 5 minutes at maximum speed to remove the excess liquid from the cutting.

1.13.8.3 Remove the Spin-Ease basket containing the cutting and discard.

1.13.9 Proceed to quantitation, as outlined in the appropriate Forensic Biology Procedures Manual.

2 MANUAL PURIFICATION OF DNA

2.1 Equipment

- Pipettes – 2 µL, 10 µL, 100 µL, 200 µL, and 1000 µL
- Microcentrifuge
- Microcentrifuge tube rack
- DNA concentrator/evaporator
- Freezer, -20 °C
- Vortex mixer
- Heat block or incubator, 56 °C
- Magnet Sphere Stand (Promega Catalog Number Z5342)

2.2 Materials

- Transfer pipettes
- Microcentrifuge tubes, 1.5 mL
- Microcentrifuge tubes, 2.0 mL
- Sterile ART tips for pipettes – 10 µL, 20 µL, 100 µL, 200 µL, and 1000 µL
- Microcon® 50 Concentrator Assembly
- Microcon® 100 Concentrator Assembly
- Kimwipes
- Gloves

2.3 Reagents

- Phenol-chloroform-isoamyl alcohol (PCIAA), pre-warmed to room temperature
- 1X TE^{-4} buffer
- Sterile Type I Water
- 0.39*M* Dithiothreitol (DTT)
- DNA IQ™ Lysis Buffer
- DNA IQ™ Resin
- DNA IQ™ Wash Buffer
- DNA IQ™ Elution Buffer

2.4 DNA IQ™ Manual Purification Procedure

2.4.1 Depending on the type of sample being purified, add the following volumes of DNA IQ™ Lysis Buffer with DTT to the sample.

2.4.1.1 Non-sperm fractions and tissue – Remove 100 µL of the non-sperm fraction/tissue lysate and place it into a new labeled 1.5 mL microcentrifuge then add 220 µL of DNA IQ™ Lysis Buffer with DTT. Note: if more than 100 µL of the non-sperm fraction is used add proportional volumes of DNA IQ™ Lysis Buffer with DTT.

2.4.1.2 Sperm pellet – add 220 µL of DNA IQ™ Lysis Buffer with DTT.

2.4.1.3 Hair samples (optional: envelopes, stamps, cigarette butts, flakes of blood, and other low level samples) – add 220 µL of DNA IQ™ Lysis Buffer with DTT. Note: this should be increased proportionally depending on the volume of ProK based digestion buffer used.

2.4.1.4 Blood Stains and buccal cell type samples – add 100 µL of DNA IQ™ Lysis Buffer with DTT.

2.4.2 Vigorously vortex the bottle of DNA IQ™ Resin for 30 seconds prior to dispensing. Then add 8 µL of the DNA IQ™ Resin to each tube.

NOTE: If the stock bottle of DNA IQ™ Resin has settled for a prolonged period of time between samples re-vortex before further use.

2.4.3 After adding resin vigorously vortex each sample for several seconds.

2.4.4 Place the tubes in a microcentrifuge rack and allow the samples to incubate at room temperature for a minimum of 5 minutes allowing the DNA to adhere to the resin. After incubation, pulse spin in a microcentrifuge.

2.4.5 Transfer the sample tubes to a magnet sphere stand. Open the caps and without disturbing the resin pellet remove the liquid in each tube and discard.

2.4.6 Add 100 µL of Lysis buffer with DTT into each tube. Remove the tubes from the magnetic stand and vortex vigorously for several seconds. Pulse spin in a microcentrifuge.

2.4.7 Place the tubes back into the magnet sphere stand and without disturbing the resin pellet remove the liquid in each tube and discard.

2.4.8 Add 100 µL of 1X DNA IQ™ Wash buffer to each tube. Remove the tubes from the magnetic stand and vortex vigorously for several seconds. Pulse spin in a microcentrifuge.

2.4.9 Place the tubes back into the magnet sphere stand and without disturbing the resin pellet remove the liquid in each tube and discard.

2.4.10 Repeat steps 2.4.8 and 2.4.9 two additional times.

2.4.11 After the last wash step, open the cap on each tube and allow the samples to completely air-dry. Note: This will take approximately 5 minutes depending on the volume of liquid remaining in the tube.

2.4.12 Add 40 µL of the DNA IQ™ Elution buffer to each tube to remove the DNA from the resin. Vortex each tube vigorously for 5 seconds.

2.4.13 Place the tubes in 56 °C incubator or heat block for 5 minutes and then pulse spin in a microcentrifuge.

2.4.14 Place the tubes back into the magnet sphere stand and remove the supernatant. **DO NOT DISCARD. THE SUPERNATANT CONTAINS THE ISOLATED DNA SAMPLE. IF DNA IQ™ RESIN IS REMOVED WITH THE SUPERNATANT, THIS MAY CAUSE INHIBITION AND PREVENT THE SAMPLE FROM AMPLIFYING.**

2.4.15 Place the supernatant (free of any DNA IQ™ Resin) into a clean, labeled microcentrifuge tube.

2.4.16 Proceed to quantitation as outlined in the appropriate Forensic Biology Procedures Manual.

2.5 MICROCON® Purification Procedure

NOTE: The Microcon® purification procedure may be used when the biological stain extracted was deposited on a substrate known to cause inhibition during amplification (i.e., denim or velvet), the substrate released an excessive amount of dye during the extraction process, or the biological stain/material was minute in size (e.g., hair root).

2.5.1 Add 500 µL phenol-chloroform-isoamyl alcohol to each tube.

NOTE: The volume of phenol-chloroform-isoamyl alcohol (PCIAA) should be approximately equal to the volume of buffer used to extract/incubate the sample. For example, if 800 µL of stain

extraction buffer was used to extract a bone sample, approximately 800 µL of PCIAA should be added to the tube in step 2.5.1.

2.5.2 Cap the tube **tightly** and mix thoroughly by hand or light vortexing until the solution has a milky appearance.

2.5.3 Spin the tube for 3 minutes at a minimum of 10,000 rpm to separate the two phases.

2.5.4 Insert a Microcon® 100 concentrator (or Microcon® 50 concentrator for minute biological stains/material) into a labeled filtrate vial (microcentrifuge tube provided with the Microcon® assembly) and add 100 µL of sterile Type I Water to the concentrator.

2.5.5 Transfer the aqueous phase (top layer containing DNA in step 2.5.3) to the Microcon® concentrator, avoiding transfer of any of the organic solvent (bottom layer in step 2.5.3) and place the cap from the filtrate vial on the concentrator.

2.5.5.1 This step, along with step 2.5.6, may be repeated as necessary to accommodate larger volume extractions or the combining of multiple samples into one due to the extraction of a diffuse stain in multiple tubes.

2.5.6 Spin the Microcon® assembly in a microcentrifuge for 10-40 minutes at approximately 5,000 rpm until the volume is reduced.

2.5.7 Carefully remove the concentrator unit from the Microcon® assembly and discard the fluid from the filtrate vial. Return the concentrator to the top of the filtrate vial.

2.5.8 Add 200 µL of sterile Type I Water to the concentrator. Replace the cap and spin the Microcon® assembly in a microcentrifuge for 10-30 minutes at approximately 5,000 rpm until the volume is completely reduced.

NOTE: If the concentrator filter becomes stained, steps 2.5.7 and 2.5.8 may be repeated additional times to reduce the potential of the dye or material causing the stain inhibiting the PCR amplification.

2.5.9 Remove the cap from the concentrator and add 30 µL 1X TE^{-4} buffer.

2.5.10 Remove the concentrator from the filtrate vial and discard the vial. Carefully invert the concentrator and place into a new labeled retentate vial (same type of tube as the filtrate vial).

2.5.11 Spin the Microcon® assembly with the inverted concentrator in a microcentrifuge for 5 minutes at 5,000 rpm.

2.5.12 Discard the concentrator unit and place the cap on the retentate cup.

2.5.13 Proceed to quantitation as outlined in the appropriate Forensic Biology Procedures Manual.

3 LOADING SAMPLES FOR ROBOTIC ISOLATION/PURIFICATION OF DNA

NOTE: Unless otherwise specified/requested, samples loaded into the 96 deep square well plate for the regular automated method will be carried through quantitation, the normalization wizard amplification setup and placement into a thermal cycler for amplification by the robot operator. Additionally, the remaining extracts will be transferred to 1.5 mL tubes for long term storage.

