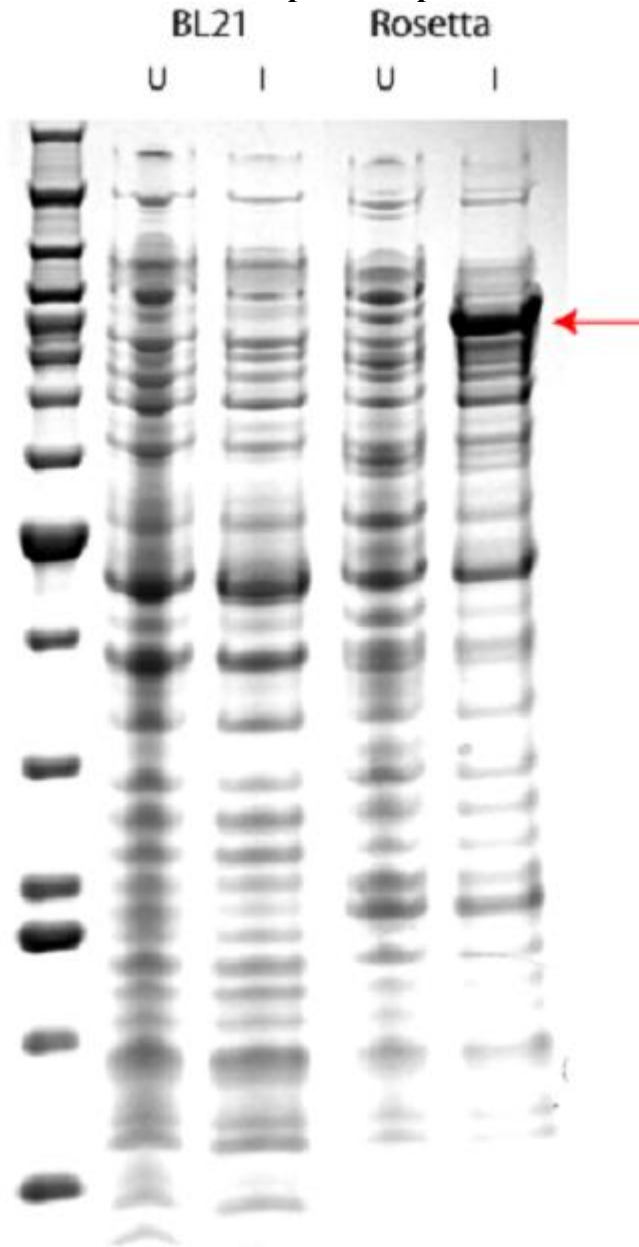


Cloning and Expression Optimization Group

Expression and solubility effects of fusion tags, E. coli strains, and temperature

- [Rare codon strain improves expression](#)
- [Effect of expression temperature](#)
- [Effect of fusion tags on expression level](#)
- [Effect of fusion tags on solubility](#)

