VBCF – Protein Technologies	**Standard Operation Procedure** **E.coli small-scale expression and purification**

1. **Aim:**
   Test expression and affinity purification of several constructs/different E. coli strains in parallel
2. **Applicability:**
   Sequence-verified plasmids suitable for expression in E. coli (DE3) cells
3. **Responsibilities:**
   Main Responsible: Anita Lehner and Christian Gruber
4. **Required materials:**
   Chemically competent E. coli cells (self-made)
   LB media
   TB media + Phosphate Buffer
   Lactose
   Appropriate Antibiotics
   Culture tubes (Falcon 352059)
   24 Well plates (round or conic bottom)
   250 ml flasks with baffles
   48 well plates
   96 well plates (preferably square shapes wells)
   Appropriate buffers
   FastBreak cell lysis reagent (ProMegaV847C)
   Benzonase (MBS)
   Airpore sealing tape (ROTH EN84.1)
   Sealing tape for PCR plates (ThermoFisher 15036)
   Repligen Robocolumns 100 µl

5. **Procedure:**

Day 1 Transformation of plasmids/ Set up of overnight culture

Transform 0.5 – 1ul of expression plasmid into the appropriate E. coli strains

- Use a GFP construct as positive control (e.g. GFP-pETM14) for each condition/ cell strain
- Label 1.5 ml Eppis accordingly. Put 0.5 µl plasmid into each Eppi.
- Get appropriate chemical competent E. coli cells (50 µl per transformation, 1 Aliquot contains 100 µl) from the -80° C freezer.
- Let the cells thaw on ice and add 50 µl to each Eppi.
- Incubate 15 min. on ice.
- Heat shock for 45 sec. at 42° C.
- Incubate for 2 min. on ice.
- Add 400 µl of room temperature SOC media and incubate shaking at 37° C (for some cell lines temperatures might differ, e.g Shuffle at 30° C) for at least 1h*
- Label culture tubes accordingly and add 5 ml of LB media + appropriate Antibiotic

- Pipette 50 µl of transformation reaction into culture tubes prepared in the previous step.
- Incubate shaking at 37° C (for some cell lines Temp. might differ, e.g Shuffle at 30° C) over night.

* Longer incubation times are possible. For Ampicillin resistant plasmids a shorter incubation time is also possible but not recommended. For lower incubation temperatures increased times are advisable.

Day 2 Set up of expression plates

Expression in 24 well plates using TB media

- Each condition should be set up in duplicates!
- Create an Excel sheet indicating the position of each sample in the plate, save in appropriate project folder including the date in the file name.
- Mix 900 ml TB media with 100 ml Phosphate buffer supplied by the media kitchen.
- Add 1.5% w/v Lactose (15 g/l)
- Add appropriate antibiotics
- Pipette 2 ml of prepared media into the required number wells of a 24 well plate
- Label the plate with date and expression temperature
- Dilute overnight culture 1:100 in expression media (20 µl/ 2 ml)
- Seal plate with Airpore seal.
- Incubate plate shaking at 250 rpm at required temperature.
- Conditions routinely used:
    - 25° C for 24 h
    - 1h at 37° C then shift to 18° C for 23 h

Expression in flasks using LB media Induction with IPTG

- Add appropriate antibiotic to LB.
- Put 50 ml of media in 250 ml baffled flasks
- Dilute overnight culture 1:50 in expression media (1 ml/ 50 ml)
- Incubate shaking at 220 rpm at 37° C measure OD600 after 1h
- Conditions routinely used:
    - 37° C for 4 h, induction with 400 µM IPTG** at OD600: 0.6-0.8
    - 37° C to OD_{600} of 0.5 then shift to 18° C, Induction 30 min later at OD_{600} of 0.6-0.8
- Harvest 37° C culture 4h of induction. Harvest 2x 15 ml culture (4000 rpm 15-30 min.) discard supernatant and aspirate residual liquid with vacuum pump.
- Store at - 20° C till further use.

