

Ethanol/EDTA 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: 5 μ L of 125mM EDTA, pH 8.0.
2	Pipette the contents of each sequencing reaction into the 1.5mL microcentrifuge tube. Vortex and spin briefly.
3	Add 60 μ L of 100% ethanol to each tube. Vortex and spin briefly.
4	Incubate at room temperature for 15 min.
5	Spin for 20 min at maximum speed. Slowly aspirate the solution away from the pellet.
6	Quick spin again and carefully 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 hood.

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.