IN VIVO GENOMIC FOOTPRINTING: GENOMIC DNA ISOLATION

Day One:

Solutions:

Solutions I, II, and III. Store at 4°C.

	[Stock]	Solution I	Solution II	Solution III
60mM Tris-Cl,pH8.2 but 8.0 is O.K.	1M	3mL	3mL	3mL
60mM KCl	3M	1mL	1mL	1mL
15mM NaCl	4M	188µl	188µl	188µl
0.5mM Spermidine	0.1M	250µl	250µl	250µl
0.15mM Spermine	0.1M	75µl	75µl	75µl
0.5mM EDTA	0.5M	50µl	50µl	50µl
0.3M Sucrose		5.1g	5.1g	----
1% Nonidet P-40 (NP-40)----		500µl	----	
ddH_2O		45.5mL	45mL	45.5mL
		50mL	50mL	50mL

Nuclear Lysis Buffer.

	[Stock]	
50 mM EDTA	0.5M	1 mL
1% Lauroylsarcosine (Sarcosyl)		0.1g
or 1% SDS	10%	1 mL
500µg/mL RNAse A	10mg/mL	500 µl
ddH_2O		7.5 mL
		10 mL

1. Heat Shock cells in tissue culture plates. Use a minimum of 2 x 10^7 cells/procedure. (About 2 x 10^7 cells are on a 80% confluent 10 cm plate)

For cells in suspension:

a. Spin down cells in 50mL Falcon tube.
b. Resuspend pellet in 1mL of media.
c. Transfer sample to 15mL Falcon tube.
d. Place tube on ice to chill to 20°C.
   * From this point on, all solutions etc. containing DMS must be collected for proper disposal.
e. Under fume hood, add 1 mL of 4µl/ml DMS in DME (for a concentration of 2µl/mL). Since the DMS doesn't go into solution easily, DMS is dissolved in DME before adding to cells. It must be prepared just before adding.
f. Incubate for 4 min at RT.
g. To stop reaction, add 10mL cold PBS.
h. Immediately spin down cells in Sorvall, 2500 RPM, 3 min, 4°C.
i. Wash pellet with 10mL cold PBS.

j. Spin down cells in Sorvall, 2500 RPM, 3 min, 4°C.

For cells attached to plate:

a. Aspirate media.
b. Add 3mL media.
c. Under fume hood, add 1 mL of 8µl/mL DMS in DME (for a final concentration of 2µl/mL). The DMS doesn't go into solution easily, so pipette repeatedly to dissolve.
d. Incubate for 4 min at room temp.
e. Wash plates 2X with 25mL cold PBS.
f. Add 5mL cold PBS per plate.
g. Scrape cells off plate using disposable scraper.
h. Transfer cells in PBS to 15mL Falcon tube.
i. Spin down cells in Sorvall, 2500 RPM, 3 min, 4°C.

2. Resuspend pellet in 1.5mL Solution I. The pellet should be a yellowish color.
3. Add 1.5mL Solution II for cell lysis.
4. Mix well.
5. Incubate on ice, 5 min. This time is not crucial.
6. Spin down nuclei, 3000 RPM, 5 min, 4°C. Aspirate off supernatant. The pellet should be white in color.
7. Wash pellet with 3mL Solution III, ie. resuspend pellet and spin down nuclei as in step 6.
8. Resuspend pellet in 500 µl of Solution III.
9. Once in solution, add 500 µl of Nuclear Lysis Buffer.
10. Incubate at 37°C, 3 hours. This time is not crucial.
11. Add 25 µl Proteinase K to concentration of 250µg/mL, stock is 10mg/mL.
12. Incubate overnight at 37°C.

Day Two:

1. 2X Phenol extraction, hand mixed NOT vortexed. To separate layers, spin in Sorvall, 10000 RPM, 10 min, 4°C. Aqueous layer on top. Remove the phenol from bottom and **leave the interface with sample**.
2. Phenol: Chloroform extraction, hand mixed NOT vortexed. To separate layers, spin in Sorvall, 10000 RPM, 10 min, 4°C. Aqueous layer on top.

3. Transfer the aqueous layer into 15 mL Falcon tube and add 100 µl of 3M NaOAc and 3 mL of EtOH, spin in Sorvall, 10000 RPM, 20 min, 4^{O}C.
4. Leave tube inverted for 30 min to dry pellet.
5. Add 1mL TE.
6. Leave overnight at 37^{O}C to dissolve pellet or incubate at 65˚C at least for 3 hours.

<u>Day Three</u>:

1. O.D. the sample. The DNA can now be stored at -20^{O}C.
2. Digest 50µg of DNA with an appropriate enzyme (Eco RI) in 400µl volume.
3. Incubate overnight at appropriate temperature.

<u>Continuing Day Three or Day four</u>:

Solutions:

2M Piperidine.
Dilute stock 1:5 with ddH_2O. The stock is actually the Fisher chemical. Use hood

To restriction digest add:
1. 2X Phenol: Chloroform extraction.
2. Chloroform: Isoamyl Alcohol extraction.
3. Add 45µl of 3M NaOAc.
4. Precipitate DNA with 900µl EtOH, -20^{O}C for 30 min, spin in microfuge 20 min.
5. Wash pellet with 80% EtOH.

Continue after O.D. determination,
6. Transfer 100 µg of DNA into 1.5 mL tube, add water up to 50µl and add 50µl of 2M Piperidine (a final Piperidine concentration should be 1M).
7. Incubate at 90^{O}C for 30 min.
8. Chill on ice and quick spin to collect everything at bottom.
9. 2X Phenol: Chloroform extraction and 1X Chloroform extraction.
10. Add 11µl 3M NaOAc.
11. Precipitate DNA with 350µl EtOH, spin in microfuge 20 min. Remove supernatant.
12. Resuspend pellet in 20µl ddH_2O.
13. O.D. sample.

References:

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