Dream Gene: *IL6*

Interleukin 6 is a protein encoded by the *IL6* gene.

“Recombinant Production of Human Interleukin 6 in *Escherichia coli*” by Nausch *et al*

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0054933

*Introduction*

* Useful in treating a range of diseases, including cancer
* Needs a large-scale method of production—difficult to extract from human tissues
* Has been expressed in *E. coli* many times, but tends to aggregate, forming multimers with reduced activity (intramolecular bonds were messed up, so intermolecular bonds formed)
* Reduced activity probably a consequence of misfolded disulfide bridges
* Goal: efficient, quick resolubilization
* 2 methods of promoting disulfide bridge formation:
  + Oxidizing environment improves formation of disulfide bridges 🡪 use *E. coli* strain Origami 2 which has mutations in two oxidoreductases VS. strain BL21—reducing cytoplasm
  + Expression of chaperones: “protein aggregation can result from chaperone limitation” 🡪 fuse IL6 to Glutathion S-Transferase from *Schistosoma japanicum*, which doesn’t interfere with its activity
* Add *N-*terminal His-tags/GST-His-tags and *C*-terminal hexahistidine residue
  + Improves purification efficiency
  + Increases circulation half-life by enabling PEGylation
* Use recombinant protein to increase production of IL6 antibodies and quickly remove IL6 from the bloodstream (?)

*Materials & Methods*

* Gene + appropriate tags = 558 bp long
* See Figure 1 for list of plasmids used and what genes they contain
* Protein products produced by inoculating a culture, growing up to OD600 0.5, then 1 mM IPTG was added and they were incubated at either 37ºC or 22ºC (*why??* Is there a difference in protein expression at these two temperatures?) 🡪 cells pelleted
* Western Blot used to check that the right protein was being expressed
* Protein concentration measured by Bradford method
* Quantification of IL6 measured using ELISA (Enzyme-linked Immunosorbent Assay)
* Biological activity of IL6 measured with the hybridoma proliferation assay

*Results*

* BL21 (reducing cytoplasm) reached the correct OD600 faster and produced more correctly-formed IL6
* Overexpressed cytoplasmic chaperones (DnaK, DnaJ, GrpE, GroES, and GroEL) increased IL6 yield
* 22ºC incubation temperature increased the amount of soluble IL6 in the presence of chaperones
* Improved solubilization with 0.1 mM IPTG
* Extract soluble proteins with lysozyme digestion and centrifugation
* Yield of IL6 highest in BL21 strain with co-expressed chaperones

*Discussion*

* “…there remains a need to develop an effective and cost-efficient method to express biologically active IL6, in order to provide a large-scale production system of IL6 under cGMP [current good manufacturing processes] condition.”
* BL21 exhibited highest yield, lowest stress response

**Figure 1** **Figure 2**

