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DNA Extraction from FANS sorted nuclei

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Neurodegeneration Met... Complex Disease Epige...

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We use this protocol and it's working

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Abstract

Here we describe an optimised protocol for the extraction of genomic DNA from frozen nuclei samples collected from different neural cells using Fluorescence-assisted Nuclei Sorting (FANS) of post mortem human brain. The same protocol is also suitable for DNA extraction from cells allowing optimal recovery in terms of DNA purity and yield.

Materials

Reagents required

- **Slagboom buffer (50mL):**
  10x STE buffer (5mL)
  5% SDS (5mL)
  Water (40 mL)

- **RNAase A** (stock concentration: 10mg/ml)
- **Proteinase K** (stock concentration: 20 mg/mL)
- **Majiik mix** (1:1 ratio yeast Reagent 3 (Autogen Bioclear, Caine, Wiltshire, UK) + 100% ethanol)
- **100% Isopropanol**
- **80% Ethanol**
- **TE or water**

Note

NOTE- Slagboom buffer can be made in advance while 80% Ethanol should be prepared fresh the same day.

Troubleshooting

DNA extraction from FANS purified nuclei

1 Defrost frozen FANS sorted nuclei contained in a 1.5ml eppendorf tube On ice

Note

NOTE 1-2 nuclei aliquots (~200,000 nuclei/tube) per each nuclei population should yield between 300-500ng DNA).

2 To each sample add either 250µL / 500µL/ 1mL of Slagboom buffer (SB) depending on size of cell pellet. Normally 250µL is sufficient for 200,000 nuclei aliquot or 1×10^6 cells

3 Add 500µL of SB to each nuclei sample (if nuclei pellet)*

Note

* If nuclei are stored in running buffer: calculate the volume of 10x STE and 5% SDS to add to each sample to be consistent with the composition of SB (1x STE; 0.5% SDS).
e.g. If you have collected a 400µL sample you would add 50µL 10x STE and 50µL 5% SDS to make the sample up to 500µL

4 Add 1µL of RNase A per 500uL buffer

5 Incubate at 37 °C for 00:45:00 (using a heat block)

6 Add proteinase K to a final concentration 2mg/mL (e.g 5µL for every 500µL of SB)

7 Mix by inverting 10 times (do not vortex or pipette mix)

8 Incubate at 60 °C for 01:00:00 (water bath)

9 Move samples from the water bath and leave at Room temperature for 00:05:00

10 Briefly spin down tubes (pulse spin)

11 For every 500µL of SB used, add 100 µL of Majiik Mix (e.g. 200 µL for 1 mL of SB)

12 Mix by vigorous inversions (**DO NOT** vortex)

13 Centrifuge at 17000 x g for 00:10:00 at Room temperature

14 Carefully recover supernatant and transfer it to a new labelled 1.5ml tube (leaving ~50µL at the bottom of the tube)

15 Repeat step above by adding another 100µL of Majiik Mix to the new tube

16 Mix by vigorous inversions (**DO NOT** vortex)

17 Centrifuge at 17000 x g for 00:10:00 Room temperature

18 Recover supernatant and transfer to a new labelled 1.5ml tube leaving ~50ul at the bottom of the old tube.

Note

NOTE - If supernatant exceeds 1mL, split into 2 tubes

19 Add an equal volume of 100% Isopropanol to each tube (e.g. 1mL supernatant + 1 mL 100% Isopropanol)

20 After adding the Isopropanol, add 0.7-1µL Glycogen Blue per tube (this step is optional – but advised as it helps to visualize DNA pellet at the end)

21 Mix by inversion (10 times)

22 Centrifuge at 17000 x g for 00:15:00 at Room temperature (hinges of tubes facing upwards)

23 Carefully remove supernatant and discard

Note

NOTE - Care must be taken when pouring supernatant since the DNA pellet only weakly adheres to the side of the tube

24 Proceed by adding 500µL of 80% ethanol to each tube

25 Mix gently by pipetting

26 Centrifuge at 17000 x g for 00:05:00 at Room temperature

27 Carefully discard supernatant

28 Pulse centrifuge to collect remaining liquid at the bottom of the tube

29 Recover and discard residual liquid from the bottom of the tube making sure **not to** disturb the DNA pellet

30 Leave DNA pellets to air dry for 5-15 minutes (leave lids open)

Note

OPTIONAL - Leave to dry for additional 00:10:00 at 37 °C but be careful not to over-dry pellets

31 Resuspend pellet in 20uL of ddH_2O or 1xTE (15µL per tube if expected very low yield). Avoid pipette mixing, only gently flicking

32 Pulse spin tubes to help pellet resuspend/ dislodge from the tube wall

33 Leave tubes at 4 °C overnight to fully resuspend before quantifying DNA samples (Nanodrop or Qubit measurements).