Small-scale protein expression

- BL21 DE3 cells (New England BioLabs) were transformed with the expression plasmid. (use the transformation protocol).
- Overnight cultures of single colonies grown in LB media supplemented with maintenance antibiotics were diluted 1000-fold into fresh media (10 mL) with maintenance antibiotics and grown at 37 °C with shaking at 200 r.p.m. to OD600 ~0.4–0.6 before induction with 0.05-0.2% arabinose
- Keep one tube aside as uninduced control
- Cells were grown for a further 3 h at 37 °C with shaking at 230 r.p.m
- Cells were isolated by centrifugation at 5,000*g* for 10 min (Keep cell pellet)
- The resulting pellet was resuspended in 300 µL 10mM TrisHCl pH 8+Lysozyme 0.1mg/ml PMSF 1mM mixed with 150mg of 100µm zirconia beads and lysed on a bead beater (3x 40s speed 6.0 with 1x 60s on ice in between incubated on ice) before centrifugation at 10,000*g* for 2 min (Keep supernatant)
- After separating the beads from the supernatant, another centrifugation follows 13,000*g* for 10 min (Keep both pellet AND supernatant)
- The supernatant was collected as the soluble fraction, and the resulting pellet was resuspended in an additional 300 µL 10mM TrisHCl pH 8+Lysozyme 0.1mg/ml PMSF 1mM to obtain the insoluble fraction
- To 37.5 µL of each fraction was added 12.5 µL 4 × Laemmli sample loading buffer (Bio-Rad) containing 2 mM dithiothreitol (DTT; Sigma-Aldrich)
- After vortexing, the fractions were incubated at 95 °C for 10 min
- 12 µL of each soluble fraction and 6 µL of each insoluble fraction was loaded into the wells of a cast gel (12%). 6 µL of Protein Ladder (Thermo Scientific PageRuler Plus Prestained Protein Ladder) was used as a reference
- Samples were separated by electrophoresis at 180 V for 35 min in 1x SDS running buffer. Gels were stained with Coomassie Blue reagent for 30min (to overnight), then washed several times with H2O (30min to overnight) before imaging with a gel dock