REDUCED VOLUME MAGATTRACT DNA EXTRACTION FROM BLOODSTAINS AND OTHER CASEWORK SAMPLES		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 1 OF 15

Reduced Volume Magattract DNA Extraction from Bloodstains & Other Casework Samples

CAUTION: DO NOT ADD BLEACH OR ACIDIC SOLUTIONS DIRECTLY TO THE SAMPLE-PREPARATION WASTE. Buffers MW1 and MTL contain guanidine hydrochloride/ guanidine thiocyanate which can form highly reactive compounds when combined with bleach. If liquid containing these buffers spill, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean with suitable laboratory detergent and water first and then with 1% sodium hypochlorite followed by water.

Sample size for the extraction should be approximately 1/3 of a swab or a 3x3 mm cutting of the stain**. This extraction is applicable for all casework samples EXCEPT semen samples.**

All bloodstain cuttings should be placed in 2.0mL screw cap sample tubes.

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A. Setting up M48 Test Batch and Saving Sample Name List

1. Open file on the M48 computer. Save this document by going to File → Save As and save the document to the "SampleName" folder on the desktop with "File Name" in MMDDYY.HHMM format and the "Save As Type" set to CSV (comma delimited)(*.csv).

2. Click "Save".

3. A window stating "The selected file type does not support workbooks that contain multiple sheets" will open. Click "OK".

4. A second window asking "Do you want to keep the workbook in this format?" opens. Click "Yes".

5. Close the Excel Worksheet.

B. Sample Preparation and Incubation

1. Remove the extraction rack from the refrigerator. Extract either evidence or exemplars. Do not extract both together.

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2. Sample preparation should be performed under a hood.

3. Obtain two empty 2.0 mL screw top sample tubes for the extraction negatives and manually label one as Extraction Negative 1 and the other as Extraction Negative 2.

4. **Have a witness verify your samples by reading the tube-top label and the entire input sample ID number for each sample. This will be your "Extraction" witness.**

5. For large runs, prepare master mix for N+2 samples as follows, vortex briefly, and add 200uL to each of the tubes in the extraction rack and the pre-prepared extraction negative tubes. For smaller runs, you may add Proteinase K and G2 Buffer to each tube individually:

Reagent	**1 sample**	**6 samples**	**12 samples**	**18 samples**	**24 samples**
Digestion Buffer (Buffer G2)	190 µL	1520 µL	2660 µL	3800 µL	4940 µL
QIAgen Proteinase K	10 µL	80 µL	140 µL	200 µL	260 µL

NOTE: If Buffer does not cover the substrate (such as those from a scraping), an extra 200 µL of buffer may be added to the tube once. If this is the case, the sample will be split and the sample name will have to be changed. The imported sample names on the instrument must also be updated.

6. Shake at 1000 rpm at 56°C for a minimum of 30 minutes. Record the thermomixer temperature.

C. BioRobot M48 Software and Platform Set-Up

1. Double click on the "BioRobot M48" icon on the desktop.

2. Click the "Start" button. **Note: The door and container interlock must be closed to proceed.**

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3. "Trace TD v1.1C1" protocol should be selected for casework samples. If not selected, click on the arrow in the middle of the screen and then select "Forensic" □ "gDNA" □ and "Trace TD v1.1C1"

4. Click on the "select" button and select "1.5 mL" for the size of the elution tubes.

5. Select the number of samples: 6, 12, 18, 24, 30, 36, 42, or 48.

6. Set sample volume to 200 µL (cannot and should not change).

7. Set elution volume to 50 µL.

8. 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 ethanol. When the catcher is clean, replace the tray, close the door, and click "OK" in the window.

