

Klenow Label Genomic DNA Cy3 &/or Cy5

NOTES:

For each reaction label 1ug fragmented genomic DNA (fragment by sonication or other method to average size of 500-1000bp will improve labeling efficiency).

We use high concentration Klenow (50units/ul) from New England Biolabs. This provides better incorporation of labeled dUTP.

PROTOCOL

1. Mix DNA and random hexamer

X ul frag genomic DNA (~1ug)
2 ul random Pd(N)₆ hexamer @ 5mg/ml (Pharmacia)
Z ul MQH₂O
20 ul total volume

2. Heat sample to 95°C for 2 min. and transfer to ice water for 5 min.
3. Set up Labeling Mix

5 ul 10X klenow buffer (Eco/Pol, NEB)
5 ul 10X dNTP (5mM dATP, dCTP & dGTP)
17 ul milliQ H₂O

4. To DNA/hexamer on ice add 27ul of labeling mix
5. Add 1.5 to 2 ul Cy dye labeled dUTP + 1ul Klenow DNA polymerase (50U/ul)
6. Incubate 2-6 hours at 37°C in dark
7. Add 2.5 ul 0.5M EDTA pH 8.0 to stop the reaction

PURIFY Target sample PRIOR TO HYBRIDIZATION!

Unincorporated dNTPs need to be removed prior to hybridization to minimize background fluorescence.

- Add 470ul milliQ H₂O to each labeling reaction.
- Transfer this to a Microcon 30 (Amicon) filtration unit in a collection tube.
- Spin at 12,000 RPM 8-10 minutes (trying to achieve a volume of 20-30ul)
- Add additional 450ul milliQ H₂O, mix and repeat spin
- Add one more volume (450ul) milliQ H₂O, mix, and concentrate to <10ul
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Note- if you are hybridizing Cy3 AND Cy5 labeled targets to the same array you should spin one sample into a clean tube after the first wash. Do this by inverting the filtration unit into a clean tube and spinning at max speed for 1 min. Mix this sample with the second sample and proceed with washes. Target a final sample volume of 10-15 ul. Proceed to hybridization

Recommended Reagents and Suppliers

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|------------------------------|---------|-----------------|------------------|
| Cyanine3-dUTP: | 25nM | PerkinElmer/NEN | Cat# NEL578 |
| Cyanine5-dUTP: | 25nM | PerkinElmer/NEN | Cat# NEL579 |
| Klenow DNA Pol | 50U/ul | NEB | Cat # M0210M |
| Pd(N) ₆ (Hexamer) | 5mg/ml* | Amersham | Cat# 27-2166-1 |
| 100 mM dNTP set | 10X** | Amersham | Cat # 27-2035-02 |
| Microcon YM-30 columns | | Amicon | Cat # 42410 |

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