

Plasmid (virus) prep from (50ml culture) Sulfolobus

1. Pellet cells (10 minutes at 5000rpm) and resuspend in 0.8 ml solution 1 (see below)
2. Add 1.6 ml solution 2, mix by shaking 5 times, put on ice for 5 minutes.
3. Add 1.2 ml ice-cold solution 3, vortex briefly and leave on ice for 3-5 minutes.
4. Spin mixture for 30 minutes at 20,000rpm in Sorvall centrifuge (SS-34), place supernatant in a new (phenol resistant) tube.
5. Add 1 volume (4ml) Phenol, shake 30-40 times, spin 30 minutes at 20,000rpm in SS-34 rotor.
6. Decant aqueous phase (and a little bit of the interface) and add 1 volume Phenol/Chloroform/Isoamyl-alcohol to it, shake 30-40 times, spin for 20 minutes at 20,000rpm in SS-34 rotor.
7. Decant aqueous phase and add 1 volume Chloroform to it, shake 30-40 times, spin for 10 minutes at 20,000rpm in SS-34 rotor.
8. Remove aqueous phase and add 0.7 volumes Isopropanol to it. Leave for 10 minutes at room temperature, then spin for 30 minutes at 20,000rpm in SS-34 rotor.
9. Wash pellet with 1ml 70% ethanol and allow to air dry.
10. Dissolve pellet in 50 microliters TE + RNase (10µg/ml)
11. Cut 2-5 µl of plasmid with restriction endonuclease

Solution 1

50 mM Glucose
25 mM Tris/HCl pH 8.0
10 mM EDTA

Solution 2

0.2M NaOH
1% (w/v) SDS

Solution 3

60 ml 5M potassium acetate solution
11.5 ml Acetic Acid
28.5 ml ddH₂O