

CHEAP CLEAN-UP PROTOCOL

To clean BigDye reactions:

1. Combine and mix MgCl₂/ethanol cocktail.
2. Add 148 µL of cocktail to 20 µL of BigDye reaction.
3. Vortex or mix well.
4. Incubate at room temperature in the dark for 20 minutes.
5. Spin at 6 kRPM for 30 minutes.
6. Carefully pour off supernatant.
7. Air dry on a Kimwipe or pulse spin upside down.

MgCl₂/ethanol

- 1 µL 0.5M MgCl₂
- 1000 µL 70% ethanol

To prep samples for ABI 3100 sequencer:

1. Add 15-17 µL of formamide to cleaned, dried BigDye reactions.
2. Spin to collect sample at the bottom of the tube. Make sure there are no air bubbles in the sample.
3. Denature at 95°C for 3 minutes.
4. Incubate at -20°C for 5 minutes.
5. Transfer all of the sample to an ABI 96-well plate. Check to make sure there are no air bubbles in the samples. Start loading plate at position A1.
6. Cover plate with rubber top.
7. Spin to collect sample at the bottom of the plate.

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