Streamlined ex vivo engineering of human T cells: a single-step approach to activation and lentiviral transduction

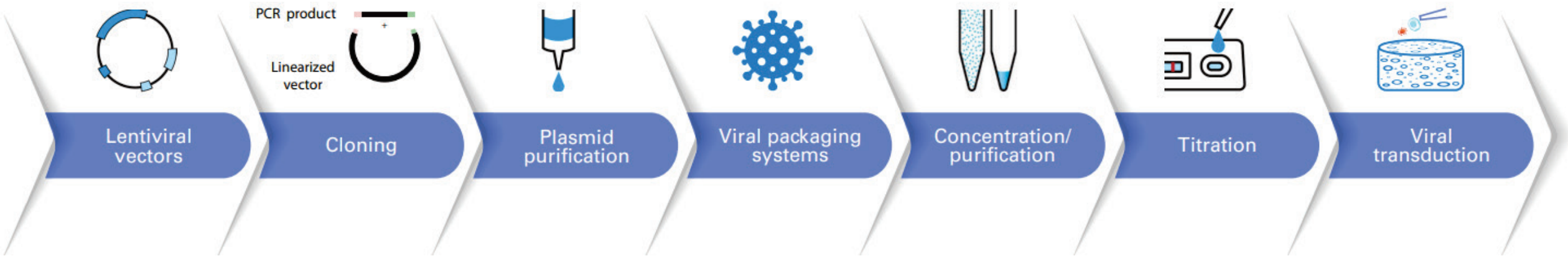
Thomas Quinn

R&D Group Leader

For Research Use Only. Not for use in diagnostic procedures.
© 2025 Takara Bio Inc. All rights reserved. All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Additional product, intellectual property, and restricted use information is available at takarabio.com.

that's GOOD science!®

Takara Bio's comprehensive product line meets lentiviral transduction needs



- Constitutive promoter
- EF-1 alpha promoter
- IRES bicistronic
- Fluorescent protein
- Tet-inducible
- Whole-cell labeling
- Organelle labeling
- Tet-On® 3G transactivator

- In-Fusion® Snap Assembly Master Mix with Competent Cells

- NucleoSpin Plasmid
- NucleoBond PC 20

- Lenti-X™ Packaging Single Shots
  - VSV-G
  - Ecotropic
  - Integrase deficient
  - Envelope free
- Lenti-X 293T cells

- Lenti-X Concentrator
- Lenti-X Maxi Purification Kit

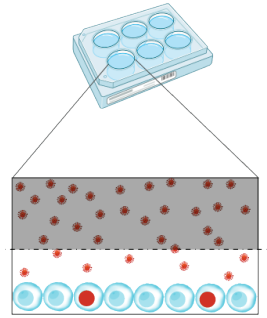
- Lenti-X GoStix™ Plus
- Lenti-X qRT-PCR Titration Kit
- Lenti-X p24 Rapid Titer Kit
- Lenti-X p24 Rapid Titer Kit (Single Wash)
- Lenti-X Provirus Quantitation Kit

- RetroNectin® reagent
- Lenti-X Accelerator
- Ecotropic Receptor Booster
- Lenti-X Integration Site Analysis Kit
- Lenti-X Transduction Sponge
- **Lenti-X T-Cell Transduction Sponge**

© 2025 Takara Bio Inc.

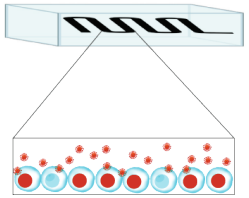
Lenti-X Transduction Sponge

Small molecule/chemical approaches



- ✗ Stress-inducing spinoculation required
- ✗ Unknown downstream impact of chemicals on cells
- ✗ Limited transduction efficiency
- ✗ Minimal colocalization
- ✗ Specific to cell type

