ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: Klenow labelling of DNA		**PAGE: 1 of 1**
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1. PURPOSE

This procedure describes labelling of DNA using Klenow enzyme.

2. SCOPE

Klenow labelling is the preferred method of cydye labelling when DNA (usually PCR product) is the starting material.

3. MATERIALS

Klenow enzyme (Roche;cat# 1008404)
Cy3-dUTP (Amersham Pharmacia cat# PA53022)
Cy5-dUTP (Amersham Pharmacia cat# PA550220)
100mM dNTP set (Sigma; cat#dNTP-100A)
Hexanucleotide mix (Roche; cat# 1277081)

4. PREPARATION

- dNTP mix
- Take 1.6ul of 2.5mM dATP, dGTP, dCTP
- Add 2.2ul of 2.5mM dTTP
- Split into 2 equal volumes of 1.9ul each per tube
- Add 0.3ul Cy3-dUTP (1mM) to one tube and 0.3ul Cy5-dUTP to the other tube and keep away from light

5. PROCEDURE

Caution: Keep light out as much as possible.
Make sure the DNA to be labeled is in a 15ul volume (10ng - 3µg)
Denature the DNA by heating to 95^{O}C for 5 min and then putting on ice
Add 2ul hexanucleotide mix
Add 2ul dNTP mix (see below)
Add 2ul Klenow
Mix by pipetting and shake by hand
Incubate at 37^{O}C for at least 2 hrs (up to 20hrs to increase yield)
Stop reaction with 2ul 0.5M EDTA 8.0
Purify the probe using either the Millipore Multiscreen Plate or the Qiagen PCR purification kit.
Determine incorporation using the nano-drop.