DNA Extraction from sorted cells

Virginia Rich

Abstract

Modified after V. Rich, 2/10/07: DNA extraction protocol from 25 mm 0.2 µm filters – Low-throughput, using DNeasy columns (based on lab Steripak filter extraction protocol and the Suzuki et al. 2001 protocol)

Citation: Virginia Rich DNA Extraction from sorted cells. **protocols.io**
dx.doi.org/10.17504/protocols.io.c38yrv
Published: 04 Jan 2016

Guidelines

Materials:

Product	Vendor	Cat #
Sucrose, molecular biology grade	FISHER	
Lysozyme	Sigma	L7651-1G
Proteinase K, PCR grade	Roche	
10% SDS		
96-100% EtOH		
Microcons 30 (now amicon)	Millipore	
Qiagen DNeasy Tissue Kit	Qiagen	

Materials

Lysozyme from chicken egg white L7651-1G by Sigma Aldrich

Protocol

Lysis Buffer

Step 1.
Prepare 2x lysis buffer and add to the volume of sample so that its final concentration is 1x.

Final concentration, 1x	For 10 ml 2x lysis buffer	For 2 ml 2x lysis buffer
40 mM EDTA	1.6 ml 0.5 M EDTA	0.32
50 mM Tris (pH 8.3)	1.0 ml 1 M TrisHCl (pH 8.3)	0.2
0.75 M Sucrose	5.13 g Sucrose	1.026
	QS to 10 ml (MQ water)	QS to 2 ml

Lysis Buffer

Step 2.

Filter-sterilize through 0.2 µm.

Lysis Buffer

Step 3.

Prepare 2x Lysis buffer with Lysozyme & RNase (1): right before use add to 1 ml aliquot of lysis buffer:

Final concentration, 1x	For 1 ml, 2x	For 5 ml, 2x
1.15 mg/ml lysozyme	3.30 mg lysozyme (0.003 g)	16.5 mg (0.0165 g)
200 mg/ml RNase 100 mg/ml	4 ml RNase 100 mg/ml	20 ul

Lysis Buffer

Step 4.

Shake to dissolve thoroughly, then filter-sterilize again.

Lysis Buffer

Step 5.

Dilute the 2x lysis buffer 1:1 with MQ water.

Lysis Buffer

Step 6.

Weigh out minimum amount of ProtK, then add the appropriate amount of lysis buffer:

Final concentration, 1x	For 1 ml, 1x	For 100 ml, 1x
10 mg/ml	10 mg ProtK	1 mg ProtK (0.001 g)

Lysis Buffer

Step 7.

Filter sterilize again.

Cell lysis & RNA removal

Step 8.

Add 1 volume of 2x Lysis buffer with Lysozyme & RNase to 1 volume of sorted cells. Note down volumes and resulting volume V1.

Cell lysis & RNA removal

Step 9.

Incubate at 37°C for 30 min, rotating end-over-end at angle (OR: in the shaking incubator @ 100 rpm, vertical), for optimal mixing with minimal frothing; alternatively, shake vertically @ 100 rpm on shaking incubator and invert 10 times every 10 min.

DURATION

00:30:00

Protein Degradation

Step 10.

Add V1*0.07 µl of ProtK lysis buffer (2) to a final concentration of 0.65 mg/ml. Note down volumes, including resulting volume V2.

Protein Degradation

Step 11.

Add V2/9 µl of 10% SDS to a final concentration of 1%. Note down volumes.

Protein Degradation

Step 12.

Incubate at 55°C for 2 hours, rotating end-over-end at angle (OR: in the shaking incubator @ 100 rpm, vertical; invert every 20 min 10 times)

DURATION

02:00:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 13.

Add 600 µl buffer AL (= buffer without the EtOH)

AMOUNT

600 µl Additional info:

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 14.

Mix thoroughly by vortexing

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 15.

Incubate at 70°C for 10 min (for heat block use 1.5 ml tubes!)

DURATION

00:10:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 16.

Add 600 µl of 96-100% EtOH

AMOUNT

600 µl Additional info:

REAGENTS

Ethanol BE-BDH1156 by P212121

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 17.

Mix by vortexing vigorously

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 18.

Check pH of lysate, must be <7 to get max. binding efficiency

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 19.

Pipette max. possible volume onto spin columns

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 20.

Spin 3 min at 8000xg; discard flow-through

DURATION

00:03:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 21.

Pipette additional lysate onto spin columns

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 22.

Spin 1 min at 8000xg; discard flow-through and collection tube, transfer column to new collection

tube

DURATION

00:01:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 23.

Add 500 µl of buffer AW1

AMOUNT

500 µl Additional info:

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 24.

Spin 2 min at 8000xg; discard flow-through

DURATION

00:02:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 25.

Add 500 µl of buffer AW2

AMOUNT

500 µl Additional info:

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 26.

Spin 3 min at 8000xg; discard flow-through and collection tube,

DURATION

00:03:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 27.

To dry columns, spin at 8000xg for 3 min

DURATION

00:03:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 28.

Discard potential flow-through; transfer column to new collection tube

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 29.

Add 200 µl pre-heated 70°C buffer AE or water

AMOUNT

200 µl Additional info:

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 30.

Incubate 1 min at room temp.

DURATION

00:01:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 31.

Spin at 8000xg for 2 min to elute

DURATION

00:02:00

DNA purification through Qiagen DNeasy tissue kit-binding column

Step 32.

Repeat with second 200 µl.

Final DNA cleanup by size exclusion columns

Step 33.

Transfer eluted DNA to the Amicon Ultra 30 K

Final DNA cleanup by size exclusion columns

Step 34.

Centrifuge at 10,000xg for 10 min

DURATION

00:10:00

Final DNA cleanup by size exclusion columns

Step 35.

Rinse DNA with 500 µl PCR water

AMOUNT

500 µl Additional info:

REAGENTS

PCR Grade Water AM9935 by Contributed by users

Final DNA cleanup by size exclusion columns

Step 36.

Centrifuge at 10,000xg for 10 min

DURATION

00:10:00

Final DNA cleanup by size exclusion columns

Step 37.

Add 20 µl dilute TE, pipette up and down 20 times and transfer to clean tube for storage

Final DNA cleanup by size exclusion columns

Step 38.

Centrifuge at 1000xg for 3 min upside down, in fresh tube to retrieve

DURATION

00:03:00

Final DNA cleanup by size exclusion columns

Step 39.

(Optional: repeat with another 20 µl to ensure all retrieved)