Easy and Efficient Lentiviral Transduction with a Macroporous Transduction Sponge

Thomas P. Quinn*, Mei Fong, Lily Lee, Adrian Martin, Myn Uddin, Michael Haugwitz & Andrew Farmer

Takara Bio USA, Inc., San Jose, CA 95131 USA *Corresponding author: thomas_quinn@takarabio.com

Abstract

The advancement and approval of engineered cellular therapies using lentiviral vectors are revolutionizing the treatment landscape for diseases resistant to traditional therapeutics. To optimize lentiviral transductions and mitigate inefficiencies, various strategies have been employed, such as spinoculation, the use of reagents (polybrene, RetroNectin® reagent) to enhance cell-virus contact, or modification of cellular responses during transduction (rapamycin, prostaglandin E2). Despite their efficacy, many enhancers have limitations due to their bioreactivity that impacts cell behavior. Moreover, lentiviral transductions in standard cell culture vessels face challenges as the virus must traverse considerable distances before reaching cells, leading to significant viral waste due to insufficient transport and virus degradation. Microfluidic systems, by spatially constraining cells and virus to small areas, address these biotransport issues. However, previous applications of microfluidics for lentiviral transduction necessitated special chips and hardware to facilitate the flow of cells and virus through microfluidic channels. To overcome these requirements, we developed the Lenti-X™ Transduction Sponge, which is a macroporous 3D alginate sponge, eliminating the need for additional hardware. The microfluidic sponge is made from calcium-crosslinked alginate, a GMP-compliant, FDA-approved biomaterial with high biocompatibility and low toxicity. The crosslinked alginate undergoes a gentle cryogelation process, yielding a sponge (5mm radius) with uniform pore sizes ranging from 20–300 nm. The simple workflow involves a mixture of cells and virus applied to the sponge for absorption, followed by a short incubation of 1 hr, media addition, and harvesting 24 hr later. The release of transduced cells is facilitated by adding a chelating release buffer that depolymerizes the sponge, ensuring the efficient release of transduced cells. Sponge-based transductions demonstrate flexibility and can accommodate a wide range of cell amounts (from 1 x 10^5–1 x 10^7 cells) and types, transduction volumes (50–150 µl), and incubation times (4–16 hr). This straightforward workflow is designed to maximize transduction efficiency across a diverse array of cell targets, including both suspension and adherent cell types. The alginate sponge demonstrated efficient transduction of over 15 cell targets, including human primary T cells, CD34+ HSCs, NK cells, and adherent cell lines, with high viability. Sponge-based transductions are consistent, with a coefficient of variation <15%. The innovative ex vivo Lenti-X Transduction Sponge is user-friendly, scalable, and compatible with diverse cell types, providing efficiencies equal to or surpassing conventional protocols.

1 Lenti-X Transduction Sponge workflow

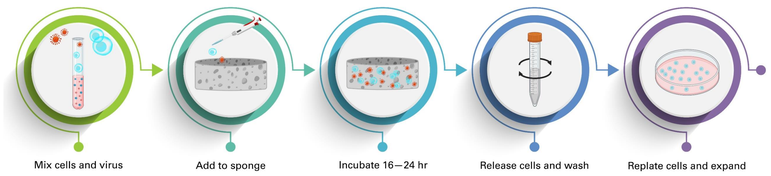

Figure 1. Simple transduction workflow using the Lenti-X Transduction Sponge. Cells and virus are mixed prior to application to the sponge. The transduction mixture is incubated for 1 hr, followed by the addition of media and incubation for 16–24 hr. No spinoculation is required. The next day, healthy, transduced cells are released using an optimized Release Buffer that depolymerizes the alginate matrix. Cells are then ready for subsequent analysis or continued culture.