** Concentration of IPTG can be varied from 100 µM to 1 mM. E.g. if protein is prone to insoluble expression low amounts of IPTG might be beneficial.

Day 2/3 Preparation of buffers

Refer to Notes. “how to choose an appropriate buffer”

- For IMAC:
  Equilibration buffer and wash I should contain 20 mM Imidazole
  (e.g. 50 mM Hepes, 300 mM NaCl, 20 mM imidazole pH 7.5)
  Wash II: should contain 40 mM Imidazole
  (e.g. 50 mM Hepes, 300 mM NaCl, 40 mM imidazole pH 7.5)
  Elution: should contain 250- 500 mM Imidazole
  (e.g. 50 mM Hepes, 300 mM NaCl, 500 mM imidazole pH 7.5)

- For Strep- Affinity
  Equilibration and wash buffer: no additive
  (e.g. 50 mM Hepes, 300 mM NaCl, pH 7.5)
  Elution: should contain 2.5 or 5 mM d-Desthiobiotin
  (e.g. 50 mM Hepes, 300 mM NaCl, pH 7.5, 5 mM d-Desthiobiotin)

- For GST- Affinity
  Equilibration and wash buffer: no additive
  Elution: should contain 10 mM GSH (reduced Glutathione)
  (e.g. 50 mM Hepes, 300 mM NaCl, pH 7.5, 10 mM GSH
  pH of buffer needs to be adjusted after addition of GSH!

Day 3 Harvest cultures

- Centrifuge 24 well plates at 4000 rpm for 30 min.
- Aspirate media with vacuum pump, be careful not to disturb pellet
- Check size of pellets, write down if size of pellets differs
- Seal plate with sealing tape for PCR plates
- Store at -20° C till further use
- LB culture: harvest 2x 15 ml culture (4000 rpm 15-30 min.) discard supernatant and aspirate residual liquid with vacuum pump.
- Store at - 20° C till further use.

If purification is done at the same day store pellets at -20 ° C or -80° C till pellets are frozen. The freeze-thaw cycle enhances cell lysis.

Day 3/4 Purification and SDS-PAGE analysis

Samples are processed in columns of 8. Only 8 samples can be processed in parallel, therefore it is advisable to lyse only 8 samples each. If more than 8 samples are pro-cessed start lysis of 2nd round as soon as 1st round is loaded onto the columns.
Plan the loading scheme accordingly and save in the Excel file created for expression.

Prepare the robot and columns

- Run the flush/ wash program. Check if there are air bubble in the translucent tubes. Repeat flush/wash till air is removed completely
- Prepare a 48 deep well plate according to the scheme below

	1	2	3	4	5	6
A	dH_2O	Equilibration	dH_2O	Equilibration	dH_2O	Equilibration
B	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
C	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
D	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
E	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
F	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
G	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
H	dH2O	Equilibration	dH2O	Equilibration	dH2O	Equilibration
	1st row of columns		2nd row of columns		3rd row of columns	
3 ml of buffer per well for 3 rows of columns						

- Put required number of mini columns into the column holding plate
- Choose program, 24 well sample, 48 well equilibration, wash and elute
- De- select option column regeneration
- Put 48 well onto the "Equilibrate" position
- Change the settings of the program to:
  - Select column 1-6 if required
  - Number of robo columns: 8 (choose 8 even if you are using a lower number of col-umns!)
  - Type of robo column: 100 µl
  - Equilibration: 1500 µl
  - Number of collected equilibration fractions: 0
  - Number of Equilibration cycles: 2 replace with screen shot
- Set all other volumes, fractions and cycles to 0
- Start the program
- The robot will equilibrate the 24 columns in row 1-3

While equilibrating check if liquid is dripping out of all columns, if not column might be blocked and needs to be replaced. If 6 rows of columns are required de-select row 1-3 and select rows 4-6. Add additional liquid to the plate and re-run the same program.

