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SHORT PROTOCOL No. 43

Automated gDNA Purification from Forensic Samples with Qiagen® MagAttract® DNA Mini M48 Kit on ep*Motion*® M5073 and 5075m

Introduction

This protocol describes the configuration and pre-programmed methods for automated genomic DNA purification from forensic samples using Qiagen MagAttract DNA Mini M48 Kit. Setup for two platforms, ep*Motion* M5073 and 5075m, are described for smaller number of samples (in tube format), and for larger number of samples (in plate format) respectively. The protocol applies for general forensic samples, additional hints for difficult samples for better extraction yield are provided. Samples are pretreated with lysis buffer and proteinase K manually, followed by automated purification protocol to process 200 µL lysed sample. Automated DNA purification by ep*Motion* helps to reduce workload needed for the routine procedure.

Instructions and Results

Sample pretreatment (manual preparation)

Samples (not more than 40 mg of solid materials) are added into 2.0 mL tubes. As forensic samples are normally available in low amount, Eppendorf DNA LoBind tubes are recommended throughout the protocol for their low binding characteristic to nucleic acids to reduce DNA loss. Buffer G2 (mixed with distilled water in a 1:1 ratio) is added to samples to a total volume up to 190 µL. Buffer G2 serves as digestion buffer for absorbent samples. For sample types that tend to be very absorbent (e.g. fabrics or cigarette butts), a greater volume of buffer is necessary. Buffer G2 should completly cover the samples to ensure the whole sample is digested. 10 µL of Proteinase K is added and the tubes are vortexed for 10 s using MixMate®. For semen stain or soft bone, additional DTT and extra amount of proteinase K are needed but total volume of the sample should not exceed 300 µL. Samples are then incubated at 56°C for 1 hr with mixing at 800 rpm using the Eppendorf ThermoMixer® C.

For chewing gum, 15 min incubation with mixing is sufficient. Semen stain or soft bone need longer incubation time, and mixing during incubation is recommended. For hair, bone and soil sample, overnight incubation with mixing is needed. Samples are then centrifuged at 15,000 x g for 5 min. Supernatant is transferred to new 2.0 mL tubes (for automated purification on ep*Motion* M5073, see Part A) or to a new 2 mL deepwell plate (for automated purification on ep*Motion* 5075m, see Part B). Transferred supernatant should be approximately 200 µL. If the supernatant is less than 200 µL, buffer G2 can be added to the volume. Transferred supernatant should not exceed 300 µL in each tube/well. In case of solid material, the material can be pressed against inside of the tube wall using forceps to obtain maximal sample volume.

A) Automated DNA purification on ep*Motion* M5073
(tube format, maximum 24 samples per run)

Worktable Layout

Position	Item
TMX/A1	PrepRack with Eppendorf DNA LoBind Tubes 2.0 mL containing 200 µL lysed sample. Mind the direction of tube placement, starting from position 1, 2, 3….
B1	1000 µL Filtertips
B2	50 µL Filtertips
C1	Reservoir rack with tubes and reservoirs containing reagents (Figure 2)
C2	Rack 24 with fresh 1.5 mL or 2.0 mL tubes for eluted DNA

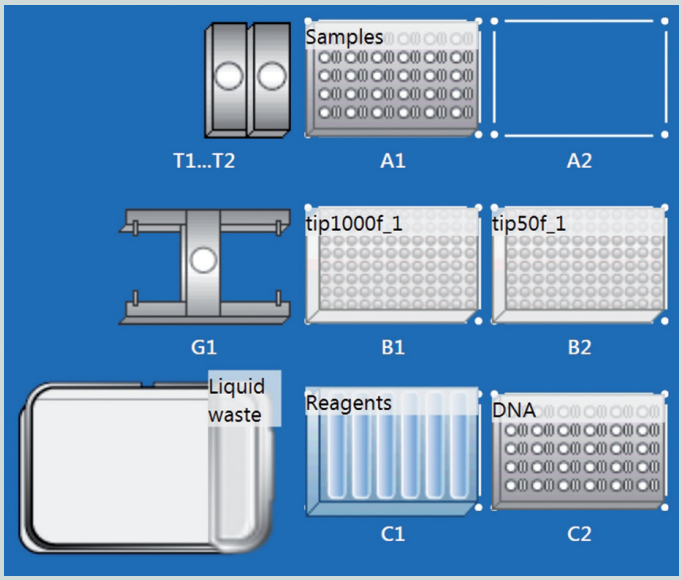


Figure 1: ep*Motion* M5073 worktable layout.