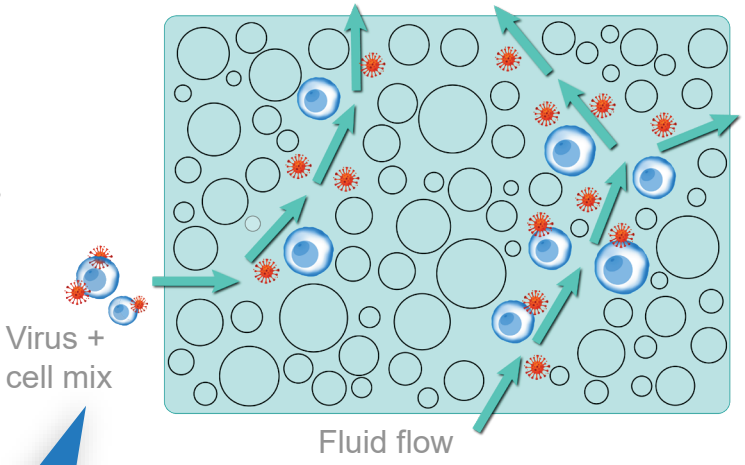
Microfluidic approaches

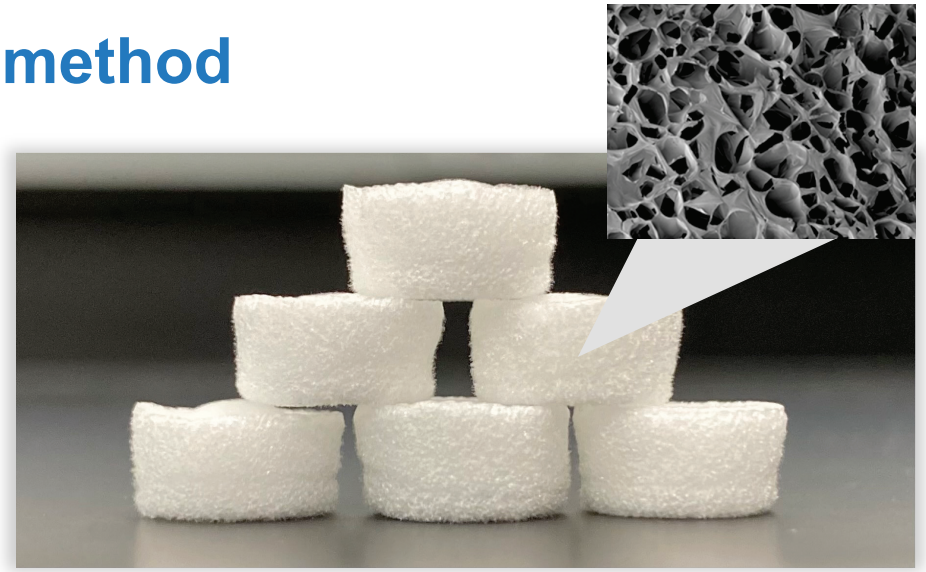


- ✗ Expensive instruments required
- ✗ Expertise needed to operate

Biocompatible and nontoxic—use your cells with confidence

Sponge method



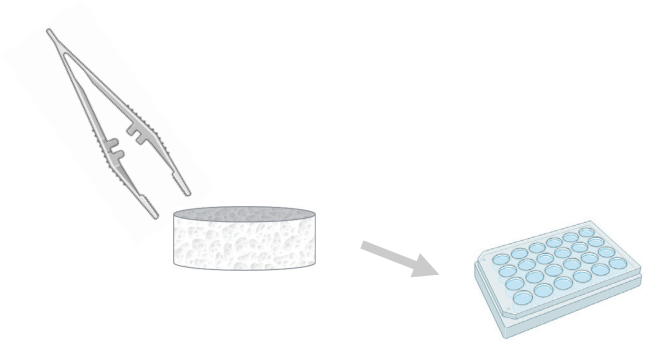


- ✓ Spinoculation-free method for enhanced lentiviral transduction
- ✓ High transduction efficiency
- ✓ Flexible, cell-type agnostic
- ✓ Capital cost savings while ensuring a microfluidic approach
- ✓ Gentle handling, resulting in high cell viability

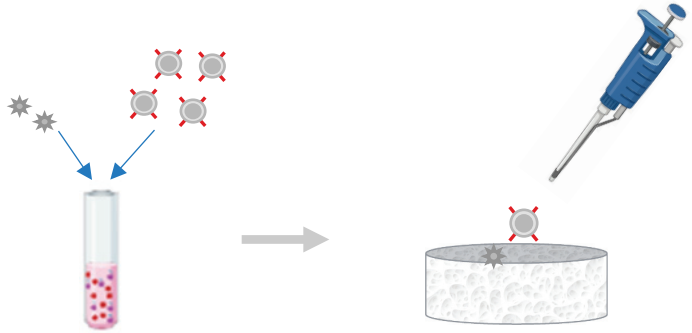
© 2025 Takara Bio Inc.

Lenti-X Transduction Sponge workflow—simple, spinoculation-free protocol for high transduction

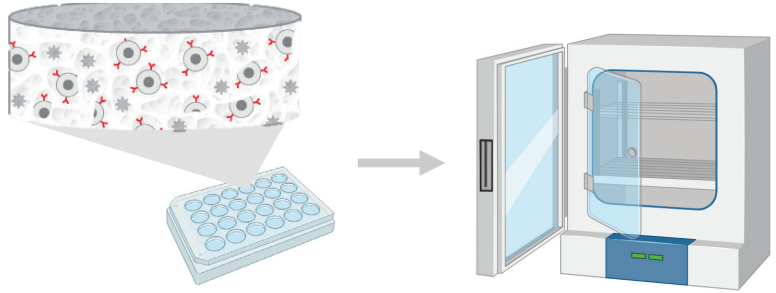
1 Transfer sponge to cell culture plate



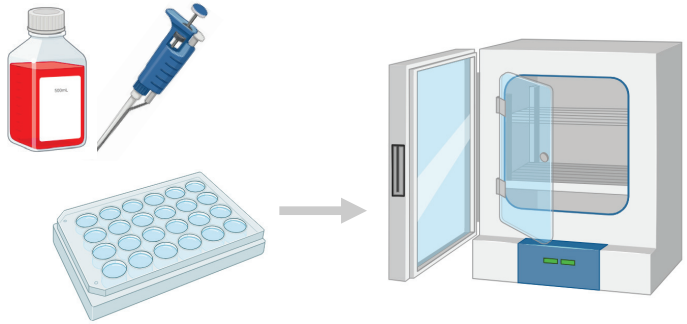
2 Mix cells and virus then add to sponge



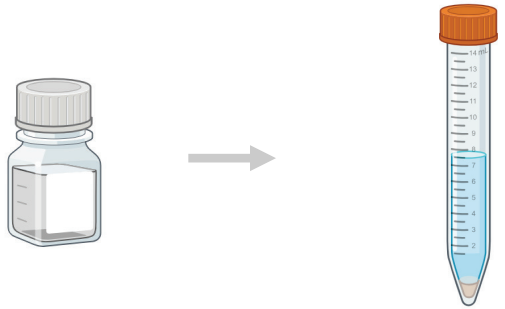
3 Incubate for 1 hr



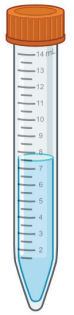
4 Add media and incubate 16–24 hr



5 Transfer sponge, add Release Buffer, and centrifuge to dissolve the sponge



6 Wash twice with phosphate-buffered saline. Your cells are ready!



Analysis

Expansion

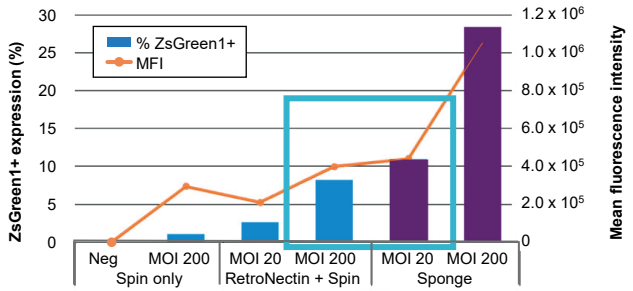
Created with BioRender.com

© 2025 Takara Bio Inc.

