Small scale genomic DNA isolation Method

Experimental considerations

- This protocol is easy and relatively brief and multiple phenol extractions are not required.
- The volumes and amounts detailed here work well for cells isolated from one-100mm plate or up to 10^7 cells. For larger numbers of cells, the protocol can be scaled up and larger tubes are required - making the whole process more tedious.
- Cells or tissues can be rapidly frozen after harvesting and kept at –20°C for extended periods of time; this is very helpful when a large number of samples are collected, or when a time course experiment is being carried out, so that all samples can be processed at the same time.

Protocol

- Harvest cells from one 100 mm tissue culture plate (or up to 10^7 cells).
- Spin cells down at 1200 rpm (500xg) for 10 minutes.
- Resuspend cells in 1.5 ml 1xPBS (transfer to an eppy tube).
- Spin for 5 minutes at 4000 rpm in microfuge.
- Resuspend cells in 100 ul 1xPBS
  (Note: Cell suspension can be frozen now and kept at -20^0C).
- Add 200 ul 1.5xLysis Buffer plus Proteinase K [Note: Proteinase K is labile and must be added fresh (make 10mg/ml Proteinase K in H_2O and add 15ul of this solution per ml of 1.5x Lysis Buffer)].
- Mix well by gentle pipetting up and down a few times.
- Incubate samples for 12 hours at 55°C.
- Spin samples briefly.
- Add 200 ul 1.5xLysis Buffer (without Proteinase K).
- Add 1 volume (500 ul) of phenol/$CHCl_3$/IAA and vortex well.
- Spin samples at high speed for 5 minutes in microfuge.
- Transfer top aqueous layer to new tube.
- Add 1 volume (500 ul) of $CHCl_3$/IAA and vortex well.
- Transfer top aqueous layer to new tube.
- Add 1/2 volume (200 ul) of 7.5 M ammonium acetate and 2 volumes (800 ul) of absolute ethanol.
- Mix well, by inverting the eppy tubes a few times (the DNA should immediately precipitate).
- Spin samples at high speed for 5 minutes in microfuge.
- Wash DNA pellet with 900ul cold 70% ethanol (stored at –20°C)[i.e. add 70% EtOH, vortex, spin 5 minutes (high speed in microfuge) and take off EtOH].
- Resuspend DNA into 100 ul 1x TE, quantitate and keep it at 4°C (do not freeze).

Note: For Southern-blots, we do not remove residual RNA, but instead, add 2 ul RNase (10mg/ml) to digestion reaction.

If residual RNA needs to be removed (e.g. if PCR is to be performed), follow the procedure below:

- Add 2 ul 10mg/ml RNase to entire sample and incubate for 30 minutes at 37°C.
- Bring volume up to 400 ul with 1x TE.
- Repeat phenol extraction above.

- Add 1/10 volume (40 ul) 3M sodium acetate and 2 volumes (880 ul) absolute ethanol.
- Mix well and Spin samples at high speed for 15 minutes in microfuge.
- Wash DNA pellet with 900ul cold 70% ethanol (stored at –20^{o}C)[i.e. add 70% EtOH, vortex, spin 5 minutes (high speed in microfuge) and take off EtOH].
- Dry and resuspend DNA (RNA free) into 100 ul 1xTE and keep it at 4^{0}C.

Solutions

1.5 x Lysis Buffer		50 ml
0.3 M Tris pH 8.0	(see Trizma table)	15 ml 1M Tris pH 8.0
0.15 M EDTA pH 8.0		15 ml 0.5M EDTA pH 8.0
1.5% SDS		7.5 ml 10% SDS

Add fresh Proteinase K (10mg/ml) up to 0.15 mg/ml [15 ul per ml]