2 Easy-to-handle, convenient format

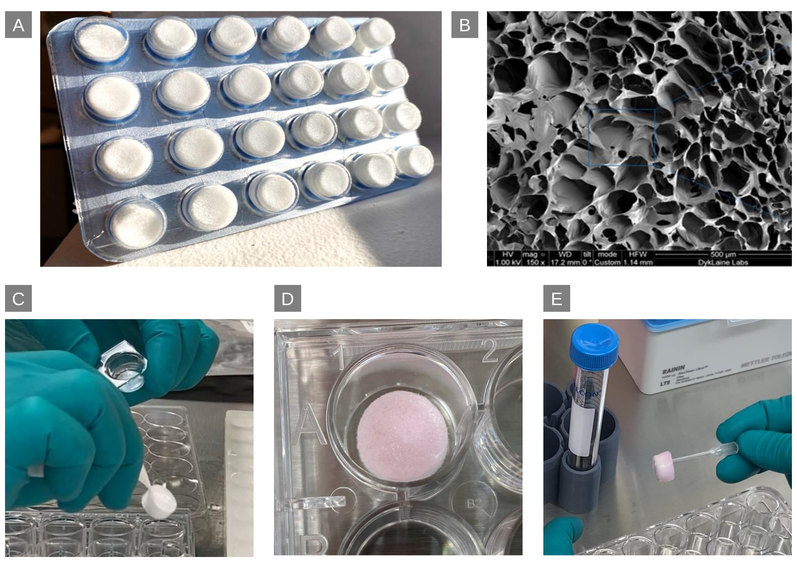

Figure 2. Convenient format for use with a 24-well tissue culture plate. Panel A. Each kit comprises 24 individual sponges, each capable of facilitating transduction of up to 1 x 10^7 cells. **Panel B.** Every sponge features a complex microfluidic pore structure with pore sizes ranging from 20–300 µm. The SEM image shown is at 150X magnification. **Panel C.** Simple placement of the transduction sponge into a well before the application of the sample. **Panel D.** The transduction mix, consisting of 1 x 10^5–1 x 10^7 cells and virus, is added to the sponge and incubated for 1 hr before the addition of media and incubation for 16–24 hr. **Panel E**. Cell release is facilitated by transferring the sponge to a 15 ml conical tube and adding Release Buffer, followed by a short wash step.

3 Transduction efficiency compared to spinoculation

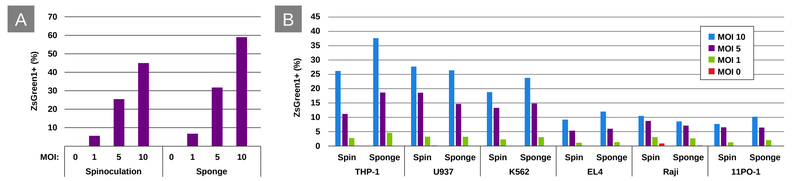

Figure 3. The Lenti-X Transduction Sponge facilitates transduction at equivalent levels to spinoculation in multiple cell types. Panel A. 1 x 10^6 Jurkat cells were transduced with lentivirus expressing ZsGreen1 at the indicated multiplicities of infection (MOIs) using spinoculation or the Lenti-X transduction sponge. The spinoculation cultures were centrifuged for 90 min at 1400x*g* at 32°C in the presence of 8 µg/ml polybrene. Sponge cultures were treated as described in Figure 1. All transductions were analyzed at 48 hr post-transduction for ZsGreen1 expression by FACS. **Panel B**. To further compare sponge transduction to spinoculation ("Spin" in Figure 3B), a variety of cell lines were transduced with ZsGreen1 lentivirus at several MOIs, as described in Panel A.

4 T-cell transduction efficiency and viability

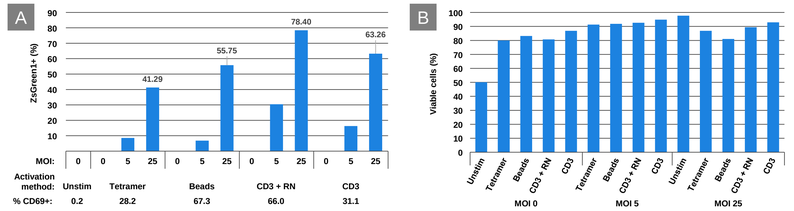

Figure 4. The Lenti-X Transduction Sponge enhances primary T-cell transduction efficiency while preserving viability. Panel A. Human primary T cells were activated using an antibody tetramer, anti-CD3/CD28-coated beads, RetroNectin reagent (RN) with CD3, or CD3 only. Activation levels indicated by percentage CD69+ expression at 48 hr are displayed. Then, 1 x 10^6 activated cells were transduced with lentivirus encoding ZsGreen1 at the specified MOI using the transduction sponge for 24 hrs. FACS analysis was performed at 48 hr post-transduction to determine %ZsGreen1 expression. **Panel B** Viability at 48 hr post-transduction as determined by 7-AAD staining and FACS

