MagAttract DNA Extraction from Bloodstains and Exemplars

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 not applicable to cigarette butts.**

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

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.

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

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.**

3. "F Trace MTL" protocol should be selected. If not, click on the arrow in the middle of the screen and then select "New Dev" □ "gDNA" □ and "F Trace MTL".

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 200 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 70% ethanol. When the catcher is clean, replace the tray, close the door, and click "OK" in the window.

9. Make sure that the chute to the sharps container bin is clear for the tips to be discarded. 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 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 stored between runs in its original stock bottle to prevent evaporation. Vortex the magnetic resin solution well, both in the stock bottle and in the reservoir, before adding it to the metal rack. If you notice crystallization in any of the solutions, discard the solution, rinse the container out with distilled water, 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 **add approximately 10% to the volumes recommended below to account for the use of the large bore pipette tips:**

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.

# of samples	Large reservoir Sterilize or UltraPure Water (mL)	Large reservoir Ethanol (mL)	Large reservoir Buffer MW1 (mL)	Large reservoir Buffer MTL (mL)	Small reservoir Buffer MW2 (mL)	Elution buffer (TE^{-4}) (mL)	Small reservoir Magnetic Resin (mL)
6	10.0	11.8	7.2	5.9	3.5	2.5	1.5
12	18.4	22.6	12.9	10.3	5.9	3.7	1.7
18	26.9	33.4	18.6	14.7	8.4	4.9	1.9
24	35.3	44.2	24.3	19.0	10.8	6.1	2.1
30	43.7	55.0	30.0	23.4	13.3	7.3	2.3
36	52.2	65.8	35.7	27.8	15.7	8.5	2.5
42	60.6	76.6	41.4	32.1	18.2	9.7	2.7
48	69.0	87.4	47.0	36.5	20.6	10.9	2.9

Place each reservoir 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)
Small **reservoir**	S4	ReaS4	(empty)
Small **reservoir**	S3	ReaS3	Wash Buffer 2 (Buffer MW2)
Small **reservoir**	S2	ReaS2	Elution Buffer (TE^{-4})
Small **reservoir**	S1	ReaS1	Magnetic Particle Resin

13. 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".

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

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

16. 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".

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

18. Place **labeled**, empty 1.5 mL sample collection tubes in the 8 degree (front) cold block for collection of final samples.

19. At this point, the samples should be near the end of the incubation period (From Section B, Step 6). Spin all tubes in a microcentrifuge for 1 minute at 10,000 to 15,000 x g.

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

21. 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".

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 200 µL of sterile or UltraPure H_2O 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.

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 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. Discard used pipette tips, sample tubes, and sample preparation plate(s). Remove reservoir rack.

3. Replace the lid on the magnetic resin reservoir and vortex remaining resin thoroughly. Transfer the Magnetic resin to the stock bottle immediately with a 1000uL pipette. 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. Wipe down the robotic platform and waste chute with 70% ethanol. **DO NOT USE**

SPRAY BOTTLES.

6. 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.

7. Click "Next".

8. When prompted, "Do you want to perform a UV sterilization of the worktable?", click "Yes".

9. Select 1 Hour for the time of "UV sterilization" then click "yes" to close the software upon completion. **THE UV STERILIZATION MUST BE PERFORMED FOR AT LEAST 15 MINUTES BETWEEN RUNS.** The UV light can be manually turned off.

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

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

12. As needed, pipette aliquots of neat and/or diluted extract into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration (refer to Section 4 of the STR manual).

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

H. BioRobot M48 Platform Diagram

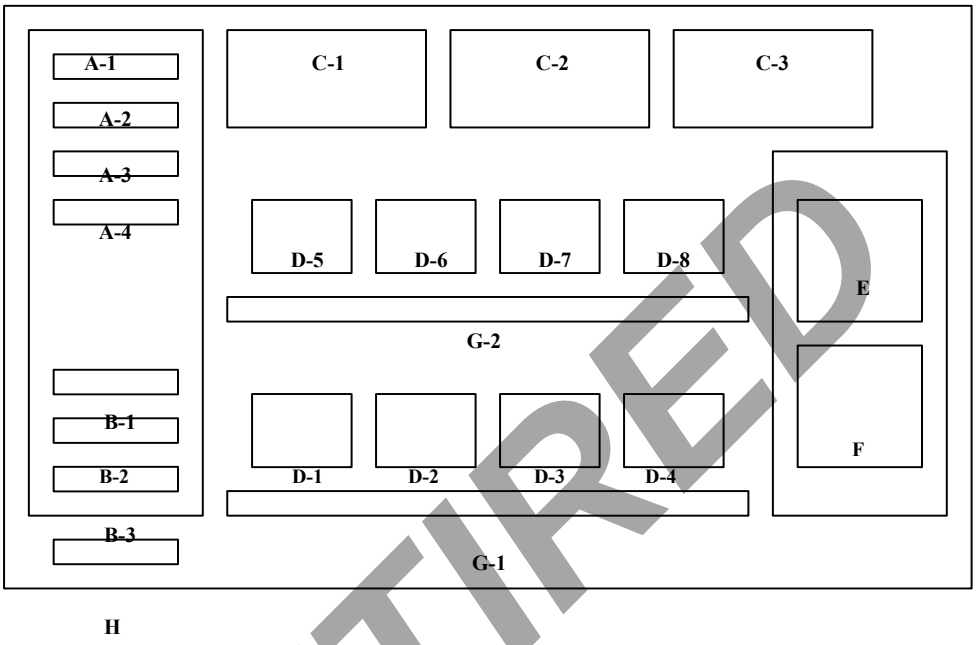

Figure 1. Diagram of Robotic Platform of the QIAGEN BioRobot M48.

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

I. 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