The Rise of Garage Science: Making Recombinant Protein Production as Cheap as Chips!

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1. Introduction.

Most of the *Escherichia coli* expression systems used for recombinant protein production (RPP) require an inducer molecule, such as IPTG (isopropyl-β-D-thiogalactoside), to switch on expression of the target protein. However, such inducer molecules can be extremely expensive, with IPTG costing up to £46,000 per kg (Fig. 1). To reduce the cost of RPP, we have engineered completely new *E. coli* RPP expression systems, which use cheap and freely available inducer molecules, such as nitrate and urea. Using our systems we show that target proteins, such as human growth hormone (hGH), can be expressed to high levels, comparable to standard RPP systems. As nitrate and urea are present in many fertilizers, found in garages and garden sheds, we demonstrate that controlled, high level RPP can be achieved using unconventional inducers, making RPP "as cheap as chips"!

IPTG £46,060 kg^{-1} | **Gold £ 31,397 kg^{-1}** | **$NaNO_3$ / Urea fertilizers ~£0.25 kg^{-1}** | **Chips £2.17 kg^{-1}**

Figure 1. Comparison of the cost of IPTG to various other commodities.

2. Regulation of *E. coli* gene expression in response to nitrate

In *E. coli* the response to nitrate is mediated by the NarL transcription factor (Fig. 2). As NarL-dependent promoters are complicated, being controlled by many other transcription factors [1,2], we have optimised various NarL-dependent promoters and used them to express GFP and hGH, with nitrate and common household fertilizers as inducers (Fig. 3).

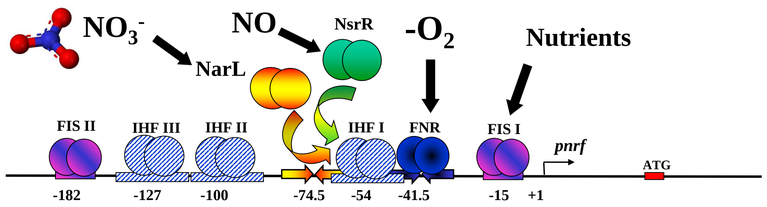

Figure 2. Regulation of the *E. coli nrf* operon promoter by NarL and other transcription factors [2].

3. Controlled nitrate-mediated RPP in *E. coli*.

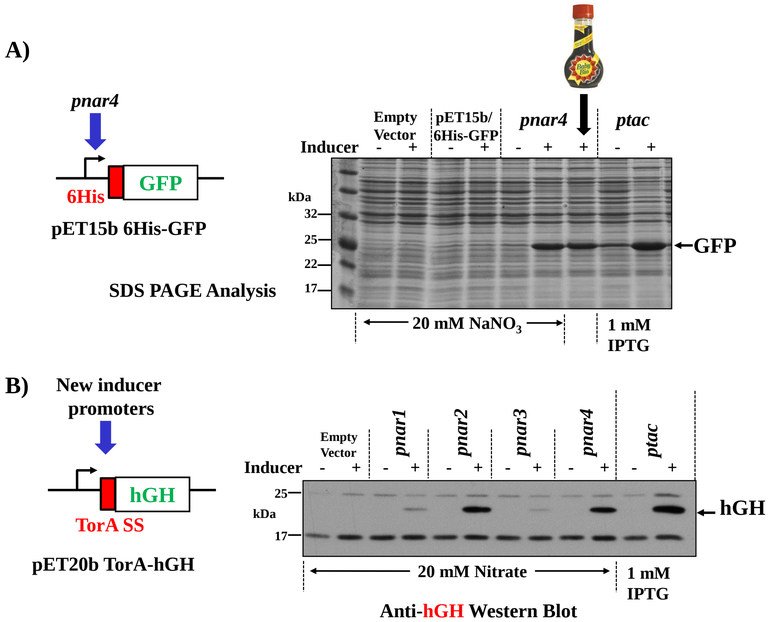

Figure 3. Expression of 6His-GFP and TorA-hGH-6His protein fusions, using nitrate and fertilizers as inducers. A) Expression of 6His-GFP in *E. coli* K-12 cells, using the nitrate-inducible promoter, *pnar4*, cloned into pET15b. 6His-GFP expression was analysed by SDS PAGE. **B)** Expression of a TorA-hGH-6His protein fusion in *E. coli* K-12 cells, using various nitrate responsive promoters (*pnar1 to pnar4*) cloned into pET20b. TorA-hGH-6His expression was analysed by Western blotting with an anti-hGH antibody. Note that the *torA* signal sequence directs the hGH-6His moiety to the twin arginine translocon (*i.e.* the Tat system) for periplasmic targeting [3]. In both panels, cells were grown in minimal salts medium until an OD_{600} of ~0.4 when RPP was induced by the addition of 20mM $NaNO_3$, 1% Baby Bio or 1 mM IPTG (where indicated) for 3 hrs.

