

Isolation of *P. falciparum* genomic DNA
July 16, 2003

1. Remove extra media from 12-15 50 ml flasks.
2. Collect RBCs in a 500 ml conical tube, filling the remaining space in the conical with PBS.
3. Pellet RBCs by centrifugation at 2000 RPM for 5 minutes, **NO** brake.
4. Wash with pre-warmed 1x PBS (does not have to be sterile).
5. Repeat steps 3 and 4, 2 more times, transferring RBCs to progressively smaller tubes, ending with two 50 ml conicals.
6. After final centrifugation, remove 1x PBS. Lyse RBCs by resuspending the pellet in an equal volume of 0.1% saponin in 1x PBS (final concentration of saponin: 0.05%). After completely resuspending RBCs in 0.1% saponin/PBS, fill the conical with 1x PBS, to better visualize the parasite pellet after centrifugation. Do not add more than 1 volume of 0.1% saponin as this may lyse the parasites along with the RBCs.
7. Pellet parasites by centrifugation at 2000 RPM for 5 minutes, low brake.
8. Transfer supernatant to a new container if desired, or aspirate to remove. Wash parasite pellet with 1x PBS by resuspension and followed by centrifugation at 2000 RPM for 5 minutes, low brake.
9. Lyse parasites with 10 ml of 1x Lysis buffer/ 0.5 ml of parasite pellet overnight at 37°C. (4 x lysis buffer: 40 mM Tris-HCl, pH 8.0; 80 mM EDTA, pH 8.0; 2% SDS, add Proteinase K (PK-10 mg/ml stock) right before use. 1x Lysis Buffer: 10 mM Tris, 20 mM EDTA, 0.5% SDS, 50 ug/ml PK). Resuspend pellet in appropriate amount of ddH₂O before adding 4x lysis buffer and PK.

Pellet Volume	ddH ₂ O volume	4x Lysis Buffer	10 mg/ml PK
0.5 ml	7.5 ml	2.5 ml	50 ul
0.75 ml	11.175 ml	3.75 ml	75 ul
1.0 ml	15 ml	5 ml	100 ul
2 ml	30 ml	10 ml	200 ul

10. After incubation at 37°C overnight, dilute 1:2 with ddH₂O. If working with more than 10 ml, split solution into multiple 50 ml conical tubes and dilute to 20-25 ml per conical.
11. To clean up the DNA, phenol (Tris-saturated, **pH 6.6 +/- 0.2**, Fisher BP1750-400)/chloroform extract (equal volume of phenol:chloroform to aqueous volume) with a 50 ml Phase-lock tube, centrifuge 10 minutes, 3000 K, high brake in the table top centrifuge. Transfer the aqueous (top) phase to a new 50 ml conical.

12. Repeat step 11 2 times, for a total of 3 phenol/chloroform extractions, using 50 ml conicals, not phase-lock tubes. Separate the phenol/chloroform from the aqueous solution by centrifugation in the table top centrifuge for 10 minutes, 3000 K, low brake.
13. After the final phenol/chloroform extraction, transfer the aqueous phase to a new 50 ml conical. Add 5 ul of RNase to the solution and incubate at 37°C for 30 minutes.
14. Remove RNase by repeating step 11. Transfer aqueous phase to new 50 ml conical.
15. Precipitate DNA by adding 0.1 volume of 3 M NaOAC(pH 5.0). Mix well. Overlay with 2.5 volumes of ethanol. Mix gently by inversion.
16. Make glass hook with Pasteur pipet and remove DNA to 1.5 ml eppendorf tube.
17. Wash gDNA with 1 ml 70% ethanol.
18. Let gDNA dry at room temperature, uncapped, for 1-3 hours, depending on the size of the pellet.
19. Resuspend DNA in 0.5 -1 ml of ddH₂O overnight at room temperature.
20. Quantitate amount of gDNA that was isolated by taking an OD₂₆₀ and an OD₂₈₀.