Nick Translation (CGH)

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National Institutes of Health

Reagents

Biotin-16-dUTP
Boehringer Mannheim, Cat. 1093070
Bovine serum albumin (BSA)
Dig-11-dUTP
Boehringer Mannheim, Cat. 1093088
dATP, dTTP, dGTP, dCTP
Boehringer Mannheim, Cat. 105 1440, 105 1458, 105 1466, 105 1482
DNase I from bovine pancreas
Boehringer Mannheim, Cat.104 159, 100 mg
EDTA, 0.5 M
Glycerol
Lambda HindIII DNA marker
Magnesium chloride ($MgCl_2$), 2 M
β–Mercaptoethanol, 99%
Polymerase (Kornberg)
Boehringer Mannheim, Cat. 104 485
NaCl, 1 M
Tris-HCl, 1 M, pH 8.0
Water, sterile

Preparation

dNTP		final conc.
100 mM dATP, dCTP, and dGTP	5 µl of each	0.5 mM
100 mM dTTP	1 µl	0.1 mM
Sterile water	984 µl	
Total	**1000 µl**	

(equals 0.5 mM each of dATP, dCTP, and dGTP, and 0.05 mM dTTP)
*Aliquot and store at -20°C

DNase I stock solution, 1mg/ml		final conc.
DNase I	10 mg	1 mg/ml
NaCl, 1M	1.5 ml	0.15 M
Glycerol	5 ml	50%
Sterile water	bring up to 10 ml	
Total	**10 ml**	

*Aliquot and store at -20°C

10X NT-Buffer		final conc.
Tris-HCL, 1 M, pH 8.0	500 µl	0.5 M

$MgCl_2$, 2 M	25 µl	50 mM
BSA, 10 mg/ml	50 µl	0.5 mg/ml
Sterile water	425 µl	
Total	**1 ml**	

*Aliquot and store at -20°C

0.1M ß-Mercaptoethanol		final conc.
99% solution (14.4 M)	34.7 µl	0.1 M
Sterile water	bring up to 5 ml	
Total	**5 ml**	

*Aliquot and store at -20°C

Procedure

1. For each DNA sample, add to an eppendorf tube:
   2 µg DNA
   10 µl 10X NT-Buffer
   10 µl dNTP mix
   10 µl 0.1 M ß-Mercaptoethanol
   4 µl BIO-16-dUTP or 4 µl DIG-11-dUTP (1 mM)
   X µl sterile water
   (The total volume including reagents added in step 3 should be 100 µl)

2. Vortex, centrifuge, and place tubes on ice.

3. Add 2 µl Polymerase (Kornberg) first, and then 3-8 µl Dnase (1 mg/ml) 1:1000 (Dnase amount will vary from different batches).

4. Vortex and centrifuge and centrifuge.

5. Incubate at 15°C for 2 hr (1.5-2 hr).

6. Prepare gel electrophoresis.

7. Run about 5 µl of each sample and the Lambda HindIII DNA marker; ideally the length of the DNA should be 500-900 bp after nick translation.

8. If DNA is too large, add more DNase and incubate at 15°C for 10-30 min.

9. Stop the nick translation with 1 µl of 0.5 M EDTA and incubate at 65°C for 10 min.

10. Store DNA at -20°C or precipitate the same day.