

**Purpose:** To prepare fragmented and labeled DNA for chipload 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

- 5M TMACL (Tetramethyl Ammonium Chloride): Sigma; P/N T3411
- 10% Tween-20: Pierce; P/N 28320 (Surfactamps); diluted to 3% in molecular biology grade water
- 12X MES (see Affy protocol for details on how to make)
- DMSO: Sigma; P/N D5879
- EDTA: Ambion; P/N 9260G
- Denhardt's Solution: Sigma; P/N D2532
- HSDNA (Herring Sperm DNA): Promega; P/N D1815
- Human Cot-1: Invitrogen; P/N 15279-011
- Oligonucleotide Control Reagent: Affymetrix; P/N 900440, available in the Xba I and Hind III GeneChip® Centurion Assay Kits, P/N 900482 and P/N 900483, respectively

B. Equipment needed

- 1.5 mL Eppendorf tubes

C. Procedure

1. Label the 1.5 mL Eppendorf tubes for chipload (CHIPLO) according to lab conventions.
2. Prepare the Hybridization Cocktail Master Mix as follows. Make enough for one plus the number of samples being processed.

Amount for 1 sample	
12X MES	12 $\mu$ L
100% DMSO	13 $\mu$ L
50X Denhardt's Solution	13 $\mu$ L
0.5M EDTA	3 $\mu$ L
10 mg/mL hsDNA	3 $\mu$ L
Oligonucleotide Control	2 $\mu$ L
Human Cot-1 (1mg/mL)	3 $\mu$ L
Tween-20 (3%)	1 $\mu$ L
5M TMACL	140 $\mu$ L
<b>Total</b>	<b>190 <math>\mu</math>L</b>

3. Aliquot 190  $\mu$ L into Eppendorf tubes.
4. Add 70  $\mu$ L of labeled DNA into each tube.
5. These CHIPLO tubes can be used immediately or stored at -20C.