

Ethanol/EDTA/Sodium Acetate Precipitation

Reference: BigDye Terminator v1.1 Cycle Sequencing Kit Protocol, Applied Biosystems 2002.

BigDye Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems 2002.

ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits, Original and Version 2.0,

Applied Biosystems, 2000.

Step	Action
1	Prepare a 1.5mL microcentrifuge tube containing: 2 μ L of 3M sodium acetate, pH 5.2 and 2 μ L of 125mM EDTA, pH 8.0.
2	Pipette the contents of each sequencing reaction into the tube of sodium acetate/EDTA. Vortex briefly.
3	Add 50 μ L of 100% ethanol to each tube. Vortex and spin briefly.
4	Incubate at room temperature for 15 min.
5	Centrifuge for 20 min at maximum speed. Carefully aspirate the solution from the pellet with a pipette tip and discard.
6	Quick spin again and remove any residual solution, if required. IMPORTANT! The supernatants must be removed completely as unincorporated dye terminators are dissolved in them.
7	Rinse the pellet by adding 250 μ L of 70% ethanol. Vortex briefly.
8	Spin for 5 min at maximum speed. Carefully aspirate liquid away from the pellet. Quick spin again and carefully remove any remaining solution, if required.
9	Dry the pellets completely. Air dry on the bench or in a laminar flow.

Note: If you have problems with 'Dye Blobs' then try repeating the 70% wash step (Step 7) or increase the volume of 70% ethanol used in this step.