ONE COLOR FISH

Nick Translation (BioNick Labeling System *GibcoBRL*)

*IMPT: Always keep the enzyme in the freezer
Keep the total reaction volume at 50ul
[BAC] = 1ug DNA

1. Labeling Rxn Example:

BAC DNA	5.5ul
10x Enzyme	5.5ul
10mM dNTPs	5.0ul
water	to 50ul total volume

Pulse spin labeling reaction
Nick translate 1 hour at 16°C

2. Check 10ul reaction on a 2.0% agarose gel
   - should be a smear between 200 and 500bp
   - keep nick translation reaction on ice until adding 5ul stop Buffer to stop the reaction

3. Add 5ul Stop Buffer

4. Add 20ug COT-1 DNA and vortex to mix

5. Add 1/10 volume 3M NaOAc and 2 volums 100% EtOH to reaction and mix. Place in a -80°C freezer for 30min.

6. Centrifuge in a 4°C microcentrifuge for 30min. at high speed.

7. Decant supernatant and wash pellet with ice cold 70% EtOH.

8. Dry pellet in Speed Vac for 5-10min.

9. Resuspend pellet in 3ul ddH_2O and 7ul hybe mix containing:

		X 3
50% Formamide	3.5	10
2X SSC	1	3.1
10% Dextran Sulfate	2.??	9

   a. Pulse spin
   b. Vortex to mix and resuspend
   c. Pulse spin
   d. Place in 37°C water bath to resuspend pellet (about 10min.)

10. Denature probe by heating to 70°C for 7.5-8min.

11. Preanneal probe at 37°C for the appropriate amount of time: Cosmids: 45-60min
    BAC: 1-5h (2.5-3h)
    YAC: 1-5h (2.5-3h)