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Genomic DNA isolation from fixed cells

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Herschel Dhekne[1], Suzanne R Pfeffer[1], Ebsy Jaimon

[1]Department of Biochemistry, Stanford University

Suzanne R Pfeffer
Stanford University School of Medicine

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Attachments

DOCX

iugzbc32f.docx

14KB

Materials

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

Troubleshooting

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

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

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.

> Note
>
> Each tube is parafilm sealed to ensure safety.

6 Add 250 µL, 100% molecular biology grade ethanol; mix slowly using a slow vortex for 00:00:05. 5s

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 Spin at 6000 x g for 00:01:00 in a fixed angle tabletop microfuge; aspirate and discard flow-through. 1m

9 Add 500 µL Qiagen **buffer AW1** to the column, spin again at 6000 x g for 00:01:00, aspirate and discard flow-through. 1m

10 Add 500 µL Qiagen **buffer AW2**, spin at 8000 x g for 00:01:00, aspirate and discard flow-through. 1m

11 Spin once more using microfuge to remove excess ethanol at 13000 x g for 00:01:00. 1m

12 Transfer column into a new, 1.5 mL collection tube.

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

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

14 Incubate for 00:01:00, then spin at 13000 x g for 00:01:00. 2m

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

16 Incubate at Room temperature for 00:01:00, then spin as before at 13000 x g for 00:01:00. 2m

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

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

> Note
>
> Theoretically, 1X10^{6}cells should yield 6 µg DNA.