

Linearization of plasmid DNA to make templates for transcription

Overview:

Linearize plasmids as templates for RNA synthesis

Notes:

1. Perform under RNA sterile conditions
2. For hLDLR-pT7Ts, use XbaI and SacI, NEB Buffer 4 and BSA
3. For BACT-Topo antisense RNA use ScaI and XmnI, Buffer 3 , BSA

Materials:

1. Restriction Enzymes and Buffers (-20°C)
2. Plasmid DNA prepared by midiprep
3. TAE, 1X from 10X (on bench)
4. Agarose (room temp chemicals)
5. Ethidium Bromide (on bench) – CAUTION- MUTAGEN
6. Sample buffer (on bench)
7. 3M NaOAc (on bench)
8. Phenol:CHCl₃:isoamyl alcohol (4°C)
9. ETOH (-20°C)

Procedures:

1. Assemble reaction as follows:

DNA (30 ug)	x uL
10X Buffer	20 uL
BSA, 100X	2 uL
Enzyme 1 (250 U)	10 uL
Enzyme 2 (200U)	10 uL
H2O (up to 200 uL)	<u>uL</u>
	200 uL

2. Incubate 37°C, 1hr. 30 min.
3. Analyze aliquot on agarose gel
 - a. Prepare 1% TAE agarose gel + Ethidium Bromide (2.5 µl/ 50 ml gel)
 - b. Prepare samples:
 - 9 µl sample + 1 µl 1X loading buffer
 1. old linearized (0.75 µg)
 2. new linearized (6 uL) (sample taken after digestion, before precipitation with NaAcetate, EtOH)
 3. Supercoiled DNA (0.75 ug)
 - DNA Ladders: 5 µl each
 1. 100 BP
 2. 1 kB

c. Run gel

- 90 mv

4. Extraction and precipitation

a. Extract with Phenol:CHCl₃:isoamyl (RNA only in 4°C)

- Equal volume

b. Precipitate with NaOAC

- To DNA ADD

1. 3M Na Acetate 0.1 V
2. 100% EtOH, ice cold 3.0 V
 - a. Mix by pipetting
 - b. Incubate -80°C for minimum of 15 min.
 - c. Pellet in microfuge 4°C, 5-15 min.
 - d. Remove supernatant
 - e. Wash pellet with 100 µl 70% EtOH ice cold
 - f. Recentrifuge
 - g. Remove supernatant
 - h. Allow pellet to air dry, 15-20 min.
 - i. Resuspend in 30 µl 1mM Tris, pH 8
 - j. Read absorbance on spec