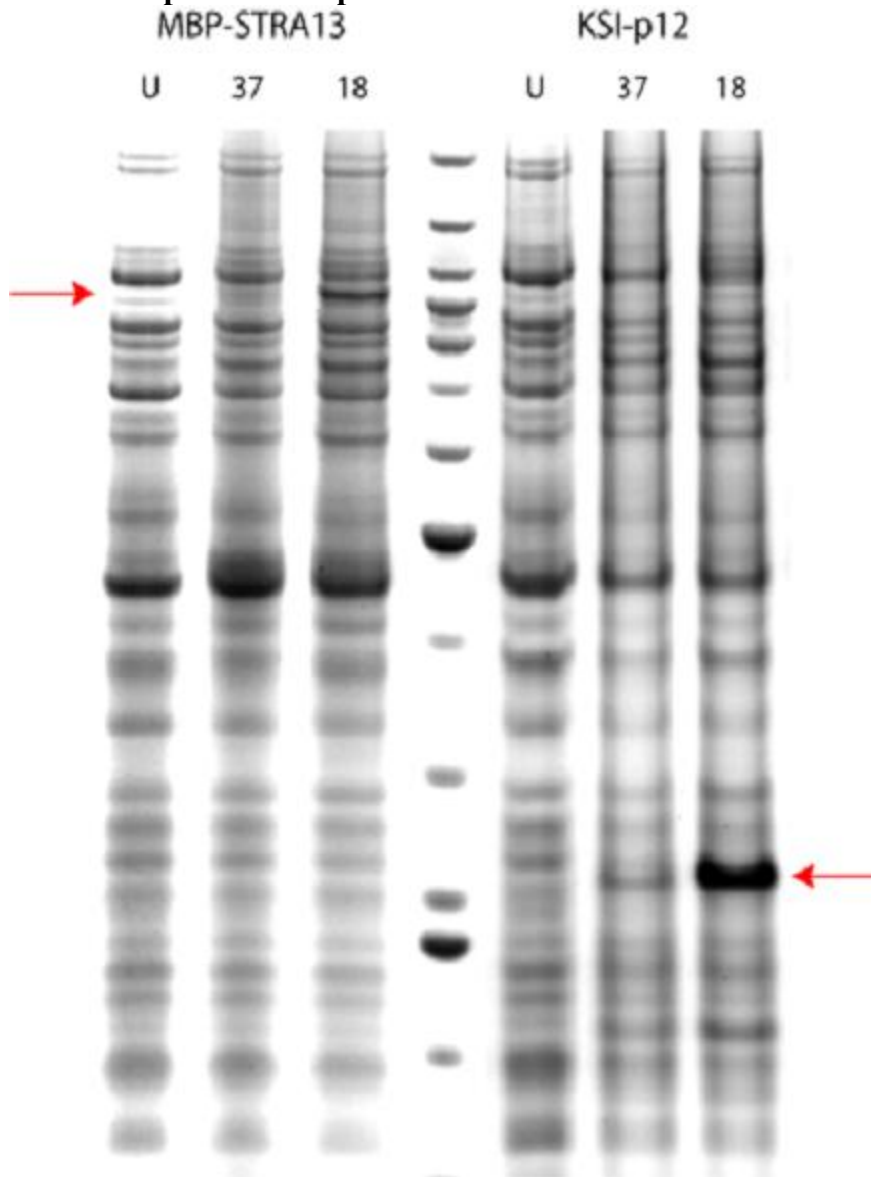
Rare codon strain improves expression



Expression optimization:
E. coli strain (rare codons)

Many mammalian genes contain codons which are rarely used in E. coli. Special E. coli strains are available that over express the tRNA genes for these rare codons, which can in some cases dramatically improve expression. Other E. coli strains are available to improve folding, reduce protease activity, or enhance solubility. The gel shows a gene which fails to express in a normal BL21 strain, but expresses a high level of protein in a strain called Rosetta which encodes the rare tRNA genes. Samples are whole-cell extracts of uninduced (U) and induced (I) cultures.