TaKaRa

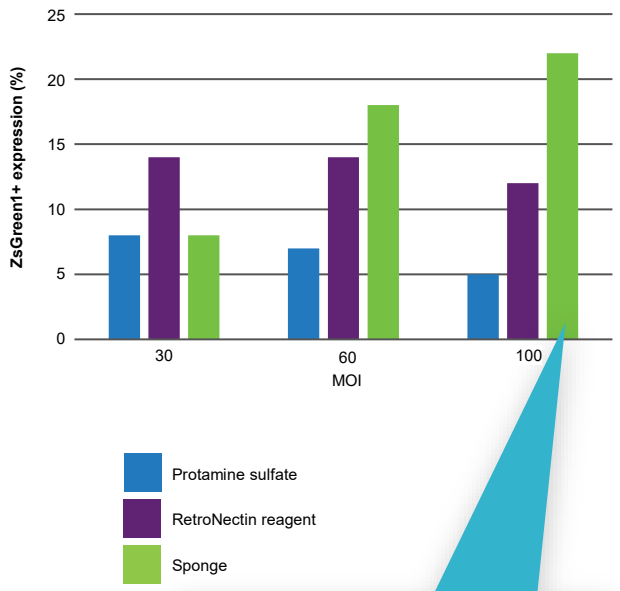
Successfully transduce primary cells

Efficient transduction of human CD34⁺ HSCs



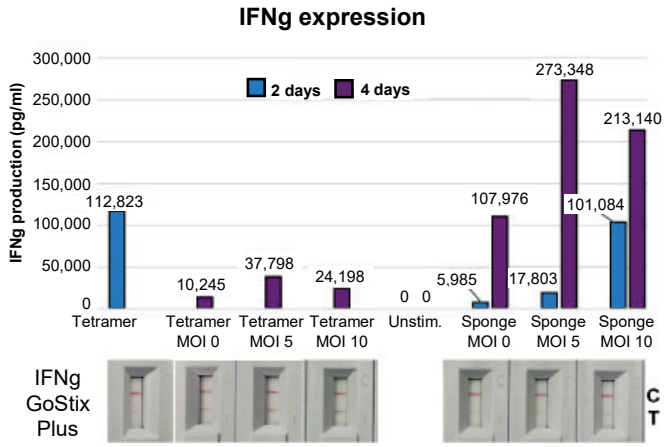
Sponge reduces virus requirement

Efficient transduction of primary human NK cells



Sponge effectively utilizes applied virus

Efficient transduction of T cells activated by different methods



Sponge effectively transduces T cells!

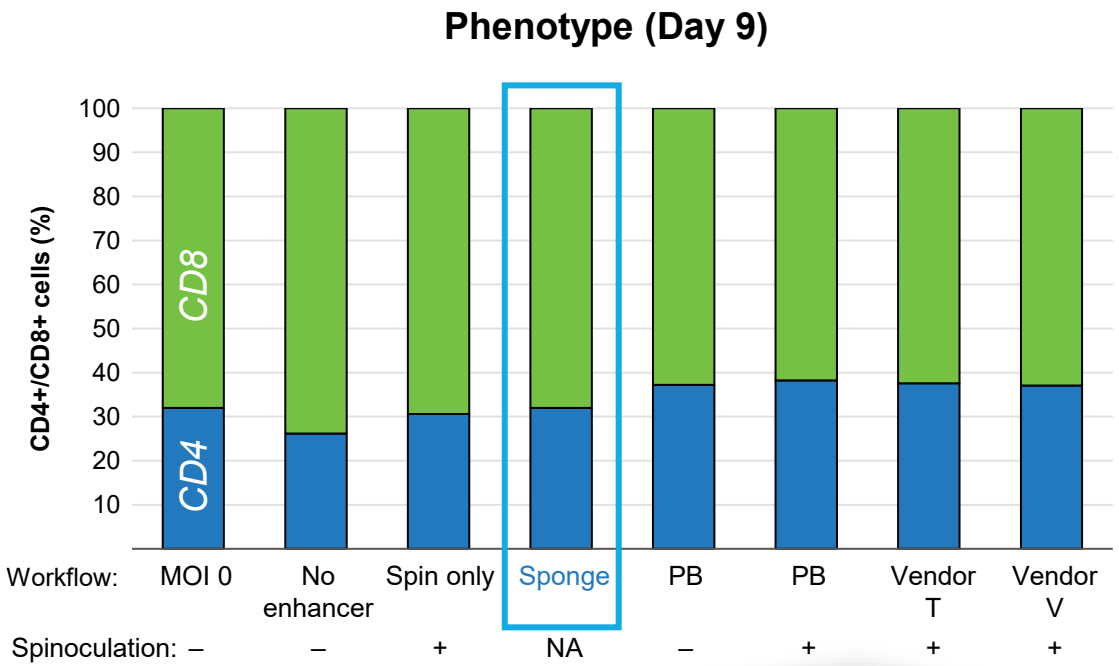
Cell viability greater than 80% for all primary cells tested

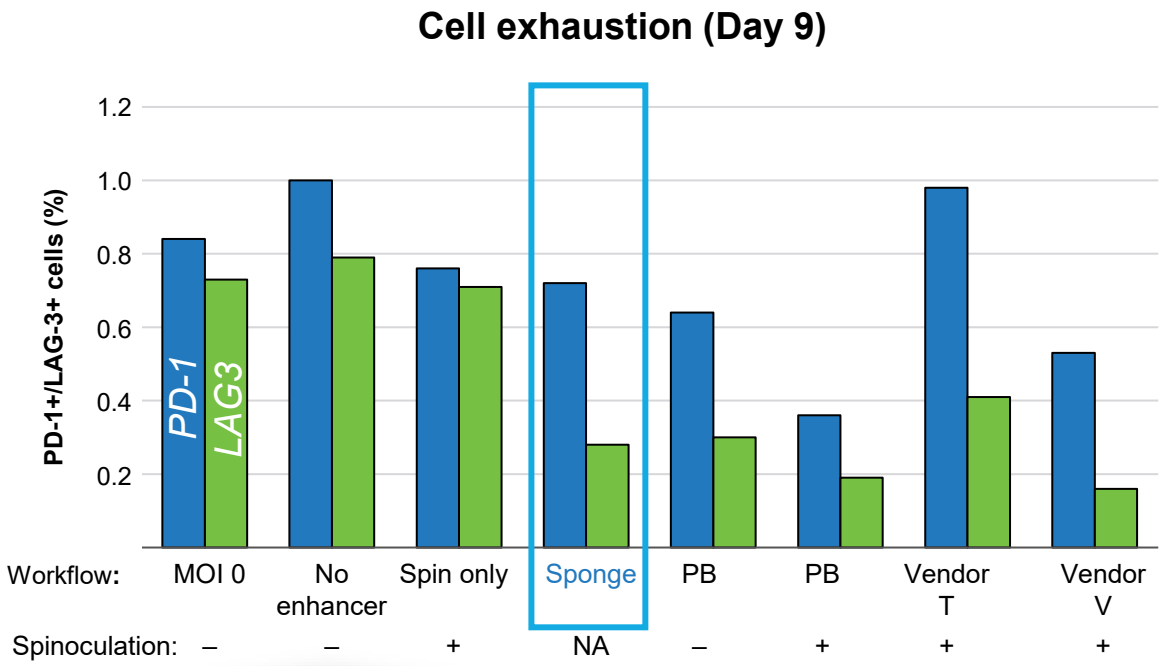
© 2025 Takara Bio Inc.

