Purification of DNA-dependent RNA Polymerase from Synechococcus elongatus PCC 7942

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Abstract

This protocol can be used to purifiy DNA-dependent RNA polymerase from *Synechococcus elongatus* PCC *7942* by heparin and ion-exchange chromatography.

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Materials

HiTrap Heparin HP affinity column 17040701 by Ge Life Sciences

Mono Q® 5/50 GL GE17-5166-01 by Ge Life Sciences

Protocol

Prepartions

Step 1.

Culture:

To get a reasonable amount of RNA polymerase it is recommended to use at least 8 litre (OD_{750nm} = 1) culture or in other words 10 gramm of cell dry cell pellet.

Prepartions

Step 2.

Lysis buffer:

10mM Tris-HCl pH 7.9

150mM NaCl

1 protease inhibitor cocktail tablet (Roche) per 50ml

0.1 mM DTT

0.1 mM EDTA

0.5% Glycerol

Prepartions

Step 3.

Loading buffer:

10 mM Tris-HCl pH 7.9

150mM NaCl

0.1 mM DTT

0.1 mM EDTA

0.5% Glycerol

Prepartions

Step 4.

Washing buffer:

10mM Tris-HCl pH 7.9

300mM NaCl

0.1 mM DTT

0.1 mM EDTA

0.5% Glycerol

Prepartions

Step 5.

Elution buffer:

10mM TrisHCl pH 7.9

600mM NaCl

0.1 mM DTT

0.1 mM EDTA

0.5% Glycerol

Prepartions

Step 6.

Column cleaning buffer:

10mM Tris-HCl pH 7.9

2M NaCl

Prepartions

Step 7.

RNA polymerase storage buffer:

50% glycerol

200mM KCl

40mM Tris 7.9

1mM EDTA

1mM DTT

NOTES

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All buffers are filtrated by vacuum pump filtration to avoid that air is getting into your HPLC system. Air bubbles will disturb your chromatogram detection.

Sample Preparation

Step 8.

The dry pellets are resuspended in lysis buffer and subjected to 6 minutes of 2 second on/off sonication at a 40 % amplitude. After sonication the lysat is incubated with 0,5 µl of Benzonase® nuclease for 30 min on ice.

REAGENTS

Benzonase® Nuclease E1014 SIGMA by Sigma-aldrich

Sample Preparation

Step 9.

Cell Lysat undergoes two centrifugation steps of 15 minutes at 15000 rcf and 20 minutes at 18000 rcf

until a clear supernatant is obtained. The supernatant was passed through a 0.45 µM PFDF filter.

Heparin Chromatography

Step 10.

Next step includes, using a high pressure liquid chromatography (HPLC). Heparin column was equilibrated with 5 column volumes (CV) of loading buffer. The lysate is loaded, and column is washed with ten CV of loading buffer. Followed by 10 CV of washing buffer. Elution is done by 10 CV of elution buffer. The column is washed with column cleaning buffer.

