

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 1 OF 18

## DNA Extraction of Bone Samples

### Bone Processing

#### A. Cleaning

1. Before extraction, a bone or tooth specimen should be cleaned entirely of soft tissue and dirt using a range of methods, such as scraping (cut glove required), rinsing and sonication. A combination of sterile scalpels, sterile toothbrushes and running water should be used to clean the specimen.
2. Once excess material is removed, use a toothbrush and water to scrub away cement and dirt-like material from bone.
3. Rinse bone with water and place in a labeled weigh boat with lint free wipes. Seal the weigh boat and place in the 56°C incubator for a minimum of 3 hours (until completely dry).
4. In comments section of exam sheet, record that cleaning was performed along with initials and date.

#### B. Consumption guidelines

Some bones will be consumed due to weight.

**For bones up to ~1.0g:** Bones will be consumed and must be documented under “*comments*” on exam sheet.

**For bones ~1.0g to 1.50g:** Consumption will be determined by the nature of the bone and whether significant weight will be lost during the processing steps. If the nature of the bone will make the weight drop below the availability to be re-tested (at least 0.50g) then the bone should be consumed and noted in “*comments*” of exam sheet.

Factors to consider: spongy, brittle, non-compact bone or where embedded cement and dirt-like material are contributing a portion of the overall weight.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 2 OF 18
------------------------------	---	-----------------

### C. Cutting/ Sonication

**Protective eyewear, lab coats, cut resistant gloves, sleeve protectors, and HEPA-filtered facial masks should be worn when cutting bone. Avoid breathing bone dust. All cutting of bone must be done under a biological hood.**

**Bones that are too small to be cut should proceed to the sonication step.**

1. Prior to sampling, document the description/appearance, weight after cleaning and measurements of the bone/tooth. Initial and date that examination/cutting was performed.
2. Prepare Tergazyme solution: fill a 50mL conical tube with 3g of Tergazyme powder and fill to the 50mL mark with dH<sub>2</sub>O. Suspend the powder with inversion and transfer to Erlenmeyer flask with stir bar. Place on heat/stir plate (use minimal heat). Solution is ready for use when reagent has completely dissolved and solution is clear.

**Once prepared, Tergazyme solution will only be effective for up to 16 hours.**

3. Using a cordless Dremel tool, cut 0.65g to 0.80g of bone in ~1/4 inch square pieces.

**0.50g of dust is optimal for large volume extraction procedure. Due to the nature of each bone, a larger portion may need to be cut to account for loss during the sonication and milling procedures (ex: spongy or brittle bone, non-compact bone and/or bone containing dirt/cement-like material). Bones that do not have enough volume for more than one extraction should be consumed, even if the total bone weight is over the 0.50g recommended for cutting.**

4. Place the bone pieces in a **new, labeled** 50mL conical tube. Label new conical tube with FB case number, PM item# and (v) initials.
5. Cover bone cuttings with 5% Tergazyme solution. Place labeled 50mL conical tubes into a tube rack. Secure tube to tube rack with tape and put tube rack into the sonicator water bath. Place weighted ring over the top of the rack to submerge and sonicate for 30-45 minutes. **Ensure water level in the sonicator is 1-2 inches from the top.**

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 3 OF 18
------------------------------	---	-----------------

6. Bones that are not being consumed should be placed in a new, labeled 50mL conical tube. Label new conical tube with FB case number, ME#, PM item #, (v) initials. (Original container should be discarded.)
7. Once sonication is complete, place a paper towel over the drain of a clean sink and decant the Tergazyme solution. Add water to the tube and gently shake. Decant water from the tube and repeat until the water runs clear and the Tergazyme solution is removed.

**Note: Some bone pieces may need to go through the sonication process twice. Repeat only when necessary. Bleach out sink when finished.**

8. Place the clean cuttings in a weigh boat on a few small lint free wipes. Cover with additional lint free wipes and another weigh boat. Label the weigh boat with the FB case number, PM item# and (v) initials. Seal weigh boats with evidence tape.
9. Dry in a 56°C incubator for a few hours or overnight. After sufficient drying, weigh bone cuttings. **The bone sample must be completely dry before milling.**

### D. Milling

**Some small bone fragments may not be suitable for milling. Consider going straight to extraction after cleaning if the fragment may not yield an attainable clump of dust after milling.**

1. Obtain mill parts and label end cap with the FB# (only use blue sharpie)
2. Weigh the dry bone pieces and record weight on exam sheet under "*weight of fragments to be milled*"
3. Transfer bone pieces to assembled mill tube containing impactor using decontaminated forceps. Cover with metal top. The top plug should be placed on to the tube with the rounded side facing out.

