

Economical parallel protein expression screening and scale-up in *Escherichia coli*

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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 ($A_{600} \sim 20$) *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 1 l 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*.

Keywords Ultra Yield™ flask · Vertiga · Protein expression · *E. coli* fermentation structural proteomics · High-density fermentation

Introduction

The advent of structural proteomics initiatives in public-funded programs and biotechnology start-ups over

the past number of years has fueled the evolution of structure-based drug design (SBDD) efforts throughout the industry. Recent advances in parallel cloning, expression and purification strategies (e.g. [1–3]), and high-throughput protein crystallographic approaches (e.g. [4–8]), 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, encompassing multiple boundaries, mutations, tags, tag cleavage sites, and/or co-expressions with ligand, chaperones etc, in order to find a product that is optimized for co-crystallization efforts in drug discovery.

Since many recombinant protein constructs generated by molecular biology techniques are often unsuitable for structural studies, as a result of low expression, poor solubility or the generation of soluble aggregates, it is advantageous to have a high-throughput screening procedure early in the process to identify the most tractable derivatives for scale-up. When *Escherichia coli* is used as the expression host, the minimal yields of tractable proteins are equal to, or greater than, 2–5 mg of purified protein from a 1 l culture. Thus, for screening purposes, a traditional culture volume of 10–25 ml is required to provide the desired ~50 µg of purified protein necessary to conduct a suitable biophysical analysis that might include SDS-PAGE, mass spectroscopy analysis, and analytical size-exclusion and/or ion-exchange chromatographies. Such volumes are not amenable to plate-based parallel screening approaches. In conjunction with the Thomson Instrument Company (Carlsbad, CA) and engineers at Glas-Col (Terre Haute, IN), we developed a novel shaker device termed Vertiga that allows

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E. coli cultures to grow to high cell densities ($\sim A_{600} = 20$) in 96×2 -ml deep-well blocks (see also [9, 10]). 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 ($\pm 1^\circ\text{C}$). 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 the appropriate biophysical characterizations prior to scale-up.

The protein expression yields obtained from cultures grown in Vertiga are highly predictive of scale-up results when similar culture conditions are deployed in the latter process. However, there are currently only a limited number of methods that will accommodate growth to such high cell densities in a parallel fashion. One of these, the GNFermentor, developed by the Genomics Institute of the Novartis Research Foundation (GNF), utilizes a proprietary oxygen-infused 96-position airlift manifold to achieve the desired cell culture densities in an array of 96×100 ml custom test tubes [8, 9]. The GNFermentor has a capacity of 64 ml per tube [2] and multiple tubes are often pooled in order to achieve the desired biomass for downstream processing. However, the GNFermentor is not commercially available. Alternatively, DASGIP market the “cellferm-pro” system for parallel *E. coli* fermentations at the 1 l scale, which we have validated in-house to achieve similarly high cell culture densities as Vertiga when cells are grown in oxygen-sparged TB. However, the cellferm-pro system is based on a more traditional fermentation format and, as such, requires an appreciable amount of set-up time. The cellferm-pro system is expensive and appears more suited to conditions scouting prior to large volume fermentation. In a more pragmatic approach, Millard et al. [11] (see also [12]) have described the use of disposable 2-l plastic bottles as a means to generate high density *E. coli* cell cultures in 250 ml volumes.

To integrate with the Vertiga device, the application of the Ultra Yield™ flask as a disposable, off-the-shelf low-cost solution to high-density parallel fermentation at the 1 l culture volume scale is now reported. This novel flask includes a baffled base and a more vertical wall construction compared to traditional shake flask designs. Unlike the GNFermentor and the cellferm-pro systems, oxygen supplementation is not required and

the Ultra Yield™ flask fits directly into standard floor model laboratory shakers. Traditional rotation speeds (250 rpm) are sufficient to allow cultures to achieve the required high cell densities. The Ultra Yield™ flask has the additional advantage of allowing growth in culture volumes up to 1 l, thereby reducing the experimental footprint and eliminating much of the requirement for sample pooling prior to downstream processing.

