

P080 QuickLyse System

Expressing recombinant proteins in the QuickLyse system**Cloning you cDNA of interest into a QuickLyse expression vector**

Subclone the cDNA encoding your protein to be expressed into a suitable QuickLyse expression vector using any of the unique restriction sites available in the multiple cloning site.

pEAS-1a

The multiple cloning site of this vector supports blue/white selection. Subcloning of your insert results in disruption of the coding sequence for the α fragment of β -galactosidase. Consequently, vectors carrying the insert give rise to white colonies when grown in an *E. coli* strain expressing the ω fragment of β -galactosidase (XL1-Blue or similar strains) on IPTG/X-gal medium.

Sequence the resulting construct to ensure the absence of any point mutations in the insert. The vector pEAS-1a may be sequenced using M13 forward and reverse primers.

Expression of recombinant protein

Transform your expression construct into an appropriate *E. coli* expression strain, such as BL21 or XL1-Blue.

1. Pick a colony from the primary plate and inoculate a 5 ml LB culture supplemented with 50 μ g/ml ampicillin
2. Grow the culture overnight at 28°C with shaking (250 rpm) to saturation
3. Inoculate the overnight culture 1/100 into a 200 ml LB culture supplemented with 50 μ g/ml ampicillin
4. Grow at 28°C with shaking (250 rpm) to an OD600 of 0.5
5. Induce protein expression by adding IPTG to 1 mM final concentration
6. Grow the induced culture for 1.5 hours at 28°C with shaking (250 rpm)
7. Induce autolysis of cells by shifting the culture to 38°C
8. Grow for 2 hours at 38°C with shaking (250 rpm)
9. Shift the culture back to 28°C and grow for another 2-4 hours

Note

The optimal post-lysis incubation time depends on the recombinant protein being expressed and may have to be optimized in a small scale trial experiment. For most proteins, incubation times between 2-6 hours are optimal.

10. Centrifuge the culture at 5000x g for 10 minutes
11. Transfer the culture supernatant to fresh tubes, discard the cell pellet
12. Your recombinant protein can now be purified from the culture supernatant