ReservoirRack Layout

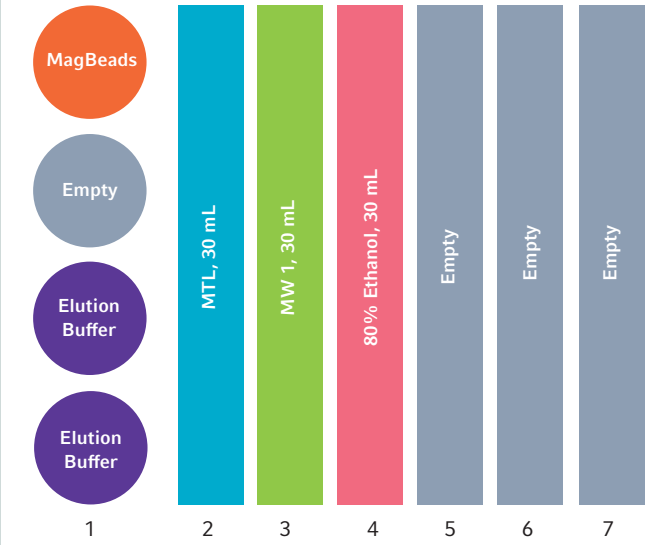
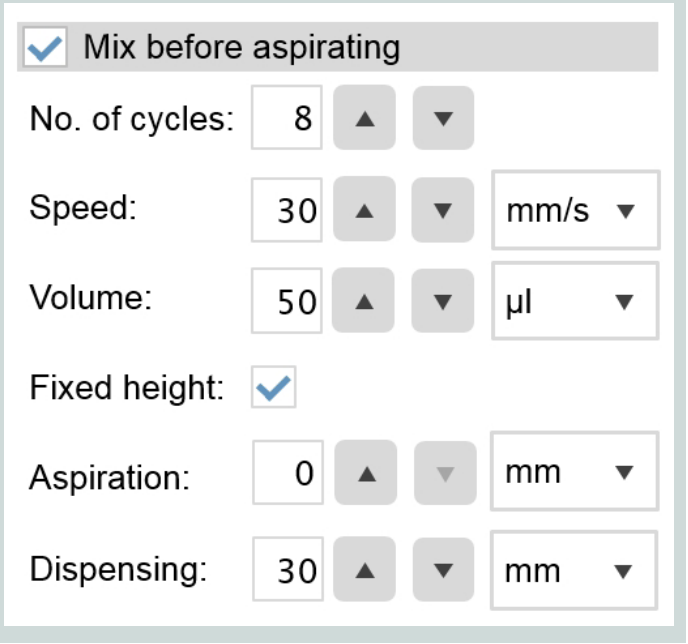


Figure 2: Reservoir rack layout on position C1 of ep*Motion* M5073 worktable. Position 1 contains a TC module with 4 x 2.0 mL tubes. Position 2 - 4 are filled with Reservoir 30 mL.

Prior to starting the automation, 26 mL of 100% ethanol is added into the Buffer MW1 bottle of the extraction kit. Buffer MTL and MW1 should be shaken thoroughly before use each time. The magnetic beads must be thoroughly resuspended before use by shaking the MagAttract Suspension B bottle and vortexing for 1 min (vortexing for 3 min before first use). Transfer sufficient amount of the reagents into ReservoirRack according to Figure 2.

The ep*Motion* starts the automated extraction by adding Buffer MTL to sample tubes in a MTL: supernatant ratio of 3:1 (e.g. if supernatant is 200 µL, 600 µL MTL is added). After that, 300 µL magnetic beads are added followed by mixing for a duration of 15 min. Following a 2-3 min of magnetic separation, the supernatant is removed. Buffer MW1 (500 µL) is added, followed by mixing, magnetic separation and supernatant removal. Repeat this washing step with Buffer MW1. 500 µL of 80% ethanol is then added, followed by mixing, magnetic separation and supernatant removal. The tubes are air dried for 5 min at room temperature or at 65 °C with mixing.
30 µL of double distilled water (elution buffer) is added and the tubes are mixed intermittently at 65 °C for 10 min. Volume of the elution buffer can be adjusted based on the initial sample amount so that the eluted samples are not too diluted or concentrated. Magnetic separation is performed and the supernatant containing purified DNA is transferred into fresh tubes 1-24 on position C2. (Figure 1)

If the magnetic beads are difficult to mix, the mixing parameters can be changed as follows:



B) Automated DNA purification on ep*Motion* 5075m
(plate format, maximum 96 samples per run)

Worktable Layout

Position	Item
A2, A3, B1, B2 & B5	1000 µL Filtertips
B4	50 µL Filtertips
B0	Reservoir 400 mL as liquid waste reservoir
TMX	Eppendorf 2 mL Deepwell plate containing 200 µL lysed sample in each well + Thermoblock DWP 2000
B3, C2-C4	Rack 24 with fresh 1.5 mL or 2.0 mL tubes for eluted DNA
C1	Magnum FLX® Magnet Adapter
C5	Reservoir rack with reservoirs containing reagents (Figure 3)

