

Recombinant protein expression troubleshooting & optimization

First things first:

- **Determine whether the protein is being expressed** but isn't soluble and/or properly folded – or, if it's not being expressed at all.
 - *For inducible expression, can check by running SDS-PAGE on pre- and post-induction samples. For others, you can do western blot, etc. on samples after harvest*
 - *If misfolded, it might go to inclusion bodies (basically aggregates) – you can try isolating them from here & refolding them, but no guarantees!*
- **Check your construct/vector (e.g. plasmid) for hidden, unwanted mutations.** These can sometimes arise during cloning so sequence the DNA you put in to make sure there aren't any typos
- **Try one more time** (sometimes, things happen and you never figure out what but waste a ton of time trying to!)

Then, try changing things up . . .

- **Temperature** (e.g., for bacteria, try lowering the temperature to 16°C before inducing expression, and then let it express overnight instead of a shorter period at higher temp)
- **Media** (the cell food) - for bacteria, maybe try TB instead of LB
- **Expression time** - how long are you letting the cells grow after telling them to make your protein?
- **Induction strength** – if you're inducing expression, such as with IPTG, you may want to try lowering the IPTG concentration so you don't overwhelm the cells
- **Codon usage** – if you change your spellings codon-wise to match the cells' preferences, you might have better luck with expression. We call this "codon optimization"
- **Vector (e.g. plasmid)**, especially which **promoter** is used (that's the sequence that helps determine when and how much mRNA gets made)
- **Cell line/strain** – different cells have different machinery and you might need to experiment, even within a certain "expression system."
 - *For example, there are lots of different strains of bacteria you can use for expression and some have bells and whistles that might help in various circumstances, such as if your protein is toxic to certain cells. There are also strains with plasmids with tRNAs for rare codons (an alternative to codon optimization) and strains that co-express chaperones.*
- **Consider using a different expression system**
 - Bacterial expression is cheapest & easiest (if it works) but bacteria don't have all the same protein-processing machinery as our cells. If you have a protein that's more

complex (needs special chaperones, gets modified post-translationally with things like glycosylation (sugar addition), etc,) you might need to use more “complex cells”

- **Yeast** cells, ***insect*** cells (e.g. Sf9, Sf21, S2, High-5), **mammalian** cell lines like (CHO (Chinese Hamster Ovary), 293 (human), etc.).
- There are also **CFE (Cell Free Expression) systems** you can try, especially if your protein is toxic to the cells

You may need to change the protein construct. You can try to:

- **Add a fusion partner** (SUMO, GST, MBP, etc.) to the N-terminus to help with expression, folding, & solubility
- **Change affinity tag and/or its location** (N or C terminus, linker length)
- **Cut off regions of the protein that are predicted to be disordered** (such as long loosey-goosey tails). Such “truncations” can help with expression, but remember that you’re removing part of the protein and even though it’s disordered, it might be important for what you want to test