3.1 Equipment

Pipettes – 2 µL, 10 µL, 100 µL, 200 µL, and 1000 µL

3.2 Materials

- 96 deep square well plates – Thermo Scientific WebSeal 96-well Non-Coated Plastic Microplates (V-bottom Wells) – P/N 60180-P202 – Cat#03-252-155
- Kimwipes
- Tape
- Microscope slides
- Sterile ART tips for pipettes - 10 µL, 100 µL, 200 µL, and 1000 µL

3.3 Loading of Samples into the 96 Deep Square Well Plate for the Regular Automated Method

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

3.3.1 Prepare a Biomek® NX^P Automation Workstation loading sheet (96 deep square well plate loading sheet) to reflect in which well each sample will be loaded being sure to follow all requirements below regarding placement of samples.

NOTE: The Biomek® NX^P Automation Workstation loading sheet (96 deep square well plate loading sheet) is a populatable page in the CE Populatable Worksheets specifically for use with the Biomek® NX^P Automation Workstation (Form 210-F1103 for PP16, Form 201-F1106 for Fusion). When entering sample information for each applicable well on the extraction page of the worksheets, volume information is also included. For optimal yields, the volume of lysate per sample should not be underestimated. It is better to overestimate the volume and round up to the nearest 50 µL than to underestimate.

3.3.2 Depending on the sample type, pipette the following volume of sample into the appropriate well of a clean 96 deep square well plate. 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:

3.3.2.1 For buccal cell type samples and bloodstains in DNA IQ™ Lysis buffer, pipette up to 800 µL of the lysate into the 96 deep square well plate.

3.3.2.2 For non-sperm fractions, pipette up to 400 µL of the lysate into the 96 deep square 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.

3.3.2.3 For sperm fractions, pipette the entire sample (this is approximately 30-50 µL of the sperm cell suspension) into the 96 deep square well plate. Take care to transfer the entire sperm pellet. If desired, DNA IQ™ Lysis buffer with DTT (~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.

3.3.2.4 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 96 deep square well plate.

3.3.2.5 For tissue samples, pipette up to 400 µL of the lysate into the deep well plate. Caution **must** be used to prevent aspirating any of the pelleted debris and loading the debris into the 96 deep square well plate. If necessary, centrifuge the sample for several minutes to pellet the debris, prior to loading the lysate into a 96 well plate for robotic DNA extraction.

3.3.3 Loading evidence samples:

3.3.3.1 Columns 11 and 12 (shown in red in the figure below) must be left blank/empty.

3.3.3.2 Once a column of evidence samples has been loaded into the 96 deep square well plate, a piece of tape may be placed over the wells to prevent other samples from being pipetted into the same well. Another option is to place a glass microscope slide over empty wells while loading the plate.

3.3.3.3 Evidence samples will be loaded into the 96 deep square well 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 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 1 column 8.

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 below).

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

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

3.3.4 Loading known/reference samples:

3.3.4.1 If known/reference samples are loaded into the evidence sample 96 deep square 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 figure above (i.e., wells A8 through H8).

3.3.4.2 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.

3.3.4.3 Alternate known samples are loaded with the evidence samples.

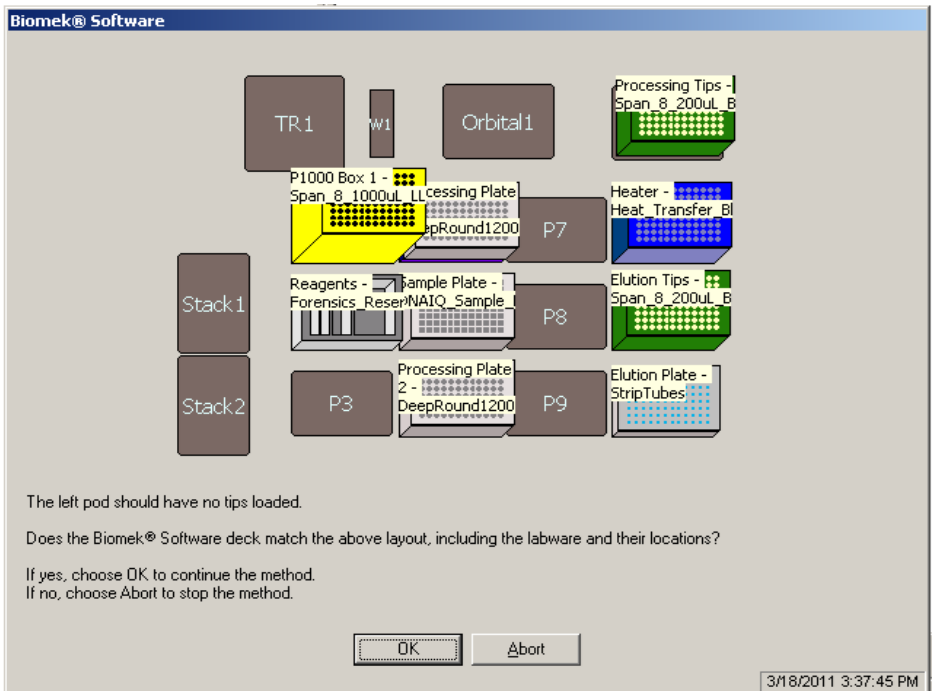
4 ROBOTIC ISOLATION/PURIFICATION OF DNA USING THE BIOMEK® NXP AUTOMATION WORKSTATION

Small differences in the well formation or depth of the well can cause the tips to "bottom out". When the tips are in contact with the bottom of the well, a vacuum can form and affect the accuracy of pipetting. Therefore, any substitution of plates or consumables must meet the specifications of the specific products listed below.

If samples are in the DNA IQ™ Lysis buffer, the volume range the extraction method can accommodate is from 200-800 µL.

If the samples are in a Proteinase K containing buffer (aqueous), the volume range the extraction method can accommodate is from 50-400 µL.

Detailed schematic view of the Biomek® NXP Workstation deck:



4.1 Equipment

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

4.2 Materials

- ABGene 96 deep round well plates
- 96 deep square well plates – Thermo Scientific WebSeal 96-well Non-Coated Plastic Microplates (V-bottom Wells) – P/N 60180-P202 – Cat#03-252-155

- 8-Reaction tube strips, 0.2 mL
- 8-Cap strips, 0.2 mL
- P250 Tips – aerosol resistant – (Beckman catalog #379503)
- P1000 Tips – aerosol resistant – (Beckman catalog #987925)
- Quarter module reservoirs divided by length (Beckman catalog #372788)
- Quarter module reservoirs (Beckman catalog #372790)
- Half module reservoirs (Beckman catalog #372786)
- Tape
- Microscope slides (optional)
- Sterile ART tips for pipettes - 100 µL, 200 µL, and 1000 µL

4.3 Reagents

- DNA IQ™ System Reaction Kit (Promega catalog #DC6700 = 400 rxns or DC6701 = 100 rxns) (stored at RT) which contains:
  - DNA IQ™ Lysis Buffer
  - DNA IQ™ Wash Buffer
  - DNA IQ™ Resin
  - DNA IQ™ Elution Buffer
- 0.39*M* DTT

4.4 BIOMEK® NXP Automation Workstation Operating Procedure for DNA Isolation/Purification

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

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

4.4.1 Turn on the computer.

4.4.2 Turn on the Biomek® NXP Automation Workstation using the power button located on the back right side of the unit under the power cord.

