Smartphone-based titration of baculoviral and AAV vectors

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Abstract

Baculoviral expression systems are well-established tools for manufacturing diverse proteins, including viral vaccines and gene therapy vectors such as AAV. Recombinant baculovirus is engineered to express a protein of interest. Successful expression of a recombinant protein relies on knowing the infectious titer of the baculovirus preparation. This permits calculation of the multiplicity of infection (MOI), which can influence the recombinant protein's final expression level. Current titer methods are time-consuming and labor-intensive, requiring hours or days to obtain results.

Here, we present GoStix™ Plus, a smartphone app for iOS and Android that delivers results in 10 minutes.

The two-step assay analyzes gp64 in viral supernatant. First, the sample is added to a lateral flow device, where test and control bands soon appear. Then, the bands are imaged via smartphone camera and analyzed using the GoStix Plus app. Titer is calculated with the help of an automatically downloaded, lot-specific standard curve.

We tested this assay system by constructing and transfecting a baculoviral vector engineered to express ZsGreen1. We found that baculovirus could be detected as early as 4 days post-transfection (P0). Additionally, were able to screen viral clones (plaques) for titer using the GoStix Plus assay after one round of amplification (P1). The resulting titer values were within 3-fold of current titration methods, with coefficients of variation less than 15%.

Adding to the utility of these lateral flow-based tests, we could detect baculovirus-produced AAV particles using an AAV capsid-specific antibody. This method will be the focus of our work moving forward.

In summary, this highly convenient, lateral-flow-based titration technology can quantify both

1 Timelines for different baculoviral titration methods

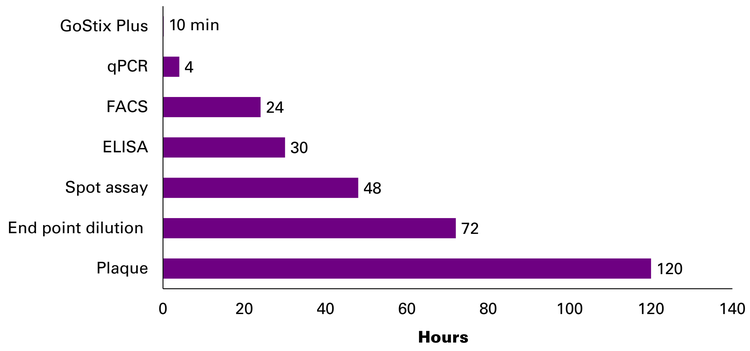

Figure 1. The timelines associated with most commonly used methods of baculoviral vector titration are measured in hours. Baculo GoStix: a lateral-flow-based method for the detection of baculoviral gp64. **qPCR:** quantitation of viral DNA genomes by quantitative PCR. **FACS:** measurement of gp64 by FACS analysis. **ELISA:** measurement of p35 protein from baculovirus-infected cells. **Spot Assay:** Antibody staining of gp64 in infected cells overlayed with agarose. **End Point Dilution:** measurement of TCID50 value. **Plaque:** Quantitation of the number of plaques observed in a monolayer of agarose-overlayed insect cells.

2 Workflow of baculovirus titration using GoStix Plus software

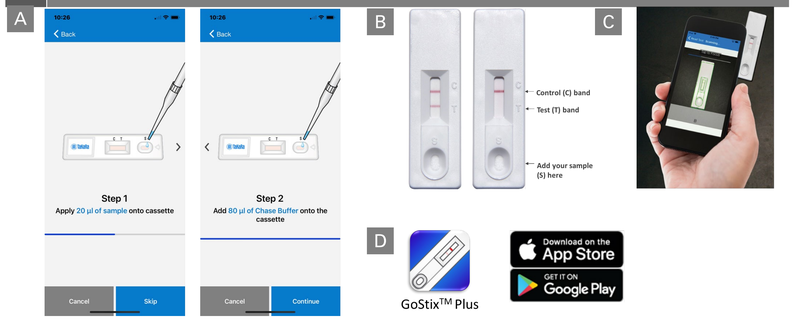

Figure 2. Baculovirus titration is fast and easy with the GoStix Plus app. Panel A. This lateral-flow assay detects gp64 present in samples by simply applying 20 µl of culture medium, followed by the addition of Chase Buffer and incubation at room temperature for 10 minutes. **Panel B.** Test and control bands develop during the 10-minute incubation time. **Panel C.** Band intensities are analyzed and quantitated using the GoStix Plus smartphone app. **Panel D.** The GoStix Plus App can be downloaded from the App Store or Google Play.

