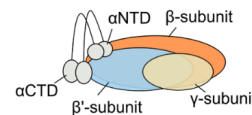


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Purification of DNA-dependent RNA Polymerase from *Synechococcus elongatus* PCC 7942 V.3

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Materials

MATERIALS


 HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

 Mono Q® 5/50 GL **GE Healthcare Catalog #GE17-5166-01**

STEP MATERIALS

 Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**


 Benzonase® Nuclease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E1014 SIGMA**


 HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

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Prepartions

1 Culture:

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

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

3 Loading buffer:

10 mM Tris-HCl pH 7.9
150mM NaCl
0.1 mM DTT
0.1 mM EDTA
0.5% Glycerol

4 Washing buffer:

10mM Tris-HCl pH 7.9
300mM NaCl
0.1 mM DTT
0.1 mM EDTA
0.5% Glycerol

5 Elution buffer:

10mM TrisHCl pH 7.9
600mM NaCl
0.1 mM DTT
0.1 mM EDTA
0.5% Glycerol

6 Column cleaning buffer:

10mM Tris-HCl pH 7.9
2M NaCl

7 RNA polymerase storage buffer:





50% glycerol
200mM KCl
40mM Tris 7.9
1mM EDTA
1mM DTT

Note


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

- 8 The dry pellets are resuspended in 25 mL lysis buffer. Add 0,5 µl of Benzonase® nuclease and 1 mg/mL lysozyme. Let the suspension incubate for 30 min on ice. 1d 6h
 Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**

 Benzonase® Nuclease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E1014 SIGMA**
- 9 Sonicate the samples for 6 minutes of 2 seconds on/off at a 40 % amplitude. 6m
- 10 Cell Lysate 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. 35m

Heparin Chromatography

- 11 The next step includes using high-pressure liquid chromatography (HPLC). The heparin column was equilibrated with 5 column volumes (CV) of loading buffer. The lysate is loaded, and the column is washed with 10 CVs of loading buffer. Followed by 10 CVs of washing buffer. Elution is done by 10 CVs of elution buffer. The column is washed with a column cleaning buffer.
 HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**
- 12 During the elution step, one should see that the chromatogram peaks several times. These fractions are loaded onto SDS gel and visualized with coomassie blue staining.

