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Thanks!

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## **Nicktranslation of DNA probe**

(This has been adapted from the Roche protocol)

### Nick/labeling reaction:

1ug - DNA  
4ul - Nucleotide mix \*  
6ul - Biotin -16-dUTP (Roche Cat# 11 093 070 910) or  
Digoxigenin-11-dUTP (Roche Cat# 11 093 088 910)  
4ul - Roche Nicktranslation Mix (Roche Cat# 11 745 808 910)  
Bring up to 20ul with H2O

Incubate for 2½ hours in a 15°C water bath.

### Stop reaction:

2ul - 0.5M EDTA  
2ul - 5% SDS  
Heat at 80°C for 10 minutes

### Precipitate:

2ul - ssDNA + tRNA (Sigma D-9156)  
2.6ul (0.1 volume) - 3M Sodium acetate  
71.5ul (2.5 volume) - 95% cold ETOH  
Place in -20°C for 1 hour or overnight

Spin tubes at 10,000 rpm for 30 min  
Rinse pellet with 70% cold ETOH  
Air dry or speed vac the pellet

Resuspend in 80ul of H2O (use 5ul of this concentration in hyb reaction)

Store at -20°C

### \* Nucleotides Mix:

6ul	dATP (100mM stock Roche)
6ul	dCTP (100mM stock Roche)
6ul	dGTP (100mM stock Roche)
982ul	H2O