

Economical Parallel Protein Expression Screening and Scale-Up in *Escherichia coli*

Oleg Brodsky¹, Samuel Ellis², and Ciarán N. Cronin¹

¹Pfizer, Inc., La Jolla, CA 92121 and ²Thomson Instrument Company, Carlsbad, CA 92054



Abstract

A novel microfermentation and scale-up platform for parallel protein production in *Escherichia coli* is described. The vertical shaker device Vertiga, which generates low-volume high density (A600 ~20) *Escherichia coli* cultures in 96-position deep-well plates without auxiliary oxygen supplementation, has been coupled to a new disposable shake flask design, the Ultra Yield™ flask, that allows for equally high cell culture densities to be obtained. The Ultra Yield™ flask, which accommodates up to 1L in culture volume, has a baffled base and a more vertical wall construction compared to traditional shake flask designs. Experimental data is presented demonstrating that the Ultra Yield™ flask generates, on average, an equivalent amount of recombinant protein per unit cell culture density as do traditional shake flask designs but at a substantially greater amount per unit volume. The combination of Vertiga and the Ultra Yield™ flask provides a convenient and scalable low-cost solution to parallel protein production in *Escherichia coli*.

Introduction

Recent advances in parallel cloning, expression and purification strategies, and high-throughput protein crystallographic approaches has led to aggressive timelines for the structure determination of therapeutically-validated protein targets. It is now commonplace to carry out preliminary characterizations of multiple constructs (20-80) of the same target in order to find a product that is optimized for co-crystallization efforts in drug discovery. Traditionally, protein expression screening has been carried out using 3 mL cultures grown in test tubes with scale-up operations being conducted in either shake flasks or in large-scale fermentors. Recently, parallel approaches to screening has been carried out using plate-based approaches. In conjunction with engineers at Glas-Col (Terre Haute, IN), we developed a novel shaker device termed Vertiga that allows *E. coli* cultures to grow to high cell densities (~A600 = 20) in deep-well blocks [Cronin *et al.*, 2004]. Vertiga is relatively inexpensive and holds from 1 to 8 deep-well blocks allowing for 1-768 independent clones to be cultured. The device uses high speed rotation (900 rpm) within a short radius (2 mm) of gyration to achieve these high cell densities when cells are cultured in rich medium (Terrific Broth). Supplementation with oxygen is not required and the chamber temperature may be set from 15°C to 45°C (±1°C). As a result of the high cell densities, individual cultures (0.75 mL each) grown in Vertiga yield sufficient amounts of recombinant protein from suitably expressing clones in order to conduct various biophysical characterizations prior to scale-up, including analytical size-exclusion chromatography.

Until now, the construct expression behavior identified in Vertiga has generally been scaled up for parallel expression by using the GNFermentor, which supports growth of *E. coli* to similarly high cell culture densities [Lesley & Wilson, 2005]. However, the GNFermentor has some limitations such as a restricted individual culture volume of 64 mL, and that a full complement of 96 cultures is necessary for operation of the unit, although its most significant limitation is that it has not been made available commercially. We now report the integration of a novel flask design, the Ultra Yield™ flask, as a disposable, off-the-shelf low-cost solution to high-density parallel fermentation at the 1L culture volume scale. This novel flask includes a baffled base and a more vertical wall construction compared to traditional shake flask designs. Traditional rotation speeds (250 rpm) are sufficient to allow cultures to achieve the required high cell densities (~A600 = 20). The current report offers validation of the Ultra Yield™ flask as a direct scale-up platform for the most promising clones identified in Vertiga. The combination of Vertiga and the Ultra Yield™ flask provides for a simple low-cost solution to parallel protein expression microscreening and scale-up in *E. coli*.

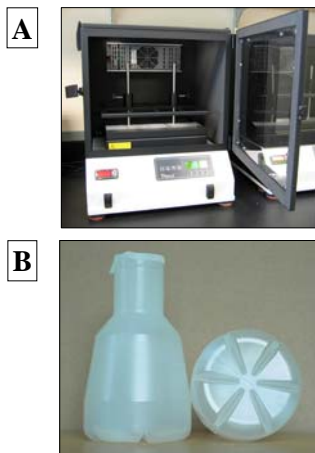


Figure 1: (A) The Vertiga shaker device that is used for microfermentation. (B) The Ultra Yield™ flask showing the baffled base and novel wall construction.