This report describes the coupling of the Vertiga device with the Ultra Yield™ flask as a simple low-cost solution to parallel protein expression microscreening and scale-up in *E. coli*. The advantages of using the Ultra Yield™ flask for improved recombinant protein production over more traditional shake flask fermentations are demonstrated.

Materials

Benzonase nuclease (25 U/ μl ; Novagen), rLysozyme (43.2 kU/ μl ; Novagen), and hen egg white lysozyme (Sigma L-6876) were obtained from the sources indicated. Terrific Broth (TB; 12.00 g/l casein peptone, 2.31 g/l KH_2PO_4 , 12.54 g/l K_2HPO_4 , 24.00 g/l yeastolate, 4.0 ml/l glycerol; product #46-055-CM) was obtained from Mediatech. Oligonucleotides and DNA sequencing were provided by Retrogen. Vertiga, Ultra Yield™ flasks and AirOtop Seals were obtained from the Thomson Instrument Company. Fernbach flasks with baffled bases (product #4425-2XL) were obtained from Corning.

The DNA sequences encoding the human protein kinases p38 α (full-length, residues 1–360; GenBank accession no. AAH31574) and p38 δ (full-length, residues 1–365, GenBank accession no. AAH00433), and the kinase domains of human JNK-2 (residues 1–364; GenBank accession no. AAH32539) and MK-2 (residues 47–343; GenBank accession no. AAH52584), were amplified from a pooled mixture of a variety of human tissue Marathon cDNA libraries (Clontech). The cDNA for human hAG-2 (GenBank accession no. AF038451) [13] was kindly provided by Dr D.A. Thompson (SGX Pharmaceuticals), and the mature sequence (residues 21–175) was amplified for expression. Expression sequences were cloned into pET30 or pET28 (Novagen) to generate polyHis tags at the C-terminus, and/or into pET47 (Novagen) to generate polyHis tags at the N-terminus. Cloning into pET30 utilized the *Nde* I and *Xho* I cloning sites of the vector and the 3' amplification primers encoded a C-terminal 6 \times His purification tag followed by a stop codon. The presence of *Nde* I sites within the p38 α and hAG-2

sequences required an alternate strategy and the amplified ORFs were cloned into the *Nco* I/*Xho* I sites in pET28. Cloning into pET47 utilized the *Xma* I and *Xho* I cloning sites of the vector to generate fusion proteins that contained a HRV3C protease-cleavable N-terminal 6×His purification tag. The 3′ amplification primers encoded a C-terminal stop codon. N-terminal polyhistidine tagged protein expression constructs in pET28 (Novagen) for *E. coli* 4-amino-4-deoxychorismate lyase (*PabC*; full-length, residues 1–269 with S126N mutation; GenBank accession no. AAC74180) [14], *E. coli* 4-amino-4-deoxychorismate synthase (*PabB*; full-length 1–453 with L12P mutation; GenBank accession no. AAC74882) [14], and *E. coli* isochorismate synthase (*EntC*; residues 5–395 with G72S, M78I, A108T, and A142E mutations; GenBank accession no. AAB40793), were a kind gift from Dr Michael D. Toney (UC Davis), who also provided an expression clone for *Bacillus stearothermophilus* alanine racemase (AR; full-length, residues 1–382; GenBank accession no. AAW37022) [15] in pET23 (Novagen). All expression vector constructs were transformed into *E. coli* BL21(DE3) for expression analysis.

Methods

Frozen cell stocks

Confluent LB cultures (800 μ l) were mixed with sterile LB/Glycerol (200 μ l; 75% glycerol, 25% LB) and transferred into 1.5 ml cryo-vials (Altech). The vials were stored at -80°C .