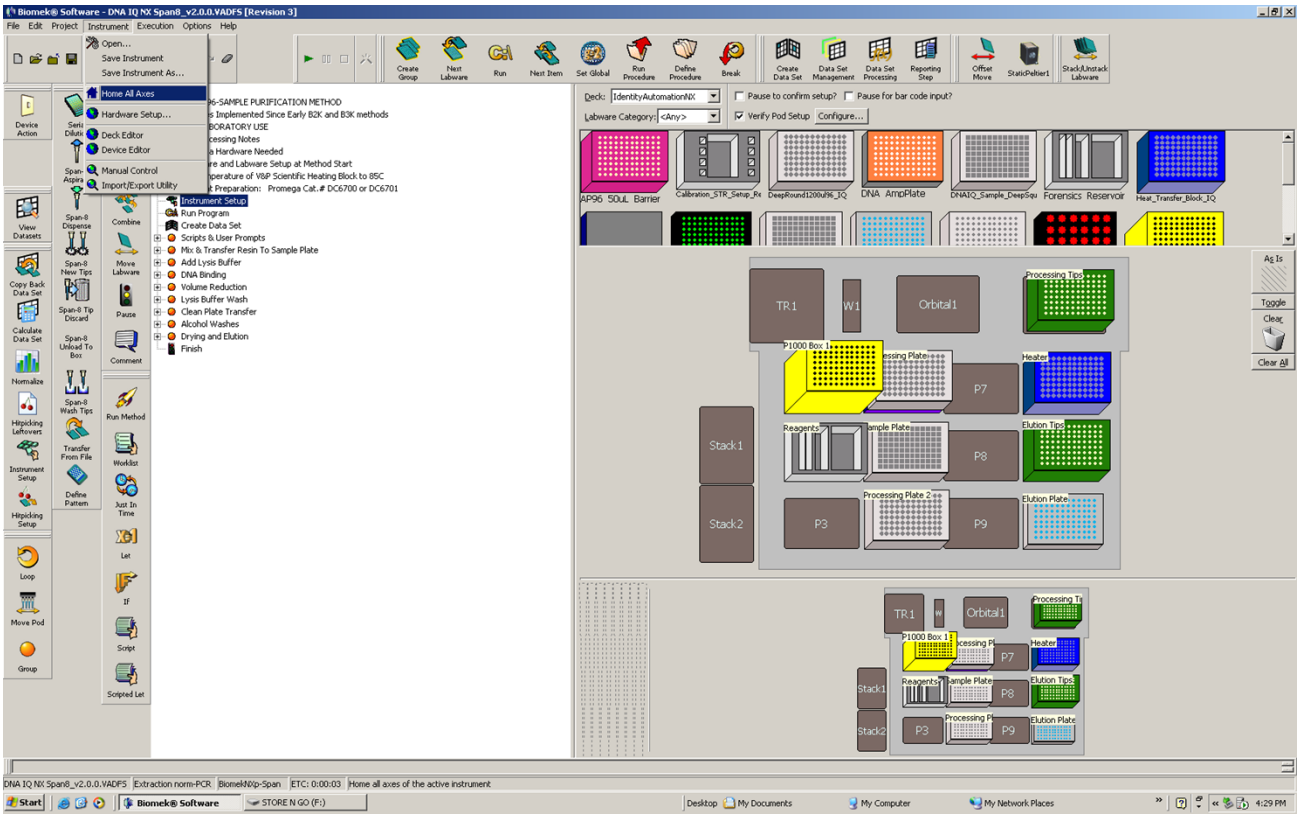
4.4.3 Turn on the Watlow electronic heating unit using the switch located on the front of the unit.

4.4.4 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 square 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).

4.4.4.1 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 deep square well plate loading sheet.

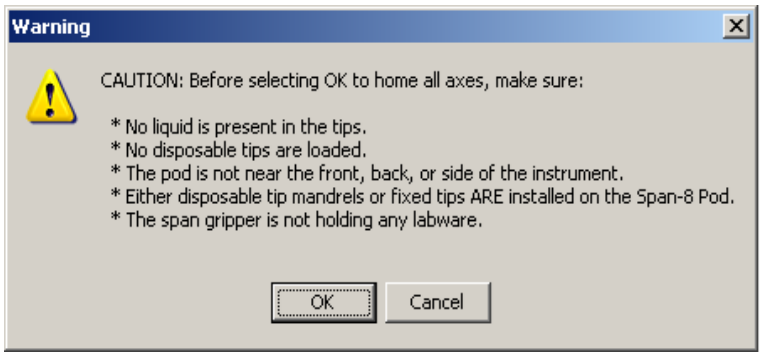
4.4.5 Click on the desktop shortcut for the NX software.

4.4.6 When the NX software opens up the following window will appear:



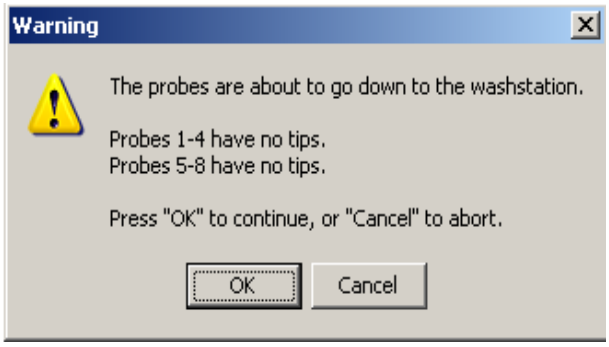
4.4.6.1 Under the instrument heading drop-down window, select **Home All Axes**.

4.4.6.2 The robot will display the following prompt:



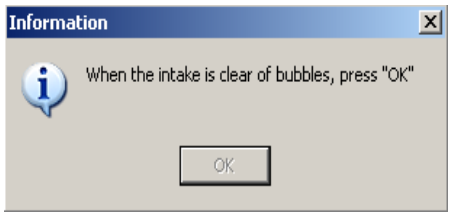
4.4.6.3 Ensure that all of the conditions are met and then click OK. If the pod is near the front, back or side of the instrument, gently slide the pod over by pushing the metal bars above the pod, not the pod itself.

4.4.6.4 The robot will home the axes and then pause and show the following:



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

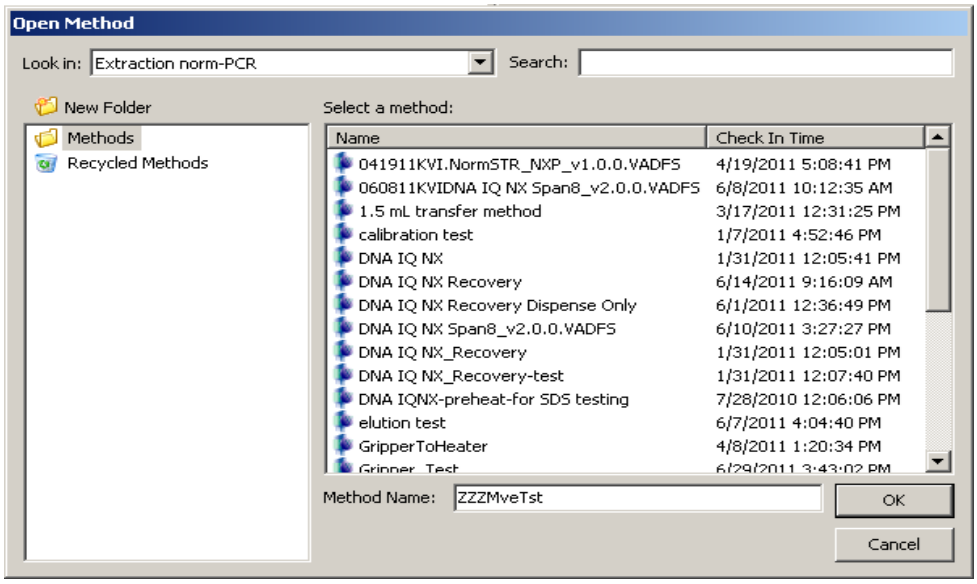
4.4.6.6 After the plungers have pushed any bubbles out, which usually only takes a few iterations of the plungers, click on the OK in the box shown below to stop purging the lines.