9. Confirm that there is a means of collection for the tips that will be discarded during the run. Click "Next".

10. The next prompt has software that calculates the number of tips necessary for the run and asks, "Do you want to reset any of the tip racks?" Click "Yes tip rack ..." for all tip racks and ensure that the tips were actually replaced and that **the pipette tips are correctly seated in the rack and flush with the robotic platform**. If no tip racks need to be reset, 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

    After you are finished, click "Next"

11. Obtain stock bottles of reagents and **record lot numbers**. Fill the reagent reservoirs as

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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.

12. 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, a**dd approximately 10% to the volumes recommended below to account for the use of the large bore pipette tips.**

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# 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^{-4}) (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.

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13. 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	**4.4**	**1497.8**	1502.2
12 samples	**5.0**	**1697.5**	1702.5
18 samples	**5.6**	**1897.2**	1902.8
24 samples	**6.2**	**2096.9**	2103.1
30 samples	**6.8**	**2296.6**	2303.4
36 samples	**7.4**	**2496.3**	2503.7
42 samples	**7.9**	**2696.0**	2703.9
48 samples	**8.5**	**2895.7**	2904.2

14. The treated resin may be prepared directly in the reservoir or in a 15mL conical tube and then added to the appropriate reservoir for addition to the platform in the amount dictated by the protocol.

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)

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Size Reservoir	Rack Position	Software Tag	Reagent
Small reservoir	S5	ReaS5	(empty)
Small reservoir	S4	ReaS4	(empty)
Small reservoir	S3	ReaS3	Sterilie or UltraPure Water
Small reservoir	S2	ReaS2	Elution Buffer (TE^{-4})
Small reservoir	S1	ReaS1	Magnetic Particle Resin

15. 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".

16. Click "Next" when you are prompted to write a memo.

17. Place the sample preparation trays on the robot. One tray for every 6 samples. Click "Next".

18. Place empty, unlabeled 1.5mL elution tubes in the 65 degree (back) hot block, located on the right side of the robotic platform. Click "Next".

19. Print labels for 1.5 mL screw top tubes for final sample collection in the robot.

20. If an extra 200 µL of buffer was added to a tube to cover the substrate, that tube must be split into two separate tubes at this point.

    To do so, remove 200 µL from the original tube and place into a new tube. The original tube is renamed by adding an "a" to the end (e.g., "SampleName**a**", "SampleName**_a**", etc.); the new tube is named with the original sample name with a "b" at the end (e.g., "SampleName**b**", "SampleName**_b**", etc.). The tubes should remain adjacent to each other and the sample positions may need to be shifted to accommodate.

21. Prepare a dilution of Poly A RNA: Add 15 µL of stock (1000 ng/uL) Poly A RNA to 45 µL of UltraPure water for a final concentration of 250 ng/uL.

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22. When the samples have finished the 56° incubation, spin them down briefly and add 1 µL of the diluted Poly A RNA solution to each sample.

NOTE: For cigarette butts, if the sample submitted is a strip of the filter paper, the lysate must be transferred to a new labeled 2.0mL screw cap tube prior to adding the Poly A RNA. Discard the cigarette strip. This is important to avoid the clogging of the M48 tips.

23. Spin all tubes in a microcentrifuge for 1 minute at 10,000 to 15,000 x g.

24. **For empty positions, add a 2.0 mL sample tube filled with 200 µL of sterile or UltraPure water.**

25. Click "Yes" when asked to input sample names.

D. 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".

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E. 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	☐
Metal reservoir rack is seated properly, UNDER the interlocks	☐
Interlocks are down	☐
Sample tubes, elution tubes and sample collection tubes have been added to the platform in multiples of 6 as follows:	
Empty 1.5 mL tubes are filling empty positions for both sets of elution tubes in the cold and hot blocks	☐
2.0 mL sample tubes filled with 200uL of sterile or UltraPure H2O are in empty positions of the sample rack	☐

2. **Have a witness confirm the order and labels of the samples by reading the tube-tops for the input samples and for the output samples by reading the tube-top label and the entire output sample ID number for each sample. The analyst should be loading the samples on to the robot as they are reading the samples to the witness. The robot setup witness should also verify that all plasticware is in the correct position and correctly seated in the platform. This will be your "Robot Setup" witness.**

3. After confirming the position and set-up of the plasticware click "Confirm".

4. Click "OK" after closing the door.

5. Click "Go" to start the extraction.

6. The screen will display the start time, remaining time, and the completion time.

7. 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.

