untitled protocol

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Abstract

In vitro transcriptions assay for cyanobacterial RNA polymerase. This protocol can be used either to do an in vitro transcription with small DNA fragments with desired promoter region or to use it with a promoter region integrated in a plasmid.

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Guidelines

RNA experiments! Always wear gloves and prepare probes under RNAse free and cooled conditions!

Before start

Prepare all Buffer, measure RNAP and DNA concentrations

Materials

✓ in vitro transcription buffer by Contributed by users

Protocol

Prepartions

Step 1.

In vitro transcription Buffer:

20 mM Tris–HCl pH 7,9

40 mM KCl,

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15 mM $MgCl_2$

Prepartions

Step 2.

Polyacrylamid Gel:

10-20% 19:1 acrylamide:bisacrylamide

8M urea

1x TBE

NOTES

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For smaller fragments use higher concentrations of 19:1 acrylamide:bisacrylamide.

Prepartions

Step 3.

Loading dye should contain at least 50% formamide or 8M urea. You can use commercial RNA loading dye, also.

Example recipie:

8M urea saturated with formamide

10mM EDTA

1xTBE,

bromophenol blue and xylene cyanol (amounts are empirical, just to make it visible on the gel)

In vitro Transcription

Step 4.

Mix:

100 nM RNAP

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10 nM promoter-containing DNA

100µM NTPs

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If desired one could use P32 labelled NTPs. Then it is recommendable to decrease the concentration of the same cold NTP to 10uM, to increase labelling efficiency.

In vitro Transcription

Step 5.

Incubate the reaction at 34°C for at least 3 hours.

Stop with equal volume of the loading dye. Before loading onto denaturing PAA gel boil the Samples for 5 min at 95°C and let them cool on ice.

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If desired, one yould expand reaction duration.

Visualization

Step 6.

Visulization of the products on the gel, can be done with nucleic acid gel stain of your choice. For small products it is recommended to stain the gel over night.

REAGENTS
GelRed™ Nucleic Acid Gel Stain, 10,000X in Water G-725 by Gold Biotechnology
SYBR Gold Nucleic Acid Gel Stain S-11494 by Contributed by users

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Two common used stains are indicated.

Warnings

Harmless

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