Genomic and episomal DNA isolation protocols

Large scale using DNAzol™

This is used for isolating DNA from 60 to 100 million cells. Scale up as necessary.

1. Harvest cells by centrifugation at 4ºC in a table-top centrifuge at ~ 1,500 rpm for 10 min.
2. For cleaner results, wash the cells with cold 1 x PBS (PF) or 1 x TDB (BF) once.
3. Resuspend cells in 1 ml DNAzol completely by pipetting up and down a few times, and transfer to an Eppendorf tube.
4. Sit at RT for ~ 30 min (though the DNAzol instructions indicate a 5 min incubation is good enough, a longer incubation usually has higher yield).
5. Spin in a bench-top centrifuge at full-speed (~ 13,000 x g) for 10 min.
6. Transfer the supernatant to a new Eppendorf tube (it's important not to include any of the pellet to ensure the purify of the final DNA).
7. Add 500 ml of 100% EtOH, mix well and spin in a bench-top centrifuge at scale 5 for 5 min.
8. Wash with 70% EtOH once and spin at scale 2 for 2 min.
9. Decant the supernatant by inverting the tube on a piece of paper towel, use a vacuum aspirator to dry off any liquid on the Eppendorf tube wall.
10. Immediately add 30–50 μl of $T_{10}E_{0.1}$ (10 mM Tris*Cl, pH 8.0, 0.1 mM EDTA pH 8.0) to resuspend the DNA pellet.

There is 0.07 pg of genomic DNA per *T. brucei* nucleus: 7 μg per 10^8 cells. Because this method does not produce pure DNA, attempting to determine yield by measuring absorbance will be totally unreliable. Either use a specific fluorimetric method or simply assume the yield that should be obtained from the starting number of cells.

Small scale using DNAzol for PCR

Starting material for BF: 1–2 ml of 1.5–2 x 10^6 cells/ml (~ 0.2 μg DNA).

1. Spin cells at RT for 10 min at 1,800 rpm.
2. Decant media, leaving ~500 μl behind to resuspend parasites.
3. Transfer parasites to a 1.5 ml tube
4. Spin for 4 min at level 4 (2500g) and discard medium.
5. Add 0.500 ml of DNAzol
6. Lyse cells by repeated pipetting.
7. Incubate for 5 min at RT.
8. Add 0.250 ml of 100% EtOH
9. Invert 5-8 times until homogeneous. gDNA (and RNA) precipitates, but no white cloud is visible.
10. Incubate at RT for 5 min
11. Pellet DNA by spinning for 15 min at top speed (~ 13,000 x g) at 4°C.
12. Discard supernatant with a pipette (or carefully with aspirator)
13. Wash pellet with 70% EtOH at RT
14. Spin for 5 min at top speed.
15. Discard supernatant with a pipette (or carefully with aspirator)
16. Spin for 1 min at top speed.
17. Discard remaining 10-20 μl with a pipette.
18. Do not let DNA pellet dry.
19. Immediately resuspend it in 10-30 μl of EB buffer (QIAGEN) (about 20 ng/μl).
20. Use 1-2 μl for each PCR reaction.
21. Store DNA at 4°C.

This method also yields ‘dirty’ DNA, so note the comments at the end of the DNAzol protocol on page 1.

Genomic DNA isolation from trypanosomatid protozoa using LiCl

Ref: Rapid isolation of DNA from trypanosomatid protozoa using a simple 'mini-prep' procedure (1993) Medina-Acosta, E & Cross, G. Mol Biochem Parasitol 59:327-530.

This is for isolation of chromosomal and episomal DNA. DNA is good for restriction digestion and standard Southern blotting. This method also yields ‘dirty’ DNA, so note the comments at the end of the DNAzol protocol on page 1.

For 1.5 x 10^7 to 1.5 x 10^8 cells (1–10 μg DNA)

1. Harvest cells by centrifugation at 800–1,360 g for 10 min.
2. If necessary, resuspend cell pellet in small volume of 1 x TDB (BF) or 1 x PBS (PF) and transfer cells to an Eppendorf tube, then harvest cells.
3. Resuspend cell pellet in 150 μl of lysis buffer (TELT buffer) by sharply inverting (do not vortex) the tubes three times to completely resuspend the cells.
4. Sit at RT for 5 min, add equal volume (150 μl) of water-equilibrated phenol/chloroform and shake the tube slowly for 5 min by hand.
5. Separate the phases by centrifugation at 13,000 g for 5 min.
6. Transfer the aqueous phase to a separate tube, add 300 μl of 100% EtOH. Gently swirl the mixture for 15 sec and incubate at RT for 5 min.
7. Centrifuge at 13, 000 g for 10 min. Wash the pellet in 1 ml 100% EtOH at RT and spin again at 13,000 g for 5 min. Air dry or vacuum dry the DNA pellet.
8. Resuspend the DNA pellet in 100 μl of TE with 20 μg/ml pancreatic ribonuclease. Incubate at 37ºC for 30 min.