T cells transduced with the sponge workflow have equivalent phenotypes to other protocols

T cells transduced with the sponge workflow have similar CD4/CD8 ratios to cells transduced with other methods

T cells transduced with the sponge workflow have similar exhaustion markers as cells transduced with other methods





The CD4/CD8 ratio is unaltered by the sponge workflow

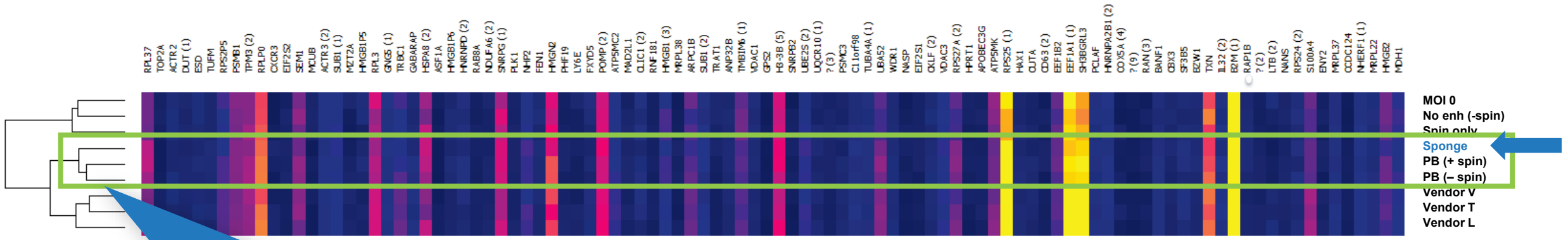
Exhaustion marker expression is <1% for all protocols tested

Viability >80% for all samples tested

© 2025 Takara Bio Inc.

Transcriptomic analysis demonstrates minimal impact of the sponge on primary T cells

No significant difference observed in expression of the top 100 differentially expressed genes using different workflows 48 hr after transduction



Expression pattern of cells transduced using the sponge workflow is similar to cells transduced with polybrene with and without spinoculation

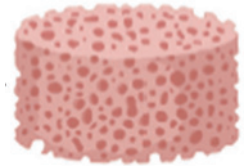
Sponge transduction is innocuous

At nine days post-transduction, all samples had similar gene expression profiles (not shown)

© 2025 Takara Bio Inc.

Introducing the new Lenti-X T-Cell Transduction Sponge

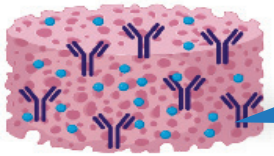
Universal Lenti-X sponge



+αCD3 +αCD28

+rhIL-2

T-cell sponge



Antibodies in the sponge allow you to activate and transduce T cells in one easy step

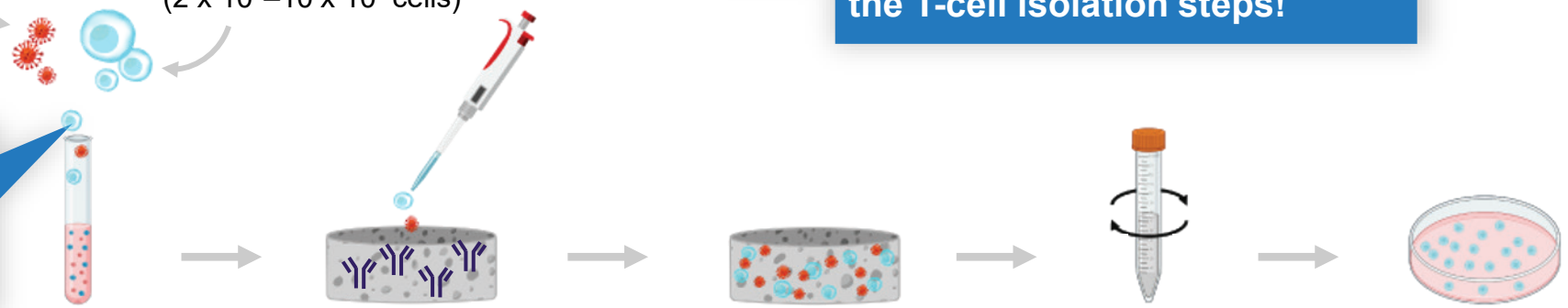
Simple, time-saving workflow

Lentivirus or retrovirus (MLV)

Unstimulated T cells or PBMCs (2×10^6–10×10^6 cells)

Starting with PBMCs eliminates the T-cell isolation steps!

