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Genomic DNA Extraction from Sorted Cells



In 1 collection

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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)
- 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	В	С
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	

- Resuspend the pellets in Δ 100 μL DPBS for every 5 x 106 cells in each sample pellet.
 - If you have 10×10^6 cells in your pellet, resuspend in $200 \, \mu L$, then split into 2 separate tubes.



5 Incubate the resuspended cells at \$\mathbb{L}^* 56 \circ\$C with agitation at \$\mathbb{\mathbb{R}}\$ 1500 rpm, 00:30:00 .



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.



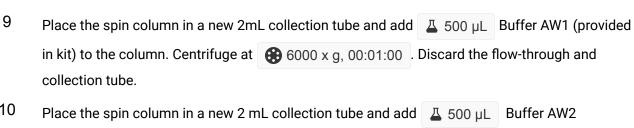
7 Transfer the lysed cell mixture to a labeled DNeasy mini spin column in a 2mL collection tube (both provided in the kit).



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

1m





₩ \

8

1m

- Place the spin column in a new 2 mL collection tube and add $\Delta 500 \,\mu$ L Buffer AW2 (provided in kit) to the column. Centrifuge at through and collection tube.
- **&** X

3m

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.
- Add Δ 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 Δ 100 μL of molecular grade water.
- Transfer any split samples (pellets with more than 5×10^6 cells) into labeled 2.0mL microcentrifuge tubes.