Labeling of microbial community DNA for microarray hybridization

1. Prepare dNTP mix: (using 100 mM dNTPs)

   5 µl each of dA/G/CTP

   2.5 µl of dTTP

   82.5 µl DEPC Water

2. Prepare the DNA/ Random primer mixture:

Random primer (Life Technologies, random hexamers, 3 µg/µL)	5.5 µL
Target or control DNA	29.5 µL
Total volume	35 µL

(1) Transfer 5.5 µL of Random primer to a PCR tube.

(2) Transfer 29.5 µL DNA to the Random primer. Usually, 500 ng to 1000 ng of DNA should be used. Adjust volume to 29.5 µL with water; or if the original concentration of a sample is very low, the DNA can be concentrated.

(3) Mix DNA and Random primer thoroughly and incubate at 99.9 °C for 5 min (using thermocycler)

(4) Immediately chill tubes on ice.

3. Prepare labeling premix

In a separate tube, for each reaction combine:

10X buffer (included with the klenow)	5 µL
dNTP mix	2.5 µL
Klenow (imer)	1 µL
CyDye* (25 nM)	0.5 µL
H_2O	6 µL
Total	15 µL

* CyDyes are light sensitive – dispense in the dark room. This can be added last

(1) Transfer 15 µL of the labeling premix to the DNA/ Random primer mixture and mix well.

(2) Incubate the reaction at 37 °C for 6 hrs.

(3) Heat-inactivate the enzyme by incubating the reaction at 95 °C for 3 min and then cool at 4 °C

*Labeled DNA can be kept at 4°C in the thermocycler if labeling is done overnight.

4. Purification

(1) Purify labeled DNA with Qiagen QIAquick Kit as specified by the manufacturer;

(2) Elute the DNA using 100 µL H_2O or EB buffer

(3) Check CyDye incorporation using Nanodrop

Minimum dye incorporation: pmol > 50 (pmol/µL * total µL)

[for example: Labeled DNA eluted with 100 µL of EB buffer and the

pmol/µL is 0.8; 100*0.8=80 – labeling is fine, proceed with hybridization]

5. Dry using a Speed Vac

Program: Temp. 45 °C, Run 2 hours, Vacuum (Level) 5.1 (preset)

Press "Auto Run"

Pellet should be dry at the end of the run.