Experimental Results

In order to validate the application of the Ultra Yield™ flask as a scale-up system, it was necessary to demonstrate that the increased cell densities generated in the flasks translated into corresponding increases in protein production per unit volume. Thus, twelve different protein expression constructs were examined for protein production following cell culture in both Vertiga and in the Ultra Yield™ flasks. The production yields of the same twelve proteins were also examined from cultures grown in traditional Fernbach flasks using both TB medium, the medium deployed routinely in both Vertiga and in the Ultra Yield™ flasks, and Luria-Bertani (LB) medium, in order to provide a comparison with standard methodologies. The twelve protein expression constructs are listed in Table 1.

As shown in Figure 2A, the average cell culture densities generated in the Fernbach flasks following overnight expression using either LB or TB media were, respectively, 2.7 (range 1.9 to 3.8) and 4.0 (range 2.2 to 6.4), whereas the average cell density in the Ultra Yield™ flask with TB medium was 14.0 (range 7.6 to 20.2). Thus, an average increase in cell density per unit volume of approximately 3.5 is obtained by using the Ultra Yield™ flask in place of a Fernbach flask.

Table 1. Protein constructs used for expression studies

Clone #	Protein	Source	Expression Vector	Purification Tag	Mass (kTag)	pI
1	Alanine isomerase (AR)	<i>S. aureus</i>	pET15b	N-His/Thrombin	44099.2	7.63
2	4-Amino-4-deoxychorismate lyase (ADCL - Pab C)	<i>E. coli</i> K-12	pET20a	N-His/Thrombin	31907.3	7.12
3	4-Amino-4-deoxychorismate synthase (ADCS - Pab B)	<i>E. coli</i> K-12	pET20a	N-His/Thrombin	53119.8	5.81
4	Isochorismate synthase (Ehr C)	<i>E. coli</i> K-12	pET20a	N-His/Thrombin	45197.5	6.38
5	p38 alpha (MAPK14 isoform 2)	<i>H. sapiens</i>	pET22b	C-His	42429.4	6.18
6	p38 alpha (MAPK14 isoform 2)	<i>H. sapiens</i>	pET47b	N-His/HRV3C	43802.0	6.09
7	p38 delta (MAPK13)	<i>H. sapiens</i>	pET47b	N-His/HRV3C	44264.9	8.20
8	JNK2 (isoform 1)	<i>H. sapiens</i>	pET22b	C-His	45103.6	6.53
9	MK-2 (isoform 2)	<i>H. sapiens</i>	pET30b	C-His	30353.9	8.48
10	MK-2 (isoform 2)	<i>H. sapiens</i>	pET47b	N-His/HRV3C	36530.0	8.48
11	hAG-2	<i>H. sapiens</i>	pET22b	C-His	19064.9	8.83
12	hAG-2	<i>H. sapiens</i>	pET47b	N-His/HRV3C	20012.9	8.83

The data from protein assay determinations, shown in Fig. 2B, demonstrate that increased culture density translates, in general, into increased protein yield. The average increase in protein recovered from the IMAC columns following purification was 6.1-fold for Ultra Yield™ flasks versus that recovered from the Fernbach flasks when using TB as the growth medium.

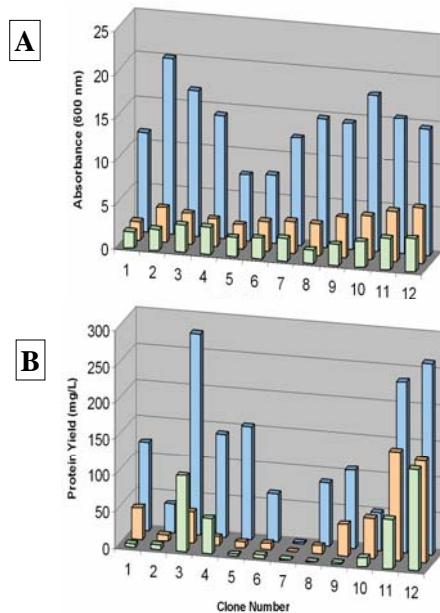
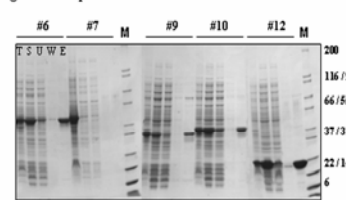


Figure 2: Effect of flask design on *E. coli* culture growth and production of recombinant protein. (A) The effect of flask type and growth medium on the observed optical densities of the cultures at A600 following overnight protein expression. Cultures carried out in Fernbach flasks using either LB medium or TB medium are shown, respectively, at the front (green) and in the center (orange). Cultures grown in Ultra Yield™ flasks in TB medium are shown at the back (blue). See Table 1 for a list of the recombinant proteins overexpressed by the various clones. (B) The yields of expressed soluble protein (determined by protein assay and recorded as mg protein per liter of expression culture) from the IMAC columns following purification of the twelve polyHis-tagged recombinant proteins, each expressed under the three conditions described in Panel A. Clone locations in Panel B are the same as those in Panel A.

