Protocols

ModA-pET21a cloning and ModA purification

Cloning

PCR

We used ex-taq polymerase for all reactions where we use the DNA for anything afterwards, and we also used ex-taq polymerase for colony PCR, plasmid PCR to check plasmids.

the annealing temperature that we used is 55°.

the template we used was synthesized by company(we should add)(modA-pUC101 and the base sequence is introduced).
we used the company protocols accordingly to PCR the ModA that includes the restriction sites(EcoRI and NotI) and a few protect bases.
(F: CCCCGGAATTCATGGCTCGTAAATGGTTG; R: GGCCGCGGCCGCCTTGATTGTAA; these were synthesized by)

Running gels

We used SybrSafe to stain the DNA and imaged in a Transilluminator. We cut out bands under a blue box.

Digests and Ligations

We used takara enzymes, EcoRI and NotI and used the companys protocols accordingly to cut the plasmid, pET21a and PCR produce.
We used takara enzyme, T4 ligase and used the companys protocols accordingly to For ligating the digested plasmid and PCR produce.

Transformation

We transformed our ligated produce into TOP10, and we used extracted plasmid PCR to prove the ligation successful.
The plasmid, ModA-pET21a was transformed into BL21 that is objective to express the protein.

Protein purification

Preparation:

20 mL cultures(Amp+) were incubated overnight in 37° 190rpm. These were diluted as less 2% overnight incubated cultures in 1.5 L the next morning and incubated until the OD reached 0.6-0.8(about 3.5h). The cultures were then induced with 0.5mM IPTG and left about 16h in 16°.

The cells were then pelleted by centrifugation,6000 rpm for 30 minutes and used for the follow work or kept in a -80ºC freezer.

Cell lysis

The frozen pellets were allowed to thaw and The frozen(centrifuged) pellets resuspended in about 80 mL of buffer A(20mM tris 500mM NaCl pH 8). The cells were lysed by probe-sonication at 65% amplitude with 40 minute cycles of 3 seconds on, 21 seconds off. The samples were kept on ice throughout the sonication process. The cell debris was pelleted by centrifugation, 16,000 rpm for 30 minutes and the supernatant removed for purification.

Purification with a Ni column:

The column was washed with buffer A to equilibrate the resin. The samples are added slowly (if necessary, the samples can be added twice)and the column is washed with Buffer B(40 mM imidazole 20mM tris 500mM NaCl pH 8)at least 50ml or the OD 280 less than 40, and then eluted with Eluted Buffer (150 mM imidazole, 20 mM Tris 500 mM NaCl pH 8) 30-50ml.

SDS PAGE:

Samples were prepared for SDS PAGE by adding 15 µL of SDS running buffer to 50 µL of the fractions of interest. 8-12 µL of each sample were run on a polyacrylamide gel against a molecular weight marker.

Native PAGE

This protocol is same as SDS PAGE except that there is no SDS in Native PAGE.