3 Production of ZsGreen1-expressing baculovirus

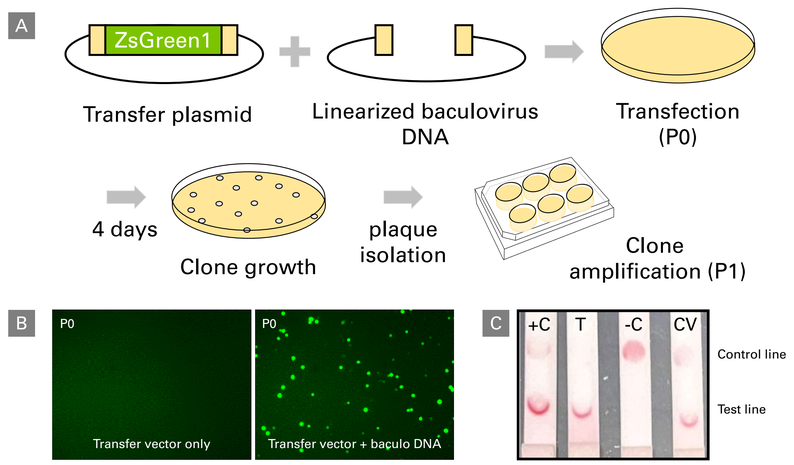

Figure 3. Baculovirus was engineered to express ZsGreen1. Panel A. ZsGreen1 was cloned into BacPAK8 vector using the In-Fusion® Snap Assembly Master Mix (Cat. # 638947). This plasmid was co-transfected with pre-linearized BacPAK6 DNA into Sf21 cells using Bacfectin in the BacPAK™ Expression System (Cat. # 631402). After 4 days, plaques containing baculovirus were selected and amplified in fresh Sf21 cells (P1). **Panel B**. Fluorescent images taken prior to the Day 4 harvest showed a significant number of ZsGreen1-expressing cells/plaques in the co-transfected population. **Panel C.** Supernatant harvested from Day 4 (P0) cultures was analyzed on lateral flow strips (spot test). A distinct signal was observed in the co-transfected cells. (**+C:** recombinant gp64 protein [20 ng]; **T:** transfected cell supernatant; **-C:** negative control, buffer only; **CV:** control virus [8.6 x 10^5 PFU])

4 FACS analysis of isolated baculovirus plaques

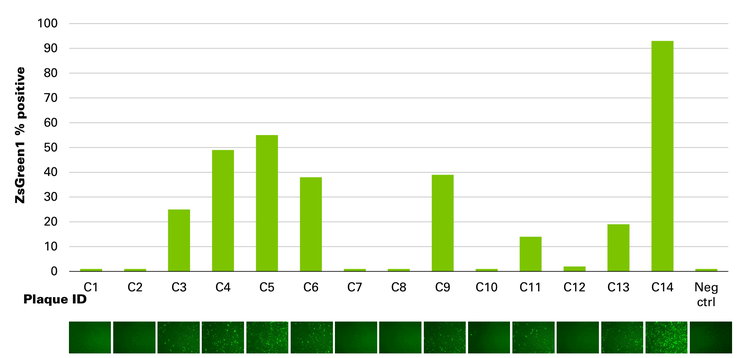

Figure 4. ZsGreen1-expressing clones were identified by FACS. Fluorescent clones were isolated from the transfected plate (P0) 4 days post-transfection and seeded onto fresh Sf21 cells. At 72 hr, cells were imaged by fluorescence microscopy (100X magnification). The corresponding wells were also analyzed by FACS at 96 hr.

6 Another analyte for lateral flow detection: AAV particles

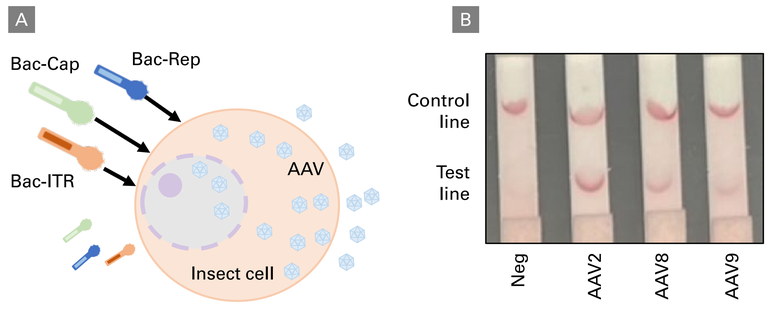

Figure 6. Lateral flow devices detected AAV particles produced in a baculovirus system. Panel A. AAV particles were produced in insect cells by co-infecting Sf9 cells with baculoviruses that express the Rep-, Cap-, and ITR-containing genome of AAV. AAV particles were then harvested and purified. **Panel B**. AAV particles of serotypes 2, 8, and 9 (~4 x 10^{11} vg equivalents) were run on lateral flow tests containing antibodies against AAV capsid protein. In each case, signals were observed for each of the 3 serotypes.

