

# Optimization of Human T Cell Activation and Expansion Protocols Improves Efficiency of Genetic Modification and Overall Cell Yield

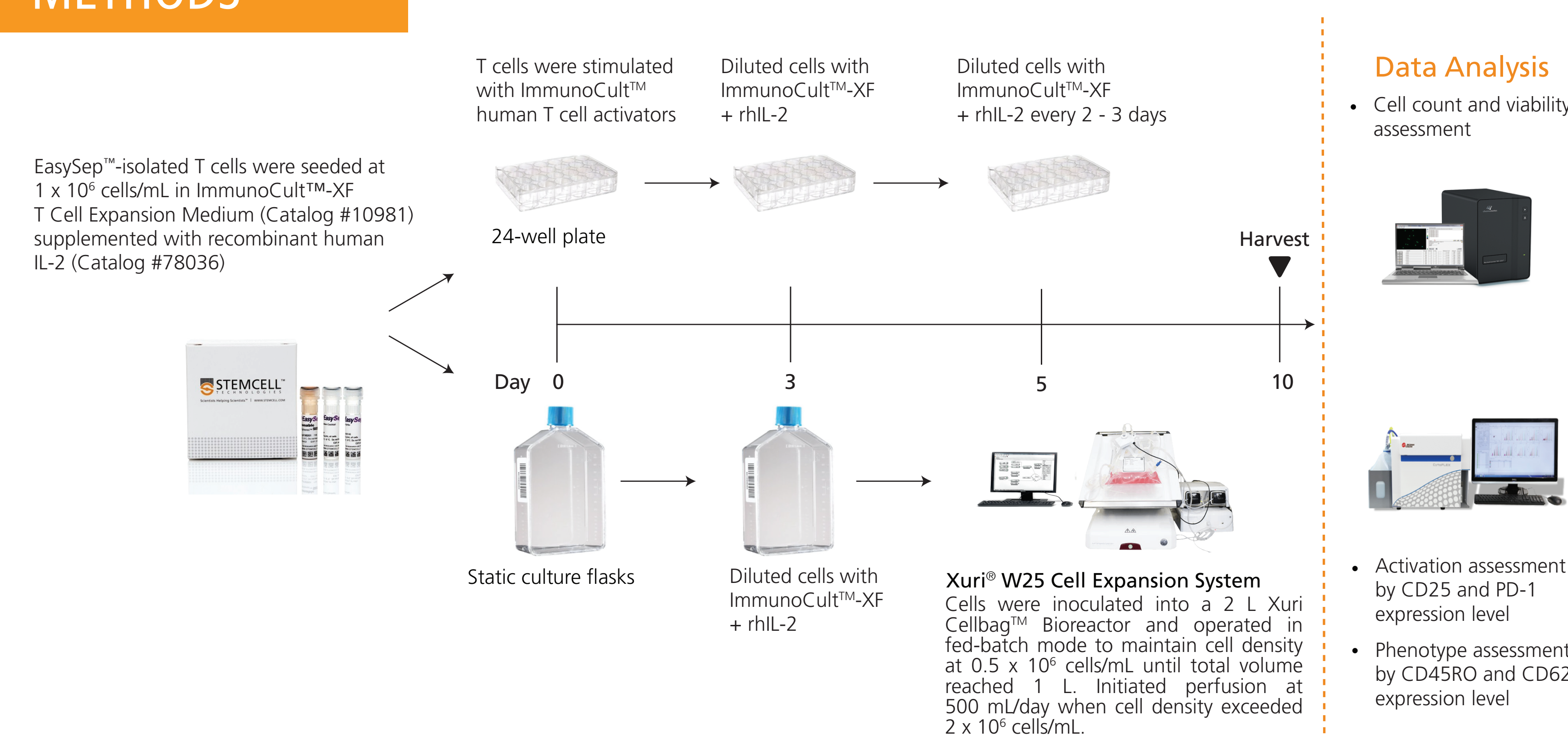
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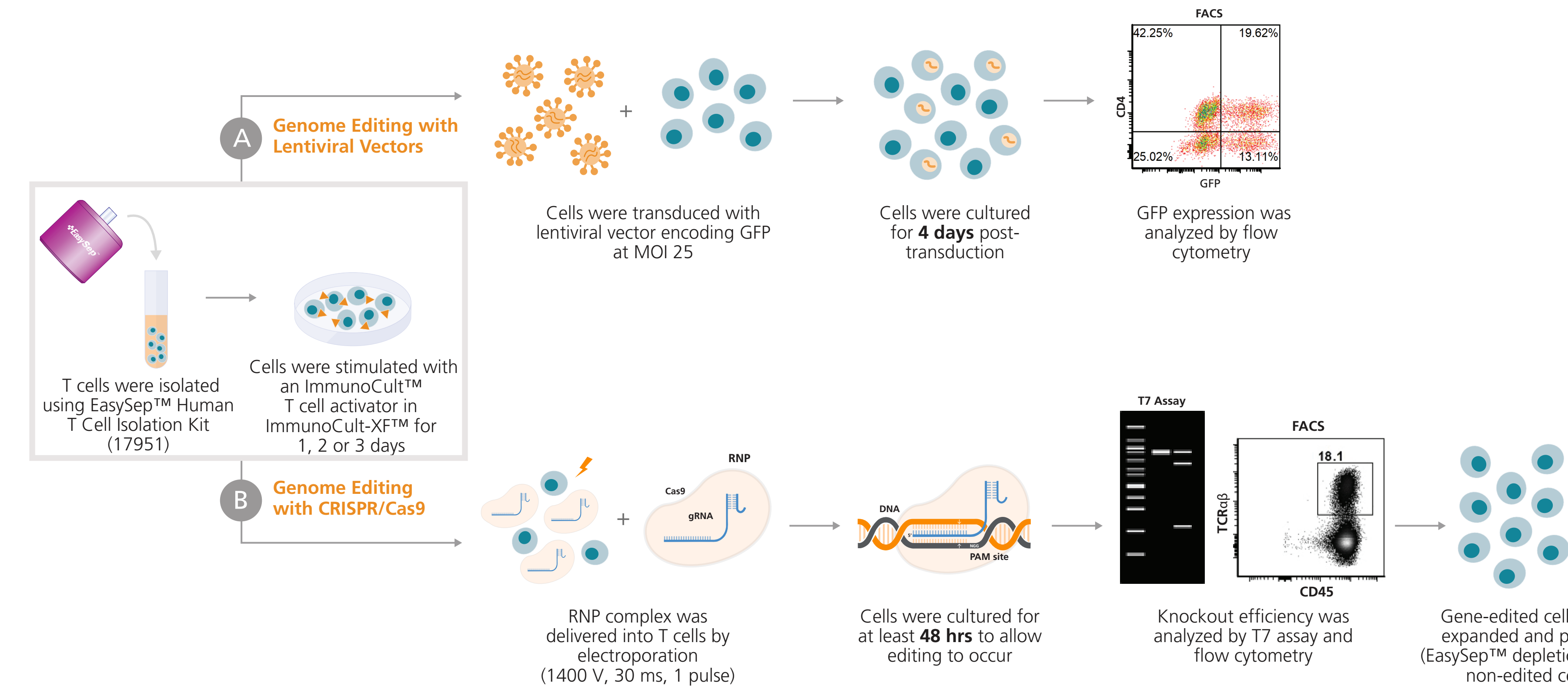
## ABSTRACT