READENTS

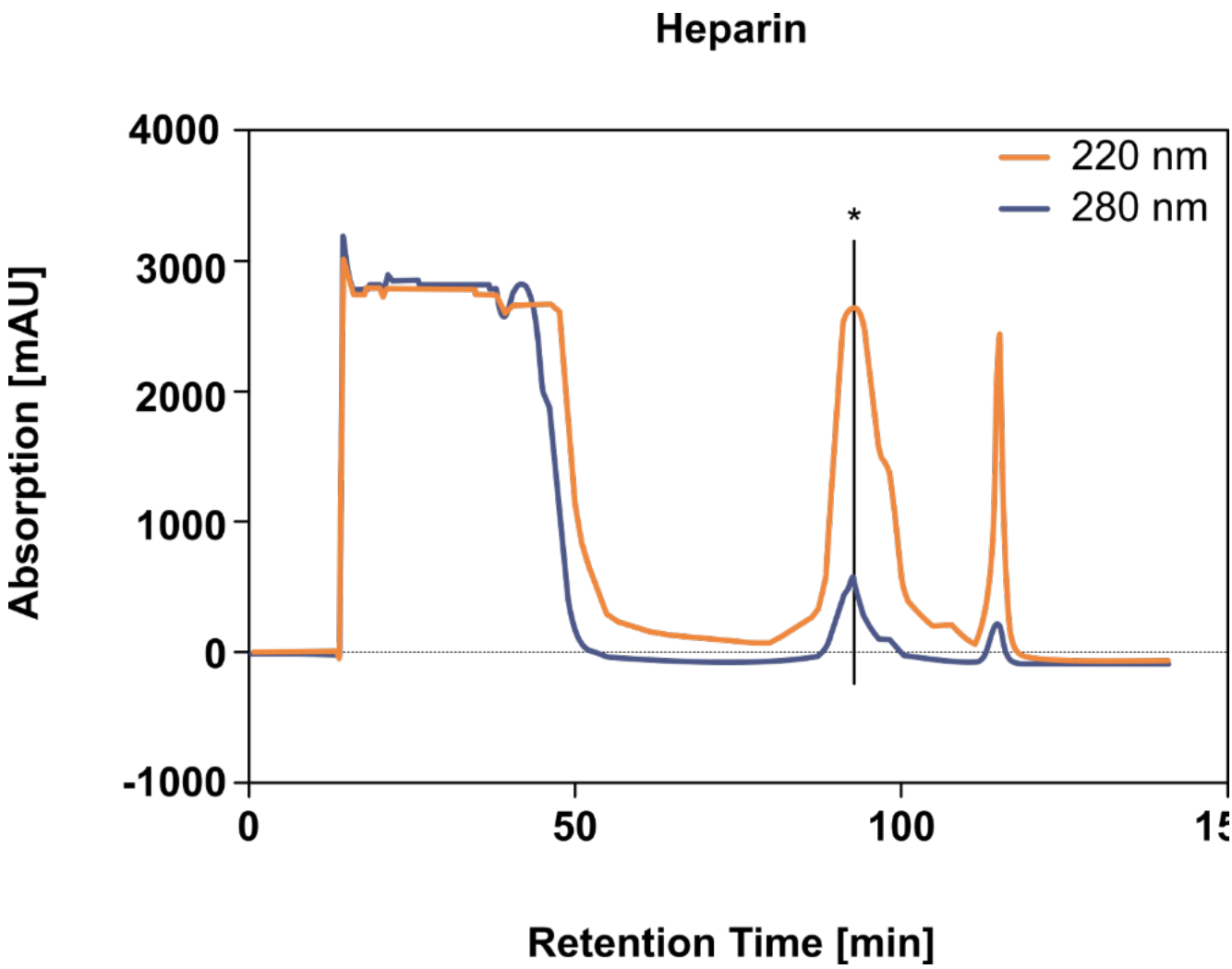
HiTrap Heparin HP affinity column 17040701 by Ge Life Sciences

Heparin Chromatography

Step 11.

During elution step, one should see that the chromatogram peaks several times. These fractions are loaded onto SDS gel and visualized with comassie blue staining.

EXPECTED RESULTS



NOTES

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An examplary image of the heparin chromatogram is shown in the 'expected results' section. The star marked line indicates the peak of the RNA polymerase. Fractions arround this peak are loaded onto a SDS gel.

Ion-exchange Chromatography

Step 12.

Fractions containing RNA polymerase were concentrated with Vivaspin® 500 µL 5K ultrafiltration spin columns washed with loading buffer several times and subjected to further purification on an ion-exchange MonoQ 5/50 GL column, using fast protein liquid chromatography.