Solutions

TELT Buffer:

50 mM TrisCl, pH 8.0

62.5 mM EDTA, pH 9.0

2.5 M LiCl

4% (v/v) Triton X-100

TE buffer

10 mM TrisCl, pH 8.0

1 mM EDTA, pH 8.0

Isolation of genomic DNA for telomere blots

For PF, a 50 ml of culture of $2x10^7$ cells/ml is more than enough.
For BF, at least 200 ml of culture of $1.5x10^6$ cells/ml is necessary.

Harvesting cells

- Harvest cells by centrifugation in a table-top centrifuge at 1,500 rpm, 10 min at 4ºC
- Wash once with cold 1 x PBS (PF) or 1 x TDB (BF) once.

Isolation of genomic DNA *(T. de Lange protocol)*

Make sure that everything is absolutely free of traces of plasmids or probes. Buffers/solutions used for genomic DNA preps should not be used for plasmid preps. Also, take a new box of pipette tips, etc. Clean your pipetmen thoroughly before you start to remove plasmid droplets from the receptor end. Avoid doing plasmid preps on the same day on the same bench!

NEVER use a VORTEX mixer to suspend your DNA! Avoid shearing of the DNA, so treat it gently throughout the procedure! Resuspend the DNA with tips that have been cut off so that there is a large opening. Use a very clean (soap, ethanol) scissors or a razorblade to cut tips.

1. Resuspend cell pellet in 1 ml TNE
2. Squirt cell suspension into a 15 ml Eppendorf Phase Lock Gel Heavy tube containing 1 ml TENS/protK (freshly prepared)
3. Incubate overnight at 37°C
4. Add 2 ml PCI (phenol-chloroform-isoamyl alcohol) and mix GENTLY by rocking for a few minutes at room temperature (the phases should mix completely
5. Spin 10 min full-speed in a table-top centrifuge at RT
6. Pour off the water-phase into a 15 ml tube containing 2 ml of iso-propanol and 0.22 ml 2M NaAc, mix gently by inverting several times
7. Fish out the bundle of DNA with a blue tip and transfer to a 2 ml Phase Lock tube containing 0.3 ml TNE+100 mg/ml RNase A (DNase free!).
8. Incubate 30 min at 37°C
9. GENTLY resuspend using a blue tip with cut off tip (razorblade)
10. Incubate 2 h at 37°C
11. Add 0.3 ml TENS/protK, mix
12. Incubate 1 h at 37°C
13. Transfer to a spun-off 2 ml Phase Lock tube, add 0.6 ml PCI, mix by inverting and spin 10 min full speed

14. Transfer upper phase to new Eppendorf tube with 0.6 ml iso-propanol and 66 μl 2M NaAc mix by inverting
15. Fish out DNA bundle with a yellow tip, transfer to new tube with 100 μl $T_{10}E_{0.1}$
16. Incubate 30 min at 37°C, resuspend with yellow tip with cut off end (razorblade)
17. Incubate at 37°C until DNA is dissolved, or put at 4°C overnight. Store samples at –20 °C.

Solutions

PBS: phosphate buffered saline, working solution 1 x buffer, pH 7.3

10X stock solution, 1 liter:
80 g NaCl
2 g KCl
11.5 g Na_2HPO_4´$7H_2O$
2 g KH_2PO_4

TNE: 10 mM Tris pH 7.4
100 mM NaCl
10 mM EDTA
(autoclave)

TENS/protK:
10 mM Tris pH 7.4
10 mM EDTA
100 mM NaCl
1% SDS
100 mg/ml proteinase K
(autoclave TEN, add SDS after autoclaving, add proteinase K right before use –will eat itself up)

2 M Na-Ac pH 5.5 (autoclave)

PCI from Fisher BP1752-400, and keep separate for genomic preps.

