Microbial Cell Factories

Poster Presentation

Monitoring protein expression levels in E. coli using a high throughput approach

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Background

We have developed a high-throughput method to rapidly identify the protein constructs which are well expressed and find out which experimental factors influence their production. From a sparse matrix designed to screen between expression strains, culture media, lysis and purification buffers for each construct, the interactions among variables leading to a higher yield of soluble recombinant protein can be easily identified.

This screening is performed by a combination of small scale fermentation in deep-well blocks, cell lysis with a 24 microtips sonicator, Ni-NTA magnetic beads purification, and an automated gel capillary electrophoresis system, which allows a high-throughput and quantitative analysis of the multiple variables in one experiment.

This technique allows one to evaluate as early as possible the expression level of the constructs, narrowing down the number of constructs subsequently going through the large scale fermentation and purification module.

Table 1:

	Expression strain	Culture medium	Lysis and purification buffer
Condition 1	Strain 2	Medium 2	Buffer 1
Condition 2	Strain 3	Medium 1	Buffer 4
Condition 3	Strain 3	Medium 2	Buffer 2
Condition 4	Strain 1	Medium 1	Buffer 1
Condition 5	Strain 2	Medium 3	Buffer 3
Condition 6	Strain 1	Medium 3	Buffer 4
Condition 7	Strain 2	Medium 2	Buffer 4
Condition 8	Strain 1	Medium 2	Buffer 3
Condition 9	Strain 3	Medium 2	Buffer 5
Condition 10	Strain 1	Medium 1	Buffer 5
Condition 11	Strain 2	Medium 3	Buffer 5
Condition 12	Strain 3	Medium 1	Buffer 3
Condition 13	Strain 3	Medium 3	Buffer 1
Condition 14	Strain 2	Medium 1	Buffer 2
Condition 15	Strain 1	Medium 3	Buffer 2

Sparse matrix design (http://www.igs.cnrs-mrs.fr/samba/samba.html): **Strain 1**: BL21(DE3)/**Strain 2**: C43(DE3)(from OverExpress™)/**Strain3**: BL21(DE3) harboring pG-KJE8 vector from Takara **Medium 1**: LB/**Medium 2**: Auto-induction/**Medium 3**: Turbo broth+ Augmedium from AthenaES™ **Buffer 1**: 40 mM HEPES;150 mM NaCl;10%glycerol;pH7/**Buffer 2**: 40 mM HEPES;400 mM NaCl;100 mM urea;pH7/**Buffer 3**: PBS pH8/**Buffer 4**: 40 mM Tris;150 mM NaCl;10%glycerol;pH8.5/**Buffer 5**: 40 mM Tris;400 mM NaCl;100 mM urea;pH8.5

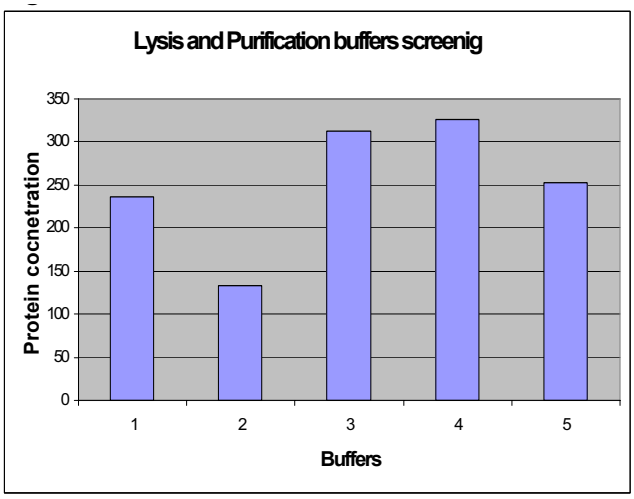

Figure 1
Quantitative analysis of a human protein purification after cell lysis and purification with 5 different buffers: The values were obtained using the ALP5100 system. The buffers 1 to 5 are described in the table1.

Results

Using a combination of robotic systems like the QIAGEN BioRobot 3000, a microsonification device (20 kHz 24 element probe from SONICS) and an Agilent ALP5100, we rapidly monitor in parallel and in a microtiter well format the level of expression in E. coli of the different protein constructs, in 15 different conditions (see Table 1). We also aim at the definition at an early stage the buffer conditions allowing protein stabilization during cell lysis and purification (figure 1).