Lysis

- Label 2 ml Eppis accordingly
- Pre- cool tabletop centrifuge to 4° C,
- Lysis buffer: 1.5 ml per sample are required, prepare 15 ml/ 8 samples. Pipette appropriate amount of 10x FastBreak lysis reagent (1.5 ml for 15 ml), add 2 µl Benzonase per ml of buffer (3 µl for 15 ml), fill up to final Volume with equilibration buffer (this leads to a dilution of buffer components by a factor of ~1,1276, e.g. 50 mM Tris is diluted to 44,34 mM)
- Resuspend each pellet in 1.5 ml of lysis luffer and transfer into Eppi
- Incubate 15 min. at room temperature, slightly shaking
- In the meantime, prepare 96 well plate for SDS- PAGE samples: dilute 6x SDS PAGE loading dye (SDS LD) 1:5 with dH2O (10 µl loading dye + 40 µl dH2O per sample required, prepare more to account for loss while pipetting)
- Distribute according to scheme below.
- Distribute 5 µl of undiluted 6x SDS LD according to scheme below

	1 (WC)	2 (SN)	3 (E1)	4 (WC)	5 (SN)	6 (E1)	7 (WC)	8 (SN)	9 (E1)	10 (WC)	11 (SN)	12 (E1)
A (sample1)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
B (sample2)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
C (sample3)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
D (sample4)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
E (sample5)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
F (sample6)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
G (sample7)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
H (sample8)	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted	10 µl SDS LD 1:5	10 µl SDS LD 1:5	5 µl SDS LD undiluted
	add 10 ul of WC sample	add 10 ul of SN sample	add 25 ul of Elution 1	add 10 ul of WC sample	add 10 ul of SN sample	add 25 ul of Elution 1	add 10 ul of WC sample	add 10 ul of SN sample	add 25 ul of Elution 1	add 10 ul of WC sample	add 10 ul of SN sample	add 25 ul of Elution 1
	1st row of columns			2nd row of columns			3rd row of columns			4th row of columns		

- Seal SDS PAGE sample plate plate with sealing tape for PCR plates (to prevent drying of the SDS LD)
- After 15 min. mix 10 µl of sample with loading dye in column 1 of 96 well plate prepared in previous step. This is the whole cell lysate (WC) sample, diluted 1:6.
- Spin down sample at 21130 rcf for 15 min in pre-cooled centrifuge
- Pipette Supernatant into column 1 of a 48 well plate (for 2nd run of 8 into column 2, for 3rd run of 8 into column 3)
- Use a multi-channel pipette to transfer 10 µl of samples to column 2 of the 96 well plate for SDS- PAGE samples. This is the soluble fraction (Supernatant = SN)
- Seal SDS PAGE sample plate with sealing tape for PCR plates (to prevent drying of the SDS LD)
- Store the plate containing the samples to be purified at 4° C till further use

Buffer plates for purification

- While samples are in the centrifuge, prepare the buffer plates according to the scheme below
- If Strep or GST purifications are done in the same run as IMAC purifications fill corresponding Wash II wells with Wash I buffer
- Wash plate:

	1	2	3	4	5	6
A	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
B	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
C	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
D	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
E	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
F	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
G	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
H	Wash I	Wash II	Wash I	Wash II	Wash I	Wash II
	1st row of columns		2nd row of columns		3rd row of columns	
	3 ml of buffer per well for 3 rows of columns					

- Put the plate onto wash 1 position
- If > 3 rows of columns are used prepare a 2nd wash plate onto put it onto position wash 2
- Elution plate:

	1	2	3	4	5	6
A	Elution	Elution	Elution	Elution	Elution	Elution
B	Elution	Elution	Elution	Elution	Elution	Elution
C	Elution	Elution	Elution	Elution	Elution	Elution
D	Elution	Elution	Elution	Elution	Elution	Elution
E	Elution	Elution	Elution	Elution	Elution	Elution
F	Elution	Elution	Elution	Elution	Elution	Elution
G	Elution	Elution	Elution	Elution	Elution	Elution
H	Elution	Elution	Elution	Elution	Elution	Elution
	1st run	2nd run	3rd run	4th run	5th run	6th run
	1 ml of buffer per well for 6 x 8 runs					