Microfermentation in Vertiga

The appropriate number of wells in a 96 \times 2 ml deep-well plate (Thomson Instrument Company), each containing 200 μ l of LB with antibiotic(s) (100 μ g/ml ampicillin or 50 μ g/ml kanamycin), were inoculated with a single frozen cell stock, the plate covered with an AirPore filter (Qiagen) and the cultures grown to saturation overnight in Vertiga at 550 rpm and 37°C . The following morning, 15 μ l of each culture were used to inoculate 750 μ l TB containing antibiotics in a second 96 \times 2 ml deep-well plate. The culture plate was covered with an AirPore filter, placed in Vertiga and grown at 37°C and 900 rpm for 4 h. The optical densities (A_{600}) of the cultures were determined by sample dilution (1:20) with medium into a microtitre plate and analyzed by using a plate reader (SpectroMax) with appropriate correction factor. The A_{600} of the cultures

averaged 3.8 (range 3.3–4.2). The cultures were induced by the addition of IPTG to 100 μ M and the plate transferred to a second Vertiga shaker maintained at 25°C and growth continued overnight at 900 rpm. The A_{600} values of the overnight cultures, determined by using the plate reader, were found to have an average value of 19.1 (range 10.8–27.3). The cultures were harvested by plate centrifugation at 5,000g for 30 min, the plate inverted to discard the medium, and the plate containing the cell pellets stored at -80°C for at least 1 h prior to processing.

Micropurification screening

Frozen 96 \times 2 ml deep-well plates containing the expression cell pellets were removed from -80°C and allowed to thaw at room temperature (RT) for \sim 10 min prior to the addition of 300 μ l of Lysis Buffer A (50 mM Tris-HCl, pH 8.0, 50 mM sucrose, 0.1 mM $\text{Na}_4\text{EDTA-NaOH}$, pH 8.0, 0.1 μ l/ml benzonase nuclease, and 0.2 μ l/ml rLysozyme). The cell pellets were resuspended by mixing with a multichannel pipet and allowed to stand at RT for 10 min. At that time, 300 μ l of Lysis Buffer B (10 mM Tris-HCl, pH 8.0, 50 mM KCl, 400 mM NaCl, 0.1 mM $\text{Na}_4\text{EDTA-NaOH}$, pH 8.0, and 10 mM MgCl_2) were added to each well and the suspensions mixed by using the pipet. A whole cell sample from each well was transferred to a microtiter plate for subsequent analysis by SDS-PAGE and the plate incubated at RT for 10 min. The plate containing the mixtures was centrifuged at 5,000g for 20 min by using a Beckman Allegra 25R centrifuge. A sample of the supernatant from each well was transferred to a microtiter plate for subsequent analysis by SDS-PAGE and the remainder of the supernatants transferred to an 800- μ l, 96-well filter plate (Thomson Instrument Company) previously loaded with 100 μ l of a 50% slurry of pre-equilibrated (50 mM Tris-HCl, pH 8.0, 400 mM NaCl) ProBond nickel affinity resin (Invitrogen). The plate was incubated for 5 min at RT over a collection plate and then centrifuged at 700g for 1 min and the flow-through collected in the collection plate. Each well was washed by centrifugation at 700g for 1 min with 3 \times 400 μ l of Wash Buffer (50 mM Tris-HCl, pH 8.0, 400 mM NaCl, 40 mM imidazole-HCl, pH 8.0, and 10% (v/v) Glycerol) and the combined Wash fractions collected in a separate collection plate. Bound proteins were eluted from the resin by centrifugation as before into a separate collection plate following the addition of 300 μ l Elution Buffer (50 mM Tris-HCl, pH 8.0, 400 mM NaCl, 250 mM imidazole-HCl, pH 8.0, and 10% Glycerol). The various fractions for each sample, including Whole Cell, Supernatant,

Flow-Through, Wash, and Elution fractions, were analyzed by reducing SDS-PAGE on a 26-lane 4–12% gradient Criterion-XT gel (BioRad) using supplied MES buffer (BioRad). Purified protein yields were quantified by using the Coomassie Plus Protein Assay Reagent (Pierce) with bovine serum albumin as standard (2 mg/ml ampoule; Pierce).

