

Cleaning Genotyping Samples using Ethanol Precipitation in 96-Well Plates

To precipitate in 96-well plates:

1. Add the following to each well of a deepwell [Phenix] plate:
 - 1- 8 μ L of your PCR product
 - 16 μ L deionized water
 - 64 μ L of non-denatured 95% ethanol

**final ethanol concentration should be 60 +/- 3%*
2. Seal the plate by applying a piece of adhesive-backed aluminium foil tape. Press the foil onto the plate to prevent any leakage.
3. Invert the plate a few times to mix.
4. Leave the plate at room temperature for 15 mins. to precipitate the extension products.

Note

 - *precipitation times <15 mins: loss of short precipitation products*
 - *precipitation times >24 hours: increase precipitation of unincorporated dye terminators*
5. Place the plate in the Eppendorf 5804 centrifuge and spin it at 2000 x g (RCF) for 30 minutes [program 1].
6. Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel folded to the size of the plate.

Important: Proceed to step 7 IMMEDIATELY. If not possible, then spin the plate for 2 minutes more immediately before performing the next step.
7. Rinse the pellet by adding 150 μ L of 70% ethanol to each well.
8. Seal the plate with adhesive tape.
9. Invert the plate a few times.
10. Remove the adhesive tape and invert the plate onto a paper towel .
11. Place the inverted plate with the towel into the tabletop centrifuge, and spin at 700 x g for 1 minute [program 4].
12. Remove the plate and discard the paper towel.

Note: Pellets may not be visible
13. Samples can be stored dry at this step, or you can proceed to sample preparation for running in the genotyper.