

Bisulfite Modification

Day 1

Prepare Fresh:

Hydroquinone : 55mg/5 ml H₂O, invert gently to get into soln*, then dilute 1:10 for working soln.
Sodium (Meta)Bisulfite : 1.88g / 5ml H₂O, invert gently to get into soln*,
then pH to 5.0 with ~5 drops 19.5M NaOH

*DO NOT VORTEX, SHAKE OR STIR AS THIS WILL INCREASE OXIDATION!!

- Dilute 1-2 ug DNA into 50ul H₂O
- Add 5.5ul 2M NaOH (diluted fresh from stock) and mix
- Incubate 37 ° C for 10 min.(complete denaturation is necessary for conversion)
- Add 30 ul diluted hydroquinone soln
- Add 520 ul Bisulfite soln.
- Mix by inversion or vortex briefly
- Add ~200 ul mineral oil to top, Incubate 50° C for ~16 hr. (In this case longer is not better)

Day 2

- Take off oil using transfer pipette
- Add DNA purification resin and follow manufacturer's instructions for use. We use the Promega Wizard DNA Clean-ups Kit. Others would probably work as well. The objective here is primarily desalting.
- Elute DNA from resin with 50ul H₂O heated to 50°-70°C
- Add 5.5 ul 3M NaOH (diluted fresh from stock)
- Incubate 5 min. at room temp.
- Add 33 ul 10M NH₄OAc, 250-300 ul EtOH
- precipitate DNA, -20°C
- pellet DNA 17kxg , 25 min, 5°C, decant EtOH (be careful! the pellets are difficult to see)
- wash pellet in 70% EtOH, decant EtOH
- air dry pellet or speed vac
- resuspend pellet in 40 ul H₂O
- store indefinitely at -80° C
- use 1-2 ul per PCR reaction for MSP or bisulfite sequencing

Reagents:

Hydroquinone - Sigma H9003
Sodium Meta Bisulfite - Sigma S8890
Promega Wizard DNA Clean-Ups - Cat. No. A7280