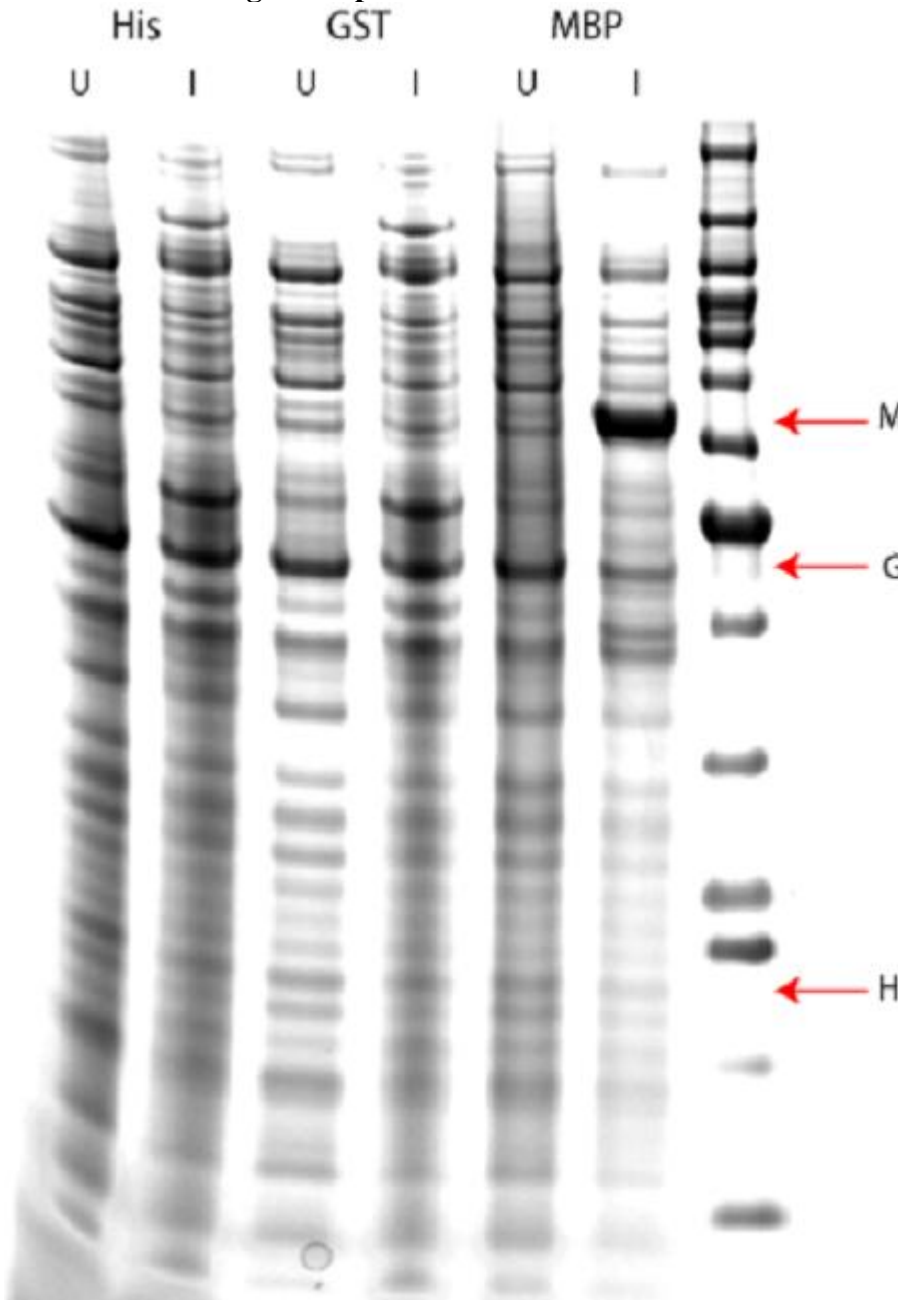
Effect of expression temperature



Expression optimization:
Temperature after
induction

Two proteins which
express at very low levels
at 37° produce more
protein at 18°. Cultures
were grown at 37°, then
split. One part was
induced with IPTG and
grown 3 hours at 37°, the
other was cooled to 18°,
induced, and grown
overnight at 18°.

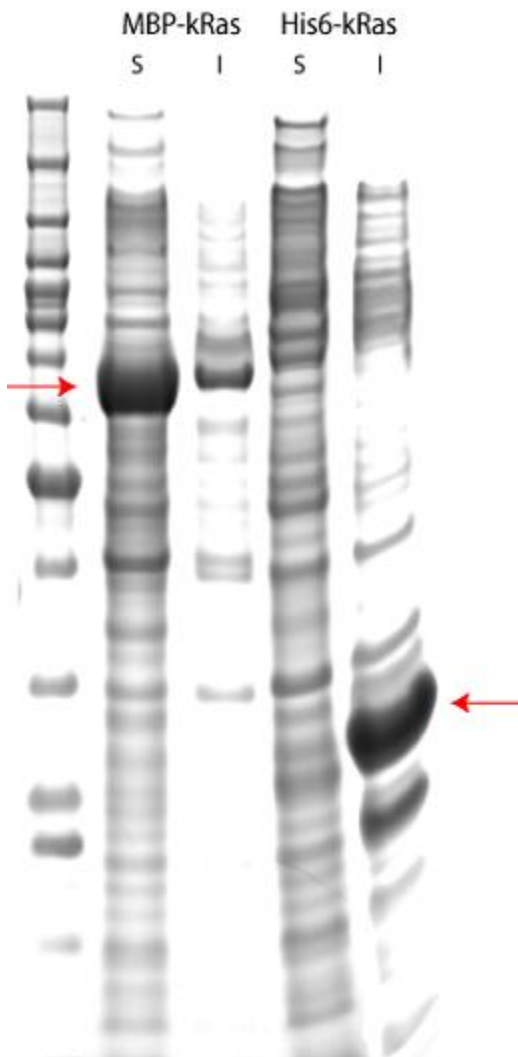
Effect of fusion tags on expression level



Expression optimization:
Fusion tags and expression level

A gene which fails to express detectable levels of protein may be converted into a strongly expressing gene with the addition of fusion tags. The gel shows three sets of uninduced (U) and induced (I) whole-cell extracts. The same gene is present in each set, but each has a different amino-terminal tag: His₆, GST, or maltose-binding protein (MBP). The predicted location of each protein is indicated by the arrows. Expression of the MBP fusion is robust, while the GST and His₆ fusions are not detectable.

Effect of fusion tags on solubility



Expression optimization:
Fusion tags and solubility

Proper protein folding and solubility can be affected by the presence of expression or solubility tags. One of the most useful tags is an amino-terminal maltose-binding protein (MBP) tag. The gel shows the same gene tagged with either His6 or MBP at its amino terminus. Upon cell lysis, most of the His6-tagged protein is found in the insoluble fraction (I). However, when tagged with MBP, nearly all of the protein is now located in the soluble supernatant fraction (S).