

Preparing Genomic DNA from *P. falciparum*

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Reagents

- At least a 5% parasitized culture
- TSE buffer: 20 ml 5 M NaCl, 20 ml 1 M Tris-HCl, 100 ml 0.5 M EDTA, water to 1000 ml
- 10% Saponin in TSE made fresh
- 0.5 M EDTA
- 10% SDS
- 2 mg/ml RNase (store in -20°C)
- 10 mg/ml proteinase K
- 6 M Na-Perchlorate
- Phenol:Chloroform (1:1)
- Chloroform
- 100% Ethanol
- 70% Ethanol

Materials

- 50 ml conical tubes
- Allegra® 6 Series and GH-3.8 Swinging Bucket Rotor
- 37°C water bath or equivalent
- Eppendorf tubes
- Microcentrifuge

1. Grow parasites to 5-8% late trophs or schizonts in 50 ml to obtain 20-30 µg of DNA.
2. Spin the *i*RBCs at 1500 rpm for 5 min.
3. Resuspend the *i*RBCs with 30 ml TSE, add 0.5 ml of 10% Saponin, and incubate for 5 min at RT. The suspension should turn transparent and rosy.
4. Pellet down the parasites and RBC ghosts by spinning 3750 rpm, 15 min, 20°C.
5. Wash the pellet twice with 25 ml TSE, spin at 3750 rpm for 5 min between each wash.
6. Resuspend the pellet with 1 ml TSE, add 50 µl of 0.5 M EDTA, 50 µl of 10% SDS, 11.2 µl of RNase (2 mg/ml stock), 20 µl proteinase K (10 mg/ml). Incubate at 37°C for 2 hours.
7. Split this solution between two Eppendorf tubes and add 50 µl 6 M Na-Perchlorate, 700 µl Phenol:Chloroform (1:1) to each tube. Rock at room temperature for 20 min.
8. Spin for 1 min, 13,000 rpm in a microcentrifuge, room temperature.
9. Transfer the upper aqueous layer into a fresh tube, and extract the solution with equal volume of chloroform two times.
10. Transfer the upper layer into 2-3 1.5 ml Eppendorf tubes, add 1 ml cold 100% EtOH, and incubate in the -20°C for 10 min.
11. Spin the tube at 15,000g for 10 min in 4°C.
12. Wash the pellet with 70% EtOH twice, and dissolve the pellet in 200-300 µl of TE.
13. Measure the concentration of DNA.