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Oral Presentation

Impact of high throughput technology on recombinant protein production

Jo J Jones*[1], Angela M Bridges[1], Andrew P Fosberry[1], Jonathan Bullock[2], Ian Hudson[2], Richard M Hall[1], Owen Jenkins[1] and Jeffrey A Cole[3]

Address: [1]Gene Expression and Protein Biochemistry, GlaxoSmithKline, New Frontiers Science Park, Coldharbour Road, Harlow, Essex, CM19 5AD, UK, [2]The Automation Partnership, York Way, Royston, Hertfordshire, SG8 5WY, UK and [3]School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

* Corresponding author

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Background

There are many challenges still to be overcome in the efficient production of recombinant protein from both eukaryotic and prokaryotic organisms. Often the problems faced are protein specific, and can result in unacceptable time delays between target screening and structural elucidation. A requirement therefore exists for rapid, parallel approaches that can identify suitable clones under a preliminary set of fermentation conditions in days rather than months.

Piccolo® is a fully automated, high throughput system, specifically developed for the rapid optimisation of protein production from both insect and microbial cells. The system is capable of expressing a maximum of 1152 cultures, at volumes between 10 ml and 100 mls, followed by protein purification using affinity chromatography. A total of four different culture media, two antibiotics and two inducers can be used, in conjunction with three post induction temperatures. The optical density of cultures is measured robotically to monitor growth, and the growing cultures can be both aerated and agitated continuously to prevent anaerobic growth. Following expression, the cells are centrifuged, lysed and the lysate bound to affinity resin. The resin is then washed, eluted and the partially purified protein stored at 6°C ready for analysis.

Results are presented that show the increased efficiency of combining the automated primary screening of Piccolo® with on-line GFP sensors to optimise the subsequent fermentation processes. The use of an on-line sensor to measure GFP-tagged protein expression in fermenters has been described previously by Jones *et al.* [1]. In the pharmaceutical industry, reducing the time taken for screening recombinant protein is becoming more important and this automated approach will undoubtedly have an important role.

Results

Early results from experiments completed using Piccolo® are encouraging. To illustrate the potential of the technology, an experiment to investigate different host strains of *E. coli* was undertaken. The expression conditions were induction at an optical density of 1.0 with 0.5 mM IPTG followed by incubation for 7 h at 30°C and for 3 h at 37°C. Six proteins all with an N-terminal 6-histidine tag were evaluated in three different host strains. The experiment used a total of 720 individual cultures, with samples in quadruplicate. Cultures were grown in special custom built culture vessel blocks (CVBs) containing 24 wells per block fitted with an aeration assembly that allowed both addition of gases and agitation. On-line growth measurements using an optical sensor were also possible. The culture blocks were incubated before induction at 37°C, moved to a post-induction incubator at the desired induction statistic for 30 minutes. After 30 minutes the CVB was removed from the incubator, IPTG was added and the CVB returned for incubation for 3 h at 37°C or 7 h at