Protein expression in Ultra Yield™ flasks

Frozen glycerol stocks were used to inoculate 50 ml of LB medium containing the appropriate antibiotic and the cultures grown to saturation overnight at 37°C. For each clone, 10 ml of the overnight culture were used to inoculate each of three flasks as follows: (a) 2.8 l baffled Fernbach flask containing 1 l LB, (b) a 2.8 l baffled Fernbach flask containing 1 l TB, and (c) a 2.5 l Ultra Yield™ flask containing 1 l TB. Each culture contained the appropriate antibiotic and all three cultures were grown in parallel in an Innova 4430 shaking incubator (New Brunswick; 3–9 flasks per incubator. Note: optimal performance of the Ultra Yield™ flask requires a rigid vertical placement. The flask holder and the shaking platform must not be loose.). Ultra Yield™ flasks were sealed with an AirOtop Seal. The cultures were agitated at 250 rpm for approximately 4 h at 37°C. At that time the incubators were set to 25°C and the agitation suspended for approximately 15 min. The optical density (A_{600}) of each culture was measured (range of 0.8 to 1.1 for LB cultures and 1.4 to 3.2 for TB cultures) and IPTG was added to a final concentration of 100 μ M. The cultures were agitated at 250 rpm overnight at 25°C. Following overnight growth, the optical density (A_{600}) of each culture was re-measured (see Results section). A 50 ml aliquot of each culture was transferred to a 50 ml Falcon tube and the suspension centrifuged at 5,000g for 30 min. The supernatants were decanted and the cell pellets stored at –80°C until processed (within one week).

Scale-up purification

To minimize differences in the isolation procedures, the proteins generated by the various expression conditions for each construct were purified in parallel. The cell mass from 50 ml of culture was resuspended in 20 ml of Preparative Lysis Buffer (50 mM Tris–HCl, pH 8.0, containing 50 mM NaCl, 1 mM MgCl₂, 0.6 g/l lysozyme, and 100 μ l/l benzonase) by using a vortex mixer, and the suspensions incubated on ice for 30 min. The concentration of NaCl was then adjusted to approximately 500 mM by the addition of 2 ml of 5 M

NaCl and the crude lysates centrifuged at 5,000g for 45 min. Each supernatant was applied to a 1 ml bed volume column of pre-equilibrated ProBond nickel affinity resin contained in a 25 ml Omni-Pac gravity flow column (BioRad). Each column was washed with 3 \times 4 ml of Preparative Wash Buffer (50 mM Tris–HCl, pH 8.0, 400 mM NaCl, 40 mM imidazole–HCl, pH 8.0, 10% Glycerol, and 0.25 mM TCEP) and the bound protein eluted with 4 ml of Preparative Elution Buffer (50 mM Tris–HCl, pH 7.4, 400 mM NaCl, 250 mM imidazole–HCl, pH 8.0, 10% Glycerol, and 0.25 mM TCEP). Preparative fractions (Whole-Cell, Soluble, Flow-Through, Wash, and Elution) were analyzed by SDS-PAGE using a 26-lane Criterion 4–12% gel (BioRad). Soluble, purified protein yields were quantified using the Coomassie Plus Protein Assay Reagent (Pierce) with bovine serum albumin as standard (2 mg/ml ampoule; Pierce).

Results

The development of Vertiga (see Fig. 1A) introduced a convenient low-cost solution to the microexpression of multiple protein constructs in parallel in order to screen their suitability to produce recombinant soluble protein for crystallization studies. The ability to generate sufficient protein for initial screening analysis in a single well of a 96-well plate format has significant advantages for parallel processing, and Vertiga achieves this by allowing cell cultures to grow to an unusually high cell density of $A_{600} \sim 20$ (see Materials and Methods for procedures used). The high density cell culture features of Vertiga have now been replicated for scale-up in a convenient, off-the-shelf, and disposable system called the Ultra Yield™ flask. The Ultra Yield™ flask, shown in Fig. 1B, is composed of polypropylene and has a more vertical wall construction than those of traditional shake flask designs. Together with the baffled base, these features provide the necessary aeration, without oxygen supplementation, to drive culture growth to similarly high cell densities as those achieved in Vertiga.

