# Construct / plasmid resource

 - name: enterovirus D68 3C protease construct bearing a C-terminal His-tag that crystallised in the space group P21<sub>21</sub>21

 # Brief description of the plasmid

 description: This protein yielded highly reproducible crystals upon microseeding, which typically diffracted to 1.7 Å resolution. This crystal system was DMSO tolerant and therefore suitable for our subsequent fragment soaking.

 # Date it was provided

 date: 2022-03-24

 # Type: One of ['plasmid', 'xray-fragment-screen', 'pdb-deposition', 'assay', 'compounds']

 type:

 #

 protocol: >

 For expression of protein used for crystallisation, the construct was transformed into the E. coli strain BL21(DE3)-RR, and cells were grown at 37°C in TB medium supplemented with kanamycin (50 g/ml). After reaching an optical density at 600 nm of around 1, the temperature was lowered to 18°C before induction of protein expression overnight by adding 0.5 mM IPTG. Harvested cells were resuspended in lysis buffer [10 mM Hepes (pH 7.5), 500 mM NaCl, 5% glycerol, 30 mM imidazole, 0.5 mM TCEP, 1% TX-100, 0.5 mg/mL lysozyme, 0.05 mg/mL benzonase]. Proteins were first purified by immobilized metal affinity chromatography (IMAC) using Ni-Sepharose 6 FF resin (Cytive) the column was washed with binding buffer [10 mM Hepes (pH 7.5), 500 mM NaCl, 5% glycerol, 30 mM imidazole, 0.5 mM TCEP] and target protein eluted using same buffer containing 500 mM imidazole. Protein was lastly purified by SEC (Superose 12 pg,Cytiva) in a buffer consisting of 10 mM Hepes (pH 7.5), 500 mM NaCl, 5% glycerol and 0.5 mM TCEP. Proteins were characterised by SDS-polyacrylamide gel electrophoresis andd mass spec and then flash- frozen in liquid nitrogen, and stored at -80°C until required.

 # Details associated with this plasmid

 # TODO: Eliminate this, since this duplicative of the AddGene site.

 details: |

 \* Vector: pNIC

 \* Entry clone accession:

 \* Cell line: E. coli Rosetta strain BL21(DE3)-RR

 \* Tags and additions: C-terminal, non-cleavable hexahistidine

 \* Construct protein sequence: ` MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQKFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVGGNGAQGFAAMLLHSYFTDTQKHHHHHH `

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