4. Regulation of gene expression in response to urea.

In many bacteria, the response to urea is controlled by the UreR transcription factor (Fig. 4) [4]. To harness this for RPP, we have engineered this regulatory system into *E. coli* K-12 cells and, by coupling this with various urea-dependent promoters, we have induced the expression of GFP and hGH, with urea, fertilizers and even human urine (Fig. 5).

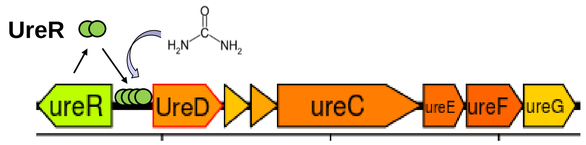

Figure 4. Regulation of urease gene expression by UreR and urea.

5. Controlled Urea-mediated RPP in *E. coli*.

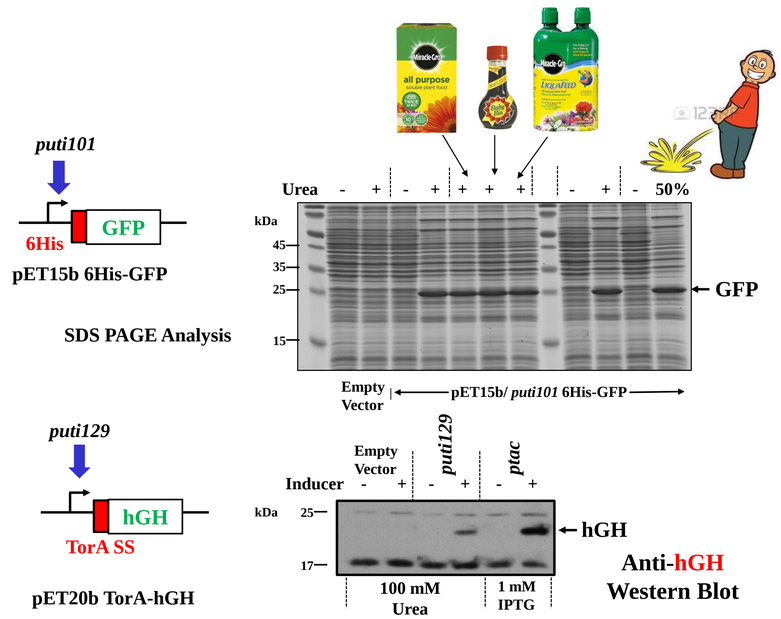

Figure 5. Expression of 6His-GFP and TorA-hGH-6His protein fusions, using urea and fertilizers as inducers. A) Expression of 6His-GFP in *E. coli* K-12 cells, using the urea-inducible promoter, *puti101*, cloned into pET15b. 6His-GFP expression was analysed by SDS PAGE. **B)** Expression of a TorA-hGH-6His protein fusion in *E. coli* K-12 cells, using the urea-responsive promoter *puti129*, cloned into pET20b. TorA-hGH-6His expression was analysed by Western blotting with an anti-hGH antibody. Note that the *torA* signal sequence directs the hGH-6His moiety to the twin arginine translocon (*i.e.* the Tat system) for periplasmic targeting [3]. In both panels, cells were grown in LB medium until an OD_{600} of ~0.4 when RPP was induced by the addition of 100 mM urea, 1% Miracle-Gro, 1% Baby Bio, 1% Miracle-Gro Liquafeed, 50% human urine or 1 mM IPTG (where indicated) for 3 hrs.

5. Conclusions.

- **We have produced a range of nitrate- and urea-responsive promoters.**
- **We have used these promoters to overexpress GFP and hGH to high levels.**
- **We have successfully used "novel" inducers to initiate high level protein production and cut the cost of RPP.**

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References.

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