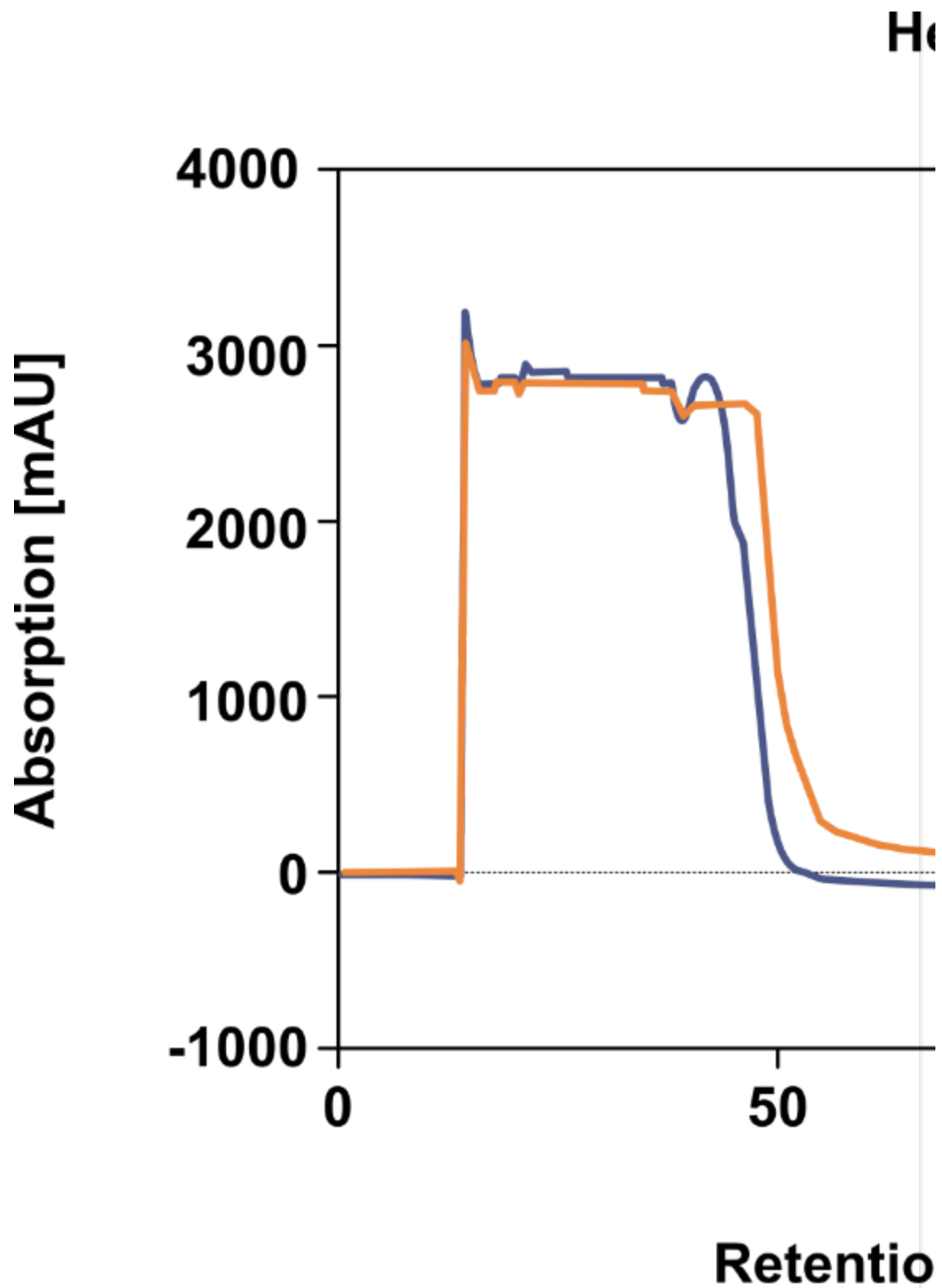


Note

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



Expected result



Ion-exchange Chromatography

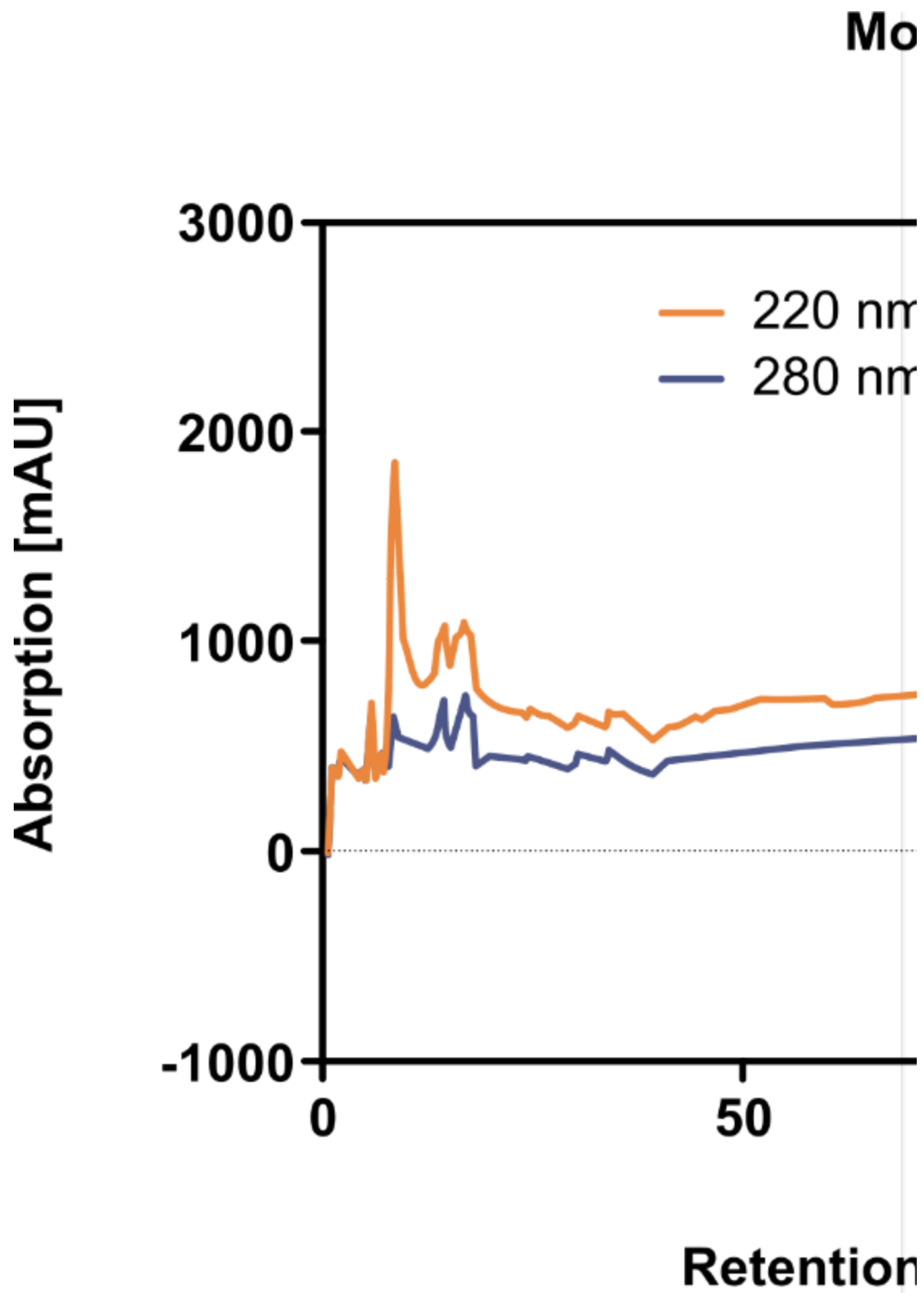
- 13 Fractions containing RNA polymerase were concentrated with Vivaspin® 500 µL 5K ultrafiltration spin columns washed with loading buffer several times and subjected to further purification on an ion-exchange MonoQ 5/50 GL column, using fast protein liquid chromatography.