5 Comparison of gp64 detection by Baculo GoStix with other titration methods

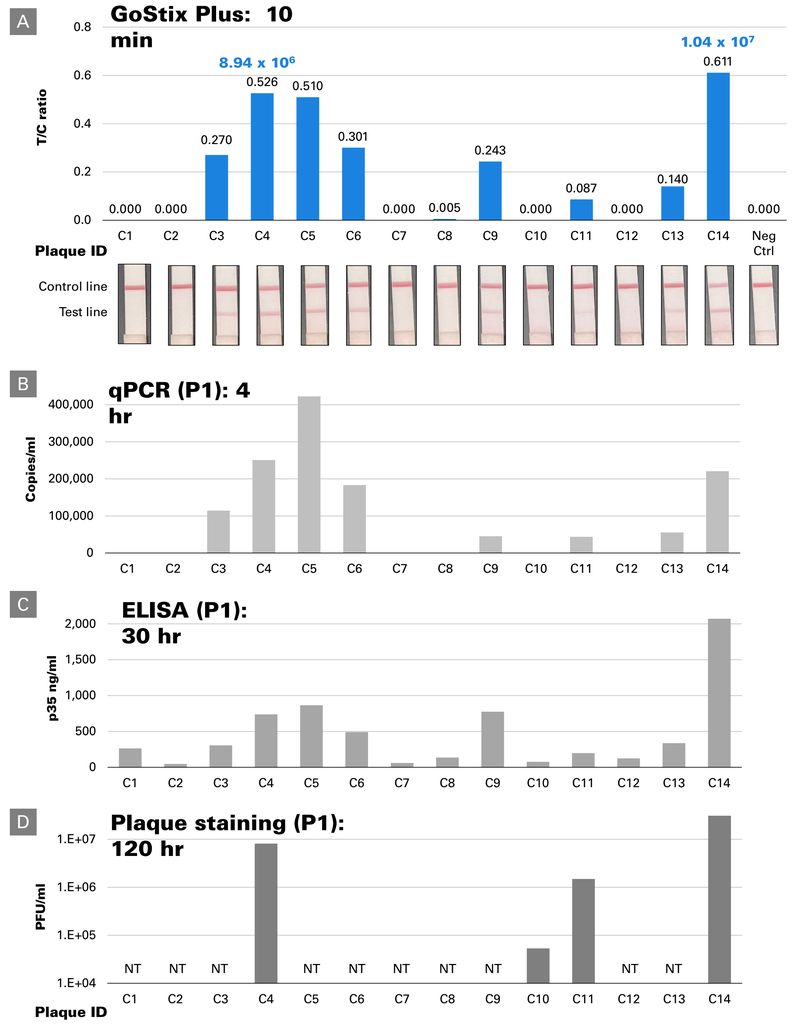

Figure 5. GoStix Plus titration is comparable to the other methods. Panel A. Twenty microliters of baculovirus-containing media were added to the GoStix lateral flow tests, chased with 80ul of Chase Buffer, and incubated for 10 minutes. The strips were imaged and then scanned using the GoStix Plus software to determine the T and C line intensities. For Clones 4 and 14, PFU/ml values (blue) were calculated using Clone 11 as a reference. **Panel B.** Baculovirus supernatants were analyzed by qPCR for viral genomic DNA using the BacPAK qPCR Titration Kit (Cat. # 631414), which uses gp64-specific primers. **Panel C.** Analysis of ZsGreen1 clones by p35-specific ELISA following infection of Sf21 cells. **Panel D.** Baculoviral supernatants were titered using the BacPAK Baculovirus Rapid Titer Kit (Cat. # 631406). The assay stains infected Sf21 cells for the expression of gp64 post-infection. There is a good correlation between the physical titration methods (GoStix and qPCR) to the functional methods that require infection (ELISA and plaque staining). The time to obtain each result is shown at the top left of each graph.

Conclusions

- The GoStix Plus method provides fast and easy titration of baculovirus preparations
- GoStix Plus results correlate well with those of ELISA, qPCR, and PFU assays but are delivered in just 10 minutes
- The speed and ease-of-use of GoStix Plus tests permit real-time monitoring of experiments and can serve as a complement or replacement for more standardized, but labor-intensive methods
- The GoStix Plus App, with its improved user interface, yields titer measurements with high reproducibility across different mobile devices
- The GoStix Plus assay is suitable for measuring samples containing baculovirus, lentivirus, adenovirus, and in the future, adeno-associated virus (AAV; Figure 6)