P080 QuickLyse System

Expressing recombinant proteins in the QuickLyse system

Cloning your 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.

Note

To avoid induction of the autolysis cassette during transformation and growth, perform only a brief heat shock (15 seconds) and grow cells at 28°C instead of 37°C for all subsequent steps. Incubation at 28°C results in a lower growth rate (i.e. colonies on LB/ampicillin plates will appear only after 20-30 hours, instead of 12-15 hours at 37°C) but does not impact on transformation efficiency.

1. Pick a colony from the primary plate and inoculate a 5 ml LB culture supplemented with 50 µg/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 µg/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 42°C
8. Grow for 2 hours at 42°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 optimzed 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

Version 1.2 - Last change 06/2008

Dualsystems Biotech AG Grabenstrasse 11a 8952 Schlieren Switzerland
phone +41 44 738 50 00 fax +41 44 738 50 05 www.dualsystems.com