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

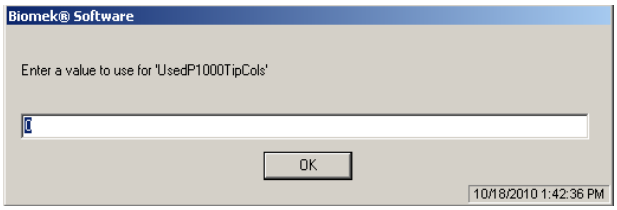
4.4.7.1 Using either the folder icon or under the File drop-down menu, open the DNA IQ NX method.

4.4.7.2 The following window will pop up:



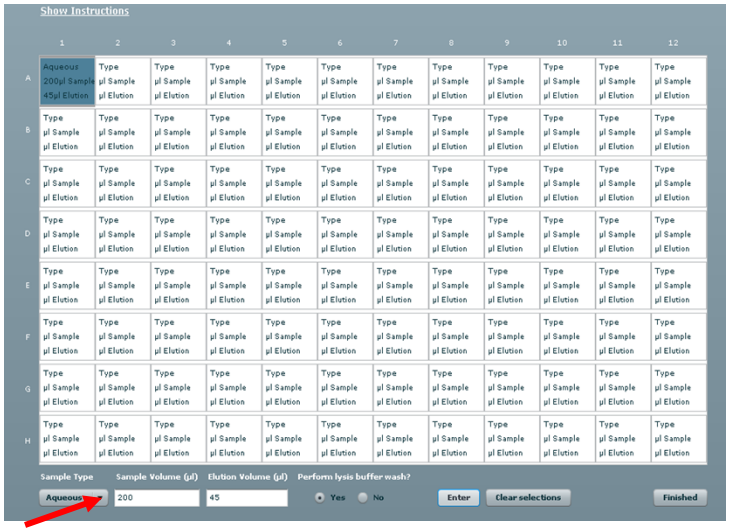
4.4.7.3 Select the DNA IQ NX method by double clicking or clicking on it and the OK button.

4.4.7.4 A series of prompts will appear as shown below:



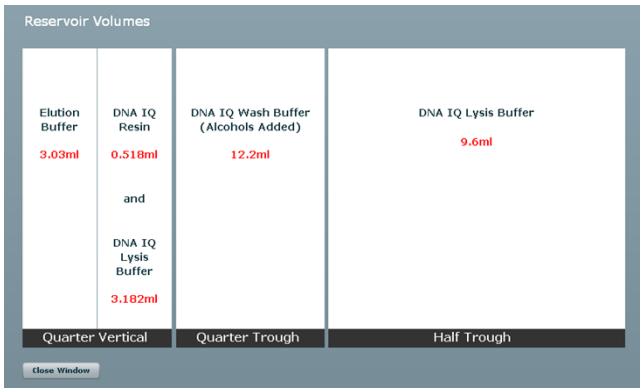
4.4.7.5 In the window shown above, enter the number of columns of tips, if any, that have already been used in the indicated P1000 tip box and click OK. A window identical to this except for the used P250 tips (the prompt says P200 which is the same) will open up, indicate the number of columns of used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

4.4.7.6 The following user interface window will open:



4.4.7.7 Highlight each well containing sample and indicate the nature of the buffer the sample is in from the drop-down menu indicated 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 key will allow the user to cherry pick all of the wells that should have the same settings. Once all applicable wells have the information entered, click on Enter. This will need to be repeated for each sample type (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 selected wells. If the entire column will have the same information entered, highlight the well in the A row and while holding down the Shift key, 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.

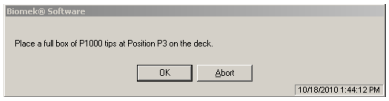
4.4.7.8 The following window will open up:



4.4.7.9 Use the volumes indicated for each of the reagent troughs shown in the window above to set up the reagents for the specific run. The values are calculated for each plate of samples from the information entered in 4.4.7.7. Click on the Close Window button when all of the reagent troughs have been filled with the appropriate reagents using the volumes indicated.

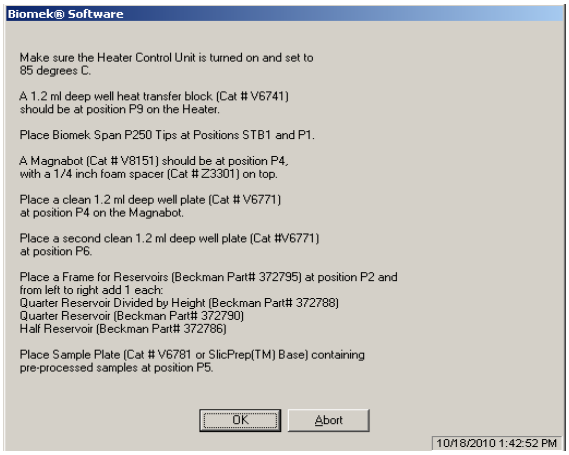
NOTE: The DNA IQ™ resin should be vortexed on high for approximately 15 seconds before pipetting into the second trough from the left. The DNA IQ™ Lysis buffer must have DTT added to it prior to use. The DNA IQ™ Wash buffer must be correctly prepared using the appropriate volumes of alcohol as indicated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

4.4.7.10 The method will then commence. Prompts regarding the tip boxes will appear as shown below:



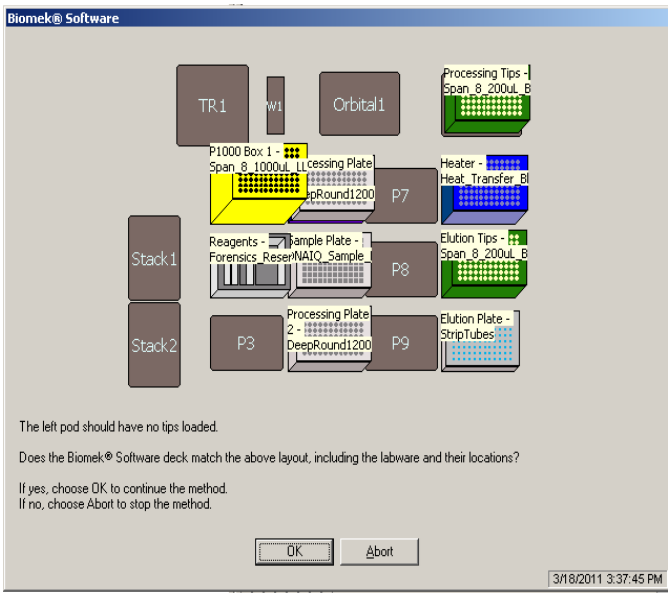
4.4.7.11 Check to be certain that the tip box is placed on the deck in the appropriate position and click OK.

4.4.7.12 The following window will pop up:



4.4.7.13 Check to make certain that all of the directions for placing labware on the deck are followed and click OK.

4.4.7.14 A prompt showing the deck layout will appear as shown below:



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).

After the DNA isolation is complete, the samples may be carried forward for quantitation as outlined in the appropriate Forensic Biology Procedures Manual.

APPENDIX A - REAGENTS

CALCIUM 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

CALCIUM CHLORIDE BUFFER, 1X

Expiration date: Twelve months from date of preparation

	MW	100 mL
1M Tris	121.1	5 mL
1 M $CaCl_2$	110.9	1 mL
Type I water		94 mL

Mix well. May be stored at room temperature.

CASEWORK DIRECT KIT, CUSTOM

Expiration date: Manufacturer's expiration date

Purchased from Promega Corporation, Madison, WI.

Refer to the Application Note #AN300 Rapid Processing of Swabs from Casework Samples Using Casework Direct Kit, Custom, Promega Corporation publication for storage conditions of Kit components (Product Insert).

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

- CASWORK DIRECT REAGENT, CUSTOM
- 1-THIOGLYCEROL
- 5X AMPSOLUTION™ REAGENT
- WATER, AMPLIFICATION GRADE

DITHIOTHREITOL, 0.39 M (DTT)

Expiration date: Twelve months from date of preparation

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

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

DTT (Molecular Biology Grade)

DNA IQ™ SYSTEM

Expiration date: Manufacturer's expiration date

Purchased from Promega Corporation, Madison, WI.