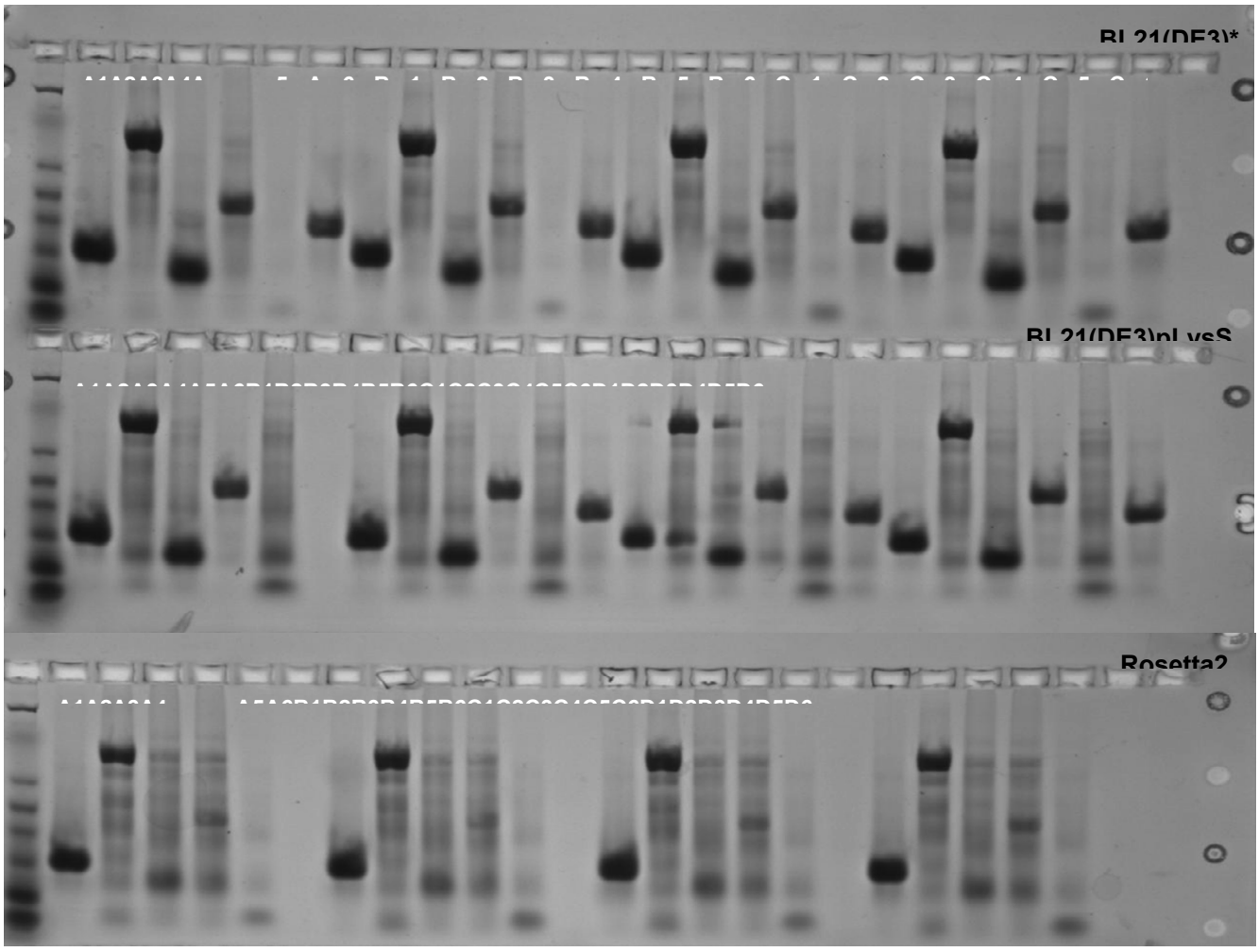

Figure 1
SDS-PAGE of six different proteins (1 to 6) analysed in quadruplicate (A to D). The proteins were expressed in *E. coli* host strains BL21(DE3)*, BL21(DE3)pLysS and Rosetta2, purified after NiNTA affinity chromatography on Piccolo®.Lane **A1** = protein 1, replicate A; **A2** = protein 2, replicate A; **A3** = protein 3, replicate A etc.; **B1** = protein 1, replicate B etc.

30°C. The CVBs were removed, decoupled from their aeration assemblies and moved to cold storage at 6°C until ready for purification.

The CVB containing culture was centrifuged, the supernatant removed and the cells lysed with lysis buffer. The CVB was again centrifuged and the lysate mixed with affinity resin to allow for binding of the affinity tag of the recombinant protein to the resin. After binding, the resin was washed twice and then eluted. The purified protein was collected in a 24 well plate and returned to cold storage at 6°C awaiting assay. Figure 1 shows some of the results obtained for the samples analysed by SDS-PAGE after purification.

Conclusion

The potential of a high throughput automated system for industry to screen a large number of conditions is demonstrated. From this experiment alone, the choice of expression host is seen to influence the amount of recombinant protein produced. Piccolo® offers the scientist the capability to screen a large number of factors in one experiment. The optimum conditions can then be scaled up into a fermenter, and if a GFP fusion protein is used, the recombinant protein expression can be followed in the fermenter on-line in real-time using a fluorescence sensor.

References

1. Jones JJ, Bridges AM, Fosberry AP, Gardner S, Lowers RR, Newby RR, James PJ, Hall RM, Jenkins O: **Potential of real-time measurement of GFP-fusion proteins.** *J Biotechnol* 2004, **109:**201-211.