GENOMIC DNA EXTRACTION USING THE MAGMAX SEMI-AUTOMATIC ROBOT

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NOTES

The following protocol is for the extraction of genomic DNA using the MagMAX Express semi-automatic robot and the Multi-sample Kit from Life Technologies (cat#413022)
This method is useful for stool samples, plate pools, cultures and other high biomass samples.
This method is not optimal for biopsies or swabs or other low biomass samples.

EQUIPMENT

- Bead based homogenizer (PowerLyzer, Medicorp Inc., #13155)
- Centrifuge
- Plate shaker
- MagMAX express semi-automatic robot

PROTOCOL

Solution Preparation:

The PK Buffer is not used in our protocol

1. Add 5.4 mL of 100% Isopropanol to Wash Solution 1
2. Add 40 mL of 100% Ethanol to Wash Solution 2
3. Prepare Binding Bead Mix
   1616 uL of DNA Binding Beads
   404 uL of supplied Water
   2020 uL Add 20 uL per well to Binding Plate, Plate#1 (Sample prep step 4)
   There is not much extra volume here, can make a bit more or keep the extra beads in case you need a bit more diluted.

4. Prepare RNaseA (this should be done last right before you run the MagMax Express)
   505 uL RNaseA solution (store in the -20C freezer)
   9595 uL supplied water
   10100 uL Add 100 uL per well to a 96 deep well plate for RNaseA Plate, Plate #4

Sample Preparation:

1. If not already done - Add 0.1-0.2g of fecal sample to a bead 2.8mm beating tube with
   0.2 g of 0.1mm glass beads
   100 uL of GES Buffer
   800 uL of 200 mM Sodium Phosphosate monobasic
2. Bead beat samples for 3 mins at 3000 rpm
3. Spin tubes for 5 mins at max speed
4. Add 160 uL of 100% Isopropanol to a 96 deep well plate (Binding Plate, Plate #1)
5. Add 200 uL centrifuged samples to each well (use adjustable 8 channel multichannel pipette)
6. Seal plate and shake at 505 rpm for 3 min on plate shaker
7. Add 20 uL of DNA binding bead mix to each well (use multidispense single channel pipette)
8. Repeat step 6

Plate Preparation:

Plate ID	Plate #	Reagent(s)	Volume (uL)	Plate Type
Binding	1	Sample isopropanol and DNA binding beads (see sample prep)	360 uL (total)	Deep Well
Wash 1	2	Wash Buffer 1	150 uL	Deep Well
Wash 2	3	Wash Buffer 2	150 uL	Deep Well
RNaseA	4	RNaseA mix	100 uL	Deep Well
Wash 3	5	Wash Buffer 2	150 uL	Deep Well
Wash 4	6	Wash Buffer 2	150 uL	Deep Well
Elution	7	DNA Elution Buffer	75 uL	Standard
Tip	8	Place tips in deep well plate	NA	Deep Well

To start the run (the protocol takes approximately 45 minutes)

1. Turn power on (button on the side)
2. Select program 4413021 DW Tissues
3. Press START and follow instructions on the machine
4. 15 minutes after the MagMax Express has started remove the RNaseA plate, Plate#4 and add
   100 uL Lysis Buffer

120 uL 100% Isopropanol

5. Return RNaseA plate (Plate# 4) to machine and press START
6. 30 minutes after step 5 remove Elution Plate (Plate#7) and add
   75 uL Elution Buffer 2
7. Return Elution plate (Plate#7) to the machine and press START
8. Once the protocol has finished running the MagMax Express with display END _OF_RUN press STOP.
9. Remove, seal and Label elution plate and store at 4°C
10. Remove remaining plates and dispose of them in the biohazardous waste bin
11. Power off the MagMax Express

Sample Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	X	X	X	X	X	X	X	X	X	X	X
B	X	NEG	X	X	X	X	X	X	X	X	X	X
C	X	X	X	X	X	X	X	X	X	NEG	x	x
D	X	X	X	X	X	X	X	X	X	X	X	X
E	X	X	X	X	X	X	X	X	X	X	X	X
F	X	X	X	X	X	X	X	X	X	NEG	X	X
G	X	NEG	X	X	X	X	X	X	X	X	X	X
H	X	X	X	X	X		X	X	X	X	X	POS

X = SAMPLE

NEG = Negative control (200 uL of DNA extraction buffer GES + sodium phosphate monophosphate)

POS = Positive control (healthy control fecal sample)