gDNA Preparation using DNeasy kit

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Reagents

- At least a 5% parasitized culture
- Qiagen DNeasy kit (cat. No. 69504)
- 10% saponin/PBS made fresh
- 1X PBS

Materials

- 50 ml conical tubes
- Allegra® 6 Series and GH-3.8 Swinging Bucket Rotor
- Eppendorf tubes

A. Saponin Lysis

1. Grow parasites to 5-8% late trophs or schizonts in 50 ml (15% trophs from a 50 ml culture yields 7 µg gDNA).
2. Spin culture 1500 rpm, 3 min, room temperature, low brake.
3. Aspirate supernatant and resuspend pellet in 50 ml fresh 0.1% saponin/PBS (500 µl 10% in 50 ml 1X PBS). Incubate at room temperature for 10 min.
4. Spin 3000 rpm, 10 min, room temperature, low brake. Aspirate supernatant.
5. Resuspend pellet briefly in 10 ml 0.1% saponin/PBS.
6. Spin 3000 rpm, 5 min, room temperature, low brake and discard supernatant.

B. Qiagen DNeasy kit (Reference Qiagen booklet)

1. Resuspend pellet in 200 µl PBS and transfer to Eppendorf.
2. Add 20 µl Proteinase K and 200 µl buffer AL, mix by vortexing.
3. Heat 70°C, 10 min.
4. Add 200 µl 100% EtOH, mix by vortexing.
5. Transfer to spin column.
6. Spin 8000 rpm, 1 min.
7. Add 500 µl buffer AW1, spin.
8. Transfer to new collection tube, add 500 µl buffer AW2, spin 14,000 rpm, 3 min.
9. Transfer to Eppendorf and elute twice with ~75 µl buffer AE, spin 8000 rpm, 1 min.