Hyman Lab
Max Planck Institute of Molecular Cell Biology & Genetics

SOP_hymanLab-02 – FUS purification

Authors: Juan M. Iglesias-Artola & Anatol W. Fritsch
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
FUS	Fused in Sarcoma
H_2O	Water
KCl	Potassium Chloride
L	Liters
min	Minutes
mL	Milliliter
mM	Millimolar
PMSF	Phenylmethylsulfonylfluorid
RT	Room temperature
TEV	Tobacco Etch Virus
X *g*	Gravity

1. Aim

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

2. Background

FUS is a prion-like protein containing low complexity domains with a strong binding affinity to RNA. Monitoring the 280/260 absorbance ratio during the purification process is therefore required to give as an indication of the removal of nucleic acids. The saturation concentration of FUS *in vitro* is 2 µM at 75 mM KCl [1]. To prevent condensation of FUS during the purification, concentration and storage processes, the salt concentration is kept very high (> 500 mM KCl).

The current protocol for the purification of FUS has been adapted from Patel et al (2015) [2]. Our construct contains a N-terminal FUS flanked by a C-terminal TEV cleavage site, an internal His6 and a C-terminal mGFP 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 FUS. Cleavage by TEV protease generates untagged FUS. The FUS fraction is separated from the His-mGFP fraction by a subsequent size exclusion chromatography step. To obtain mGFP labeled FUS, 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 up to 5 mg protein per Liter of cells infected.

3. Procedure

3.1 Buffers

3.1.1 Lysis buffer

Chemical	Concentration (mM)
Tris pH 7.4	50
KCl	500
Glycerol	5%
Imidazole	10
PMSF	1
Protease Inhibitor cocktail	1 tablet

3.1.2 Ni-NTA wash buffer

Chemical	Concentration (mM)
Tris pH 7.4	50
KCl	500
Glycerol	5%
Imidazole	20

3.1.3 Ni-NTA elution buffer

Chemical	Concentration (mM)
Tris pH 7.4	50
KCl	500
Glycerol	5%
Imidazole	300

3.1.4 MBP Wash buffer

Chemical	Concentration (mM)
Tris pH 7.4	50
KCl	500
Glycerol	5%

3.1.5 MBP elution buffer

Chemical	Concentration (mM)
Tris pH 7.4	50
KCl	500
Glycerol	5%
Arginine	500
Maltose	20

3.1.6 **Storage buffer**

Chemical	**Concentration (mM)**
Hepes pH 7.25	50
KCl	750
Glycerol	5%
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 with the following construct:

- *Used by authors: 079JieFUSno-optimizedpOCC177pOEM1-N-HIS-MBP-PS-NotI-AscI-TEV-SNAPs*
- *Available in the Hyman lab DNA library: TH0901-pOCC177-FUS-wt_opt(Nhe).ape*

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 15,000 psi

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

3.3 **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 2.5 mL/min – Collect 50 µL of flow through – Sample 2

3.3.4 Rinse the columns with 10 CV **Ni-NTA wash buffer** at 5 ml/min – Sample 3

3.3.5 Elute 1.5 ml fractions with **Ni-NTA elution buffer** at 5 ml/min

3.3.6 Pool fractions containing the protein after confirmation by Bradford – Collect 50 µL – Sample 4

3.4 **MBP Trap HP column purification, peristaltic pump at RT**

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

3.4.2 Equilibrate the columns with 10 CV **Ni-NTA elution buffer** at 5 mL/min

3.4.3 Load pooled protein fractions from the Ni-NTA elution at 1 mL/min – Collect 50 µL of flow through – Sample 5

3.4.4 Rinse the columns with 10 CV **MBP wash buffer** at 2.5 ml/min – Sample 6

3.4.5 Elute 1.5 ml fractions with **MBP elution buffer** at 5 ml/min

3.4.6 Pool fractions containing the protein after confirmation by Bradford – Collect 50 µL – Sample 7

3.5 **3C and TEV protease cleavage, overnight at 18 °C**

3.5.1 Dilute the purified protein to a concentration of less than 15 µM using **MBP elution buffer**

3.5.2 Add 3C and TEV proteases to a final concentration of 1 mg protease for 100 mg of protein substrate

3.5.3 Incubate sample overnight at 18 °C

3.5.4 Confirm complete cleavage of the MBP and SNAP tags by gel electrophoresis – Collect 50 µL – Sample 8

3.6 **SepFast GF-HS-L 26x600mm, gel filtration and buffer exchange, ÄKTA pure at RT**

