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Development of a semi-automated high throughput transient transfection system

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ABSTRACT

Transient transfection of mammalian cells provides a rapid method of producing protein for research purposes. Combining the transient transfection protein expression system with new automation technologies developed for the biotechnology industry would enable a high throughput protein production platform that could be utilized to generate a variety of different proteins in a short amount of time. These proteins could be used for an assortment of studies including proof of concept, antibody development, and biological structure and function. Here we describe such a platform: a semi-automated process for PEI-mediated transient protein production in tubespins at a throughput of 96 transfections at a time using a Biomek FX^P liquid handling system. In one batch, 96 different proteins can be produced in milligram amounts by PEI transfection of HEK293 cells cultured in 50 mL tubespins. Methods were developed for the liquid handling system to automate the different processes associated with transient transfections such as initial cell seeding, DNA:PEI complex activation and DNA:PEI complex addition to the cells. Increasing DNA:PEI complex incubation time resulted in lower protein expression. To minimize protein production variability, the methods were further optimized to achieve consistent cell seeding, control the DNA:PEI incubation time and prevent cross-contamination among different tubespins. This semi-automated transfection process was applied to express 520 variants of a human IgG1 (hu IgG1) antibody.

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1. Introduction

Transient transfection of mammalian cells is a well-established method for protein production in the biotechnology industry. The use of mammalian cells for protein expression offers an advantage over prokaryotic or other eukaryotic systems due to its ability to promote correct folding and post-translational modifications for the expressed protein (Geisse and Fux, 2009). The benefit of mammalian transient over stable expression systems is the shorter timeline for material generation. CHO stable cell line generation may take 4–5 months whereas transient expression of secreted proteins requires only 7–14 days, depending on cell type and process (Baldi et al., 2007; Geisse and Fux, 2009; Pham et al., 2006). Suspension mammalian cell lines such as Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK) 293 cells have been widely used in industry for transient transfections both at larger scales up to 100 L in bioreactors and smaller scales in 50 mL tubespins (Baldi et al., 2005; Girard et al., 2002; Stettler et al., 2007). As such, transient transfections have been performed in a high throughput fashion for decades (Bennett et al., 1991).

Manual small scale transfections using tubespins, shake flasks and plates can be highly repetitive and executed in high quantities, making them an ideal candidate for automation. As such, 293 transfections have been automated for T flasks using a CompacT SelecT and 24 well plates with a Tecan Evo. The quality and quantity of secreted mammalian proteins produced from the automated and manual T flask transfection methods were found to be comparable (Nettleship et al., 2010; Zhao et al., 2011). However, thus far, there are no reports of an automated tubespin transfection system. Based on the optimal performance of transfections using tubespins (Stettler et al., 2007), we elected to automate the process.

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This process involves the batch transfection of 96 individual tubespins organized into a tray, 8 per column and 12 per row. Several approaches were taken to automate the manual tubespin transfection process and address the potential concerns associated with performing high throughput transient transfections. As with most high throughput processes that encompass large sample sizes, protein production consistency across the 96 individual tubespins is critical. Two factors that can influence transient protein production consistency are cell density and DNA:PEI complex addition. By utilizing a spinner bag agitated on a magnetic stir plate, we were able to prevent cell settling during the cell dispensing process and thus obtain comparable cell seeding densities among different tubes. Our data, along with previous studies (Bertschinger et al., 2008; Derouazi et al., 2004), shows that the incubation period for forming DNA:PEI complexes impacts DNA:PEI complex size and resulting transfection yields. This prompted us to program an automated complex addition method using the Biomek FX^P so that each tube received the DNA:PEI complex within a 9–10 min incubation range to minimize any decrease in protein expression due to increasing complex incubation time. Experiments were also conducted to confirm that no cross contamination occurred between tubespin columns during the DNA:PEI complex addition since the Biomek FX^P utilizes fixed, non-disposable tips for liquid transfer. This resulting semi-automated transient transfection process entails automated seeding and transfection and manual transfer of the tubespin rack from the incubator to the automated workstation. It is capable of performing 96 individual transfections in a single batch, making this a valuable tool for antibody development, expression screening and structure biology studies.