Adding unstimulated cells saves time by eliminating an extra step in your workflow



Mix cells and virus → **Add to sponge** → **Incubate 48 hr** → **Release cells and wash** → **Replate cells and expand**

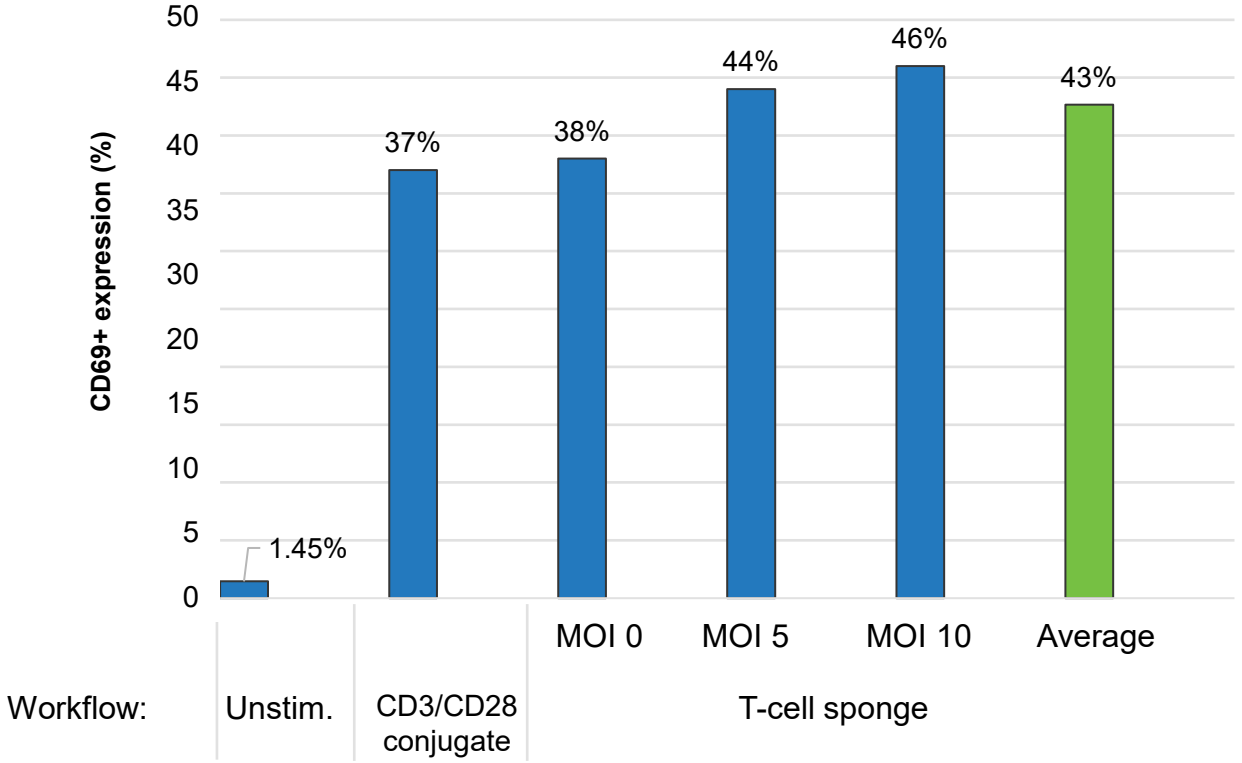
Created with BioRender.com

© 2025 Takara Bio Inc.

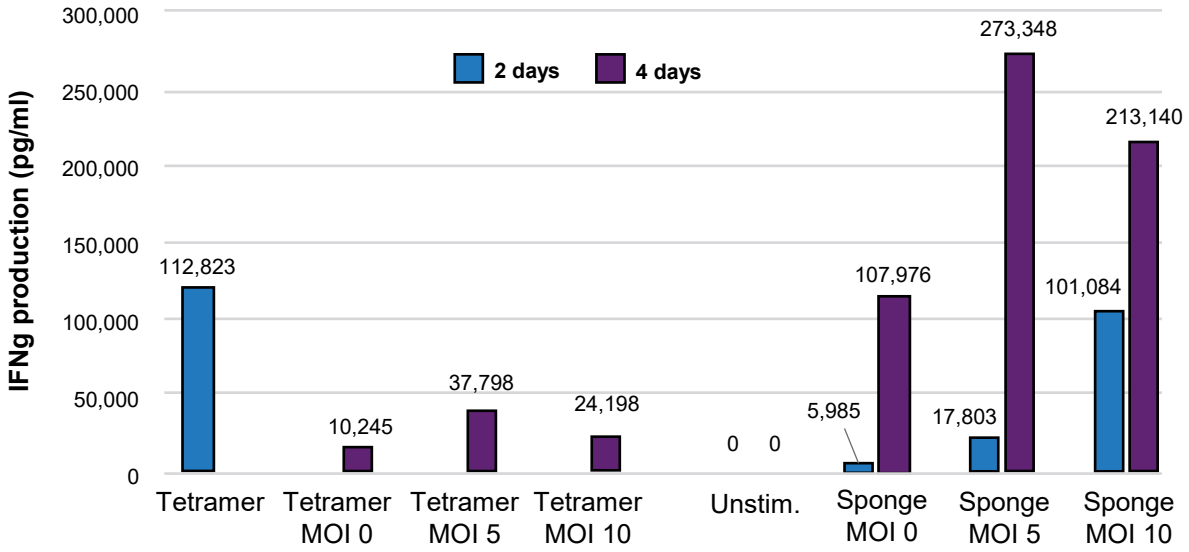
TaKaRa

Consistent activation and transduction using the T-cell sponge

T-cell sponge transduction efficiency is comparable or higher than classic anti-CD3/CD28 activation