**Shake specimen vial and ensure that the impactor can move back and forth.**

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 4 OF 18
------------------------------	---	-----------------

4. Open freezer mill lid. Add liquid nitrogen slowly into the mill up to the FILL LINE to avoid splashing and boiling over. **Cryogloves and eye protection are required and the liquid nitrogen must be at the fill mark or damage can occur.**
5. **Programming and use of the 6870 freezer mill** (see Step 6 for programming and use of the 6750 freezer mill):

- a. Adjust mill settings as follows:

Cycle	set to # of samples + 2	
Time	T1 (milling)	2.0 min
	T2 (pause)	2.0 min
	T3 (pre-cool)	15.0 min
Rate	Bones – 8-10	
	Teeth – 6-8	

- b. Place mill tubes into the mill with four in the chamber and the remaining in the basket.
- c. Place the basket into the mill.
- d. Slowly close the mill to avoid splashing.
- e. Lock the mill shut and turn on the power switch located in the back left side of the mill.
- f. Touch the screen to prompt you to the pre-set settings from the main screen.
- g. Look over the settings; freezer mill settings should be as outlined in the table from Step 5 above. If the settings need to be changed press the settings button on the screen and make changes.
- h. Change cycle number to match total number of samples plus two ( $n + 2$ ).

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 5 OF 18
------------------------------	---	-----------------

- i. When mill has been programmed, press the start button. The screen should change and status should change from “Standby” to “Pre-cool”. Allow the mill to run the cycle. You will hear the mill running when on the “run” status. When the sound ends, the cycle is over and the mill needs to be opened and samples removed.
- j. Place the next 4 mill tubes in the mill chamber from the basket and add more liquid nitrogen to bring to full level.

**The liquid nitrogen level must be checked after each cycle and filled back to level if needed to avoid damage to mill.**

6. Programming and use of the 6750 freezer mill is the same as listed above in Step 5. The 6750 freezer mill, however, can only mill one mill tube at a time while holding two other mill tubes in the chamber.
7. Inspect each sample after removal from the mill. If sample is sufficiently pulverized, remove the metal top using the Spex Certi-Prep opening device. **Samples may be reinserted into the mill for additional grinding, if necessary.**
8. Using decontaminated tweezers, remove impactor from vial and submerge in a 4L Nalgene bucket of 10% bleach.
9. Transfer the bone dust to a tared and labeled 50mL conical tube (label conical tube with FB case number and sample name). Ensure complete dust transfer by tapping bottom of cylinder. Record the weight of the dust under “*dust weight (g).*”
10. Place remaining mill parts in the 4L Nalgene bucket of 10% bleach, all parts should be submerged.
11. Place tubes of bone dust in designated area for pending extraction.
12. When finished milling, flip mill switch off and leave mill open for liquid nitrogen to evaporate.

**Cleaning mill parts:** Mill parts must be cleaned immediately after processing.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 6 OF 18
------------------------------	---	-----------------

- a. Separate all mill parts and scrub individually with toothbrush using 10% bleach.
  - b. Rinse with water and place mill parts in a bucket containing 0.1% SDS.
  - c. Brush parts with a new toothbrush in the SDS solution.
  - d. Rinse parts with water again and place in a bucket containing 10% bleach.
  - e. Rinse all parts with water.
  - f. Separate the plastic cylinders from the metal parts.
  - g. Rinse metal parts in 200 proof ethanol. **DO NOT** rinse the plastic cylinder in ethanol as it will cause the plastic cylinder to degrade.
  - h. Expose all the parts to UV light for a minimum of 2 hours-overnight. The UV light in a biological hood or a StrataLinker can be used. All parts exposed to bone dust need to be placed face up towards the UV light. The mill tubes need to be standing up.
13. Continue to Large Volume Demineralization Extraction Procedure.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 7 OF 18

## Large Volume Demineralization Extraction Procedure with Qiagen M48 Low Elution

### I. Extraction Sample Set-up

1. Set up work area; obtain samples, conical tubes for controls, and reagents (0.5M EDTA and 20mg/mL Pro K).
2. Label two extraction negative control tubes.
3. **Have a witness confirm the order of your sample set. This will be your “Bone Incubation” witness.**
4. Add 9mL 0.5M EDTA and 200  $\mu$ L ProK to each tube.
5. Vortex thoroughly and parafilm all samples.
6. Place samples in shaker and incubate at 56°C at a speed of 124 RPM overnight. Shaker should default at these settings.

