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Genomic DNA Extraction from Sorted Cells

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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 5×10^6 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 (2×0.5ml) **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 5×10^6 cells in each sample pellet.
 - If you have 10×10^6 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 .
- 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).



30m





- 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 μ L 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 μ L 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 μ L 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 μ L of molecular grade water.
- 15 Transfer any split samples (pellets with more than 5×10^6 cells) into labeled 2.0mL microcentrifuge tubes.
- 16 Quantitate with the Nanodrop, and store the samples at  -20 $^{\circ}$ C .