Activation also confirmed by IFNg expression measured by IFNg GoStix Plus

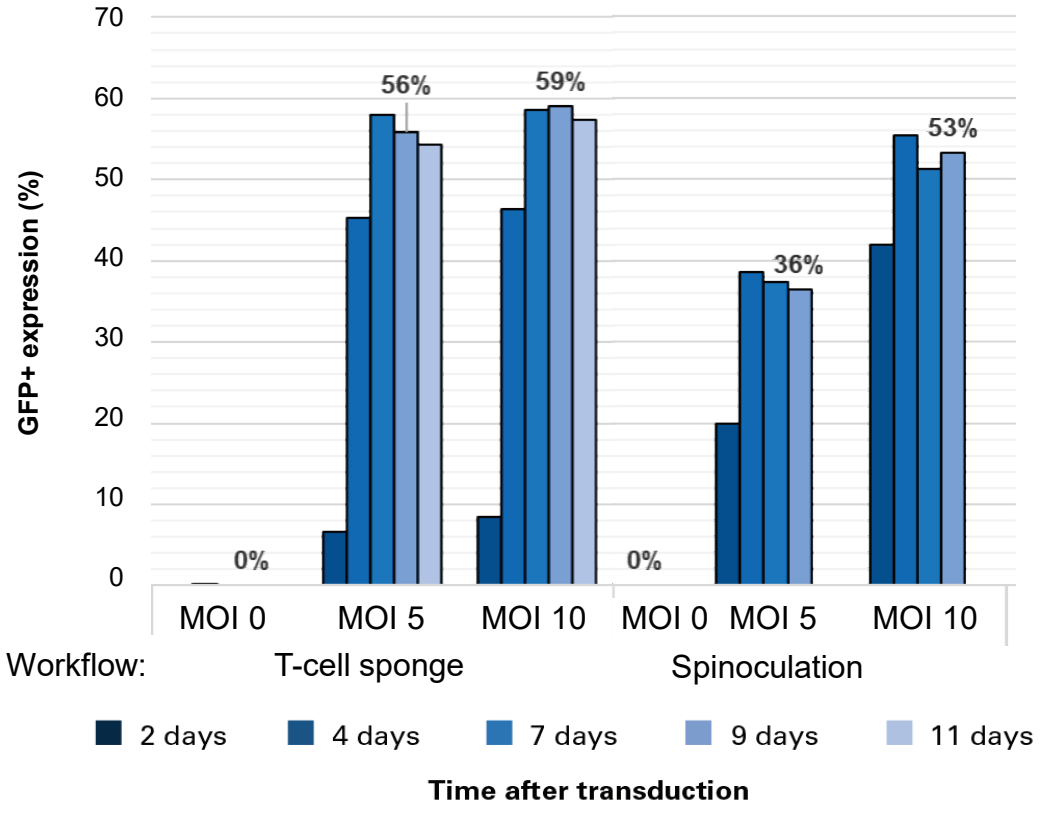


IFNg GoStix Plus

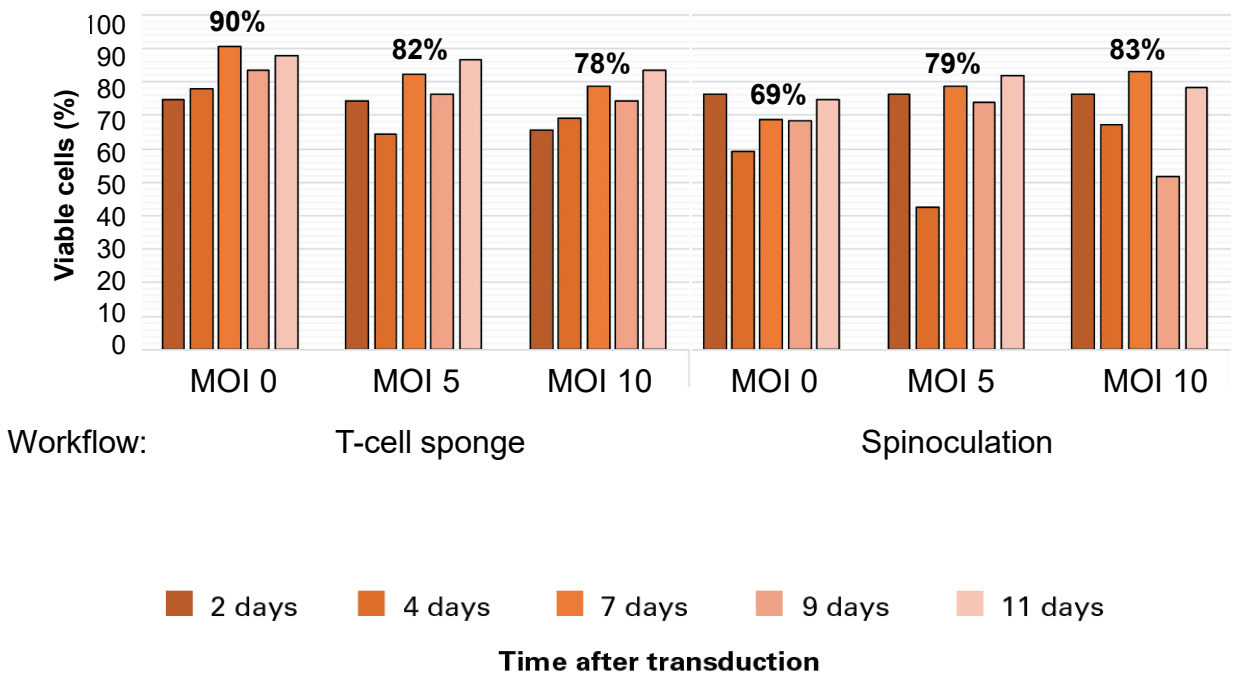
© 2025 Takara Bio Inc.

