

Purpose: To fragment and label amplified DNA 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

- Fragmentation Reagent (DNase I): Affymetrix, P/N 900131 *
- 10 X Fragmentation Buffer: Affymetrix, P/N 900422 *
- Molecular Biology Water: Bio Whittaker Molecular Applications/Cambrex, P/N 51200
- 4% TBE Gel: BMA Reliant precast (4% NuSieve 3:1 Plus Agarose); P/N 54929
- All Purpose Hi-Lo DNA Marker: Bionexus, Inc.; 50 - 10000bp; P/N BN2050
- Gel Loading Solution: Sigma; P/N G2526
- GeneChip DNA Labeling Reagent: Affymetrix; P/N 900430, *
- Terminal Deoxynucleotidyl Transferase (30 U/ μ L): Affymetrix; P/N 900426 *
- 5X Terminal Deoxynucleotidyl Transferase Buffer: Affymetrix; P/N 900425 *

* These reagents are available in the Xba I and Hind III GeneChip® Centurion Assay Kits, P/N 900482 and P/N 900483, respectively

B. Equipment needed

- Gene Amp PCR System 9700
- 12-strip tubes and caps

C. Preparation

1. Take out the fragmentation buffer, labeling reagent, and TdT buffer and thaw on ice. This step should be started during quantitation for optimal use of time.
2. Pre-heat the post-PCR thermal cycler (on the right) to 37C by running the 37C hold program.
3. Aliquot 45 μ L of each positive sample (eliminate the negative controls) into fresh 12-strip tubes. Put on ice.

D. Fragmentation

1. Add 5 μ L 10X Fragmentation Buffer to each sample in the tubes ON ICE and vortex at medium speed for 2 seconds (with tubes covered).
2. Get fragmentation reagent from the freezer and keep in enzyme block.
3. Prepare a dilution of the fragmentation reagent as follows, based on the enzyme concentration on the stock tube. If the concentration is not given below, check the 100K manual from Affy for how to calculate this step.

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| Fragmentation Reagent | 3 μ L | 2 μ L |
| 10X Fragmentation Buffer | 15 μ L | 15 μ L |
| Water | 132 μ L | 133 μ L |
| Total | 150 μ L | 150 μ L |

| Reagent | 2 units/ μ L | 3 units/ μ L |
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4. Add 5 μ L of diluted Fragmentation Reagent (0.04 U/ μ L) to each sample tube ON ICE. Pipet up and down several times to mix. DO THIS STEP QUICKLY.
5. Cover the tubes with strip caps and seal tightly.
6. Vortex the tubes at medium speed for 2 seconds, and spin briefly.
7. Place the fragmentation plate in pre-heated thermal cycler (37°C) as quickly as possible.
8. Run the “frag35” program (37C 35 min, 95 C 15 min, 4C hold)
9. After the run, spin the tubes briefly.

E. Gel run

1. Take a fresh 4% gel from the box. Peel off the two brown strips at the bottom.
2. Remove the cover of the gel.
3. Firmly attach the gel tray to the middle portion of the gel rig. Make sure the wells are away from you.
4. Add 1X TBE to cover the lips of the tray.
5. Aliquot 4 μ L of gel loading solution into 12-strip tubes equal to the number of samples to run.
6. Take 4 μ L of each fragmentation product and mix with the gel loading solution in the strip tubes.
7. Put 3 μ L marker in the first lane, and 8 μ L samples in the remaining lanes.
8. Run at 120V for 1 hr. Make sure the black lead is closer to the wells (negative, repels the DNA). While gel is running, you should start labeling.

F. Labelling

1. Prepare Labeling Mix as master mix ON ICE and vortex at medium speed for 2 seconds. Prepare the mix for the number of samples you have plus 1.

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| Amounts for 1 reaction: | |
| 5X TdT Buffer | 14 μ L |
| GeneChip DNA Labeling Reagent | 2 μ L |
| TdT (30U/ μ L) | 3.5 μ L |
| Total | 19.5 μL |
2. Aliquot 19.5 μ L of Labeling Master Mix into each tube containing 50.5 μ L of fragmented DNA .
3. Cover the tubes with strip caps.
4. Vortex at medium speed for 2 seconds, and briefly spin the plate.
5. Put on the thermal cycler and run the “labeling” program (37C 2 hrs, 95C 15 min, 4C hold)
6. Briefly spin the tubes when the program is done. The tubes can be stored at -20C if not proceeding to making chipload.