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Working

Enzo's CGH Labeling Kit for Oligo Arrays

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
PB binding buffer	19066	Qiagen

Denature DNA and anneal random primers

1 pipette the following in a nuclease free thermocycler strip:
a. DNA X µl
b. water 19 – X µl (Vial W)
b. Primers/Reaction buffer 20 µl (Vial 1)

Total volume: 39 µl

2 Add cap, flick to mix contents and briefly spin down

3 Heat at 99 °C for 00:10:00

4 Place in a cooled 96 well rack on ice 4 °C for 00:05:00

5 Centrifuge briefly

Extend Primers with Klenow Exo-DNA Polymerase

6 Add, while samples are on ice 4. °C :
a. To test sample 10 µl Cy3 (Vial 2)
b. To reference sample 10 µl Cy5 (Vial 3)
c. Klenow Exo-DNA Pol 1 µl (Vial 4)
Total 50 µl

7 Add cap, flick to mix contents and briefly spin down

8 Incubate at 37 °C 04:00:00 for

9 Add 5µl of Stop Buffer (Vial 5), mix, and briefly centrifuge

Removal of uncoupled nucleotides

10 Transfer labelled DNA's to 1.5 ml reaction tubes and keep Cy3 and Cy5 labelled DNA's separated

11 Add 275 µl of Binding Buffer (PB)

PB binding buffer
by Qiagen
Catalog #: 19066

12 Load column and centrifuge for 60 seconds at 16,000g and discard flow through

13 Add 650 µl of Wash Buffer (PE), centrifuge 60 seconds at 16,000g and discard flow through

14 Centrifuge for 60 seconds at 16,000g, discard centrifuge tube and place column in fresh 1.5 ml centrifuge tube

15 Add 10.5 µl of Elution Buffer (EB) (middle of the filter) and incubate for 1 minute.

16 Centrifuge for 60 seconds at 16,000g

17 Add 10.5 µl of Elution Buffer (EB) (middle of the filter) and incubate for 1 minute. .

18 Measure yield and specific dye incorporation with NanoDrop, Program MicroArray.
Labelled, cleaned up gDNA can be stored at -20°C in the dark for 7 days.