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, 12 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 12 proteins were also examined from cultures grown in traditional Fernbach flasks using

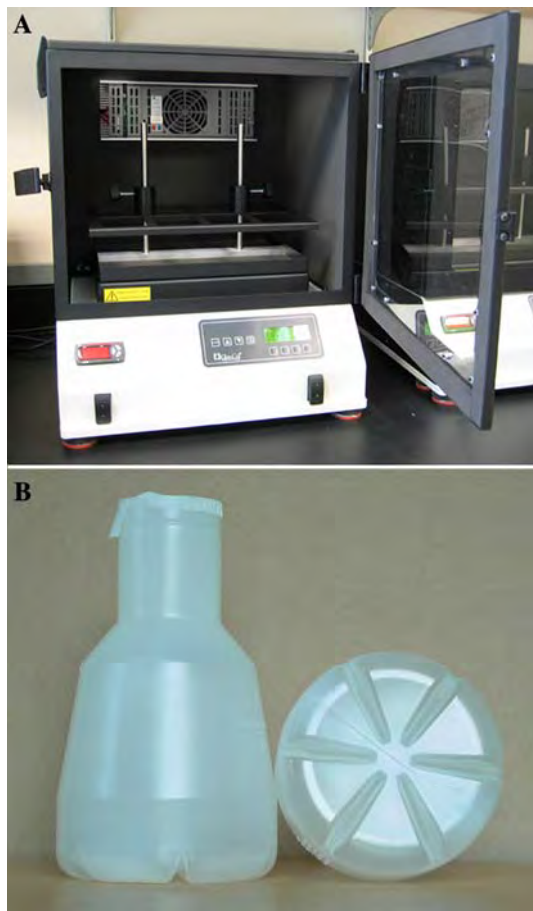


Fig. 1 Vertiga and the Ultra Yield™ flask. **(A)** The Vertiga shaker device that is used for microfermentation. **(B)** The Ultra Yield™ flask showing the baffled base and novel wall construction

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 12 protein expression constructs are listed in Table 1. The group includes four prokaryotic proteins and five

mammalian proteins, with three of the latter set included as both N-terminal and C-terminal polyHis-tagged versions. All 12 protein expression constructs were assembled in IPTG-inducible pET vectors, and all proteins were expressed in *E. coli* BL21(DE3) (see Materials and Methods section for clone construction and protein expression details).

The effects of culture medium and flask type on scale-up expression culture densities are shown in Fig. 2A. The data demonstrate that, as expected, TB medium allows for increased cell growth in Fernbach flasks when compared with LB medium in the same flask type. However, the Ultra Yield™ flasks provide for substantial increases in cell densities when cultures are grown in TB medium compared with similar cultures grown in Fernbach flasks (see Materials and Methods for expression conditions). The average cell culture densities generated in the Fernbach flasks following overnight expression using either LB or TB media were, respectively, 2.7 (range 1.9–3.8) and 4.0 (range 2.2–6.4), whereas the average cell density in the Ultra Yield™ flask with TB medium was 14.0 (range 7.6–20.2). 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.

To determine if the increases in cell culture densities obtained in the Ultra Yield™ flasks translated into concomitant increases in recombinant protein yield per unit volume, all 12 proteins expressed in each culture condition were purified and the yields assessed by protein assay and by SDS-PAGE analysis. The data from the 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.

Table 1 Protein constructs used for expression studies

Clone #	Protein	Source	Expression Vector	Purification Tag	Mass (+Tag)	pI
1	Alanine racemase (AR)	<i>S. aureus</i>	pET15b	N-His/thrombin	44989.2	7.63
2	4-Amino-4-deoxychorismate lyase (ADCL - <i>Pab C</i>)	<i>E. coli</i> K-12	pET28a	N-His/thrombin	31907.3	7.12
3	4-Amino-4-deoxychorismate synthase (ADCS - <i>Pab B</i>)	<i>E. coli</i> K-12	pET28a	N-His/thrombin	53119.8	5.81
4	Isochorismate synthase (<i>Ent C</i>)	<i>E. coli</i> K-12	pET28a	N-His/thrombin	45197.5	6.38
5	p38 alpha (MAPK14 isoform 2)	<i>H. sapiens</i>	pET28b	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	44284.9	8.20
8	JNK2 (aka MAPK9)	<i>H. sapiens</i>	pET30b	C-His	43103.6	6.53
9	MK-2 (isoform 2)	<i>H. sapiens</i>	pET30b	C-His	35353.9	8.48
10	MK-2 (isoform 2)	<i>H. sapiens</i>	pET47b	N-His/HRV3C	36353.0	8.48
11	hAG-2	<i>H. sapiens</i>	pET28b	C-His	19084.9	8.83
12	hAG-2	<i>H. sapiens</i>	pET47b	N-His/HRV3C	20012.9	8.83