- Put the plate onto Elution position

Positioning of samples and plates for fraction collector

- Put the 48 well sample plate onto Equilibration positon
- Put a 96 deep well plate on Pos. 1 of the fraction collector
- Put a 96 well plate onto Pos. 2 of the fraction collector, use the large adapter to adapt the height

Set up the for the robot for purification

- Choose program, 24 well sample, 48 well equilibration, wash and elute
- De- select option column regeneration
- Change the settings of the program to:
    - Select column 1-6 (if required)
    - Number of robo columns: 8 (choose 8 even if you are using a lower number of columns!)
    - Type of robo column: 100 µl
    - Equilibration: 1200 µl (that corresponds to the sample loading)
      Number of collected equilibration fractions: 1
      Number of Equilibration cycles: 1 replace with screen shot
    - Wash: 1500 µl
      Number of collected wash fractions: 1
      Number of wash cycles: 2
    - Elution: 400 µl
      Number of collected elution fractions: 2
      Number of elution cycles: 1
- Start the program

If error message "not enough liquid detected in tip 1-8" appears: for the samples, choose go to bottom. For Buffers choose retry and add more buffer as soon as the tips are at safe height.

The robot will collect the flow through and the washing steps in the 96 deep well plate in Pos 1. of the fraction collector. The Elutions will be collected in the 200 µl 96 well plate in position 2.

96 deep well collection plate:

	1	2	3	4	5	6	7	8	9	10	11	12
A (sample1)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
B (sample2)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
C (sample3)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
D (sample4)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
E (sample5)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
F (sample6)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
G (sample7)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
H (sample8)	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II	flow through	wash I	wash II
	1st row of columns			2nd row of columns			3rd row of columns			4th row of columns		

If more than 4 rows of columns are used plate must be exchanged after 4th run.

<u>200 µl 96 well plate:</u>

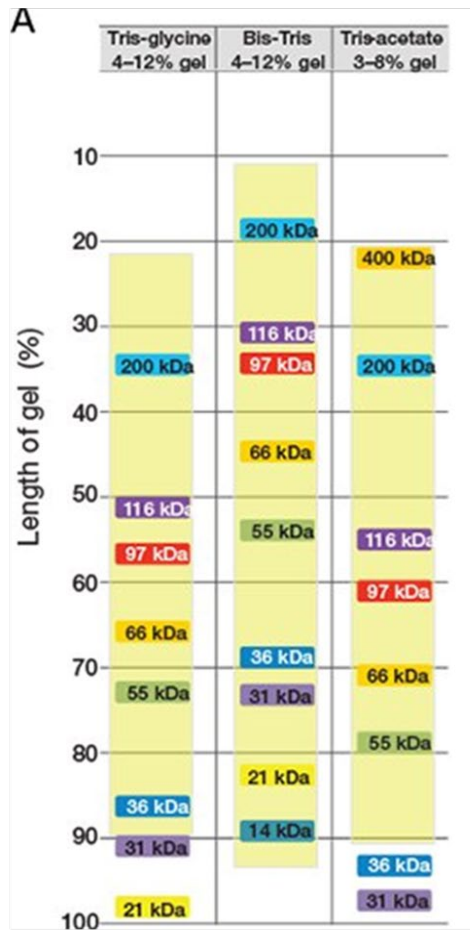
	1	2	3	4	5	6	7	8	9	10	11	12
A (sample1)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
B (sample2)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
C (sample3)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
D (sample4)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
E (sample5)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
F (sample6)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
G (sample7)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
H (sample8)	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2	Elution 1	Elution 2
	1st run		2nd run		3rd run		4th run		5th run		6th run	