5 CD34$^+$ HSC and NK cell transduction efficiency

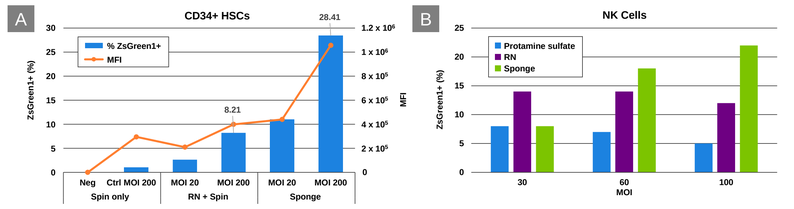

Figure 5. The transduction sponge facilitates efficient transduction of both human CD34+ HSCs and NK cells. Panel A. 1 x 10^6 human CD34+ hematopoietic stem cells (HSCs) were transduced with lentivirus encoding ZsGreen1 at the indicated MOIs using either a RetroNectin reagent + spinoculation (RN + spin) protocol or the transduction sponge for 24 hr. As controls, 1 x 10^6 CD34+ HSCs were incubated with either no virus (Neg) or transduced using an MOI of 200 (Ctrl MOI 200) using a spinoculation only (Spin only) protocol. All transductions were analyzed at 48 hr post-transduction for ZsGreen1 expression by FACS and mean fluorescence intensity (MFI) was also calculated. Viability as determined by 7-AAD staining was >99% (data not shown). **Panel B**. Primary human NK cells were cultured in a feeder-free system for 1 week and then transduced with a lentivirus expressing ZsGreen1 at the indicated MOIs using a protamine sulfate + spinoculation protocol, a RetroNectin reagent + spinoculation protocol, or the transduction sponge for 24 hr. All transductions were analyzed at 48 hr post-transduction for ZsGreen1 expression by FACS.

6 Transduction efficiency across varying cell amounts, transduction times, and sponge replicates

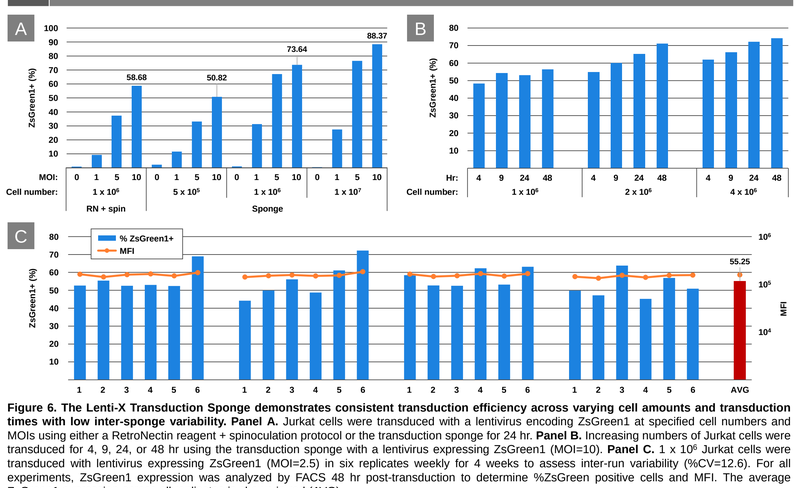

Figure 6. The Lenti-X Transduction Sponge demonstrates consistent transduction efficiency across varying cell amounts and transduction times with low inter-sponge variability. Panel A. Jurkat cells were transduced with a lentivirus encoding ZsGreen1 at specified cell numbers and MOIs using either a RetroNectin reagent + spinoculation protocol or the transduction sponge for 24 hr. **Panel B.** Increasing numbers of Jurkat cells were transduced for 4, 9, 24, or 48 hr using the transduction sponge with a lentivirus expressing ZsGreen1 (MOI=10). **Panel C.** 1 x 10^6 Jurkat cells were transduced with lentivirus expressing ZsGreen1 (MOI=2.5) in six replicates weekly for 4 weeks to assess inter-run variability (%CV=12.6). For all experiments, ZsGreen1 expression was analyzed by FACS 48 hr post-transduction to determine %ZsGreen positive cells and MFI. The average ZsGreen1 expression across all replicates is shown in red (AVG).