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

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

- DNA IQ™ LYSIS BUFFER

  Prior to use for extraction, 2.5 µL of 0.39 M DTT must be added per every 100 µL of DNA IQ™ Lysis Buffer.

- DNA IQ™ RESIN
- DNA IQ™ WASH BUFFER

  Expiration date: Twelve months from date of preparation or limiting component manufacturer's date, whichever comes first.

  Add 35 mL of reagent grade ethanol and 35 mL of Isopropyl Alcohol to 2X Wash Buffer. Be certain to accurately measure the alcohol volumes since not doing so could negatively impact the performance of the extraction. Mix contents and store at room temperature in a tightly capped container.

- DNA IQ™ ELUTION BUFFER

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.

ETHANOL, REAGENT GRADE

Expiration date: Dispose of when necessary

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)

EDTA (Disodium Salt)

8-HYDROXYQUINOLINE

Expiration date: Manufacturer's expiration date if listed, otherwise 3 years from date of receipt

ISOPROPYL ALCOHOL

Expiration date: Manufacturer’s expiration date

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
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.

PHENOL-CHLOROFORM-ISOAMYL ALCOHOL, 25:24:1

Expiration date: Manufacturer's expiration date if listed, otherwise 3 years from date of receipt

To aid visualization add approximately 0.07125 g of 8-Hydroxyquinoline/100 mL of phenol-chloroform-isoamyl alcohol solution until the phenol turns slightly yellow in color. CAUTION: Measure 8-Hydroxyquinoline under hood. Store at 2-5 C.

PROTEINASE K ENZYME (20 mg/mL)

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.

SARKOSYL, 20%

Expiration date: Twelve months from date of preparation

	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

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.

SDS (SODIUM DODECYL SULFATE), 0.1%

Expiration date: Twelve months from date of preparation

Add 5 mL of 20% SDS solution to 995 mL of Type 1 Water.

SDS (SODIUM DODECYL SULFATE), 20%

Expiration date: Twelve months from date of preparation

	MW	1 L	2 L
SDS	288.3	200 g	400 g
Type I Water		750 mL	1500 mL

CAUTION: AN AEROSOL MASK OR FUME HOOD SHOULD BE USED WHEN MAKING THIS SOLUTION.

Add the appropriate amount of SDS to Type I Water and mix until SDS is completely into solution and solution is clear, not cloudy. Adjust to final volume with Type I Water and store at room temperature.

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, 5 M - may also be purchased.

SODIUM HYDROXIDE (NaOH), 2 N

Expiration date: Twelve months from date of preparation.

	MW	2 L	20 L
10 N NaOH	40.0	400 mL	4 L
Type I Water		1600 mL	16 L

Combine components and mix well. Store at room temperature.

SODIUM HYDROXIDE (NaOH), 5 N

Expiration date: Dispose of when necessary

	MW	1 L
10 N NaOH	40.0	500 mL
Type I Water		500 mL

Begin with 60-75% of the total volume of Type I Water in a beaker on a stir plate and add the NaOH slowly. Bring up to final volume by adding Type I Water.

STAIN EXTRACTION BUFFER

Expiration date: Twelve months from date of preparation

	MW	1 L	2 L
Tris	121.1	1.21 g	2.42 g
EDTA	372.2	3.72 g	7.44 g
NaCl	58.44	5.84 g	11.68 g
Type I Water		600 mL	1200 mL

Adjust pH to 8.0 with NaOH

20% SDS	288.3	100 mL	200 mL

Adjust to final volume with Type I Water and store at room temperature.

TNE

Expiration date: Twelve months from date of preparation

	MW	500 mL	1 L
Tris	121.1	0.605 g	1.21 g
NaCl	58.44	2.92 g	5.84 g
EDTA	372.2	0.185 g	0.37 g
Type I Water		450 mL	900 mL

Combine components and mix until completely dissolved. Bring up to final volume with Type I Water and store at room temperature.

TRIS, 1 M, pH 7.5

Expiration date: Twelve months from the date of preparation

	MW	500 mL	1 L	2 L
Tris	121.1	60.55 g	121.10 g	242.2 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 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-EDTA (TE^{-4} Buffer), 1X, pH 8.0

Expiration date: Twelve months from date of preparation

	Stock	200 mL	500 mL
10 mM Tris	1.0 M	2 mL	5 mL
1mM EDTA	0.5 M	40 µL	100 µL
Type I Water		160 mL	400 mL

Add Tris to Type I Water and adjust the pH 8.0 using 37.1% HCl. Add EDTA, recheck pH and bring to appropriate final volume. Autoclave for 20 minutes and store at room temperature.

TRIS-EDTA (TE^{-4} Buffer), 100X

Expiration date: Twelve months from date of preparation

	MW	1 L	2 L	4 L	8 L
0.1 M EDTA	372.1	37.2 g	74.4 g	148.8 g	297.6 g
1 M Tris	121.1	121.1 g	242.2 g	484.4 g	968.8 g
Type I Water		1L	2L	4L	8L

Dissolve Tris in 800 mL of Type I Water and adjust the pH to 7.5 with 37.1% HCl. Add EDTA, recheck pH and bring solution to final volume. Autoclave for 20 minutes. (It may be necessary to add 10 N NaOH to adjust pH to 7.5.)

XYLENE

Expiration date: Manufacturer's expiration date

APPENDIX B – REFERENCES

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APPENDIX C - TROUBLESHOOTING

1. General Biomek® NX^P Automation Workstation Operation

    1.1 Span-8 pipetter tool

    In order to obtain the most accurate pipetting, air bubbles must be purged from the lines. This will be performed every time the Home All Axes function is performed. Perform a visual check to ensure no large air bubbles are present in the plungers. The carboy containing the system fluid should always be at least ¼ full to prevent depletion. The system fluid is tap deionized water.

2. After a malfunction or crash requiring the DNA IQ™ NX method to be aborted, follow the Biomek® NX^P DNA IQ™ Recovery Method outlined in Appendix D.

3. If it appears one or more probes are not aligned in the z-axis which can cause pipetting errors, follow the Probe alignment procedure outlined in Appendix E.

APPENDIX D - 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.

1 Reset pod 1 to prepare for the recovery method.

NOTES: 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.

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 pipetter 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.

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).

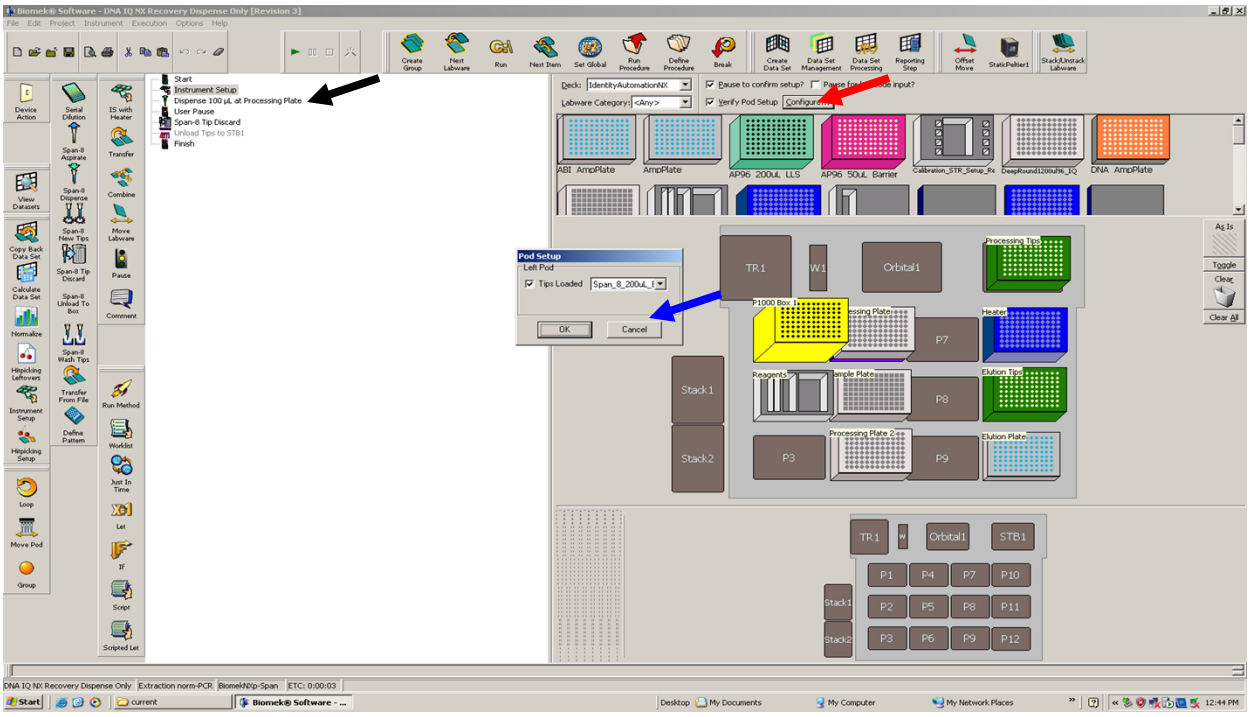

Figure 1. Shows the steps involved in selecting the tip type for the DNA IQ NX Recovery Dispense Only method.