#### Programming/using the shaker:

To program the shaker use the “Select” button to highlight the fields on the right of the control panel. Once field is highlighted the up and down arrows can be used to set field to the appropriate number. Once samples are in the shaker, close the cover and select the “Start” button. Samples should begin shaking at set RPM’s. Before opening the cover to remove samples, press the “Stop” button and allow samples to come to a stop. If shaker starts to beep after opening or closing cover hit the “Select” button once. (This beep is signaling that temperature has dropped from the setting that was selected.)

### II. Clean-up

1. Remove tubes from shaker and set temperature to 60°C, speed at 124 RPM.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 8 OF 18
------------------------------	---	-----------------

2. Add 1.0mL of 1.0M KOH to each tube. Dispose of all KOH tips in the amber hazardous waste bottle labeled “potassium hydroxide”.

*Eye protection must be worn when handling 1.0M KOH. Avoid contact of reagent with metal part of pipette when aliquotting from reagent container.*

3. Vortex thoroughly and place on shaker once it has reached 60°C for 5min.
4. Vortex all samples and place in large centrifuge at 2500 RPM for 3-5min.
5. Label 10K Amicon tubes (tops and sides) the same way the extraction sample set is labeled.
6. **Have a witness confirm the order of your samples ensuring that they are correctly set up for transfer to the correctly labeled Amicon tube. This will be your “Bone Clean-up” witness.**
7. Transfer the supernatant portion of the samples to Amicons. Throw away incubation tubes in the hazardous waste trash.
8. Spin Amicons in large centrifuge at 4000-4500 RPM for an initial 45-60min. **The Eppendorf centrifuge will only reach 4000 RPM.**
9. Continue spinning until samples are at or below the 500µL mark on the Amicon tube.
10. Once under 500µL, remove the top of the Amicon tube, pull out the filter portion and drain out the liquid in the bottom of the Amicon into a sink with running water.
11. Replace the filter in the tube. Add 5mL sterile or UltraPure water to each Amicon.
12. Spin again at 4000-4500 RPM for 10-15 until sample is at or below the 500µL mark on the Amicon tube.
13. Repeat steps 10-12 one more time for a total of 2 sterile or UltraPure water washes.



# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 9 OF 18
------------------------------	---	-----------------

14. Label stratalinked M48 tubes the same way the incubation and Amicon tubes were labeled.
15. **Have a witness confirm the order sample set to ensure that the Amicon sample will be transferred to the correctly labeled M48 tube. This will be your “M48 tube set-up” witness.**
16. Using a 200µL pipette and sterile or UltraPure water, bring the volume of the sample in the Amicon tube up to 500µL.
17. Using the pipette tip, move it across the bottom of the Amicon filter to re-suspend sample with sterile or UltraPure water. Tilt the Amicon so sample collects to one side and draw up the sample, placing it into the labeled M48 tube. Throw away Amicon tubes when finished in the biohazard trash.
18. **Samples should be processed on the M48 within 48 hrs of extraction clean-up. If M48 processing cannot be done immediately after extraction, keep samples in a freezer until procedure can be done.**

### III. M48 large volume-low elution procedure

**NOTE: G2 and ProK are NOT added to the samples and the samples are not incubated. No new controls are introduced for this procedure.**

1. Open file on the M48 computer. Save this sheet by going to File→Save As and save the sheet to the “SampleName” folder on the desktop with “File Name:” in MMDDYY.HHMM format and “Save As Type:” set to CSV (Comma delimited)(\* .csv). Use the original extraction date and time.
2. Click “Save”. A window stating “*The selected file type does not support workbooks that contain multiple sheets*” will open. Click “OK”.
3. A second window asking “*Do you want to keep the workbook in this format?*” opens. Click “Yes”.
4. Open instrument program on computer and set program to “**Large volume v1.1**”.
5. Click on the “select” button and select “1.5 ml” for the size of the elution tubes

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# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 10 OF 18
------------------------------	---	------------------

