Hyman Lab
Max Planck Institute of Molecular Cell Biology & Genetics

SOP_hymanLab-01 – PGL-3 purification

Author: Juan M. Iglesias-Artola
Edited by Martine Ruer & Mohamad Almedawar

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I. List of abbreviations

%	Percent
°C	Degree Celsius
µg	Micrograms
µl	Microliter
CV	Column Volume
DTT	Dithiothreitol
H_2O	Water
KCl	Potassium Chloride
L	Liters
min	Minutes
mL	Milliliter
mM	Millimolar
RT	Room temperature
TEV	Tobacco Etch Virus
X *g*	Gravity

1. Aim

This SOP is a systematic and written instruction on the procedure of PGL-3 protein purification in the laboratory of Prof. Anthony A. Hyman, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

2. Background

PGL-3 is an RNA-binding protein from Caenorhabditis elegans. It is a critical component of germline P granules, (Brangwynne 2009). It contains an RGG-domain and undergoes liquid phase-separation in cellular conditions. The protocol for the purification of PGL-3 has been adapted from Saha et al 2016. Our construct contains a N-terminal PGL-3 flanked by a C-terminal TEV cleavage site, an internal His6 and a C-terminal monoGFP tag. The soluble protein fraction is first purified via His affinity chromatography. The ion exchange chromatography step is necessary to remove strongly bound nucleic acid contaminants which will otherwise interfere with the phase separation behavior of PGL-3. Cleavage by TEV protease generates untagged PGL-3 The PGL-3 fraction is separated from the His-monoGFP fraction by a subsequent size exclusion chromatography step. To obtain monoGFP labeled PGL-3, TEV cleavage can be omitted and the tagged protein can be purified by size exclusion chromatography after the ion exchange step.

This purification usually gives xxx – xxx mg protein per Liter of cells infected.

3. Procedure

3.1 Buffers

3.1.1 Lysis buffer

Chemical	Concentration (mM)
HEPES pH 7.25	25
KCl	300
Imidazole	10
DTT	1
Protease Inhibitor (Roche, EDTA-free)	1 tablet

3.1.2 Ni-NTA wash buffer

Chemical	Concentration (mM)
HEPES pH 7.25	25
KCl	300
Imidazole	20
DTT	1

3.1.3 Ni-NTA elution buffer

Chemical	Concentration (mM)
HEPES pH 7.25	25
KCl	300
Imidazole	250
DTT	1

3.1.4 Dilution buffer

Chemical	Concentration (mM)
Tris pH 8.0	25
DTT	1

3.1.5 **HiTrapQ binding buffer**

Chemical	**Concentration (mM)**
Tris pH 8.0	25
KCl	50
DTT	1

3.1.6 **HiTrapQ elution buffer**

Chemical	**Concentration (mM)**
Tris pH 8.0	25
KCl	1000
DTT	1

3.1.7 **Superdex buffer**

Chemical	**Concentration (mM)**
Tris pH 7.25	25
KCl	300
DTT	1

3.2 **Insect cell transfection and lysis**

3.2.1 Infect SF9ESF (10^6 cell/mL) cells in 1 L medium three days prior to harvesting

3.2.2 Harvest cells by centrifuging at 500 X *g* for 10 min

3.2.3 Resuspend cells using 50 mL of **Lysis buffer** for every 500 mL of cells

3.2.4 Lyse cells using the LM20 microfluidizer with 2 passages at 15000 psi

3.2.5 Centrifuge in JA25.50 rotor at 20,000 rpm for 45 at 15 C – Collect 50 µL – Sample 1

3.3 **4 x 5 mL ProTino Ni-NTA column purification, peristaltic pump at RT**

3.3.1 Rinse the columns with 10 CV of H_2O at 5 mL/min

3.3.2 Equilibrate the columns with 10 CV **Lysis buffer** at 5 mL/min

3.3.3 Load crude supernatant at 3 mL/min – Collect 50 µL of flow through – Sample 2

3.3.4 Rinse the columns with 10 CV **Ni-NTA wash buffer** – Sample 3

3.3.5 Elute 1.5 ml fractions with **Ni-NTA elution buffer**

3.3.6 Pool fractions containing the protein – Collect 50 µL – Sample 4

3.4 **TEV cleavage**

3.4.1 Add 700 µl TEV protease (1 mg/ml) to pooled fractions

3.4.2 Rotate end-to-end overnight at 4 °C – Sample 5

3.5 **Anion exchange chromatography with 2 x HiTrapQ HP 5 ml (RT) ÄKTA pure**

3.5.1 Dilute the sample 6 x with **Dilution buffer** to reach 50 mM KCl for binding to the anion exchange column matrix. Rotate end-to-end overnight at 4 °C – Collect 50 µl Sample 5