Note

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

- 14 The column was equilibrated with five column volumes (CV) of the loading buffer. The suspension was loaded onto the column with loading buffer and continuously washed until the OD_{280nm} reading drops to the baseline. Then a gradient of 50 CV from 250mM to 600mM NaCl [10mM Tris-HCl pH 7.9, 0.1 mM DTT, 0.1 mM EDTA, 0.5% Glycerol] was applied. A final wash of the coexemplarylumn is done with column cleaning buffer.

Expected result



Note

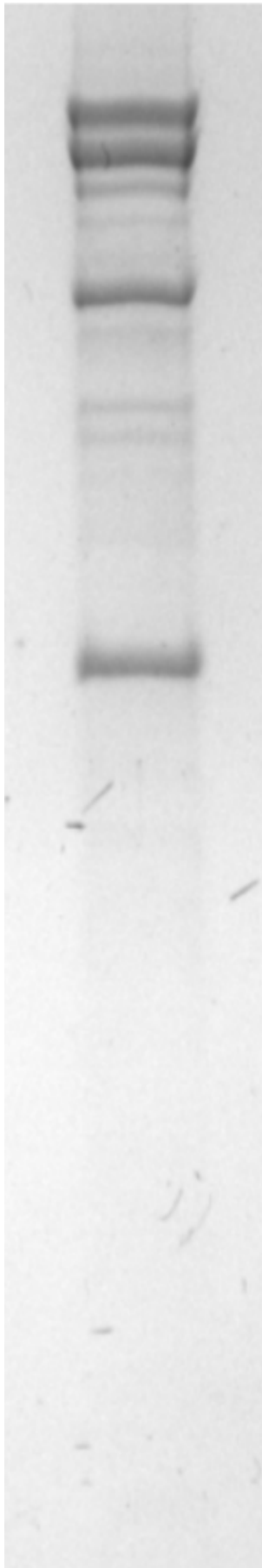
An exemplary image of the MonoQ chromatogram is shown in the 'expected results' section. The star marked line indicates the peak of the RNA polymerase. Fractions around this peak are loaded onto an SDS gel.

RNA polymerase sample preparation

- 15 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 result





Typical result after Ion-exchange chromatography