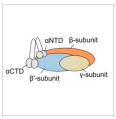


Jan 22, 2020

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

Lutz Berwanger[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.bbmfik3n

Axmann Lab CyanoWorld

Lutz LCB. Berwanger
Heinrich-Heine Universität Düsseldorf

ABSTRACT

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

MATERIALS

NAME	CATALOG #	VENDOR
HiTrap Heparin HP affinity column	17040701	Ge Life Sciences
Mono Q® 5/50 GL	GE17-5166-01	Ge Life Sciences

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Lysozyme	12671-19-1	Sigma Aldrich
Benzonase® Nuclease	E1014 SIGMA	Sigma-aldrich
HiTrap Heparin HP affinity column	17040701	Ge Life Sciences

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

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. 30h

Lysozyme
by Sigma Aldrich
Catalog #: 12671-19-1

Benzonase® Nuclease
by 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

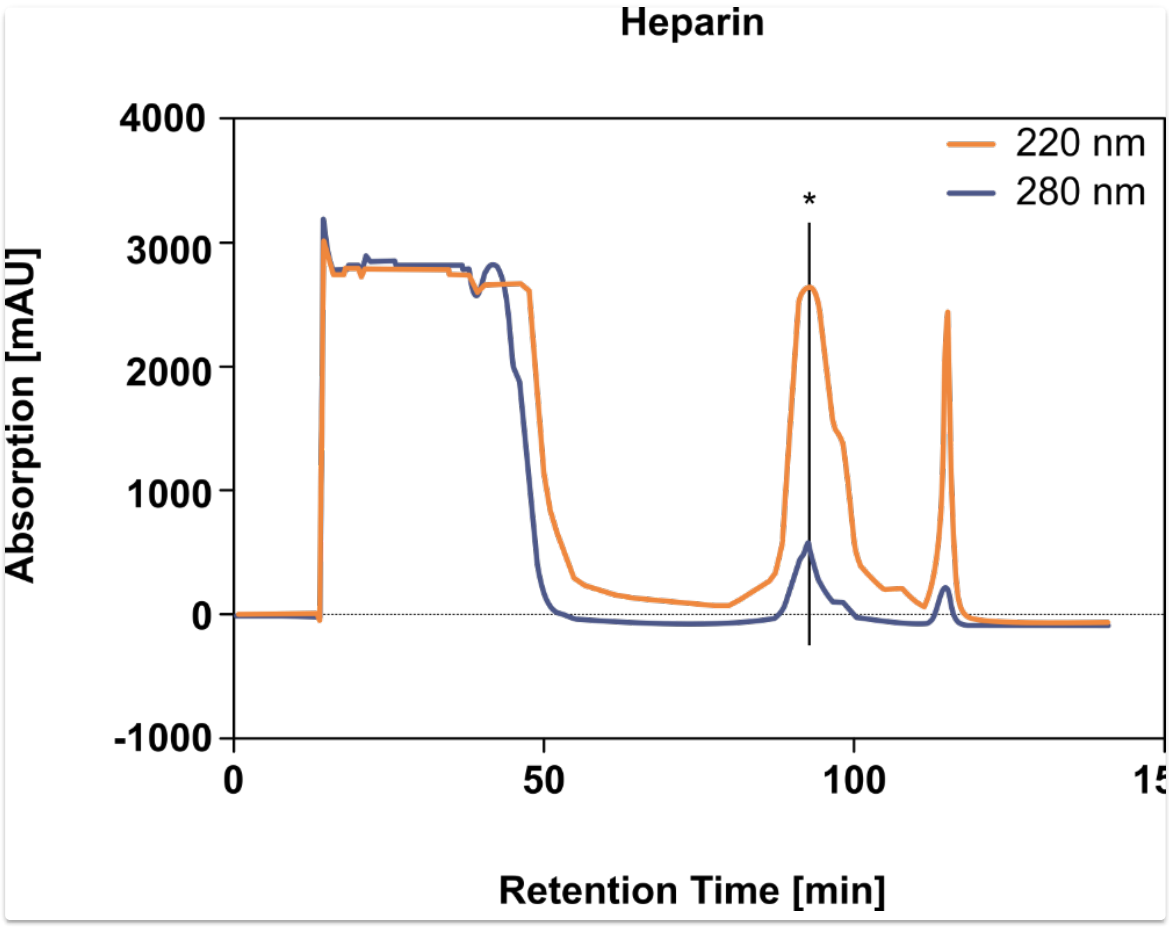
Heparin Chromatography

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
by Ge Life Sciences
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.

