Protocol

DNA Template Preparation for *in vitro* Transcription

This protocol is for the DNA Template Preparation for *in vitro* Transcription

Double stranded linear DNA with blunt or 5'-protruding ends can be used as template for *in vitro* transcription. Linearized plasmid DNA, PCR products or cDNA can be used as templates for transcription if they contain a double-stranded RNA polymerase promoter region in the correct orientation.

Consensus promoter sequences of different RNA Polymerases:

T7 TAATACGACTCACTATAGGG

T3 AATTAACCCTCACTAAAGGG

SP6 ATTTAGGTGACACTATAGAA

G will be the first base (+1) of the RNA transcript

The synthesis of sense or antisense RNA transcripts depends on the orientation of the promoter with respect to target sequence. The target sequence must be placed downstream of the promoter for sense RNA and must be inverted for antisense RNA transcription.

Plasmid Templates

Quality

Plasmid DNA quality affects transcription yield and the integrity of synthesized RNA. The greatest transcription yields are achieved with the highest purity plasmid templates. Plasmids purified by common laboratory methods can be used if the DNA is free of contaminating RNases, SDS, EDTA, proteins, salts* and RNA. DNA should have a A260/280 ratio of 1.8-2.0. The GeneJET™ Plasmid Miniprep Kit generates high purity plasmid DNA suitable for transcription.

* T7 and SP6 RNA Polymerases are inhibited by ~50% at NaCl or KCl concentrations above 150 mM and T3 RNA Polymerase – at above 250 mM.

Linearization

To produce RNA transcripts of a defined length, plasmid DNA is linearized by restriction digestion downstream of the insert. Restriction enzymes which generate blunt ends or 5'-overhangs are preferred. 3'-overhangs have been reported to generate spurious transcripts (1) and should therefore be avoided. 3'-overhangs can be blunted by T4 DNA Polymerase prior to transcription.

Due to the high processivity of RNA polymerases, circular plasmid templates generate long heterogeneous RNA transcripts in higher quantities than linear templates. Therefore, it is important to completely linearize plasmid DNA to ensure efficient synthesis of defined length transcripts. If complete digestion is unachievable, gel purify the linearized DNA template band e.g. with a DNA Gel Extraction Kit prior to transcription reactions.

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After linearization, it is recommended to purify the DNA template by phenol/chloroform extraction:

1. Add 1⁄10th volume of 3 M Sodium Acetate Solution to the DNA.
2. Mix thoroughly.
3. Extract with an equal volume of a 1:1 phenol/chloroform mixture, and then twice with an equal volume of chloroform. Collect the aqueous phase and transfer to a new tube.
4. Precipitate the DNA by adding 2 volumes of ethanol. Incubate at -20°C for at least 30 min and collect the pellet by centrifugation.
5. Remove the supernatant and rinse the pellet with 500 µl of cold 70% ethanol.
6. Resuspend the DNA in 20 µl of DEPC-treated water.

PCR Templates

PCR products can serve as templates for in vitro transcription. The RNA polymerase promoter must be located upstream of the sequence to be transcribed.

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