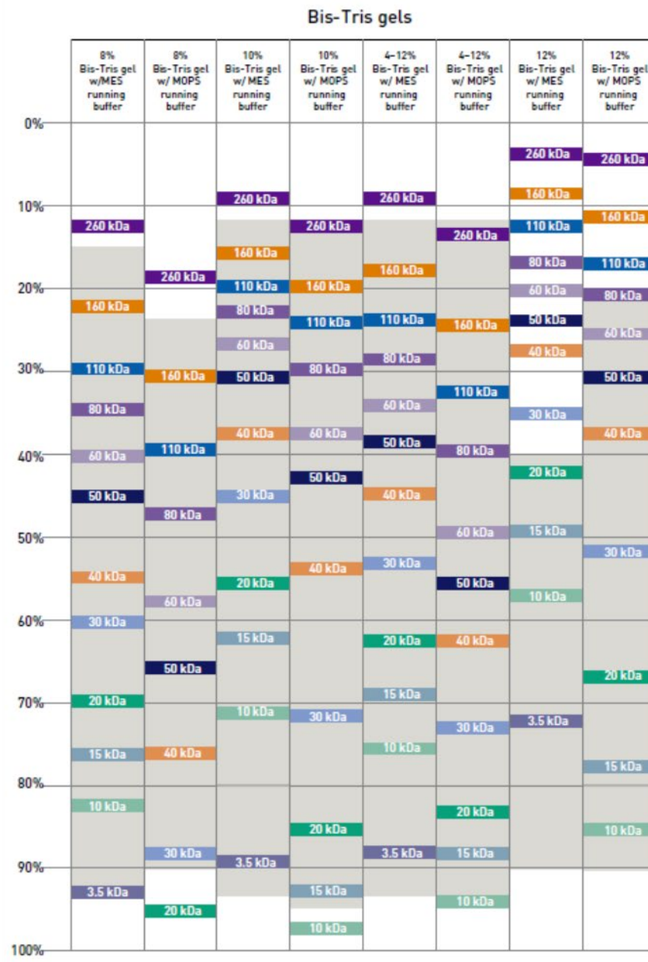
- Using a multi- channel pipette transfer 25 µl of Elution 1 (E1) into the corresponding wells of the SDS- PAGE sample plate
- Seal both collection plates with PCR sealing tape, label them accordingly and store at 4 °C.

SDS PAGE Analysis:

- Check the predicted MW of the proteins and choose gel and running buffer accordingly
  Most of the time 4-12% gels with MES or MOPS buffer are sufficient

NuPAGE 4- 12% Bis- Tris gradient gel run with MES Buffer: 3.5 – 160 KDa
NuPAGE 4- 12% Bis- Tris gradient gel run with MOPS Buffer: 15 – 260 KDa
NuPAGE 3- 8% Tris- Acetate gradient gel run with Tris- Acetate Buffer: 40- 500 kDa
Novex 10- 20% Tris- Glycine gradient gel run with Tris Glycine Buffer: 10- 100 kDa





Separation range information from ThermoFisher

- Before loading the samples, rinse the slots with running buffer by pipetting up and down
- Load 12 µl of WC and SN samples and 18 µl of E1. If possible, load all 3 samples of each purification next to each other on the gel.
- Re- seal SDS PAGE sample plate and store at 4 °C.
- Run gel according to recommendations of manufacturer
- Stain with Instant blue and proceed to reporting

Cleaning of columns

- Prepare a 48 well plate according to the scheme below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH
B	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH
C	500 mM Imidazole	dH2O	20% EtOH	500 mMImidazole	dH2O	20% EtOH	500 mMImidazole	dH2O	20% EtOH	500 mMImidazole	dH2O	20% EtOH
D	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH
E	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH
F	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH
G	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH	500 mM Imidazole	dH2O	20% EtOH
H	500mM Imidazole	dH2O	20% EtOH	500mM Imidazole	dH2O	20% EtOH	500mM Imidazole	dH2O	20% EtOH	500mM Imidazole	dH2O	20% EtOH
	1st row of columns			2nd row of columns			3rd row of columns			4th row of columns		

- Put plate onto wash position
- Change settings to:
  - Program: 24 well sample 96 well equilibration, wash and elution
  - Wash: 1500 µl
  - Number of collected wash fractions: 0
  - Number of wash cycles: 3
  - Set all other values except flow rate to 0
  - De- select column regeneration
- If > 4 rows of columns were used prepare a 2nd cleaning plate and put onto position wash 2

As soon as program is finished pipette some 20% EtOH on top of the columns and seal with the rubber seals. Make sure that all columns are sealed properly!