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.

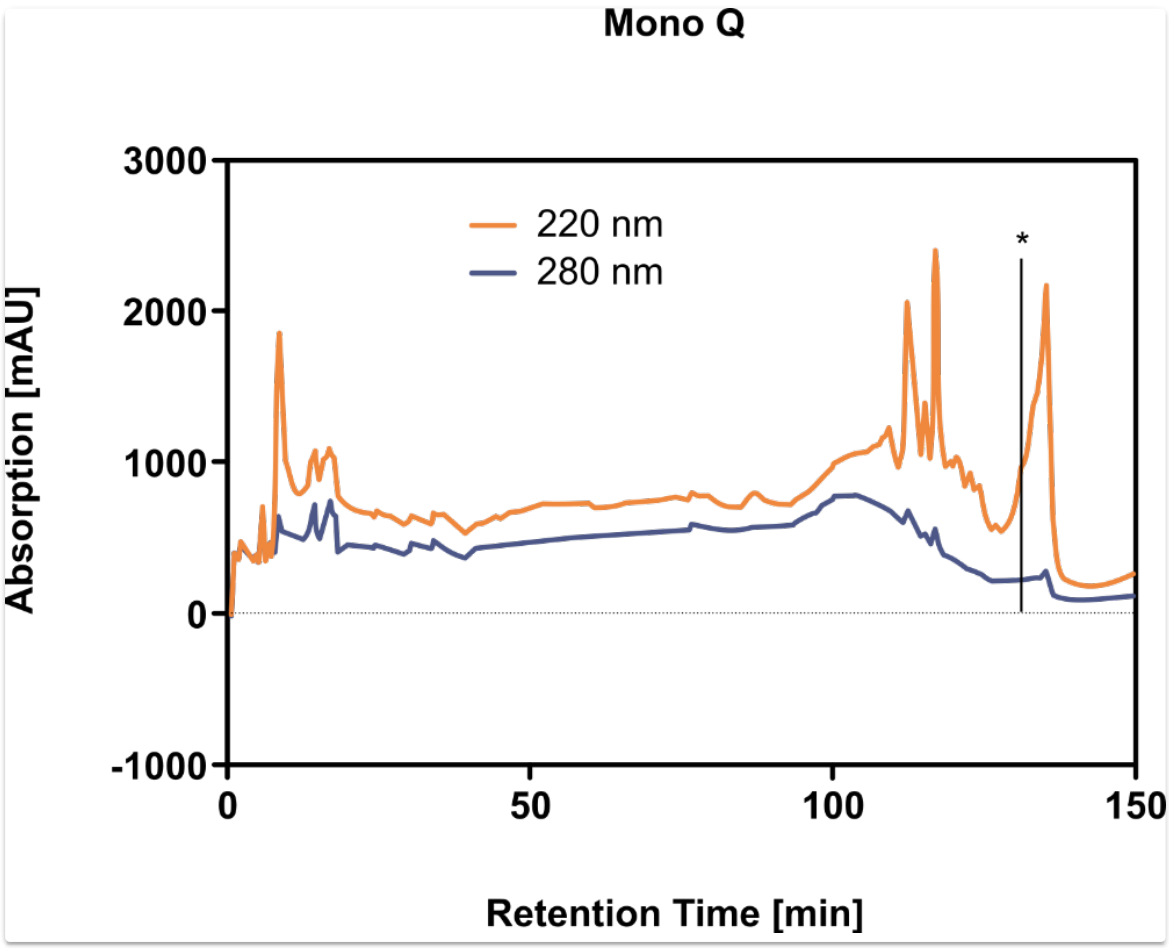


Ion-exchange Chromatography

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.

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 OD280nm 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.



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.

Typical result after Ion-exchange chromatography

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited