

Purpose: To purify and quantify PCR product for use in the Affymetrix 100K genotyping protocol.

Procedures: This procedure is taken from the Affymetrix 100K genotyping manual. The manual should also be consulted for any questions about the protocol.

A. Reagents needed

- Buffer EB (1000 mL): QIAGEN P/N120002
- Molecular biology water

B. Equipment needed

- Manifold - QIAvac multiwell unit: QIAGEN P/N 9014579, or QIAvac: QIAGEN P/N 19504
- MinElute 96 UF PCR Purification Kit: QIAGEN P/N 28051 (four plates), or P/N 28053 (24 plates)
- Biomek Seal and Sample Aluminum Foil Lids: Beckman P/N 538619
- Jitterbug 115 VAC: Boekel Scientific P/N 130000
- Vacuum Regulator for use during the PCR clean up step. QIAGEN Vacuum Regulator (use with QIAvac manifolds): QIAGEN; P/N 19530*
- 12-strip tubes and 12-strip caps
- UV spec

C. Preparation

1. Connect a vacuum manifold to a suitable vacuum source able to maintain ~800 mbar, e.g., QIAvac 96 or QIAvac Multiwell Unit (QIAGEN). Place a waste tray inside the base of the manifold. This apparatus is on the bench behind the UV spec.
2. Place a MinElute 96 UF PCR Purification Plate on top of the manifold. Cover the entire plate with the aluminum cover. Cut off a strip for the wells you will use. Mark the row with a marker so we know it is used.

D. Purification

1. Turn on the vacuum. Adjust the regulator to 800-900 mbar.
2. Pipet 200 μ L of each sample onto the appropriate well. After going through all the samples, the water should have gone down far enough that you can pipette the remaining
3. 200 μ L onto each well.
4. Maintain the ~800 mbar vacuum until the wells are completely dry.
5. Wash the PCR products by adding 50 μ L molecular biology water and dry the wells almost completely completely (taking about 20 minutes). **Repeat this step 3 times total.**
6. Switch off vacuum source and release the vacuum.
7. Carefully remove the MinElute plate from the vacuum manifold.
8. Gently tap the MinElute plate on a stack of clean absorbent paper to remove any liquid that might remain on the bottom of the plate.
9. Add 40 μ L EB buffer to each well. Cover the plate with PCR plate cover film.

10. Moderately shake the MinElute plate on a plate shaker, e.g., Jitterbug (Boekel Scientific, model 130000), for 5 minutes.
11. Recover the purified PCR product by pipetting the eluate out of each well into a fresh 12-strip.. For easier recovery of the eluates, the plate can be held at a slight angle. Make sure you take 40 μL off each well.

E. Quantitation

1. Add 4 μL of purified PCR product to 156 μL molecular water (40-fold dilution) and MIX WELL. Do this in a 12-strip tube and vortex (covered) to mix. Prepare two samples of 4 μL EB buffer added to 156 μL molecular water as blanks. Cover the strip with strip tube caps to prevent evaporation.
2. Using one of the blanks, zero the spectrophotometer. Measure the samples (10 μL each) and then check that the second blank measures 0.
3. Using the observed DNA concentration, calculate the total amount of purified PCR product left in the original strip tubes. (concentration ($\mu\text{g}/\mu\text{L}$))*36 μL .
4. Calculate the volume of EB to add to get a concentration of 40 μg per 45 μL . ((45 μL * amt of DNA / 40 μg) – 36 μL)
5. Add this amount of EB to the appropriate tubes. Make sure to cover the tubes again if not proceeding immediately to fragmentation.