6. Select the number of samples 6, 12, 18, 24, 30, 36, 42, or 48.
7. Set sample volume to 500uL
8. Set elution volume to 50uL
9. The next prompt asks to ensure the drop catcher is clean. In order to check this, click on “manual operation” and select “Drop Catcher Cleaning”. The arm of the robot will move to the front of the machine, and the drop catcher (a small plastic tray) will be right in front of you. Remove and clean with 70% ethanol. When the catcher is clean, replace the tray, close the door, and click “OK” in the window.
10. Make sure that the chute to the sharps container bin is clear for the tips to be discarded. Click “Next”.
11. The software will calculate the number of tips necessary for the run. Place tips in the tip rack(s) if necessary. **When filling racks, make sure that the pipette tips are correctly seated in the rack and flush with the robotic platform.** Tips are located in three racks. These racks may be filled one at a time, BUT you must fill a **whole rack** at a time. After a rack is filled, reset the tip rack by clicking on “Yes tip rack ...”, If no new tips are being added to the robot click “No”.

Tips needed for a run:

Samples	6	12	18	24	30	36	42	48
# Tips	30	42	54	66	78	90	102	114

12. After you are finished, click “Next”
13. Obtain stock bottles of reagents and **record lot numbers**. Fill the reagent reservoirs as stated below. All reagents are stored in their respective plastic reservoirs in the metal rack, labeled with the lot number of the reagent that they contain, and covered with Parafilm, **EXCEPT** the magnetic resin. The resin is disposed of after every extraction. Vortex the magnetic resin solution well, both in the stock bottle and in the reservoir, before adding it to the metal rack (see step 13 for preparation of MagAttract Resin). If you notice crystallization in any of the solutions, discard the solution, rinse the container out, and start again with fresh reagent.

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# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 11 OF 18

14. Remove the Parafilm and lids from the reagents, and fill the reservoirs to the appropriate level using solutions from the working solution bottles using the same lot as labeled on the reservoir. If not enough of the same lot of a solution remains, discard the remaining solution from the reservoir, rinse and re-label the reservoir with the new lot number. When filling the reservoirs **add approximately 10% extra to the volumes recommended below to account for the use of the large bore pipette tips:**

# of samples	Large reservoir Sterile or UltraPure Water (mL)	Large reservoir Ethanol (mL)	Large reservoir Buffer MW1 (mL)	Large reservoir Buffer MTL (mL)	Small reservoir Sterile or UltraPure Water (mL)	Elution buffer (TE <sup>-4</sup> ) (mL)	Small reservoir Poly A RNA - Magnetic Resin (mL)
6	10.0	11.8	7.2	5.9	3.5	1.6	1.5
12	18.4	22.6	12.9	10.3	5.9	1.9	1.7
18	26.9	33.4	18.6	14.7	8.4	2.2	1.9
24	35.3	44.2	24.3	19.0	10.8	2.5	2.1
30	43.7	55.0	30.0	23.4	13.3	2.8	2.3
36	52.2	65.8	35.7	27.8	15.7	3.1	2.5
42	60.6	76.6	41.4	32.1	18.2	3.4	2.7
48	69.0	87.4	47.0	36.5	20.6	3.7	2.9

**Note:** Bottles of MW1 require the addition of ethanol prior to use. See bottle for confirmation of ethanol addition and instructions for preparation if needed.

15. Follow software instructions to prepare reagent rack. Software will indicate the reagent, reagent position in the rack and amount of reagent to use.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 12 OF 18

Refer to the table below for amounts of 1000ng/uL Poly A RNA stock solution to add for resin preparation:

Samples	Volume of 1000ng/uL stock PolyA RNA solution added to resin (uL)	Volume of Untreated MagAttract Resin (uL)	Total Volume of RNA Treated MagAttract Resin (uL)
6 samples	<b>4.4</b>	<b><u>1497.8</u></b>	1502.2
12 samples	<b>5.0</b>	<b><u>1697.5</u></b>	1702.5
18 samples	<b>5.6</b>	<b><u>1897.2</u></b>	1902.8
24 samples	<b>6.2</b>	<b><u>2096.9</u></b>	2103.1
30 samples	<b>6.8</b>	<b><u>2296.6</u></b>	2303.4
36 samples	<b>7.4</b>	<b><u>2496.3</u></b>	2503.7
42 samples	<b>7.9</b>	<b><u>2696.0</u></b>	2703.9
48 samples	<b>8.5</b>	<b><u>2895.7</u></b>	2904.2

The pretreated resin may be prepared in a 15mL conical tube and then added to the appropriate reservoir for addition to the platform in the amount dictated by the protocol. Vortex the magnetic resin in the container before pipetting into M48 reagent container and vortex the M48 reagent container once the PolyA dilution has been added.

