

Direct Fluorescent labeling-Nick translation

1. On ice add the following:
 - 12µl template (about 1µg) DNA
 - 4µl 5X Fluorophore Labeling PreMix
 - 4µl Nick Translation Mix (Roche)
2. Mix and centrifuge
3. Incubate for 90 minutes at 15°C
4. Check labeling reaction:
 - place on ice and shield from light
 - remove 3µl and add to 7µl water
 - denature for 5 min at 95°C
 - add 2µl loading dye
 - run on 1% agarose/1X TAE gel → probe should be between 200 and 500 nt.

If necessary, reincubate the reaction at 15°C and recheck fragment length.
4. Stop reaction by adding 1µl 0.5M EDTA and incubate for 10 min. at 65°C.

5X Fluorophore Labeling PreMix

- 5µl 2.5 mM dATP
- 5µl 2.5 mM dCTP
- 5µl 2.5 mM dGTP
- 12µl 1mM Fluor-conjugated dUTP (Roche)
- 23µl water

Notes:

Fluorophore conjugated nucleotides are sensitive to light, so care should be taken to ensure tubes are shielded when fluorophore is present

If reactions are prepared in PCR tubes, 15°C and 65°C incubations may be performed in PCR machine with heated lid

Store 5X Fluorophore Labeling PreMix at -20°C shielded from light