Consistent transduction efficiency and cell viability with the T-cell sponge

Transduction efficiency of human primary T cells

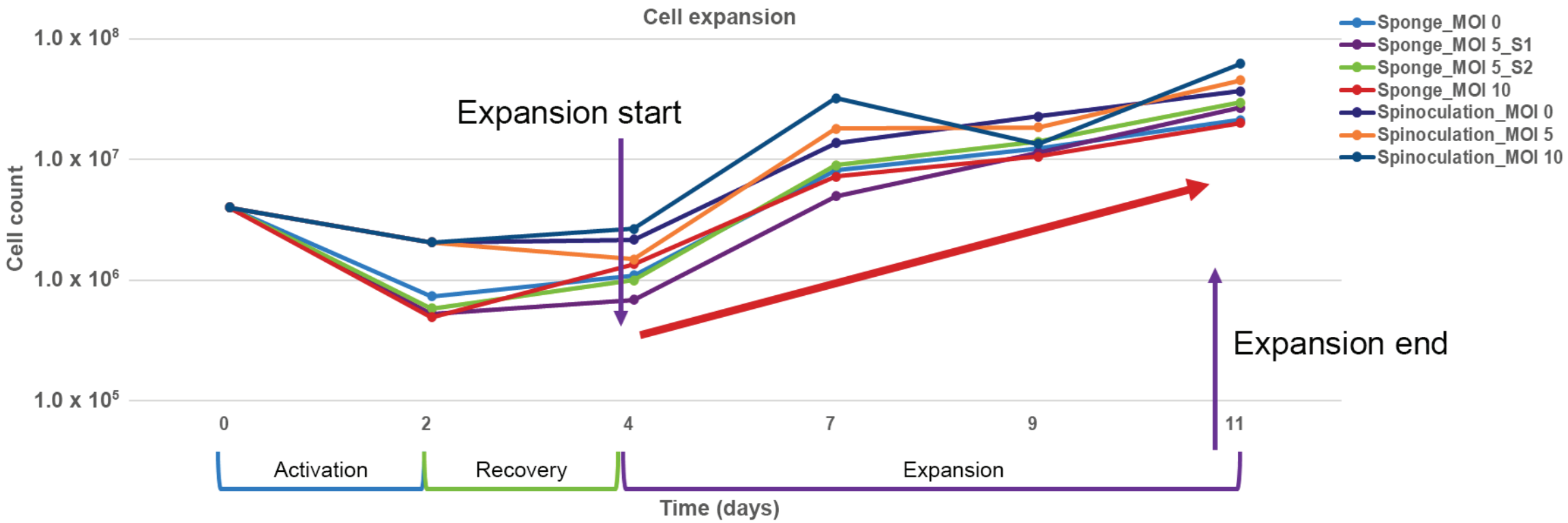


T-cell viability across a range of MOI values



© 2025 Takara Bio Inc.

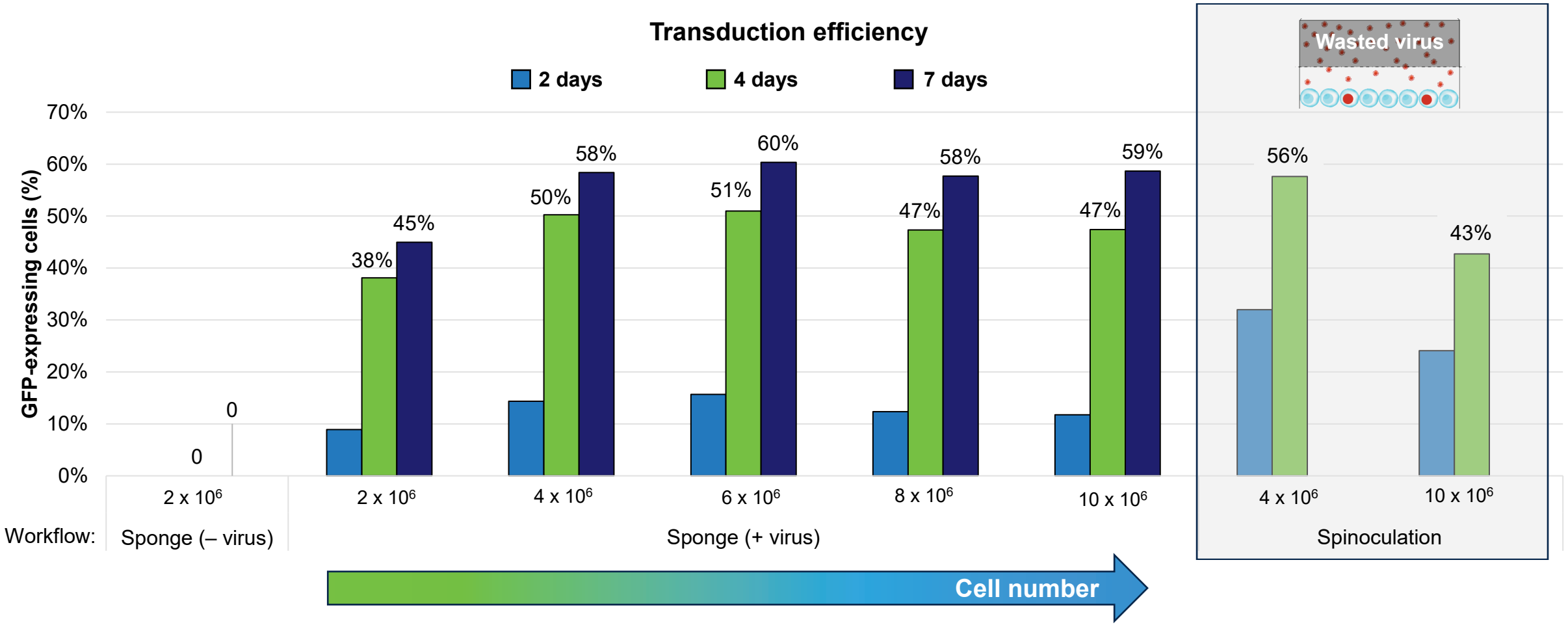
Sponge-transduced cells expand comparably to those transduced by spinoculation



Human primary T cells transduced with GFP-expressing lentivirus were analyzed at the indicated timepoints post-transduction

© 2025 Takara Bio Inc.

