1) **Prepare construct**
   a) Cloning: Clone the GST Biobrick part BBa_K608408 beforte the N-terminus of the protein you wish to purify. You may want to put a Glycine linker inbetween the two parts, for better expression and folding proterties of the fusion protein. DO NOT USE THE RFC10 STANDARD, but another, which allows fusion proteins. We recommend the Gibson Assembly, it worked conviently for us.
   b) Vector: You may want to use a vector especially designed for protein expression. The iGEM vectors are all high copy plasmids, which can lead to an overload of protein in your E. coli cells. This can result in toxic effects or inclusion bodies.
   c) Transformation: you may want to chose a E. coli strain especially designed for protein expression. It is helpful for a successfull protein purification to use a protease deficient strain such as BL21.

2) **Cell culture and Lysis**
   a) Grow cells overnight after transformation in 2ml medium.
   b) Inoculate 250mL LB in a 1l flask
   c) Optional: induce with IPTG (500 µL)
   d) centrifuging cells in 50ml falcon tubes
   e) resuspend pellets in 7,5µl ice cold PBS,
   f) sonificatie cells (4x 1 min with pause, maximal power, 1 pulse per second),
   g) Add 1,5X Protease inhibitor and 25µl of 10mM Lysozyme and let rotate at room temperature for 30min
   h) Centrifuge lysate for 10min at 500xg

3) **Preparation of glutathione sepharose beads. You may add 1X protease inhibitor to all used PBS.**
   a) Gently shake the bottle of sepharose to resuspend the matrix.
   b) Use a pipet to remove sufficient slurry for use and transfer to an 15 ml falcon tube. (Dispense 1.33 ml of original sepharose slurry per ml of final volume required.)
   c) Sendiment the matrix by centrifugation at 500xg for 5 min. Carefully decant the supernatant.
   d) Wash the sepharose by adding 10 ml of cold 1xPBS per 1.33 ml of the original slurry of glutathione sepharose dispensed. Invert to mix. (Sepharose must be thoroughly washed with PBS to remove the 20% ethanol storage solution. Residual ethanol may interfere with subsequent procedures).
   e) Sediment the matrix by centrifugation at 500xg for 5 minutes. Decant the supernatant.
   f) For each 1.33 ml of the original slurry, add 1 ml of 1xPBS. This produces a 50% slurry. Mix well prior to the subsequent pipetting steps. (Sepharose 4B equilibrated with PBS may be stored at 4°C for up to a month.

4) **Purification of fusion proteins**
   a) Transfer supernatant from step 2)h) on top of slurry from step 4)f)
   b) Rotate falcon for 30 min
   c) Centrifuge at 500xg for 5min
   d) Collect supernatant in a separate 50ml falcon (in case the protein does not attach well to the beads, this supernatant can be used for a second purification)
   e) Add 10ml of cold PBS with protease inhibitor to flask with the beads
   f) Rotate falcon for 10 min
   g) Repeat steps c) - f)
   h) Centrifuge at 500xg for 5min
   i) Collect supernatant in a separate 50ml falcon (in case the protein does not attach well to the beads, this supernatant can be used for a second purification)
   j) Eluate beads with 1ml elution buffer (50mM Tris pH 8, 10mM Glutathione) – transfer slurry into a 2ml eppi
   k) Centrifuge eppi at 13000rpm to make a stable pellet
   l) Transfer supernatant to a new eppi. Take care not to take up beads from the pellet.
   m) Optional: repeat j) – l) to wash the rest off the beads