Place reservoirs into the metal rack in the following locations. The plastic reservoirs only fit into the rack one way. Check the directions of the notches which should point **into** the robot:

Size Reservoir	Rack Position	Software Tag	Reagent
Large reservoir	L4	Rea_4	Sterile or UltraPure Water
Large reservoir	L3	Rea_3	Ethanol (100%)
Large reservoir	L2	Rea_2	Wash Buffer 1 (Buffer MW1)
Large reservoir	L1	Rea_1	Lysis and Binding Buffer (Buffer MTL)
Small reservoir	S6	ReaS6	(empty)
Small reservoir	S5	ReaS5	(empty)

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# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 13 OF 18
------------------------------	---	------------------

Size Reservoir	Rack Position	Software Tag	Reagent
Small reservoir	S4	ReaS4	(empty)
Small reservoir	S3	ReaS3	Sterile or UltraPure Water
Small reservoir	S2	ReaS2	Elution Buffer (TE <sup>-4</sup> )
Small reservoir	S1	ReaS1	Magnetic Particle Resin

16. Flip up the “container interlocks” and place the metal reservoir holder onto the left side of the robotic platform in the proper position. **DO NOT force the holder into place and be careful not to hit the robotic arm.** After correctly seating the metal holder, flip down the “container interlocks” and press “next”.
17. Click “Next” when you are prompted to write a memo.
18. Place the sample preparation trays on the robot. One tray for every 6 samples. Click “Next”.
19. Place empty, unlabeled 1.5mL elution tubes in the 65 degree (back) hot block, located on the right side of the robotic platform. Make sure tubes are in places for any blank samples. Click “Next”.
20. Print labels for 1.5 mL screw top tubes for final sample collection in the robot.
21. Place **labeled**, empty 1.5 mL sample collection tubes in the 8 degree (front) cold block for collection of final samples. Make sure tubes are in place for any blank samples.
22. **Make PolyA dilution (add 30  $\mu$ L of PolyA to 90  $\mu$ L of UltraPure water) and add 2.5  $\mu$ L of PolyA dilution to each M48 sample tube.**
23. Vortex samples and centrifuge if needed. Do not obtain a pellet in M48 tube from over centrifuging. If pellet occurs, vortex slightly to re-suspend before placing sample on M48 instrument.
24. Fill “Blanks” with 500uL sterile or UltraPure H<sub>2</sub>O.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 14 OF 18
------------------------------	---	------------------

25. Make sure all of the lot numbers are recorded. **When they are ready, have a witness confirm the order and labels of both the sample tubes and the labeled 1.5 mL final sample collection tubes. The robot setup witness should also verify that all plastic ware is in the correct position and correctly seated in the platform. This will be your “Robot Setup” witness.**
26. Click “Yes” when asked to input sample names.

#### IV. Importing Sample Names

1. At the sample input page, click “Import”.
2. The Open window will appear. “*Look in:*” should automatically be set to a default of “*SampleName*”. If not, the correct pathway to the folder is My Computer\C:\Program Files\GenoM-48\Export\SampleName. (The SampleName folder on the desktop is a shortcut to this file.)
3. Select your sample name file and click “Open”. Verify that your sample names have imported correctly. Do not be concerned if a long sample name is not completely displayed in the small window available for each sample.
4. Manually type in the word “Blank” for all empty white fields.
5. Click “Next”.

#### V. Verifying Robot Set-Up and Starting the Purification

1. In addition to confirming the *position* of all plasticware and samples, check the following conditions before proceeding:

All plasticware (tips, sample plates, tubes) is seated properly in the robotic platform	<input type="checkbox"/>
Metal reservoir rack is seated properly, UNDER the interlocks	<input type="checkbox"/>
Interlocks are down	<input type="checkbox"/>
Sample tubes, elution tubes and sample collection tubes have been added to the platform in multiples of 6 as follows:	

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 15 OF 18

Empty 1.5 mL tubes are filling empty positions for both sets of elution tubes in the cold and hot blocks	<input type="checkbox"/>
2.0 mL sample tubes filled with 500uL of sterile or UltraPure H2O are in empty positions of the sample rack	<input type="checkbox"/>

2. After confirming the position and set-up of the plastic ware click “Confirm”.
3. Click “OK” after closing the door.
4. Click “Go” to start the extraction. Check that the bag attached to the waste chute is open and clear.
5. The screen will display the start time, remaining time, and the completion time.
6. Monitor the extraction until the transfer of DNA sample from the sample tubes to the first row of sample plate wells to ensure proper mixing of magnetic resin and DNA sample.
7. At the end of the extraction, a results page will be displayed indicating the pass/fail status of each set of six samples.