3.6.1 Equilibrate column with **Storage buffer**

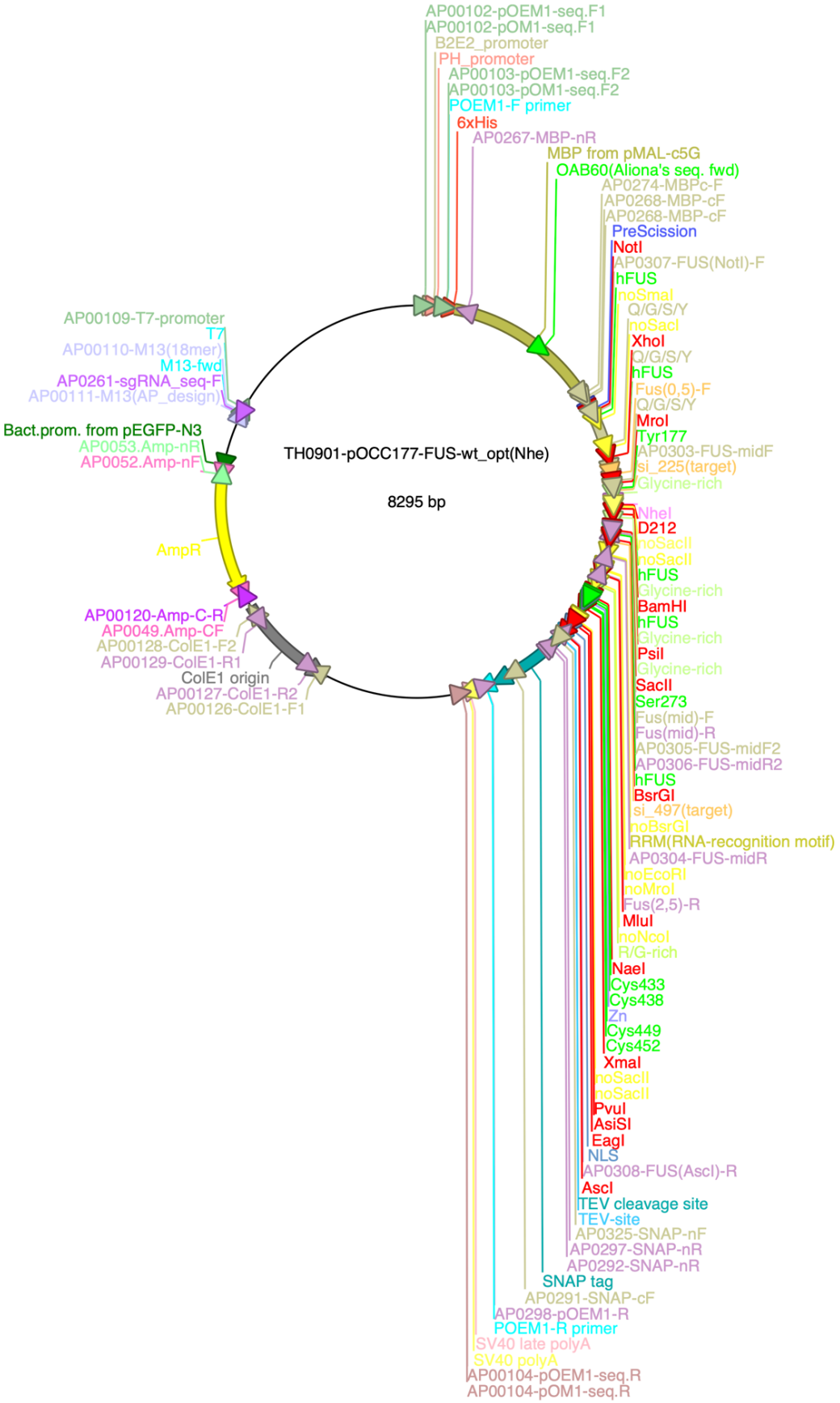
3.6.2 Concentrate sample to 12 mL and load to column