NOTES

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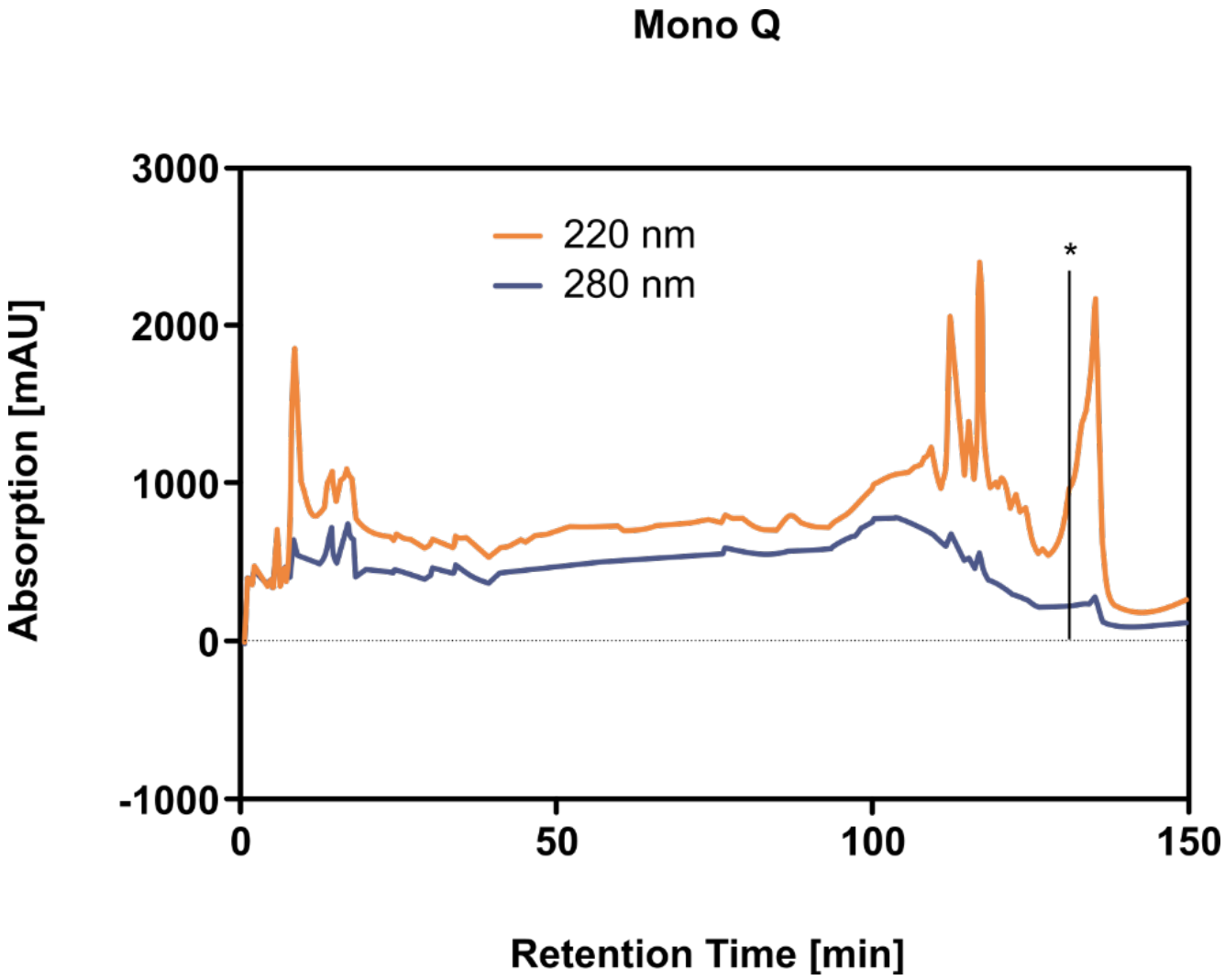
Washing step is crucial for further purification! One have to be sure that the elution buffer is fully exchanged with washing buffer to ensure binding of the RNA polymerase onto the Ion-exchange column.

Ion-exchange Chromatography

Step 13.

The column was equilibrated with five column volumes (CV) of loading buffer. Suspension was loaded onto column with loading buffer and continuously washed until the OD280nm reading drops to the baseline. Then a gradient of 50 CV from 250mM to 600mM NaCl [10mM TrisHCl pH 7.9, 0.1 mM DTT, 0.1 mM EDTA, 0.5% Glycerol] was applied. Final wash of the column is done with column cleaning buffer.

EXPECTED RESULTS



NOTES

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An examplary image of the MonoQ chromatogram is shown in the 'expected results' section. The star marked line indicates the peak of the RNA polymerase. Fractions arround this peak are loaded onto a SDS gel.

RNA polymerase sample preparation

Step 14.

Again fractions containing RNA polymerase were concentrated with Vivaspin® 500 µL 5K ultrafiltration spin columns washed with loading buffer several times. Use the same ultrafiltration spin column to change buffer and concentrate RNA polymerase with storage buffer. To validate your sample one can use western blotting or mass spectrometry. Samples are stored at -20°C.

EXPECTED RESULTS

Typical result after Ion-exchange chromatography