2. Materials and methods

2.1. Cell culture

The 293T cell line used for this study was a suspension adapted HEK293 cell line that was stably transfected with the SV40 large T-antigen. Cells were cultivated as a seed train in shake flasks under conditions of 37 °C, 5% CO_2, and 150 rpm agitation speed at a 25 mm throw diameter in an 80% humidified incubator before transient transfection. Gibco Freestyle 293 expression medium (Life Sciences, Carlsbad, CA) supplemented with 1% ultra-low IgG serum (Sigma, St. Louis, MO) was used as the seed train and production medium. Unless otherwise specified, all transient transfections were carried out in 50 mL tubespins (Stettler et al., 2007) with a 30 mL final working volume and processed in batches of 96. A Biomek FX^P liquid handling robot was used to bulk dispense cells into the 96 tubespins for efficiency. Post-transfection, cells were cultured for 7 days at 37 °C, 5% CO_2 and 225 rpm agitation speed at a 50 mm throw diameter in an 80% humidified Kuhner ISF1-X incubator.

2.2. Automation instrumentation

A system for automated cell culture process development had been previously designed and was adapted for this process. A key design decision was made early on to use 50 mL tubes as the reactors and to not use a decapper. SeptaVent™ tubes (Optimum Processing, Greenbrae, CA) are similar to the more familiar TubeSpin® 50 bioreactors (TPP, Trasadingen, Switzerland) in that they are 50 mL conical tubes with 0.2 μm filters in the cap for gas exchange. However, the SeptaVent also has a pre-slit silicone septum in the cap. This septum allows a narrow pipette or cannula to pass through the cap into the bioreactor and the slit closes as the pipette is removed. These tubes are kept in an 8 × 12 tray which fits on the deck of a Biomek FX^P robot (Beckman Coulter, Brea, CA) with a 96-channel pipetting head utilizing single-use pipette tips (96-tip head) and an 8 channel pipettor (8-tip span). The 96-tip head is equipped with Beckman's Enhanced Selective Tip option, allowing it to load fewer than 96 tips at a time. The Biomek liquid handler is inside a class II biological safety cabinet (Baker, Sanford, ME).

To work efficiently with the SeptaVent tubes, the robot is equipped with an 8-tip span with extra-long fixed tips. These tips can reach to the bottoms of the tubes to access the full culture volume, and eight reactors are accessed in parallel. Modifications to the base FX^P robot were made to enable aseptic operation with fixed tips. A selection valve was connected to the system fluid line that allows cleaning fluids such as Steriplex® (sBioMed®, Orem, Utah) to be pumped through to sanitize the fluidics and tips. This valve also allows media and cell culture fluid to be pumped through the system and into the cell culture reactors. Procedures were developed to clean and sanitize the system before and after each batch of 96 tubespins. A shorter procedure was also developed to clean the tips between reactors.

2.3. Standard and direct transfection methods

Cells were seeded at 1.0e6 cells/mL for transfection and incubated at 37 °C, 5% CO_2 for 2 h prior to transfection. Plasmid DNA encoding either a standard hu IgG1 antibody, or murine IgG2a (mu IgG2a) antibody was purified at the giga prep scale (Sigma, St. Louis, MO). For the standard transient transfection method, 30 μg of DNA was diluted in a DMEM-based medium to a final volume of 3 mL. Then 60 μL of 7.5 mM 25 kDa linear PEI was added to the DNA solution, mixed and incubated at room temperature for the indicated times before being added to the cells. For the direct transfection method (Raymond et al., 2011), the DNA:PEI ratio used was equivalent to the ratio used in the standard transfection method. 30 μg of DNA was added to a DMEM-based medium to give a final volume of 3 mL and incubated for 5 min. The DNA-media mixture was then added directly to the cells. Lastly, 60 μL of PEI was added to the cells to complete the direct transfection method process. For the serum free vs serum containing direct transfection comparison, the seeded cells were centrifuged at 25G for 10 min, and resuspended in an equal volume of serum containing or serum free media.

2.4. Cell count and protein concentration measurement

Viable cell density and viability of 293T seed train (and spinner bag post-seeding counts) were measured using a NyOne 96-well imaging system by SynenTech (Elmshorn, Germany). The NyOne instrument has been validated to produce less than 10% variability among replicates. Samples from tubespin cultures were collected using the Biomek FX^P and sampled with the NyOne employing trypan blue exclusion to determine viability and cell density. For protein concentration determination, supernatant samples were harvested from the tubespin cultures on day 7. Hu IgG1 antibody expression levels in the supernatant were determined using a Protein A HPLC assay. For the cross contamination assessment, hu IgG1 expression levels in the supernatant were measured using an intact hu IgG1 ELISA and mu IgG2a antibody expression levels were measured using an intact mu IgG2a ELISA.

2.5. Particle size and zeta potential measurement

Both DNA:PEI complex particle size and zeta potential were measured using the Brookhaven Instruments (Holtsville, NY) ZetaPALS (Zeta Potential Analyzer Utilizing Phase Analysis Light Scattering). For particle size determination, a 3 mL DNA:PEI complex was prepared in a DMEM-based complex medium, as