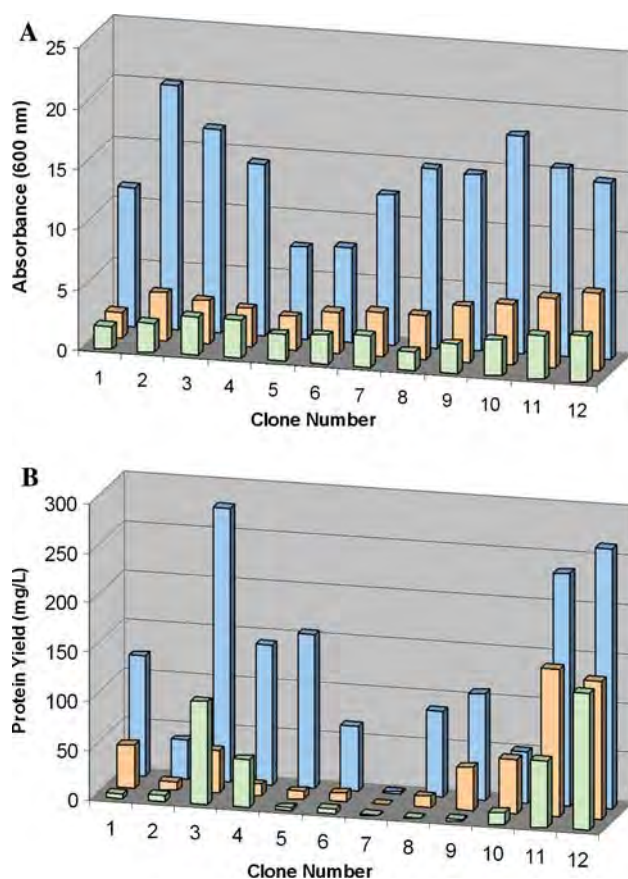


Fig. 2 Effect of flask design on *E. coli* culture growth and production of recombinant protein. **(A)** The effect of flask type and growth medium on the observed optical densities of the cultures at A_{600} following overnight protein expression. Cultures carried out in Fernbach flasks using either 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

Although this value exceeds the average increase in cell density observed for the Ultra Yield™ flasks versus the Fernbach flasks, it does not necessarily follow that the production of protein is greater in the Ultra Yield™ flasks per unit of cell density, as there are substantial variations in the fold-yield among the different proteins (see Fig. 2B) that may be attributable to this limited set of 12 constructs.

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. The Vertiga yield data less closely resemble the scale-up expression data generated in the Fernbach flasks, which is expected, since the average A_{600} under the latter conditions is substantially less than those achieved in the Ultra Yield™ flasks. Nevertheless, there is a good qualitative correlation regarding either poor or good expression between Vertiga and the scale-up fermentations carried out in the Fernbach flasks.

Discussion

There is a great deal of methodologies available to conduct small and large-scale laboratory production of recombinant proteins in *E. coli*. Traditionally, initial 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. The evolution of parallel approaches to screening and scale-up in recent years has generated a substantial number of reports describing various small-scale plate-based platforms [e.g. [3, 16, 17]]. One of the most prevalent devices in use for plate-based growth of *E. coli* cultures is the HiGro system (GeneMachines). Although the HiGro can hold up to 24 deep-well plates, the unit is relatively expensive and generally supports cell growth only to about $A_{600} = \sim 2$ in TB medium (see <http://www.genomicsolutions.com/>). This cell culture density is generally too low to allow for the isolation of sufficient purified recombinant protein from a single well in order to carry out a biophysical analysis that includes analytical size exclusion chromatography, which allows classification of the target with regard to aggregation state. This limitation is overcome when cultures are grown in Vertiga, which supports growth to $A_{600} = \sim 20$ in TB medium (see Introduction), thus allowing for the recovery of almost 10 times as much protein from the same volume.