Efficient transduction across a range of cell numbers

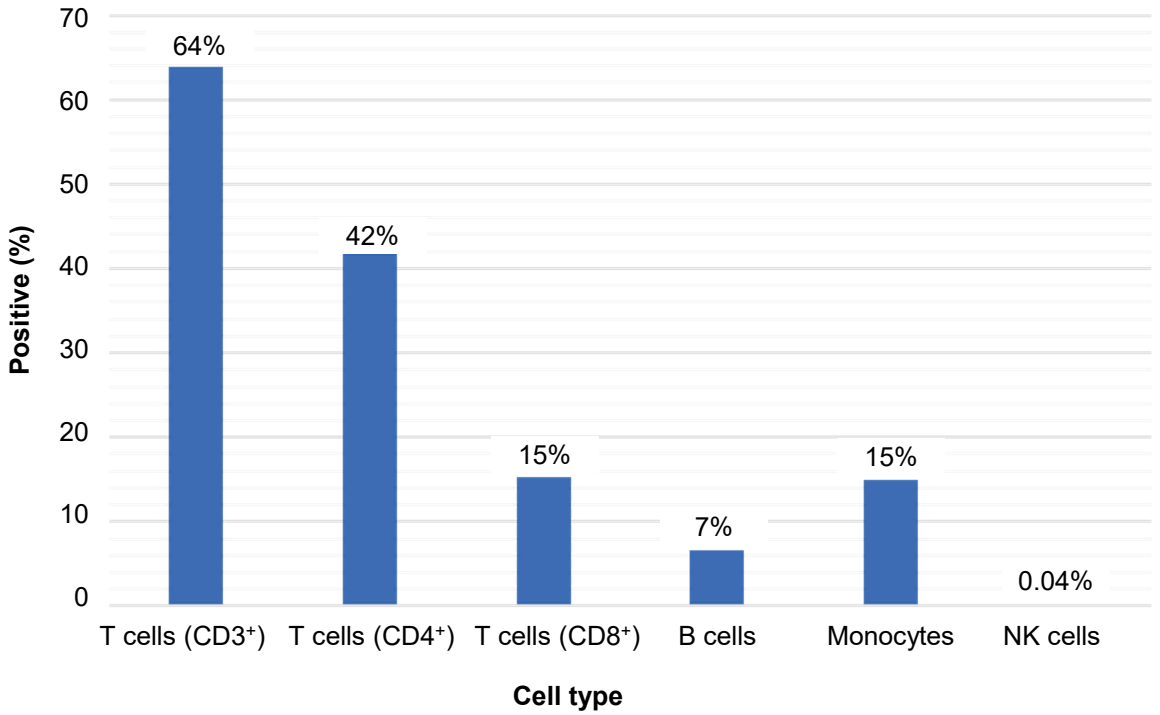


Test: Human primary CD3⁺ T cells
Virus: LV-GFP (MOI = 5)
Analysis: FACS at times indicated post-transduction

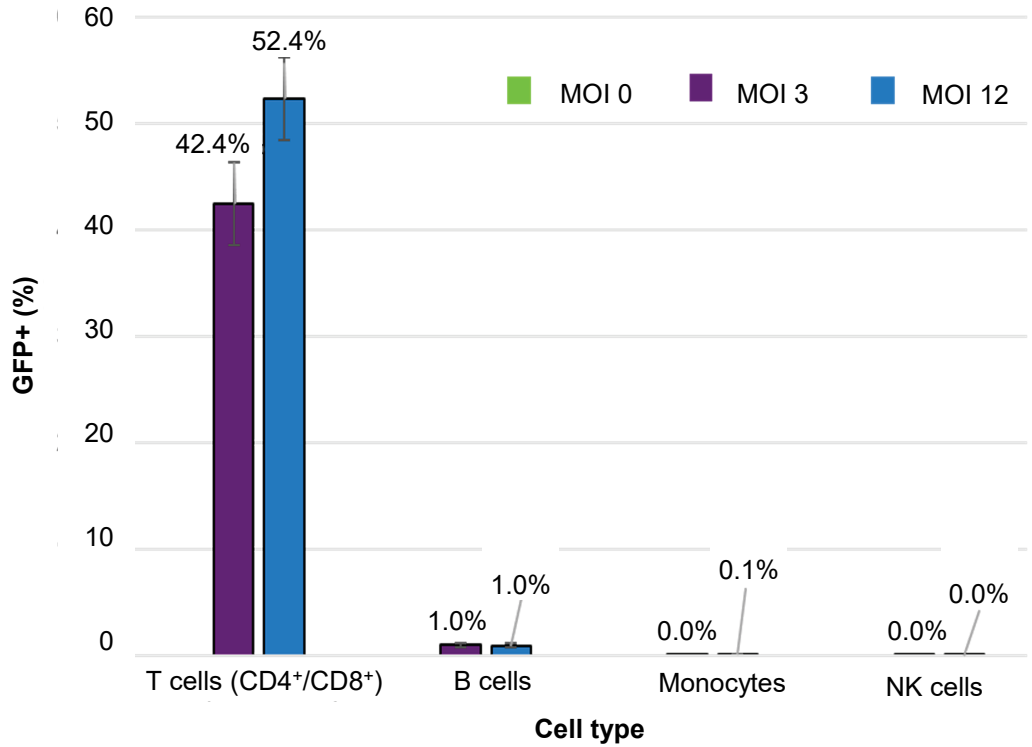
© 2025 Takara Bio Inc.

Save days in your workflow by using PBMCs as the starting material

PBMC phenotype before transduction



High T-cell transduction efficiency from PBMCs with the T-cell sponge



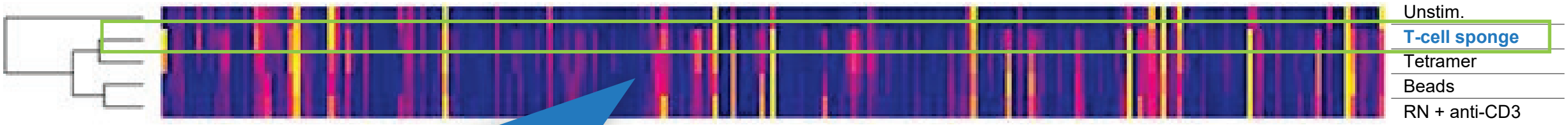
Selective activation and transduction of T cells in the PBMC population saves a half day of T-cell isolation and two days of activation prior to transduction

© 2025 Takara Bio Inc.

Transcriptomic analysis demonstrates minimal impact of the sponge on primary T cells using the T-cell sponge

No significant difference observed in the expression of the top 100 differentially expressed genes using different workflows 48 hr after transduction

Heat map and hierarchical clustering



Expression pattern of cells transduced using different workflows

Sponge transduction is innocuous

At nine days post-transduction, all samples had similar gene expression profiles (not shown)

© 2025 Takara Bio Inc.

Lenti-X T-Cell Transduction Sponge

- Gain microfluidics-driven transduction efficiency without an instrument or expensive consumables
- Achieve transduction efficiencies that are equal to or better than with current methods
- Minimize cell handling and hands-on time with a simplified workflow
- Activate and transduce T cells in a single step
- Facilitate transduction of T cells directly from PBMCs without prior T-cell isolation
- Maintain cell viability, phenotype, and yield
- Transduce a wide range of cell numbers (2×10^6–1×10^7 per sponge)

Learn more at: takarabio.com/lenti-x

© 2025 Takara Bio Inc.

TaKaRa

that's
GOOD
science!®