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8. At the end of the extraction, a results page will be displayed indicating the pass/fail status of each set of six samples.

F. Saving Extraction Report Page

1. At the results page click the "Export" button at the bottom center of the screen. 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). For instance, an extraction performed at 4:30pm on 5/14/06 would be saved as 051406.1630.csv.

3. Click "Save".

4. Drag a copy of the result file into the appropriate LIMS SHARE folder.

5. Proceed with clean-up and sterilization.

G. Post-Extraction

1. Remove samples (from the 8 degree (front) cold block) from the robotic platform and cap with newly labeled screw caps.

1. Samples can be immediately purified and concentrated if needed. See section J.

H. Clean Up and UV Sterilization

1. 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).

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2. Replace the lid on the magnetic resin reservoir and vortex remaining resin thoroughly. Discard the Magnetic resin immediately with a 1000uL pipetteman. Rinse the reagent container with de-ionized water followed by ethanol and store to dry.

3. 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.**

4. 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.

5. Click "Next".

6. When prompted, "Do you want to perform a UV sterilization of the worktable?", click "Yes". **THE UV STERILIZATION MUST BE PERFORMED FOR AT LEAST 15 MINUTES BETWEEN RUNS.** The UV light can be manually turned off.

7. Select 1 Hour for the time of "UV sterilization" then click "yes" to close the software upon completion.

8. Store the extracts at 2 to 8°C or frozen.

9. In the LIMS system, navigate to the Data Entry page, assign the samples to a storage unit (cryobox), and import instrument data.

10. Submit samples at 1/10 and/or 1/100 dilutions, as needed for real-time PCR analysis to determine human DNA concentration.

11. **COMPLETE THE M48 USAGE LOG WITH THE PURPOSE, PROGRAM, PLATE, AND ANY COMMENTS ARISING FROM THE RUN.**

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I. BioRobot M48 Platform Diagram

