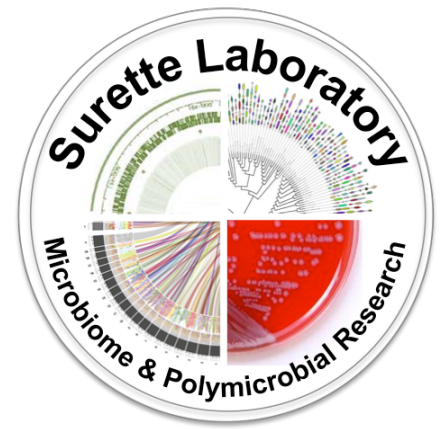


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# GENOMIC DNA EXTRACTION USING THE MAGMAX SEMI- AUTOMATIC ROBOT

## CREATED/UPDATED BY: L ROSSI



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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.

- 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:**

- 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
- Bead beat samples for 3 mins at 3000 rpm
- Spin tubes for 5 mins at max speed
- Add 160 uL of 100% Isopropanol to a 96 deep well plate (Binding Plate, Plate #1)
- Add 200 uL centrifuged samples to each well (use adjustable 8 channel multichannel pipette)
- Seal plate and shake at 505 rpm for 3 min on plate shaker
- Add 20 uL of DNA binding bead mix to each well (use multidispense single channel pipette)
- 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)**

- Turn power on (button on the side)
- Select program 4413021 DW Tissues
- Press START and follow instructions on the machine
- 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)