3.6.3 Collect the fractions containing the first peak in the chromatogram – Collect 50 µL – Sample 9

3.6.4 Concentrate the protein to a final concentration of 15 µM

4. **Attachments:**

4.1 **Construct Map: TH0901-pOCC177-FUS-wt_opt(Nhe)**


TH0901-pOCC177-FUS-wt_opt(Nhe)
8295 bp
AP00102-pOEM1-seq.F1
AP00102-pOM1-seq.F1
B2E2_promoter
PH_promoter
AP00103-pOEM1-seq.F2
AP00103-pOM1-seq.F2
POEM1-F primer
6xHis
AP0267-MBP-nR
MBP from pMAL-c5G
OAB60(Aliona's seq. fwd)
AP0274-MBPc-F
AP0268-MBP-cF
AP0268-MBP-cF
PreScission
NotI
AP0307-FUS(NotI)-F
hFUS
noSmaI
Q/G/S/Y
noSacI
XhoI
Q/G/S/Y
hFUS
Fus(0,5)-F
Q/G/S/Y
MroI
Tyr177
AP0303-FUS-midF
si_225(target)
Glycine-rich
NheI
D212
noSacII
noSacII
hFUS
Glycine-rich
BamHI
hFUS
Glycine-rich
PsiI
Glycine-rich
SacII
Ser273
Fus(mid)-F
Fus(mid)-R
AP0305-FUS-midF2
AP0306-FUS-midR2
hFUS
BsrGI
si_497(target)
noBsrGI
RRM(RNA-recognition motif)
AP0304-FUS-midR
noEcoRI
noMroI
Fus(2,5)-R
MluI
noNcoI
R/G-rich
NaeI
Cys433
Cys438
Zn
Cys449
Cys452
XmaI
noSacII
noSacII
PvuI
AsiSI
EagI
NLS
AP0308-FUS(AscI)-R
AscI
TEV cleavage site
TEV-site
AP0325-SNAP-nF
AP0297-SNAP-nR
AP0292-SNAP-nR
SNAP tag
AP0291-SNAP-cF
AP0298-pOEM1-R
POEM1-R primer
SV40 late polyA
SV40 polyA
AP00104-pOEM1-seq.R
AP00104-pOM1-seq.R
AP00109-T7-promoter
T7
AP00110-M13(18mer)
M13-fwd
AP0261-sgRNA_seq-F
AP00111-M13(AP_design)
Bact.prom. from pEGFP-N3
AP0053.Amp-nR
AP0052.Amp-nF
AmpR
AP00120-Amp-C-R
AP0049.Amp-CF
AP00128-ColE1-F2
AP00129-ColE1-R1
ColE1 origin
AP00127-ColE1-R2
AP00126-ColE1-F1

5. Comments

- Before the cleavage of the MBP tag, FUS is highly soluble and will not phase separate during the purification procedure.
- The SepFast GF-HS-L 26x600mm column is the only column currently available at the chromatography facility that can efficiently separate FUS from the MBP and SNAP proteins in one run. If using other columns, the MBP and SNAP tags have to be cleaved and filtered separately.
- We have used arginine to reduce phase separation during tag cleavage. However, it might be necessary to test other additives to the buffer that would allow to carry out the cleavage reaction at higher protein concentrations. One could test aromatic amino acids for example.
- Care should be taken when measuring the final concentration of FUS. Phase separation can be observed as an increase in the absorbance at 500 nm using a Nanodrop in UV-Vis settings. If droplets are present the estimated concentration will be highly overestimated. In this case, the stock should be diluted until no absorbance is observed at 500 nm. FUS extinction coefficient at 280 nm is ε = 92030
- In the last purification, we prepared 18 µM stocks. However, after filtration of the sample before each experiment, the protein concentration was reduced to approximately 10 µM.
- The storage buffer contains 750 mM KCl, however, it should be tested if reducing KCl to 500 mM is still possible. A reduction in salt concentration will be important for further experiments with the purified protein.
- HEPES was preferred over TRIS for the storage buffer since it has lower temperature dependence.

6. References

1. Wang, J. *et al.* A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. *Cell* **174**, 688-699.e16 (2018).

2. Patel, A. *et al.* A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. *Cell* **162**, 1066–77 (2015).

7. Change history

Version….from	Page(s)	Changes/ additions
1.1 (19.08.2020)	3,6,8	Introduction added, corrected 3.5.2 and 5
1.2 (19.08.2020)	8	References added with Papers3