3.5.2 ÄKTA HiTrapQ 5mL protocol:

a) Column wash 1: 5 CV using inlet A7 (H_2O)

b) Column wash 2: 5 CV using inlet B1 containing **HiTrapQ elution buffer**

c) Equilibration: 5 CV using inlet A1 containing **HiTrapQ binding buffer**

d) Sample application: use inlet S1 to insert the sample – Collect 50 µL – Sample 6

e) Column wash 3: 5 CV using inlet A1 containing **HiTrapQ binding buffer**

f) Elution: Linear gradient elution to 55% of inlet B1 (**HiTrapQ elution buffer**) over 25 C.V. Then, step with 100% B1 for 5 CV

3.5.3 Pool samples containing the protein – Collect 50 µL – Sample 7

3.6 **Size exclusion chromatography HiLoad 16/60 Superdex 200 (RT) ÄKTA pure**

3.6.1 Equilibrate column with 1.20 CV of **Superdex Buffer** using inlet A1

3.6.2 Apply sample using a 5 mL injection loop

3.6.3 Elute sample with 1.20 CV of **Superdex Buffer** using inlet A1

3.6.4 Pool samples containing the protein - Collect 50 µL – Sample 8

4. Results

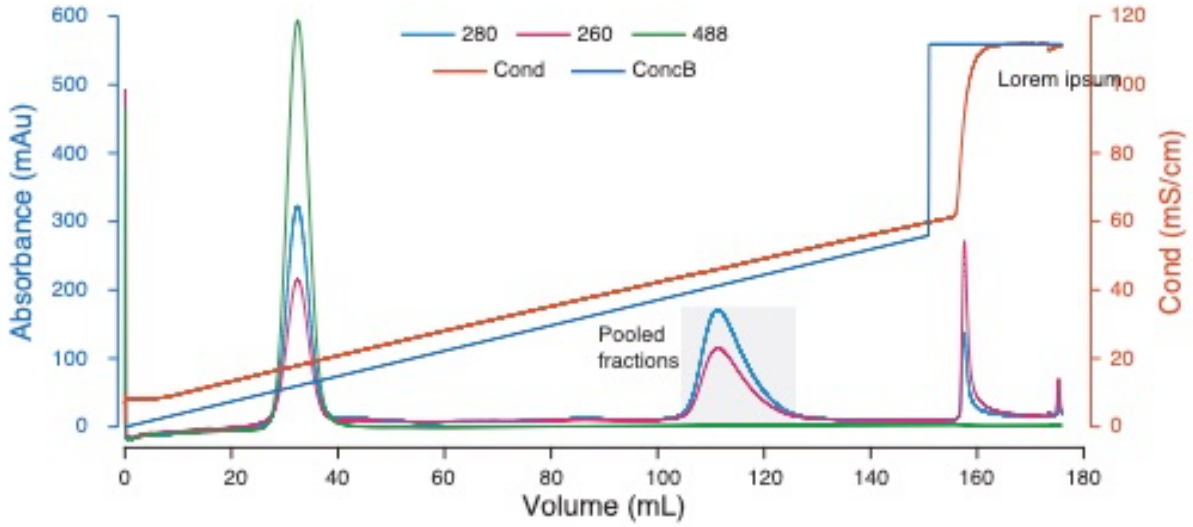

Figure 1: HiTrapQ purification chromatogram. 1 L of cells were loaded to a 5 mL HiTrapQ column after purification using a Ni-NTA column and diluting the solution 6 X to reach 50 mM KCl. It was observed that at the beginning of the run protein accumulated in a defined green region on top of the column, probably due to aggregation. The left y-axis shows the absorbance at 280 nm and the right y-axis shows the conductivity. The concentration of buffer B goes from 0 to 100 %.

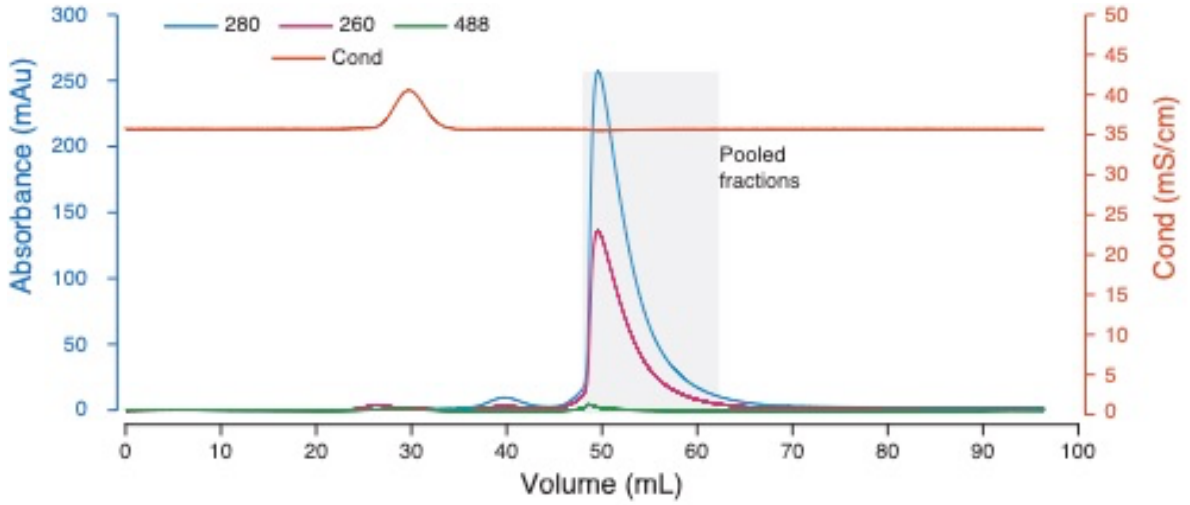

Figure 2: HiLoad 16/60 Superdex 200 chromatogram. After TEV cleavage PGL-3 was loaded on a HiLoad 16/60 Superdex 200 column to separate PGL-3 from GFP. The loss of absorbance at 488 nm indicates that the TEV cleavage has proceed to close to 100 %.