## VI. Saving Extraction Report Page

1. At the end of the extraction, a results page will be displayed indicating the pass/fail status of each set of six samples. **DO NOT** click “Next” until you have exported the results. To export results, click on the “Export” button. The Save As window will appear. “Save In:” should be set to the “Report” folder on the desktop. This is a shortcut to the following larger pathway: My Computer\C:\Program Files\GenoM-48\Export\Report.
2. In “File Name:”, name the report in the format, MMDDYY.HHMM. Set “Save As Type:” to Result Files (\*.csv). Use the original extraction data and time. For instance, an extraction performed at 4:30pm on 5/14/06 would be saved as 051406.1630.csv.
3. Click “Save”.

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

## DNA EXTRACTION OF BONE SAMPLES

DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 16 OF 18
------------------------------	---	------------------

4. Drag a copy of the result file into the appropriate LIMS SHARE folder.
5. Proceed with clean-up and sterilization.

### VII. Post-Extraction Clean Up and UV Sterilization

1. Remove samples (from the 8 degree (front) cold block) from the robotic platform and cap with newly labeled screw caps.
2. Wipe down the robotic platform and waste chute with Ethanol. **DO NOT USE SPRAY BOTTLES.** Discard used pipette tips, sample tubes, and sample preparation plate(s).
3. Replace the lid on the magnetic resin reservoir and vortex remaining resin thoroughly. Discard the Magnetic resin immediately with a 1000uL pipettman. Rinse the reagent container with de-ionized water followed by ethanol and store to dry.
4. Cover all other reagents and seal with Parafilm for storage. **MAKE SURE RESERVOIRS ARE LABELED WITH THE LOT NUMBER OF THE REAGENT THEY CONTAIN and that the lot numbers have been recorded.**
5. Replace tips on the instrument that were used during run. There are three racks, and all racks should be full. Ensure that the pipette tips are correctly seated in the rack and flush with the robotic platform.
6. Click "Next".
7. When prompted, "Do you want to perform a UV sterilization of the worktable?", click "Yes".
8. Select 1 Hour for the time of "UV sterilization" then click "yes" to close the software upon completion.
9. Store the extracts at 2 to 8°C or frozen.
10. In the LIMS system, navigate to the Data Entry page, assign the samples to a storage unit (cryobox), and import instrument
11. Submit samples at neat and/or 1/100 dilutions, as needed for real-time PCR analysis to determine human DNA concentration (refer to the STR manual).
12. **COMPLETE THE M48 USAGE LOG WITH THE PURPOSE, PROGRAM, PLATE, AND ANY COMMENTS ARISING FROM THE RUN.**