NOTES

How to choose an appropriate buffer:

Start with blasting the protein sequence and check Database entries (e.g. Uniprot) for more information. If for example, the protein binds metal Ions, it might be a good idea to add some to your buffer.

In general, for a start the chosen buffers should be kept as simple as possible.

The components of the buffer should not interfere with the affinity purification (e.g. EDTA or high concentrations of DTT and IMAC) or with any required downstream applications like concentration measurements via colorimetric assays (e.g. TCEP and BCA assay).

The buffer should also not interfere with any activity of the target protein (e.g. Tris in-terferes with activity of some enzymes).

The buffer components should not interact with each other (e.g. DTT is a weak chelator if metals ions are required use TCEP instead. Phosphate buffers form insoluble adducts with metal Ions)

More information can be found here:

https://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html

https://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers.html

If you click on the buffer substance in the diagram, more information about the substance will be shown.

If there is no additional information available, the following suggestions are a good starting point:

• **pH**: Check the pI of the proteins. If possible, use a pH that is at least 1 Unit above or below than the predicted pI. The pH must be compatible with the affinity purification (e.g. acidic buffers are incompatible with IMAC and Strep affinity purifications)

Refer to websites above to choose an appropriate buffer substance for the chosen pH value. Keep in mind that some buffers (especially Tris) change pH with temperature.

• **Salt**: usually NaCl or KCl from 150-300 mM. 300 mM NaCl is a good starting point. Salt concentration can be de- or increased depending on behavior and function of the protein. E.g nucleic acid binding proteins do prefer higher amounts of salt, 500 mM NaCl is a good starting point for those

• **Reducing agents**: if a protein contains Cystein residues it is usually recommended to add some reducing agents. 0.5-1 mM TCEP is a good starting point

Available E. coli Expression Strains

E. coli strain	Resistance	Application/ Description
Bl21 (DE3)	none	for **routine T7 expression**/ Protease deficient B strain
Rosetta 2 (DE3)	Chloramphenicol	expression of eukaryotic **proteins that contain codons rarely used in E. coli.**
Lemo21 (DE3)	Chloramphenicol	**Tunable T7 Expression** Strain for difficult targets membrane proteins, toxic proteins and proteins prone to insoluble expression Tunable expression is achieved by varying the level of lysozyme (lysY), the natural inhibitor of T7 RNA polymerase. The level of lysozyme is modulated by adding L-rhamnose to the expression culture at levels from zero to 2000 µM. When Lemo21(DE3) is grown without rhamnose, the strain performs the same as a pLysS containing strain.
LOBSTR	none	Based on BL21(DE3) **reduces** the polyhistidine-tag binding affinities of **ArnA and SlyD** the two most common E. coli contaminants
SHuffle® T7 Express	Spectinomycin and Streptomycin but **not required** for expression	E. coli B cells with enhanced capacity to correctly **fold proteins** with **multiple disulfide bonds in the cytoplasm** Constitutively expresses a chromosomal copy of the disufide bond isomerase DsbC
Rosetta gami 2 (DE3)	Chloramphenicol	K-12 derivative that carry trxB/gor mutations for **folding proteins** with **multiple disulfide bonds in the cytoplasm** supplies 7 **rare tRNA**s
BL21 AI	none	for applications that require **tight regulation** of T7 RNAP under the control of the arabinose-inducible araBAD promoter, especially useful for the expression of genes that may be toxic to other BL21 strains where basal expression of T7 RNAP is leaky

Some cell lines do grow quite slowly, especially Rosetta gami 2. If using Rosetta gami 2 do transformation in the morning and incubate overnight culture for 24 h.

The development of this SOP was supported by the programme Interreg V – A Slovakia - Austria, project CAPSID.