ReservoirRack Layout

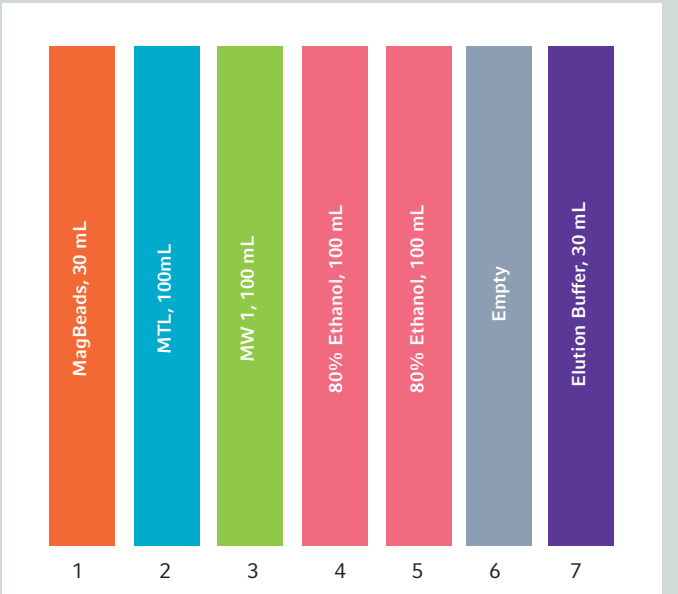


Figure 3: Reservoir rack layout on position C5 of ep*Motion* 5075m worktable. Position 1 and 7 are filled with Reservoir 30 mL. Position 2 to 5 are filled with Reservoir 100 mL.

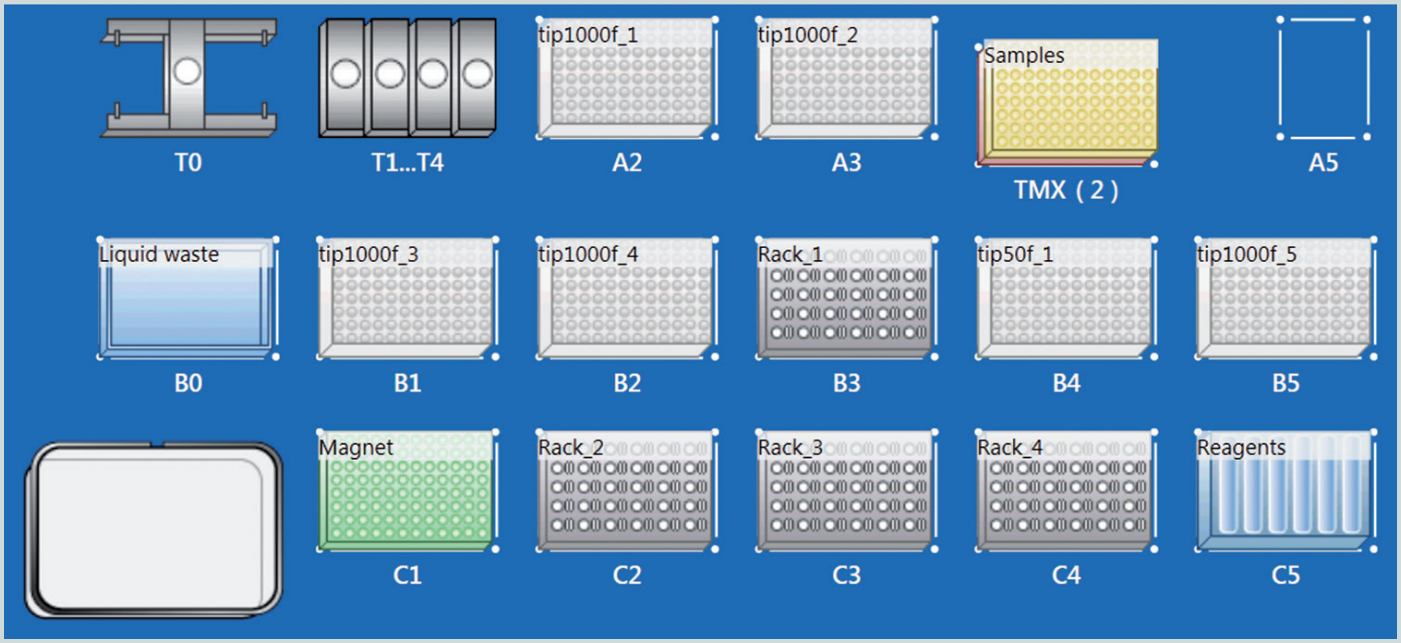


Figure 4: ep*Motion* 5075m worktable layout.