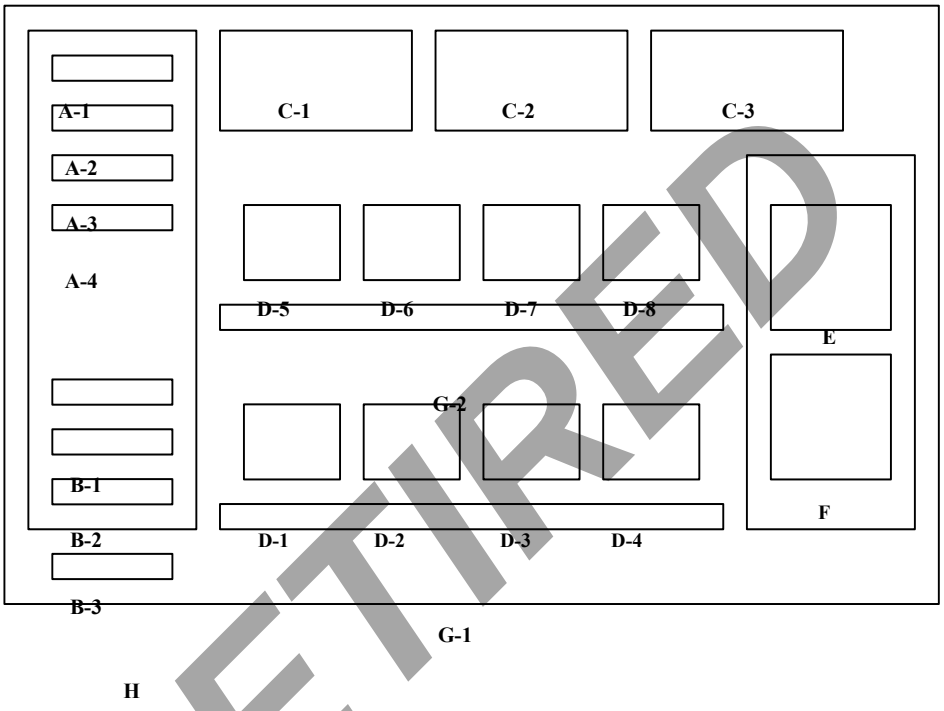

Figure 1. Diagram of Robotic Platform of the QIAGEN BioRobot M48.

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

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J. Purification and Concentration

1. Prepare Microcon® DNA Fast Flow tubes and label the membrane tube and filtrate tube cap.

2. **Witness step: Confirm the sample names and order on the documentation by reading the tube-top label and complete INPUT sample ID, also read the tube-top label and complete OUTPUT sample ID for each sample.**

3. Pre-coat the Microcon® membrane with Fish Sperm DNA in an irradiated microcentrifuge tube or 15 mL tube:

    a. Fish Sperm DNA Preparation

        i. Add 1 µL of stock Fish Sperm DNA solution (1mg/mL) to 199uL of water for each sample on the test batch.

        ii. Aliquot 200 µL of this Fish Sperm DNA solution to each Microcon® tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the documentation for calculated value.

Reagent	1 sample
Water	199 µL
Fish Sperm DNA (1mg/mL)	1µL

4. Filtration

    a. Add the entirety of each extract to its pretreated Microcon® membrane. If this is a purification/concentration assay of a sample, raise the sample volume to 200µL with dH2O. The sample tubes may be discarded.

    Centrifuge the Microcon® tube at 2400 rpm for 12 minutes. An additional 3 minutes may be required to ensure that all the liquid is filtered. However, do not centrifuge too long such that the membrane is dry. If the filtrate does not appear to be moving through the membrane, elute the filtrate and continue centrifuging the eluant into a fresh microcon with a pretreated membrane.

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If indicated on the evidence examination schedule or by a supervisor, or if the filtrate is not clear, perform a second wash step applying 400 µL of water onto the membrane and centrifuging again at 2400 rpm for 12 minutes or until the all the liquid is filtered. However, do not centrifuge to dryness. This process may be repeated, as necessary. Document the additional washes.

b. Visually inspect each Microcon® membrane tube. If it appears that more than 5 µL remains above the membrane, centrifuge that tube for 3 more minutes at 2400 rpm.

5. Elution

a. Open only one Microcon® tube and its fresh collection tube at a time.

b. Add 25 µL 0.1X TE to the Microcon® and invert the Microcon® over the new collection tube. Avoid touching the membrane.

c. Centrifuge at 3400 rpm for 3 minutes.

d. Transfer the eluant to an irradiated and labeled 1.5 mL tube. Measure and record the approximate volume in LIMS. The total volume should not exceed 30 µL and should not be less than 25 µL. Adjust the final volume to 25 µL using 0.1X TE (if less). Discard the Microcon® membrane.

e. If the eluant appears to be a dark color or is not clear, it may be necessary to purify the sample again. Prepare a fresh Microcon® tube and repeat steps 4-5.

f. Store the extracts at 2 to 8°C or frozen.

g. In the LIMS system, navigate to the Data Entry page, assign the samples to a storage unit (cryobox), and indicate which samples are completed.

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K. Troubleshooting

Error	Cause/ Remedy
Resin/sample is being drawn up into pipette tips unequally	**Report problem to QA.** Resin buffer has evaporated. O-rings are leaking and need service.
Crystallization around 1st row of wells in sample plate	Forgot to fill empty sample tubes with 200uL of sterile or UltraPure H_20
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 "Manual 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. **Report problem to QA.** O-rings require replacement or greasing. If the problem persists, contact QIAGEN Technical Services

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