7 Sponge-transduced T cells exhibit a normal phenotype

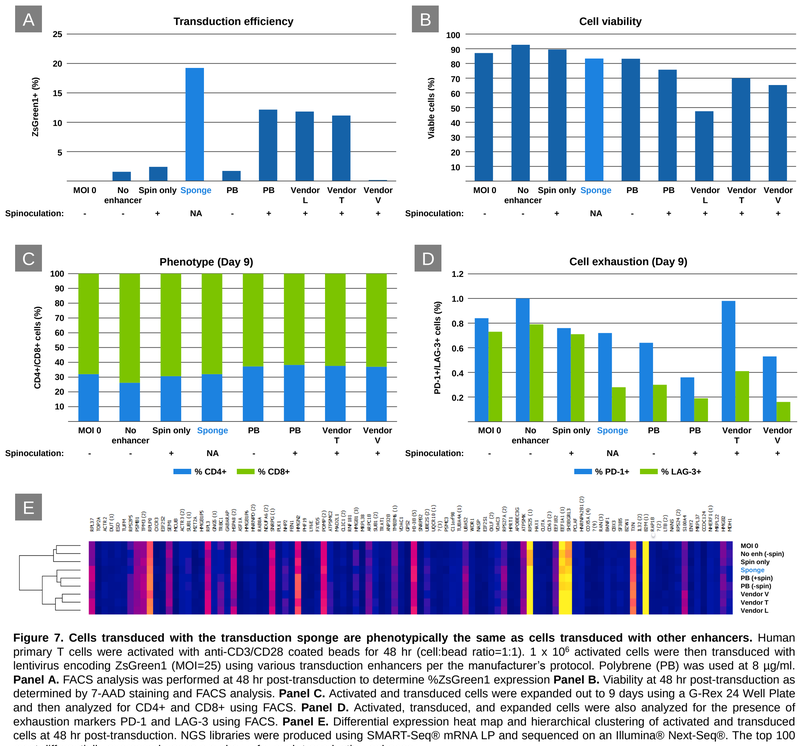

Figure 7. Cells transduced with the transduction sponge are phenotypically the same as cells transduced with other enhancers. Human primary T cells were activated with anti-CD3/CD28 coated beads for 48 hr (cell:bead ratio=1:1). 1 x 10^6 activated cells were then transduced with lentivirus encoding ZsGreen1 (MOI=25) using various transduction enhancers per the manufacturer's protocol. Polybrene (PB) was used at 8 µg/ml. **Panel A.** FACS analysis was performed at 48 hr post-transduction to determine %ZsGreen1 expression **Panel B.** Viability at 48 hr post-transduction as determined by 7-AAD staining and FACS analysis. **Panel C.** Activated and transduced cells were expanded out to 9 days using a G-Rex 24 Well Plate and then analyzed for CD4+ and CD8+ using FACS. **Panel D.** Activated, transduced, and expanded cells were also analyzed for the presence of exhaustion markers PD-1 and LAG-3 using FACS. **Panel E.** Differential expression heat map and hierarchical clustering of activated and transduced cells at 48 hr post-transduction. NGS libraries were produced using SMART-Seq® mRNA LP and sequenced on an Illumina® Next-Seq®. The top 100 most differentially-expressed genes are shown for each transduction enhancer.

8 Modified sponges facilitate simultaneous activation and transduction of unstimulated T cells

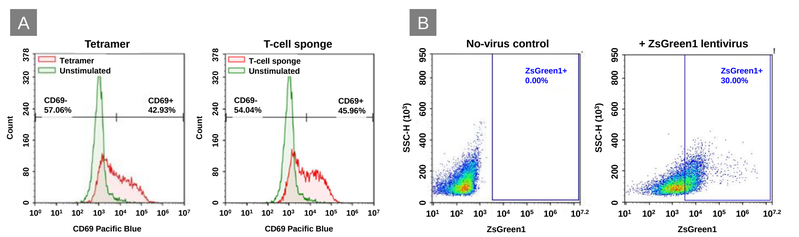

Figure 8. T-cell-specific transduction sponges containing activation reagents facilitate simultaneous activation and transduction of unstimulated T cells. Unstimulated T cells were combined with lentivirus encoding ZsGreen1 and directly applied onto a transduction sponge containing anti-CD3 antibodies, anti-CD28 antibodies, and IL-2 (100 IU/ml) for T-cell activation. After 24 hr, the cells were released from the sponge for further culture and analysis. **Panel A.** Comparison of activation methods for unstimulated T cells. T cells were activated using the T-cell-specific sponge or anti-CD3/CD28 tetramer. After 24 hr of activation, cells were analyzed for CD69+ expression via FACS. **Panel B** Transduction efficiency of T cells using the T-cell-specific sponge. Transductions were analyzed at 48 hr post-transduction for ZsGreen1 expression by FACS. A no-virus control is shown on the left.

Conclusions

- The Lenti-X Transduction Sponge workflow produces transduction efficiencies equal to or better than spinoculation.
- A simple workflow minimizes cell handling and hands-on time.
- Allows the transduction of a wide variety of cell types, including CD34+ HSCs, NK cells, and T cells.
- Can transduce a wide range of cell numbers (1 x 10^5–1 x 10^7 per sponge) enabling downstream applications requiring large number of transduced cells.
- Transduces T cells equivalently or better than other transduction enhancers, with minimal to no variance in phenotype, viability, or expression profile.
- Modified transduction sponges can be applied in cell-type specific transduction applications.

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999