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.

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).

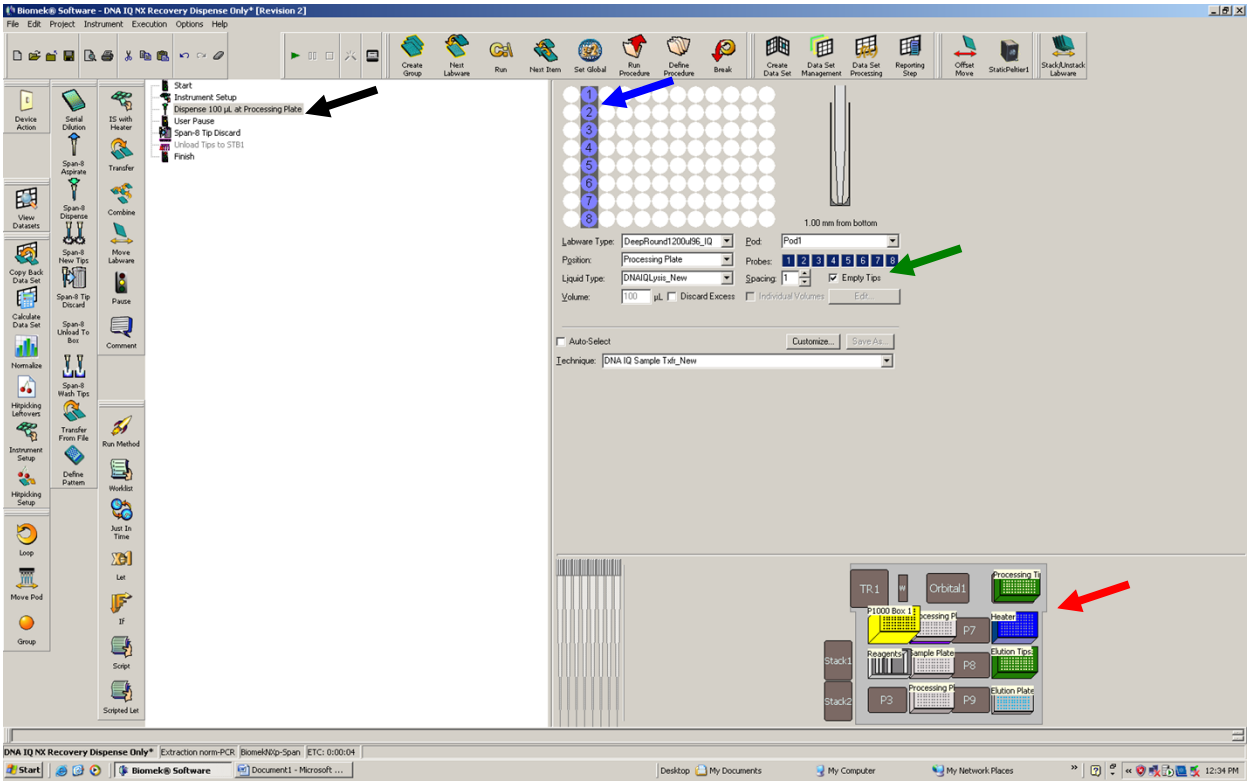

Figure 2. Shows where to designate the location, labware, and volume for the dispense step.

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).

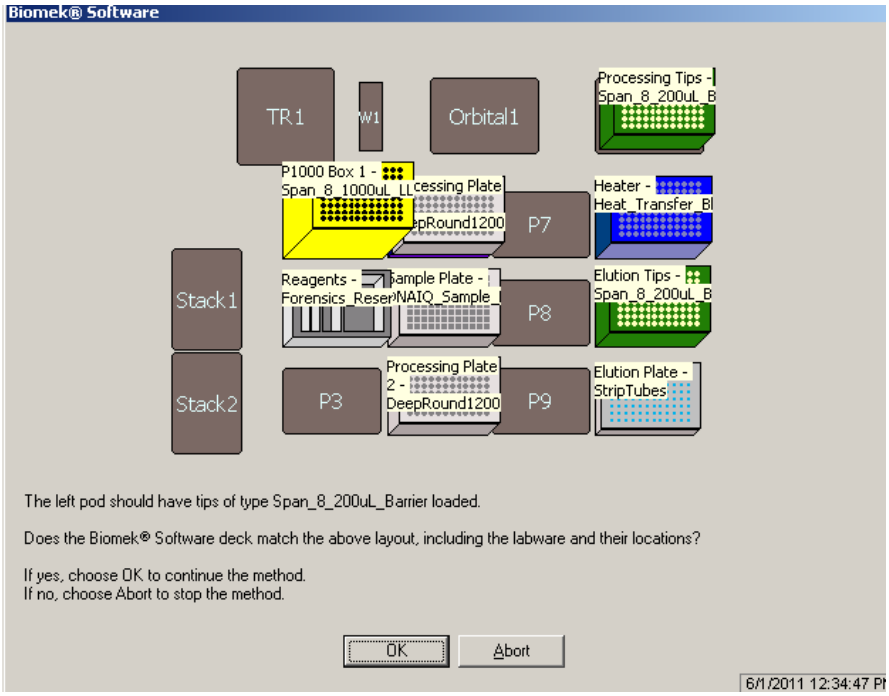

Figure 3. Deck layout for the DNA IQ NX Recovery Dispense Only method.

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

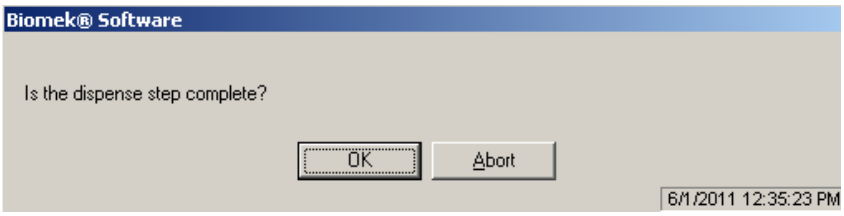

Figure 4. Dispense complete prompt.

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.

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).

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.

