

Sample Labeling

Step 1: Fluorescent Labeling of gDNA

Kit: Agilent SureTag DNA Labeling Kit

1. Set the PCR thermocycler on the AGLB program and allow the block to heat up to 94 degrees Celsius.
2. Thaw the samples on ice.
3. Dilute all of the test samples and reference DNA in a separate 1.5 ml eppendorf tube to the lowest concentration (20ul) of the samples with nuclease-free water.
4. From the diluted samples calculate the volume of DNA to obtain 1 ug of DNA. Add the appropriate volume in an 8 strip PCR tube containing 2.5 ul of random primers and the appropriate amount of water to a total volume of 15.5 ul of total reaction volume per sample. Place a cap and spin the reaction mix down.
5. For the reference sample labelling, if you have 8 test samples, then do separate labelling reaction for 8 reference samples. Place a cap on it and spin the samples down.
6. Place the samples in the PCR thermocycler and run the incubation at 94 degrees Celsius for 10 minutes and then 4 degrees indefinitely, until ready to proceed to the next step.
7. **DO STEP 7 IN DARK.** While it's incubating, thaw the reagents listed below. Prepare two labeling master mixes, one for the test samples (Cyanine-3 dUTP's) and the other for the reference sample (Cyanine-5 dUTP) in the following manner per sample in separate tubes. For 8 samples, make 2 separate master mix for 9 reactions, one using Cy3 and the other using Cy5. Store them on ice until ready.

Reagents	x 1
5X buffer	5 ul
10 X dNTP	2.5 ul
Cy3/Cy5	1.5 ul
Exo(-) Klenow	0.5 ul

8. Remove the samples from the thermocycler and spin the samples down. Remove the caps for the test samples and add 9.5 ul of the master mix containing Cyanine-3 dUTP to each of the samples. Place a fresh cap and spin them down.
9. Repeat step 8 with the reference samples, except add 9.5 ul of the master mix containing Cyanine-5 dUTP.

Place the tubes in the PCR thermocycler and proceed to the next step in the program. Incubate the samples at 37 degrees Celsius for 2 hours, 65 degrees Celsius for 10 minutes, and 4 degrees Celsius indefinitely.