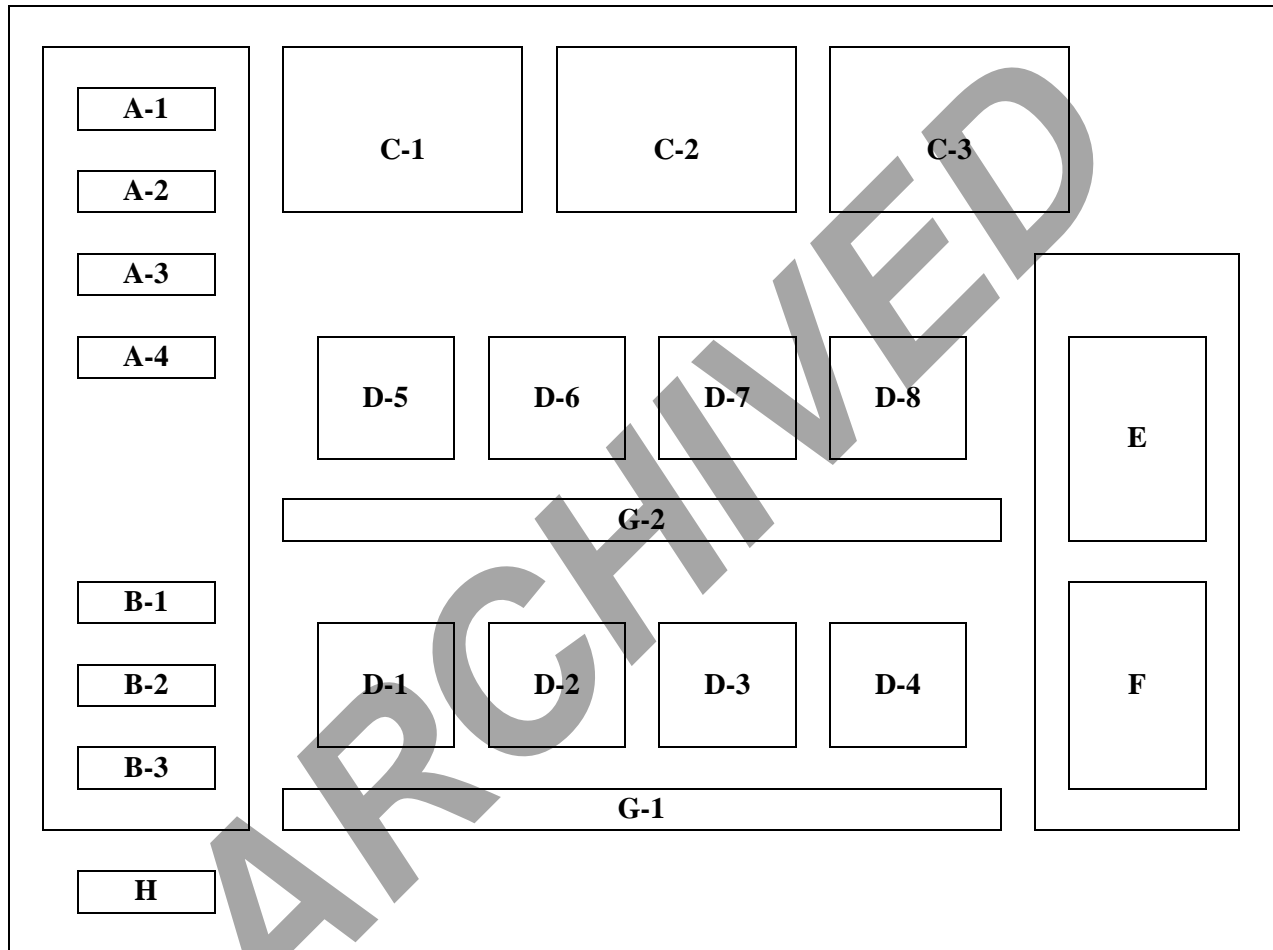
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# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 17 OF 18

## VIII. BioRobot M48 Platform Diagram



**Figure 1.** Diagram of Robotic Platform of the QIAGEN BioRobot M48.

- A (1-4) Large Reagent Reservoir Positions
- B (1-3) Small Reagent Reservoir Positions
- C (1-3) Tube Racks
- D (1-8) Sample Plate Holders
- E Hot Elution Block (65°C)
- F Cold Final Elution Block (8°C)
- G (1-2) Sample Tube Racks
- H Waste Disposal Chute

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DNA EXTRACTION OF BONE SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 18 OF 18

## IX. Troubleshooting

Error	Cause/Remedy
Resin/sample is being drawn up into pipette tips unequally	<b>Report problem to QA.</b> Resin buffer has evaporated. O-rings are leaking and need service.
Crystallization around 1 <sup>st</sup> row of wells in sample plate	Forgot to fill empty sample tubes with 500uL of sterile or UltraPure H <sub>2</sub> O
BioRobot M48 cannot be switched on	BioRobot M48 is not receiving power. Check that the power cord is connected to the workstation and to the wall
Computer cannot be switched on	Computer is not receiving power. Check that the power cord is connected to the computer and to the wall power outlet.
BioRobot M48 shows no movement when a protocol is started	BioRobot M48 is not switched on. Check that the BioRobot M48 is switched on.
BioRobot M48 shows abnormal movement when a protocol is started	The pipettor head may have lost its home position. In the QIAsoft M software, select “ <u>M</u> anual Operation/ Home”.
Aspirated liquid drips from disposable tips.	Dripping is acceptable when ethanol is being handled. For other liquids: air is leaking from the syringe pump. <b>Report problem to QA.</b> O-rings require replacement or greasing. If the problem persists, contact QIAGEN Technical Services