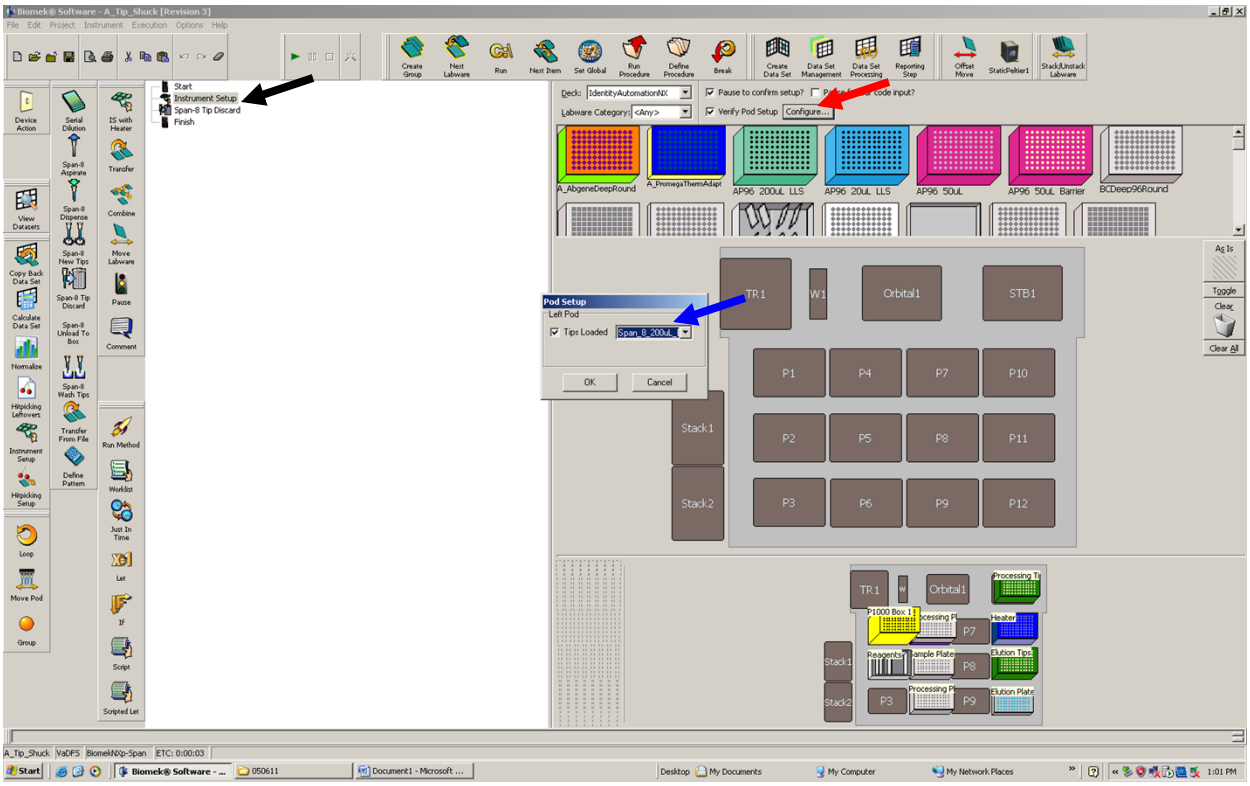

Figure 5. Shows the steps involved in selecting the tip type for the A_Tip_Shuck method.

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.

2.1 Perform the Home All Axes function following the protocol in 4.4.6.

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.

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 4.4.7), the GI Interface prompt (shown in Figure 6) will also appear.

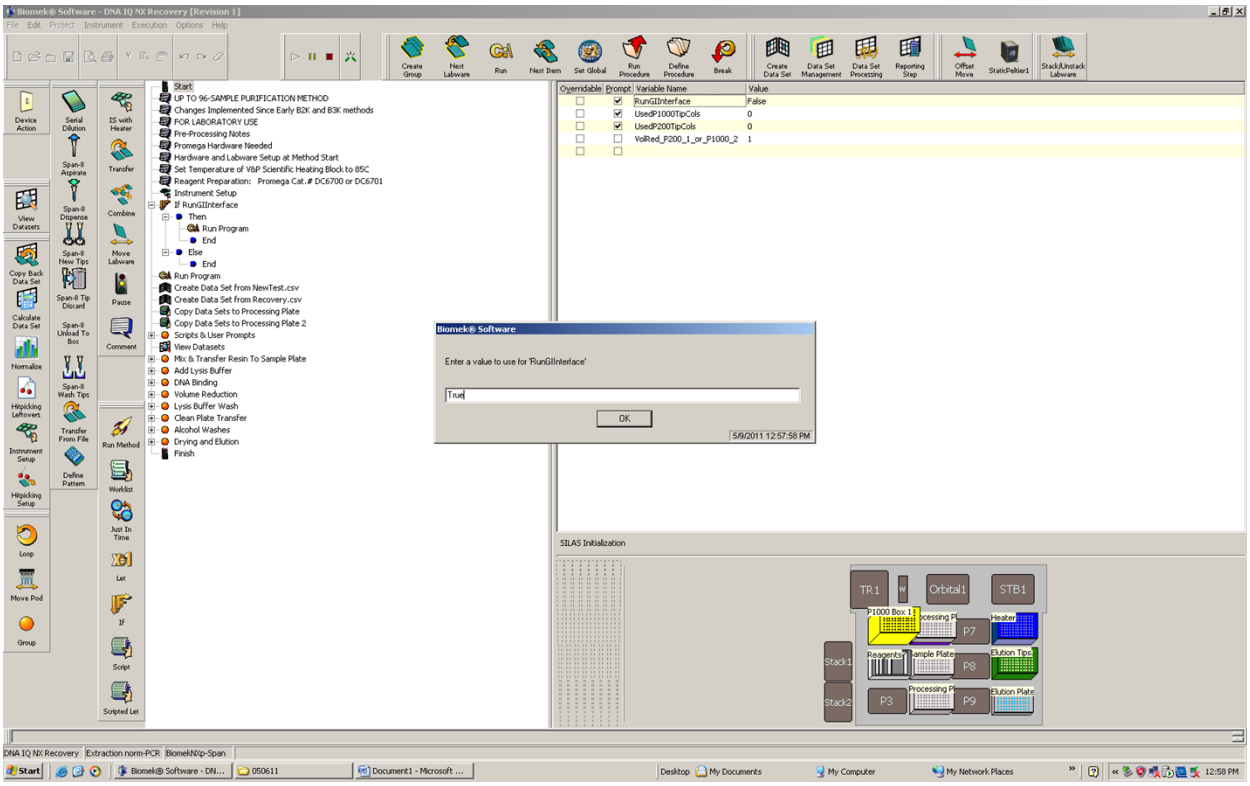

Figure 6. Prompt for running the GI Interface in the recovery method.

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® NXP 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.

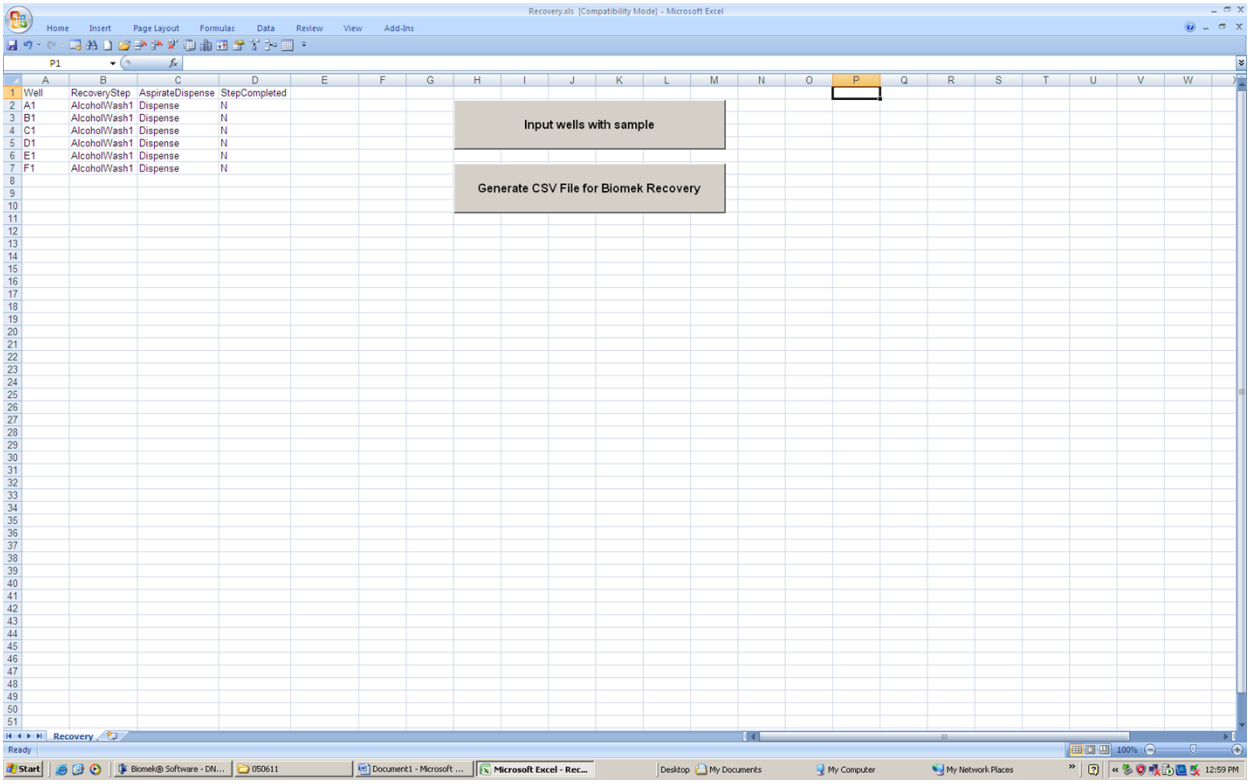

Figure 7. Recovery.xls worksheet for entering the conditions for the recovery run.

