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miRNA extraction from fecal matter V.1

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nick Heyer[1]

[1]Colorado State University

NHeyer

n nick Heyer
Colorado State University

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

> This Proticol is still being eddited

Abstract

This is a modification to the Qiagen proticol for miRNA extraction for tissues

Prep

1 Set Centrifuge to 4C if needed

2 Create 70% and 90% Et-OH

2.1 _____num samples $\cdot 0.55 \cdot 0.7 =$ _____ml 100% Et-OH
_____num samples $\cdot 0.55 \cdot 0.3 =$ _____ml Molecular Grade Water

2.2 _____num samples $\cdot 0.55 \cdot 0.9 =$ _____ml 100% Et-OH
_____num samples $\cdot 0.55 \cdot 0.1 =$ _____ml Molecular Grade Water

Lysis, chloroform extraction, and Large Nucliotide removal 27m

3 In a 1.5ml tube, homogenize _____mg of dry fecal matter in 700ul of QIAzol 10m

4 Incubate at 20C for 5min

5 Add 140ul of chloroform, **MIX WELL** and let incubate for 1 min at 20C, **MIX AGAIN,** and incubate for 3 min

6 Centrifuge at >12,000xg at 4C for 15 min 15m

7 Transfer aqueous phase and determine volume by pipetting, combine with 1x Volume of 70% Et-OH . 2m

8 Run through a "RNeasy Mini spin Column" at >8,000xg at 20C

miRNA recovery

9 **Recover flow through**, and add 0.65x volumes for 100% Et-OH

10 Run through a "RNeasy Mini spin Column" at >8,000xg at 20C, 700ul at a time discarding flow-through

11 Wash matrix with 700ul RWT Buffer

12 Wash matrix with 500 ul of RPE

13 Wash matrix _____x with ice cold 90% Et-OH

14 Dry Tube by spinning column for 5 min

15 Elute in 30ul of elution buffer