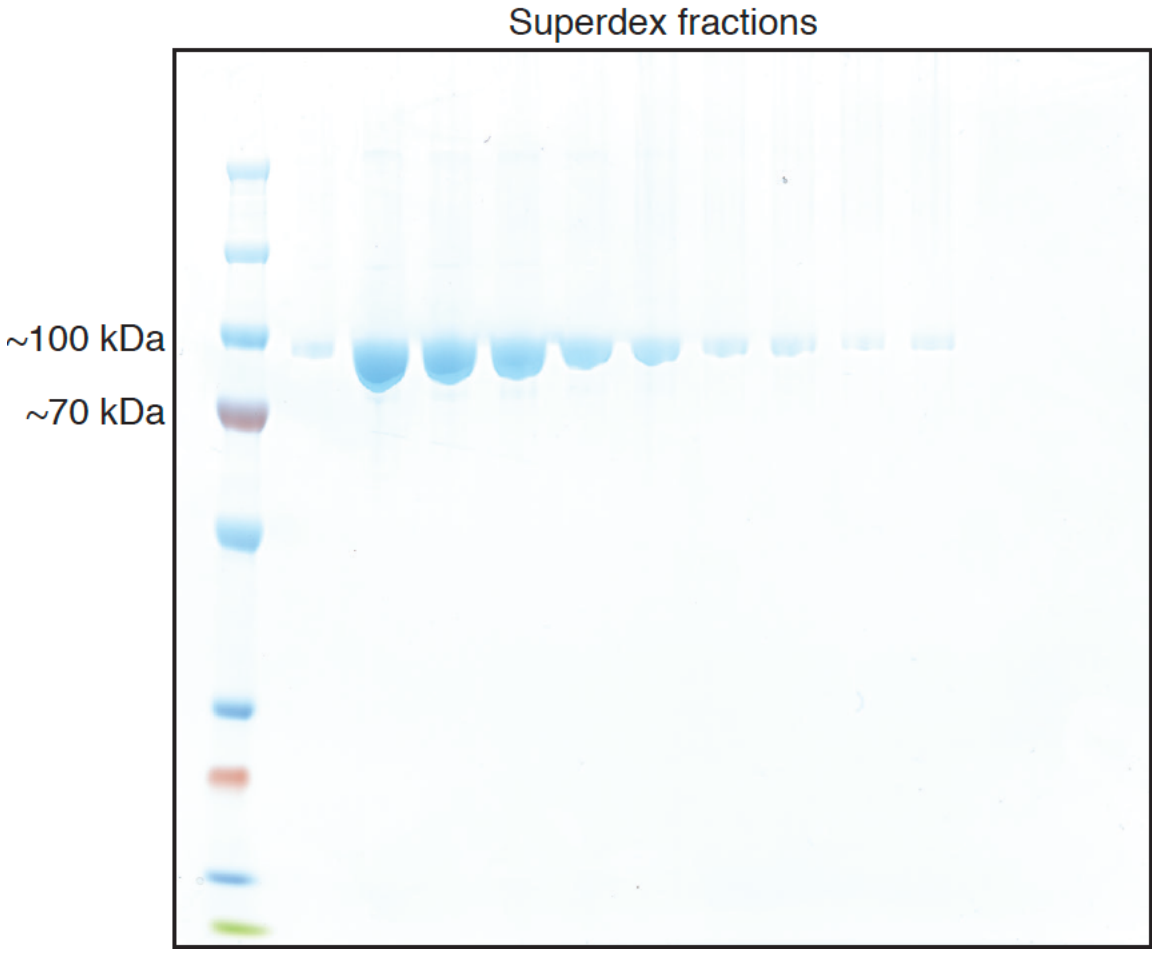

Figure 3: Gel filtration fractions. After gel filtration, the collected fractions were run on a gel to test for their purity before pooling them together. The gel was run at 180V for 50 min in MOPS buffer using a 12% premade gel.

5. References

1. Saha, S. *et al.* Polar Positioning of Phase-Separated Liquid Compartments in Cells Regulated by an mRNA Competition Mechanism. *Cell* **166**, 1572-1584.e16 (2016).

6. Change history

Version….from	Page	Changes/ additions
1.1 (27/05/2020)	8-10	Figures added, spell check
1.2 (02/06/2020)	4-10	Corrections from Martine, new logo (header)
1.3 (03/07/2020)	7-8	Graph corrected by Juan, updated by Martine