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Genomic DNA Extraction from Sorted Cells

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol details the extraction of genomic DNA from cells.

Guidelines

The DNeasy columns cannot take more than 5 x 106 cells per column. Larger pellets can be split into multiple extraction reactions, then combined after elution.

Materials

Materials

- Pelleted cells for DNA extraction (thawed, if previously frozen)
- DNeasy Blood & Tissue Kits **Qiagen Catalog #**69506
- 1.7-mL microcentrifuge tubes
- DPBS
- Monarch RNase A – 1 ml (2x0.5ml) **New England Biolabs Catalog #**T3018L
- Molecular grade water
- 2.0-mL microcentrifuge tubes
- 96-100% Ethanol

Equipment

- Benchtop centrifuge
- Thermomixer
- Vortexer
- Picofuge
- Nanodrop

Extraction of genomic DNA from cells

39m

1 Prepare a lysis master mix of the following reagents and mix by vortexing briefly:

	A	B	C
	Reagent	**Volume per sample (uL)**	**Volume for X samples (uL)**
	DPBS	100	X * 100 * 1.05
	Buffer AL	200	X * 200 * 1.05
	Proteinase K	20	X * 20 * 1.05
	RNase A	2	X * 2 * 1.05
	Total	322	

2 Aliquot 322 µL of this master mix into labeled 1.7-mL microcentrifuge tubes.

3 Resuspend the pellets in 100 µL DPBS for every 5 x 106 cells in each sample pellet.

- If you have 10 x 10^6 cells in your pellet, resuspend in 200 µL, then split into 2 separate tubes.

4 Add resuspended cells in 100 µL DPBS to an appropriately labeled tube containing the lysis master mix. Mix by vortexing.

5 Incubate the resuspended cells at 56 °C with agitation at 1500 rpm, 00:30:00.

30m

6 Spin down the lysis tubes briefly, then add 200 µL of 96-100% ethanol to the lysed cells. Mix thoroughly by vortexing. Spin the tubes down to remove drops from lids.

7 Transfer the lysed cell mixture to a labeled DNeasy mini spin column in a 2mL collection tube (both provided in the kit).

8 Centrifuge at 6000 x g, 00:01:00. Discard the flow-through and collection tube.

1m

9 Place the spin column in a new 2mL collection tube and add 500 µL Buffer AW1 (provided in kit) to the column. Centrifuge at 6000 x g, 00:01:00 . Discard the flow-through and collection tube. 1m

10 Place the spin column in a new 2 mL collection tube and add 500 µL Buffer AW2 (provided in kit) to the column. Centrifuge at 20000 x g, 00:03:00 . Discard the flow-through and collection tube. 3m

11 Place the spin column in a new 2mL collection tube and centrifuge at 20000 x g, 00:01:00 . 1m

12 Place the spin column in a new (labeled) 1.7-mL microcentrifuge tube.

13 Add 100 µL of molecular grade water to the spin column and incubate at Room temperature for 00:02:00 . Centrifuge at 6000 x g, 00:01:00 . 3m

14 Repeat Step 13 with an additional 100 µL of molecular grade water.

15 Transfer any split samples (pellets with more than 5 x 10^6 cells) into labeled 2.0mL microcentrifuge tubes.

16 Quantitate with the Nanodrop, and store the samples at -20 °C .