The construct expression behavior identified in Vertiga has, until now, generally been scaled up for parallel expression by using the GNFermentor developed at GNF, which supports growth of *E. coli*

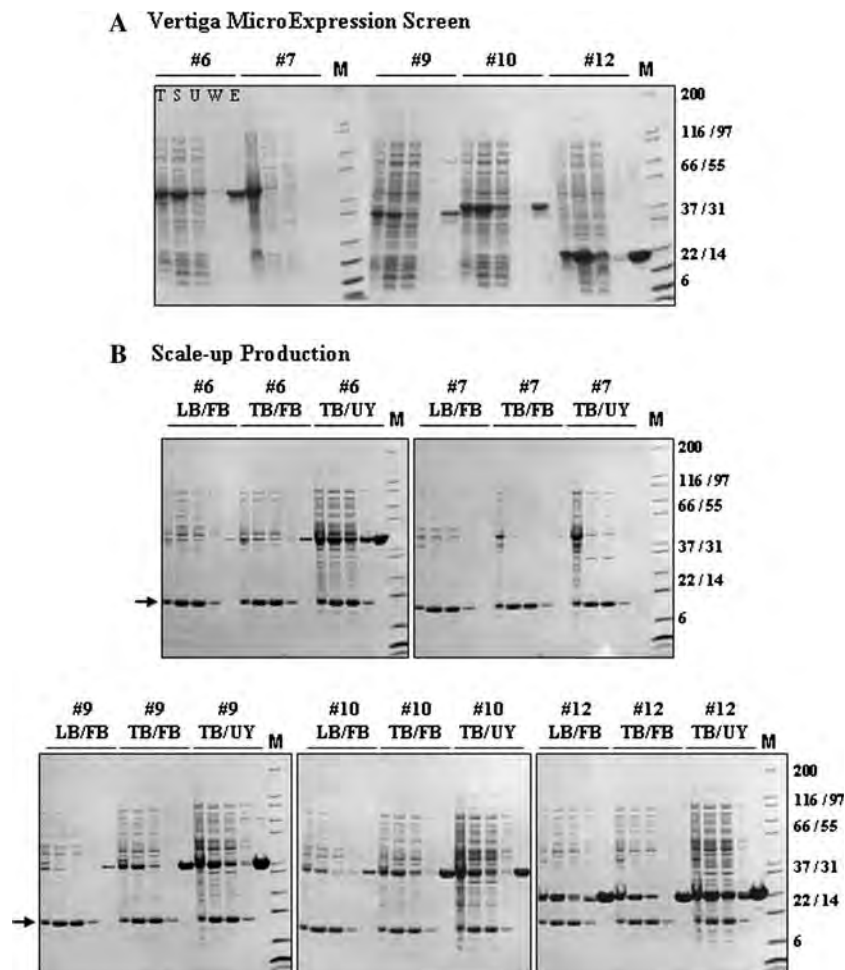


Fig. 3 SDS-PAGE analyses of recombinant protein expression in Vertiga and the Ultra YieldTM flasks. **(A)** Shown is the SDS-PAGE analysis of recombinant protein expressions carried out in Vertiga for clones 6, 7, 9, 10 and 12 (see Table 1 for clone details). The five gel loadings for each sample are comprised of: total lysate (T), soluble extract (S), IMAC unbound fraction (U), IMAC wash fraction (W) and 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 YieldTM 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

expression clones to similarly high cell culture densities [2, 8]. 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. The use of 2-l plastic bottles described by Millard et al. [11] as a means to generate high density *E. coli* cell cultures in volumes up to 250 ml provides for an economical alternative to the GNFermentor. The availability of the Ultra YieldTM flask, described above, now provides additional advantages for high density *E. coli* fermentation by reducing the experimental footprint 4-fold via its 1 l

capacity and by virtue of its validation as a direct scale-up platform for the most promising clones identified in Vertiga. The Ultra YieldTM flask is convenient to use, is inexpensive and disposable, and avoids the requirement to pool multiple smaller scale fermentations into a single paste. The combination of Vertiga and the Ultra YieldTM flask thus provides for a simple low-cost solution to parallel protein expression microscreening and scale-up in *E. coli*.

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