

Purpose: To ligate adaptor to digested DNA for use in the Affymetrix 100K genotyping protocol. This protocol is written for doing one chip (either Xba or Hind)

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

As this procedure processes DNA before PCR, precautions regarding contamination with template (human) DNA should be stringently observed.

This procedure should only be performed for one enzyme (XbaI or HindIII) at a time to avoid confusing samples.

A. Reagents needed

- T4 DNA Ligase: New England Biolab (NEB); P/N M0202L
- T4 DNA Ligase Buffer: New England Biolab (NEB); P/N B0202S (*)
- H₂O (Molecular Biology Water): BioWhittaker Molecular Applications/Cambrex; P/N 51200
- Adaptor Xba (5 μ M) Affymetrix P/N 900410(*) **OR**
Adaptor Hind (5 μ M) Affymetrix P/N 900410 (*)

*The T4 DNA ligase buffer comes with the T4 DNA ligase. The Adaptors come with the GeneChip® Centurion Assay Kits, P/N 900482 and 900483, respectively

B. Equipment needed

- 2 UV-sterilized PCR hoods
- 12-strip caps
- Thermal cycler

C. Preparation

1. Sterilize both PCR hoods with UV light for 15 minutes. Flip pipettes in hood and resterilize for another 15 minutes.
2. Thaw T4 DNA ligase buffer on ice for at least 1 to 1.5 hours. If digestion was done on the same day, you can start thawing the buffer when you set up the digestion.
3. Thaw the adaptor at room temperature. When thawed, put on ice.
4. Put the 12-strip tubes from the digestion into the PCR staging hood **AFTER** sterilization.
5. Vortex and spin briefly the buffer and adaptor to make sure they are well mixed.

D. PCR clean hood preparation

(This section is performed in the PCR clean hood, where no template DNA is ever present. Currently, it is the AirClean 600 near the door.)

1. Take T4 DNA ligase out of freezer and put on ice.
2. In the hood, prepare Ligation Master Mix for the number of samples that were digested plus 1 extra.

Amounts for 1 reaction:	
Water	0.625 μ L
T4 DNA ligase buffer	2.5 μ L
Adaptor (Xba or Hind)	1.25 μ L
T4 DNA ligase	0.625 μ L
Total	5 μL

E. PCR Staging hood procedure

This procedure is performed in the PCR Staging hood (brown Labconco hood against the wall).

1. Vortex the master mix on the vortexer in the hood to make sure it is well mixed.
2. Aliquot 5 μ L of the Ligation Master Mix to each tube that digestion was performed in.
3. Cover tubes with a new row of strip caps.
4. Vortex in the hood, and spin briefly.

F. Reaction run

1. Place the tubes in the "NO PCR" thermal cycler (the one on the left).
2. Run program "ligation" (16C 2hrs, 70C 20min, 4C hold)
3. When the run is done, proceed to PCR or store sample at -20C.