Perform the same reagent preparation as described in Part A. The ep*Motion* starts the automated extraction by adding 40 µL of magnetic beads and 600 µL of Buffer MTL into each well. The plate is mixed for 15 min at 1,200 rpm on TMX. Sample plate is then transferred onto the magnetic separator for a 2-3 min separation. Supernatant is then aspirated and discarded into liquid waste reservoir. The plate is then transferred back to TMX. 500 µL of Buffer MW1 is added into each well and the plate is mixed for 5 min at 1000 rpm.

After that, magnetic separation is performed, supernatant is discarded and the plate is placed back to TMX. The same sequence is repeated twice with 500 µL of 80% ethanol. Samples are air dried for 10 min at 75°C with mixing. Elution buffer (30-70 µL) is added and the plate is mixed at 75°C, 1000 rpm for 8 min. Magnetic separation is performed for 2 min. The supernatant containing purified DNA is transferred into fresh tubes or positions B3, C2, C3 and C4 for samples 1-24, 25-48, 49-72 and 73-96, respectively (Figure 4). Alternatively, a fresh plate may be used to substitute for tubes.

Results

Eluted DNA was measured with Eppendorf BioSpectrometer® at 260 nm (A260) and 280 nm (A280). Table 1 shows the concentration and purity of DNA purified manumally and with automated method using ep*Motion* M5073, from 200 µL lysed saliva. DNA purified using ep*Motion* is comparable to those samples purified manually in terms of yield and purity.

Table 1: DNA purified manually and with automated method using ep*Motion* M5073. DNA was purified from 200 µL lysed saliva.

Sample	Method	Concentration (ng/µL)	Purity A260/A280
Saliva	Manual	29.6	1.79
Saliva	Manual	24.8	1.86
Saliva	ep*Motion*	16.2	1.70
Saliva	ep*Motion*	19.4	1.68

Ordering information

Description	Order no. International	Order no. North America
Equipment and consumables for DNA purification on ep*Motion* M5073 (tube format)		
ep*Motion*® M5073 with Eppendorf EasyCon™, Eppendorf MagSep™ module, Eppendorf ThermoMixer®, epBlue™ software and Prep assistant, TS 50, TS 1000, PrepRack, ReagentRack, Rack for 24 Eppendorf Safe-Lock tubes, Liquid Waste Tub and waste box.	5073 000.205	5073000213
1 x Reservoir Rack Module TC, for use in ep*Motion*® Reservoir Rack, temperable, 4 x Eppendorf Safe-Lock tubes 0.5/1.5/2.0 mL	5075 799.081	960002620
Eppendorf DNA LoBind Tubes, 2.0 mL	0030 108.078	022431048
Equipment and consumables for DNA purification on ep*Motion*® 5075m (plate format)		
ep*Motion*® 5075m with Eppendorf MagSep™ module, Eppendorf ThermoMixer®, epBlue™ software, mouse and waste box.	5075 000.305	5075006024
Eppendorf MultiCon PC controller incl. keyboard	5075 001.101	–
TM 50-8 eight-channel dispensing tool	5280 000.215	960001044
TM 1000-8 eight-channel dispensing tool	5280 000.258	960001061
Gripper	5282 000.018	960002270
Rack 24 for Eppendorf Safe-Lock tubes 1.5/2.0 mL, tempering (4 pieces are needed)	5075 751.275	5075751275
Thermoblock for Eppendorf Deepwell Plate 96/2000 µL	5075 751.330	5075751330
Deepwell Plate 96/2000 µL, PCR clean, white	0030 501.306	951033405
ep*Motion*® reservoir 100 mL for use with Reservoir Rack 7	0030 126.513	960051017
ep*Motion*® reservoir 400 mL	5075 751.364	5075751364
Eppendorf Magnum FLX® Magnet Adapter	5075 751.836	960066124
General accessories and consumables required for ep*Motion*		
Reservoir Rack 7	5075 754.002	960002148
ep*Motion*® reservoir 30 mL for use with Reservoir Rack 7	0030 126.505	960051009
Eppendorf DNA LoBind tubes, 1.5 mL	0030 108.051	022431021
epT.I.P.S.® Motion 50 µL Filter	0030 014.413	0030014413
epT.I.P.S.® Motion SafeRack 1000 µL Filter	0030 014.650	0030014650
Other relevant Eppendorf products		
MixMate®	5353 000.014	022674200
Eppendorf ThermoMixer® C	5382 000.015	5382000023
Eppendorf SmartBlock™ 2.0 mL	5362 000.035	5362000035
Eppendorf µCuvette® G1.0 and Eppendorf BioSpectrometer® basic	6135 000.904	6135000923
Centrifuge 5424	5424 000.410	022620401

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