IMPACT-CN

- Preparation of Plasmids
  - ◊ Clone your protein fragment into one of the following vectors:
    - pTXB1
    - pTYB1
    - pTYB2
    - pTYB11
    - pTYB12
  - ◊ transfer the complete construct into ER2566 via electro transformation
- cell growth
  - ◊ prepare a pre-culture with some cell material within up to 10mL LB substituted with 200 µg/mL Amp and 20 µg/mL Cm
  - ◊ incubate the cultures overnight at 37°C
  - ◊ prepare the main culture:
    - 250mL LB (+Amp and Cm)
    - Inoculate the culture to an OD_{600} of 0.1 with the pre culture
  - ◊ Incubate the main culture at 37°C until an OD600 auf 0.5 to 0.8 is reached
  - ◊ Induce the protein expression via addition of IPTG (0.3-0.5mM)
    - (1M stem solution: 125 µL per culture)
  - ◊ Let the cells grow further for 30min at 37°C
  - ◊ Protein expression over night at 19°C
- Cell harvest and decomposition

- ◊ Pellet the whole culture in large centrifugal jars
    - 4,500rpm, 10-20min, 4°C
- ◊ Discard the supernatant
- ◊ Resuspend the pelleted cells in 20-30mL lysis solution
    - Retransfer them into falcon tubes
- ◊ Decompose the cells using the French Press
    - 2 times for each sample
    - Pressure of approximately 1200
    - Work with ice whenever possible
- ◊ Centrifuge the samples to divide the desired substances from the cell debris
    - 4,500rpm, 1h, 4°C
    - Transfer the supernatant into falcon tubes

- Column preparation
    - ◊ Close the lower part of the columns with Para film
    - ◊ Fill them with 5mL of water(=1volume) and mark the fill level
    - ◊ Let the water out
    - ◊ Fill the column with chitin beads up to the marking
        - An evenly surface gets better results
    - ◊ Put a filter plate onto the beads
    - ◊ **From here, all steps should be carried out at 4°C**
    - ◊ Equilibrate the columns with 10volumes of column buffer (low-salt)
- Loading of the columns
    - ◊ Put the supernatant onto the columns
    - ◊ Make sure the flow-through is less than 0.5-1 mL/min

        - E.g. with the use of clamps

- Washing of the columns
    - ◊ Wash the columns with 10 volumes of the column buffer (high salt)
- Induction of cleavage
    - ◊ Wash the columns with 3 volumes of cleavage buffer
        - Fast, so no clamps!
    - ◊ Let the columns induce at 4-23°C for at least 16 (up to 40) hours
- Elution
    - ◊ Eluate the columns with 1-2volumns column buffer (low salt)
    - ◊ Collect the eluate within falcon tubes or blue caps
    - ◊ Concentrate the eluate by using suitable protein filters
        - Centrifuge for 30-45minutes at full speed up to an end volume of about 250 µL
    - ◊ Wash the concentrated protein solution with 4mL protein wash buffer
        - Repeat this 2-3 times total
    - ◊ Optionally: wash with storage buffer to ensure a longer functionality
    - ◊ Make an Bradford Estimation
        - E.g. with Roti® Nanoquant(Roth), see our other protocols
    - ◊ Store the proteins at the freezer or refrigerator
        - Depending on the usage
- Column regeneration
    - ◊ All of the columns can be reused 4-5 times
    - ◊ Resolve residual proteins with 3 volumes of NaOH (0.3M)
    - ◊ Incubate for 30minutes

- ◊ Wash the columns with further 7 volumes NaOH
- ◊ Wash with 20 volumes of H_2O
- ◊ Wash with 5 volumes of column buffer
- ◊ Store the columns at 4°C, closed with Para film

- Solutions needed for this protocol:
  - ◊ Lysis Buffer

Substance	concentration	mass/volume in 1L
Triton X-100	0.1%	1mL
PMSF	20 µM	200 µL
TCEP	0.1-1mM	100-1000 µL
Make the following stem solutions: • 100mM PMSF • 1M TCEP Resolve the substances within column buffer (low salt)		

  - ◊ Column Buffers

substance	concentration	mass/volume in 1L
Na_2HPO_4 • 2 H_20	20mM	3,6g
NaCl	50mM (low salt) 500mM (high salt) 1000mM(high salt) 1500mM(high salt)	2,92g 29,22g 58,44g 87,66g

EDTA	1mM	10mL of stem solution
• Prepare an EDTA stem solution of 0.1M, resolve in ddH_2O • Resolve all substances in ddH_2O • Adjust the pH to 8.0 (6.0-9.0) with phosphoric acid		

◊ Cleavage Buffer

substance	concentration	mass/volume in 1L
Na_2HPO_4	20mM	3.6g
NaCl	50mM (optionally up to 1000mM)	29g
EDTA	1mM	10mL
DTT	50mM	*
• *0.93g DTT resolved in 6mL ddH_20+119mL column buffer (low salt) make up 125mL cleavage buffer • All substances resolved in H_20 • pH has to be adjusted to 7-9 with phosphoric acid • Store dark because of the DTT!		

◊ Protein wash buffer

substance	concentration	mass/volume in 1L
Na_2HPO_4	20mM	3.6g
NaCl	10mM	0.58g
EDTA	1mM	10mL

Originally from NEB, now modified