The SDS-PAGE analysis for five of the twelve expressed proteins is shown in Fig. 3B, together with the corresponding Vertiga microexpression SDS-PAGE analysis in Fig. 3A. The data demonstrate an excellent correlation between the recombinant protein expression behavior in Vertiga to that generated when scale-up expressions are carried out in the Ultra Yield™ flasks. For example, N-His tagged p38delta (sample #7) shows high expression in both systems but the majority of the expressed protein is insoluble with little to no yield from the IMAC column. On the other hand, hAG-2 (sample #12) shows high expression, high solubility and a good yield off the IMAC resin when the experiments are conducted using either Vertiga or the Ultra Yield™ flask.

A. Vertiga MicroExpression Screen



B. Scale-up Production

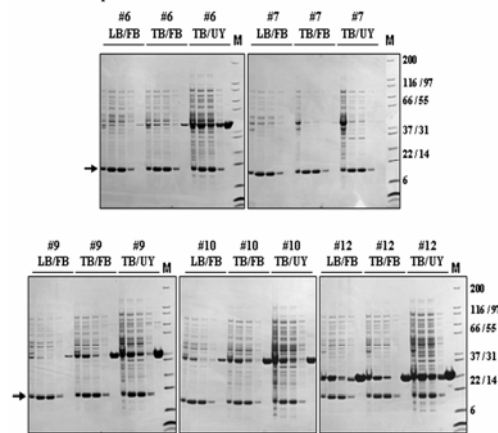


Figure 3: SDS-PAGE analyses of recombinant protein expression in Vertiga and the Ultra Yield™ flasks. (A) Shown is the SDS-PAGE analysis of recombinant protein expressions carried out in Vertiga for clones 6, 7, 9, 10 and 12 (see Table 1 for clone details). The five gel loadings for each sample are comprised of: total lysate (T), soluble extract (S), IMAC wash fraction (W), IMAC elute fraction (E). (B) Shown are the results obtained for the same five clones following scale-up expression under the three culture conditions tested: LB medium in Fernbach flasks (LB/FB), TB medium in Fernbach flasks (TB/FB), and TB medium in Ultra Yield™ flasks (TB/UY) (see Fig. 2 for growth data and protein yields). The gel loadings for each sample are the same as those described in (A). The arrow indicates the position of the hen egg white lysozyme band used to aid cell lysis.

Conclusions

The Ultra Yield™ flask allows *E. coli* expression cultures to achieve high-density cell growth similar to that observed in Vertiga. The data presented show that there is an excellent correlation between the expression data generated in Vertiga with that obtained from the Ultra Yield™ flasks. The increase in cell densities obtained in the Ultra Yield™ flask relative to those generated in traditional shake flask designs translates into increased recombinant protein production, effectively decreasing scale-up volume requirements. The combination of Vertiga and the Ultra Yield™ flask thus provides for a simple low-cost solution to parallel protein expression microscreening and scale-up in *E. coli*. For additional details see Brodsky and Cronin (2006).

References

- Brodsky, O. and Cronin, C.N. (2006) *J. Struct. Funct. Genomics*. In Press.
- Cronin, C.N., Hilgers, M.T., Velasquez, J., McManus, B., Page, R., Stevens, R., Sims, E. (2004) A Low-Cost, High-Throughput, Micro-Expression Screen for *Escherichia coli*-produced Recombinant Proteins, 7th Annual CHI-PepTalk Protein Expression Conference, (12-13 January 2004), San Diego, CA (see also Page, R., Moy, K., Sims, E.C., Velasquez, J., BMcManus, B., Grittini, C., Clayton, T.L. and Stevens, R.C. (2004) *BioTechniques* 37, 364-370).
- Lesley, S.A. and Wilson, I.A. (2005) *J. Struct. Funct. Genomics* 6, 71-79.