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.

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

NOTE: For conditions where there are two choices listed, either choice will work 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, the samples may be carried forward for quantitation as outlined in the Forensic Biology Procedures Manual, DNA Quantitation.

APPENDIX E – 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.

Open Probe Alignment method

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.

2 Follow the directions in 4.4.6 for Homing All Axes.

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.

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.

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

5 Open the Service project folder.

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!**

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.

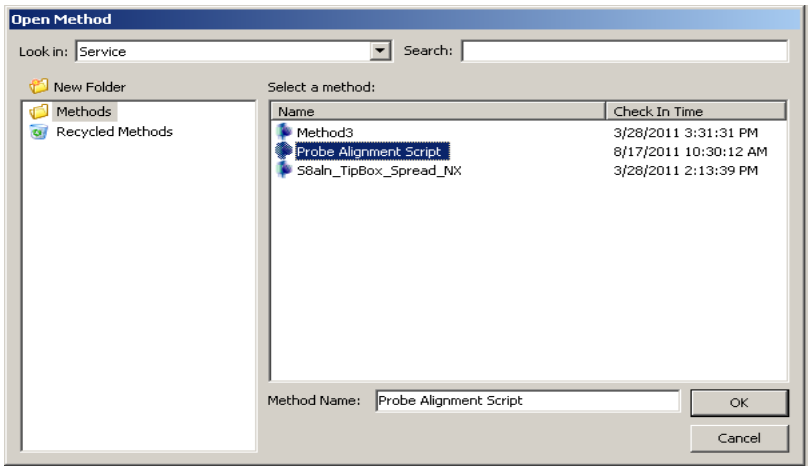

Figure 1. Opening Probe Alignment Script method.

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.

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

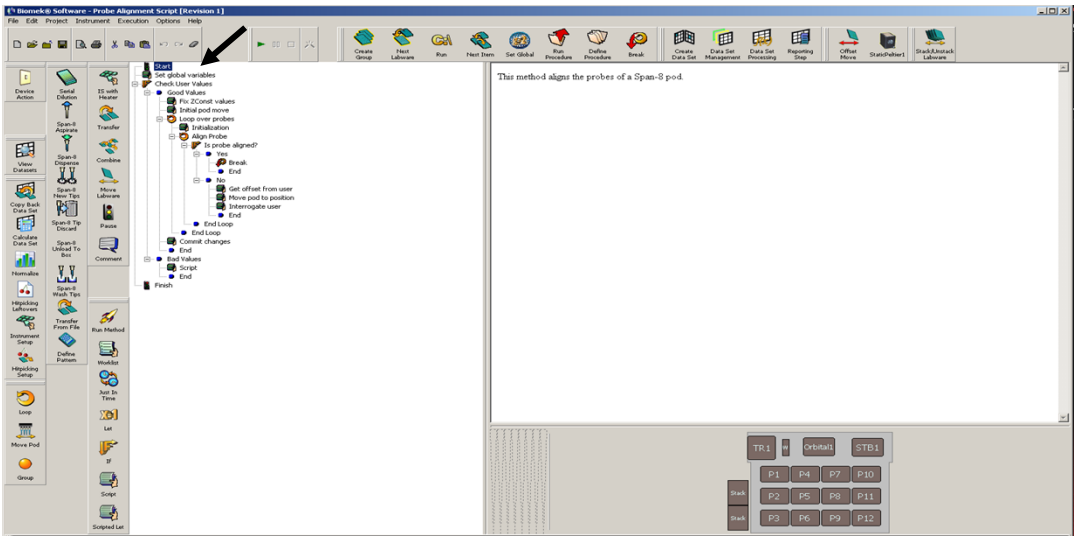

Figure 2. Initiating the Probe Alignment Script method.
Arrow points to green arrove for starting a method.

10 The following prompt will pop up.

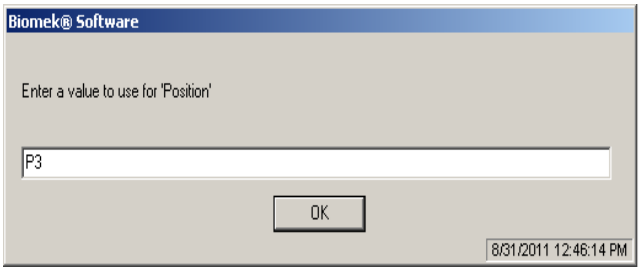

Figure 3. Positioning Pod 1 over the Magnabot.

11 Type in P3 and click OK.

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

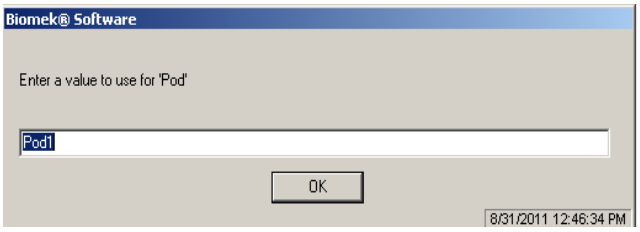

Figure 4. Selecting the Pod.

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.

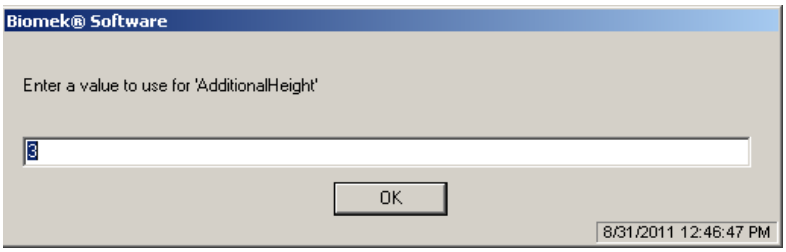

Figure 5. Pod 1 z-axis position.

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.

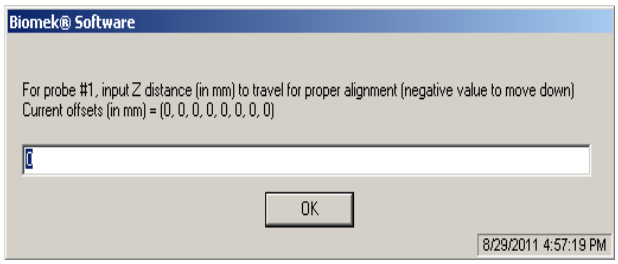

Figure 6. Moving the probe (in mm) up or down for proper alignment.

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

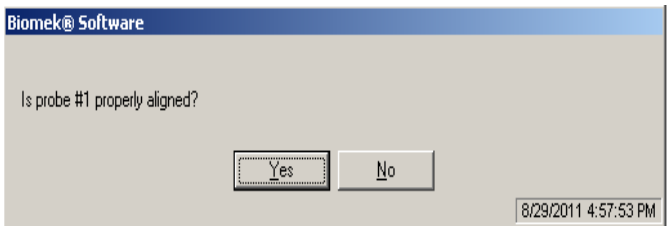

Figure 7. Determining if the probe is properly aligned.

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.

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.