Oliver's telomeric DNA isolation protocol for overhang assays

1. Grow 100–400 ml of bloodstream forms in HMI-9 to a density of $2x10^6$ /ml.
2. Precool centrifuge and rotor to 4°C.
3. Tubes intended for harvesting are washed with 70% ethanol and rinsed with sterile H_2O. I do this because people generally use these tubes for E. coli cultures and maxipreps.
4. Prepare 100 ml of 1x TDB and cool on ice.
5. Put TNE on ice (2 ml per sample).
6. Harvest cells by centrifugation at 4°C for 10 minutes at 3,500 rpm.
7. Wash 2x with ice-cold TDB.
8. Resuspend cells in 1ml TNE: transfer to an Eppendorf Phase-Lock tube.
9. Add 1ml of TNES + 200 µg Proteinase K (10 µl of 20 mg/ml stock solution).
10. Incubate overnight at 37°C. (DO NOT incubate at 55°C, as parts of the DNA will denature and increase background hybridization signal).
11. Add 2 ml of PCI (Phenol:Chloroform:Iso-Amyl Alcohol 50:48:2) and shake gently until homogenous.
12. Spin at 12,000 rpm for 5 min
13. Repeat PCI extraction.
14. Decant aqueous phase into a 15 ml Falcon tube containing 300 µl 3 M NaOAc pH 5.2 and 2 ml isopropanol. Gently invert tube to precipitate genomic DNA.
15. Prepare glass hooks by burning the tip of a Pasteur pipette into a little hook.
16. Gently fish the DNA bundle out of solution and wash DNA by dipping it into 75% ethanol.
17. Immediately transfer DNA (still on the hook) into 300 µl $T_{10}E_1$. It is crucial not to let the DNA dry on the hook: if it does, resuspension will be difficult.
18. Add 100 µg/ml DNase-free RNase A (30 µl of a 1 mg/ml RNase solution). Incubate 1 h at 37°C.
19. Add 200 µg Proteinase K and incubate overnight at 37°C.
20. Add 200 µl $T_{10}E_1$ and transfer to a 2 ml Phase Lock tube.
21. Add 500 ml PCI and shake gently by inverting the tube. Once homogenous, mix for 1 more min.
22. Centrifuge 12,000 rpm for 5 min at 4°C.
23. Pour aqueous phase into new Eppendorf tube, precipitate DNA by adding 40 µl 3 M NaOAc pH 5.2 + 800 µl isopropanol.
24. Centrifuge 2 min at 3,000 rpm at RT. Very carefully aspirate supernatant.
25. Wash 1x with 75% ethanol and resuspend pellet in 200 µl $T_{10}E_1$
26. Measure DNA concentration by fluorimeter.

Extraction of episomal DNA from procyclics

(*Hirt* method from Pradeep: EMBO J. 1993 Jun;12(6):2529-3)

Starting material: $3x10^7$ PF cells

1. Spin cells at 2,800 rpm for 10 min
2. Wash 1x in PBS
3. Resuspend cell pellet in 70 µl of Hirt-Buffer A
4. Add 80 µl of Hirt-Buffer B
5. Add 40 µl of 5M NaCl
6. Place tubes on ice and leave them in the cold-room for 16 h
7. Spin at 8,000g for 10 min (pellet: white 'cloud'; supernatant ≥130 µl)
8. Extract 3x with PCI (supernatant ≥110 µl)
9. Add 2 volumes of 100% EtOH (220µL) and place tubes at -70°C for 90 min.
10. Spin at top speed for 30 min at 4°C
11. Wash with 1ml of 70% EtOH
12. Spin at top speed for 15 min at 4°C
13. Air-dry for 10 min
14. Resuspend in 5 µl of EB buffer (QIAGEN)
15. 2-3 µl are used in the subsequent PCR reaction

Solutions

Hirt-Buffer A (10 mM Tris pH 9.0, 10 mM EDTA, 10 mM EGTA)

Tris 1M	pH 9.0	500 µl
EDTA 500 mM	pH 8.0	1 ml
EGTA 500 mM	pH 8.0	1 ml
H_2O		47.5 ml

Hirt-Buffer B (10 mM Tris pH 9.0, 10 mM EDTA, 10 mM EGTA, 2% SDS)

Tris 1M	pH 9.0	500 µl
EDTA 500 mM	pH 8.0	1 ml
EGTA 500 mM	pH 8.0	1 ml
SDS 20%		5 ml
H_2O		42.5 ml