EDTA/NaOAc/Ethanol Precipitation of Cycle-Sequenced Products
Prepared by Amy Smith

This protocol continues from protocol **BDTv3.1 Cycle-Sequencing**

After Cycle Sequencing is complete, remove plate from thermocycler and place at 4˚C or –20˚C until ready to precipitate: Do not let plate sit in thermocycler overnight (low sample volume leads to easy evaporation).

EDTA helps to stabilize extension products during precipitation, and also helps to wash out unincorporated dyes from the completed reaction. NaOAc helps to prevent loss of small fragements due to EDTA, but does not effectively remove all unincorporated dyes. I only recommend using NaOAc if good signal from base 1 is absolutely necessary (very long fragments or no sequence overlap available). If NaOAc is not necessary, refer to the protocol **EDTA/Ethanol precipitation of Cycle-Sequenced Products**.

This protocol assumes a 10µl cycle-sequencing reaction. For larger (or smaller) volumes, alter volumes below proportionately.

1. To each well add 1µl 125mM EDTA to each well. Make sure EDTA dissolves into samples. Mix with pipette tips.
    *EDTA is a known XXX, handle with care. Prepare 125mM ahead of time: dissolve into 18MΩ water and filter sterilize. EDTA takes about 1hr to dissolve. EDTA can be dispensed into sterile boats or 0.2ml tubes for multi-channel pipetting, but do not save used aliquots.

2. Add 1µl 3M NaOAc to each well. Make sure NaOAc dissolves into samples. Mix with pipette tips.
    *NaOAc should be prepared regularly, but need not necessarily be made fresh. NaOAc is a known XXX, and is at pH 4.8 by glacial acetic acid. NaOAc solution is very vaporous; do not leave open on benchtop for prolonged periods.

3. Add 25µl 100% EtOH (from freezer) to each well. Mix with pipette tips.
    *you may use 95% EtOH, but will need to add more (32µl) to maintain final EtOH concentration (67-71%).

4. Replace sealing tape, seal very well and vortex lightly. Incubate at room temp for 15min.

5. Spin @ 2500g 30min.

6. Immediately flick plate contents into sink and lightly tap dry on paper towels.
    *if you do not immediately empty plate once spin has finished (5min or less), give samples a quick spin (2500g, 2min) to re-precipitate pellet

7. Invert plate onto paper towel and spin 185g for 1min to dry.

*This is to remove any excess NaOAc, which is a critical step. Start timer immediately.

8. Add 35µl 70% EtOH to each well and mix with pipette tips.
*make EtOH fresh to ensure accurate concentration.

9. Spin @ 2500g, 15min

10. Flick plate contents into sink, invert plate onto paper towel and spin 185g for 1min to dry.
*start timer immediately.

11. Resuspend samples in 10µl formamide. Cover plate with sealing tape, vortex thoroughly (15sec) and denature in thermocycler (5min@95˚C, 4˚C for a minimum of 3min).
*plates waiting to be run may be stored at –20˚C, but probably should be re-denatured before running on the genetic analyzer. Stored plates should be wrapped in foil to protect dyes from light.