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Genomic DNA isolation from fixed cells

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ABSTRACT

This protocol details the procedure of genomic DNA isolation from fixed cells.

ATTACHMENTS

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MATERIALS TEXT

Reagents:

- Proteinase K (Zymo #D3001-2-20) (20mg/ml stock in storage buffer).
- RNase A (Sigma #70856) (10mg/ml stock).
- 100% Molecular biology grade Ethanol.
- Silica spin columns (Econospin).
- Qiagen buffer AL.
- Qiagen buffer AW1.
- Qiagen buffer AW2.
- Nuclease free water.

Buffer AL (storage: room temperature (RT))

A	B
Tris-HCl (7.4)	50 mM
Guanidine HCl	5.5 M
EDTA	20 mM
Triton X-100	1.3%

Buffer AW1 (storage: RT)

A	B
Guanidine HCl	1 M
EtOH, pH 5.5	57%

Buffer AW2 / PE (storage: RT)

A	B
Tris-HCl (pH 7.5)	10 mM
Ethanol	80%

Buffer AW2 / PE (storage: RT)

A	B
Tris-HCl (pH 9)	10 mM
EDTA	0.5 mM

Genomic DNA isolation from fixed cells 38m 5s

1 Resuspend 1-3 X10^6 cells in **250 µL** of PBS and transfer to a 2 mL tube (this would represent one **3.5 cm** dish of 3T3 cells).

2 

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Add **200 µg** Proteinase K (from a **20 mg/mL** stock) and **200 µg** RNase A (from a **20 mg/mL** stock).

3 30m

Incubate cells at **37 °C** for **00:30:00** in a water bath.

4

Add **250 µL** Qiagen AL lysis buffer per **250 µL** of the protease and RNAase-containing cell suspension and mix thoroughly.

5

Place tubes in an incubator at **56 °C** with shaking at **800 rpm** **Overnight** , capped.

Each tube is parafilm sealed to ensure safety.

6 5s

Add **250 µL** , 100% molecular biology grade ethanol; mix slowly using a slow vortex for **00:00:05** .

7 With a razor blade, trim the tip of a **1 mL** pipet tip to enlarge the opening. Use this tip to pipet out DNA from the ethanol solution and apply it onto a silica spin DNA binding column (e.g. EconoSpin 1920-250).

8 1m

Spin at **6000 x g** for **00:01:00** in a fixed angle tabletop microfuge; aspirate and discard flow-through.

9 1m

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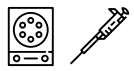
Add **500 µL** Qiagen **buffer AW1** to the column, spin again at **6000 x g** for **00:01:00** , aspirate and discard flow-through.

10  1m

Add **500 µL** Qiagen **buffer AW2**, spin at **8000 x g** for **00:01:00** , aspirate and discard flow-through.

11  1m

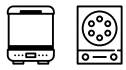
Spin once more using microfuge to remove excess ethanol at **13000 x g** for **00:01:00** .

12 

Transfer column into a new, 1.5 mL collection tube.

13 Elute with pre-warmed, **100 µL** nuclease-free **water** or **TE**.

> Volume depends on starting number of cells: use **100 µL** per 1 million cells.

14  2m

Incubate for **00:01:00** , then spin at **13000 x g** for **00:01:00** .

15 

Add another **50 µL** **nuclease free water** to accomplish a second elution.

16  2m

Incubate at **Room temperature** for **00:01:00** , then spin as before at **13000 x g** for **00:01:00** .

17 **The two flow-through fractions contain the genomic DNA**.

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18 Perform Nanodrop and Qubit HS DNA estimation to calculate yield.

Theoretically, $1X10^6$cells should yield **6 µg** DNA.

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