

Bisulfite Modification of Genomic DNA

A. Reagents

- Sodium metabisulfite powder (ICN Biomedical).
- Hydroxyquinone crystals (ICN Biomedical).
- 10M NaOH
- 3M NaOH
- Paraffin oil
- Geneclean kit (BIO 101)
- 20 mg/ml glycogen (Boehringer) (store @ -20°C)
- 10M ammonium acetate (made up clean with sterile water, but not autoclaved)
- 100% ethanol
- 75% ethanol
- Sterile DNase-free water.
- Plugged tips

B. Procedure

1. Dilute or transfer <math><1\ \mu\text{g}</math> of extracted genomic DNA in 20 μl H₂O to a 1.5 ml screw cap and shear by pipetting with a plugged tip. Don't overload the reaction with too much DNA.
2. Add 2 μl of 3M NaOH to denature the gDNA in 0.3N NaOH for 20 min at 75°C. This is harsh and will destroy some DNA but is absolutely essential for efficient modification.
3. Prepare fresh 4.8 M sodium metabisulfite (pH 5.0) by adding 4.55 g of Na₂S₂O₅ and 0.4 ml of 10 N NaOH to 8.2 ml H₂O and mix gently by inversion. Do not vortex or heat! Some insolubles at the bottom of the tube are OK.
4. Prepare fresh 10 mM hydroquinone by adding 11 to 10 ml H₂O. Pipette the crystals out of the weighing dish with a plugged tip and some sterile water. Mix gently by inversion.
5. Transfer the denatured gDNAs to ice and add to each sample 250 μl of 4.8 M sodium metabisulfite and 14 μl of 10 mM hydroquinone. Mix gently then cover the reaction with paraffin oil.
6. Incubate the reactions for 4-5 hr at 55°C, shielded from light.
7. Purify the modified DNA using the Geneclean kit. Can add glassmilk directly to the bisulfite reaction and go from there. Two washes with NEW wash are sufficient. The first is always a pain due to residual oil. Elute DNA twice with 20 μl of sterile water each time. Some glassmilk in the eluate is OK.
8. Add 4.5 μl of 3M NaOH to desulfonate the modified DNA in 0.3N NaOH for 20 min at 37°C.
9. Add 11 μl of 10M NH₄ acetate, 2 μg glycogen and 110 μl 100% ethanol to precipitate each modified and desulfonated sample overnight @ -20°C.
10. Pellet the DNAs for 30 min at full speed in a 4°C microfuge. Wash pellet once with 75% ethanol, air dry briefly and resuspend in 20 μl H₂O. Store @ -20°C or -80°C. Use 1-4 μl per PCR reaction to amplify prior to sequencing or MsSNuPE.