Cancer immunotherapy using chimeric antigen receptor (CAR) T cells is a rapidly progressing field, and manufacturing these cells is a complex process that requires multiple optimization steps. We have developed reagents for the isolation, activation, and expansion of human T cells that will be available for clinical cell therapy manufacturing. Soluble ImmunoCult™ Human T Cell Activators induce T cell activation via cross-linking CD3 and co-stimulatory molecules on the surface of cells. Activated T cells then can be genetically modified and subsequently expanded in serum- and xeno-free ImmunoCult™-XF T Cell Expansion Medium. Here, we present several optimization strategies with ImmunoCult™ products in order to obtain high transfection efficiency and maximum cell yield. By evaluating activation dynamics of T cells and determining the optimal transfection time points, the transfection efficiency can be substantially improved in both CRISPR/Cas9- and lentiviral-mediated gene modification methods. Our study also suggests that diluting T cells to a lower cell density after the third day following activation greatly improves cell viability and cumulative cell growth, resulting in a more than 1000-fold expansion of total human T cells with > 85% viability over 10 - 12 days of culture. Expanded T cells co-express CD45RO<sup>+</sup>CD62L<sup>+</sup>. As an example, we applied the workflow described here to generate T cell receptor alpha beta (TCRαβ) knockout (KO) T cells from healthy donors with up to 90% knockout efficiency. The purity of TCRαβ KO cells can be further increased with the use of an EasySep™ Human TCRαβ depletion kit. Taken together, the processes outlined in this study can be easily and rapidly implemented to improve T cell manufacturing efficacy.

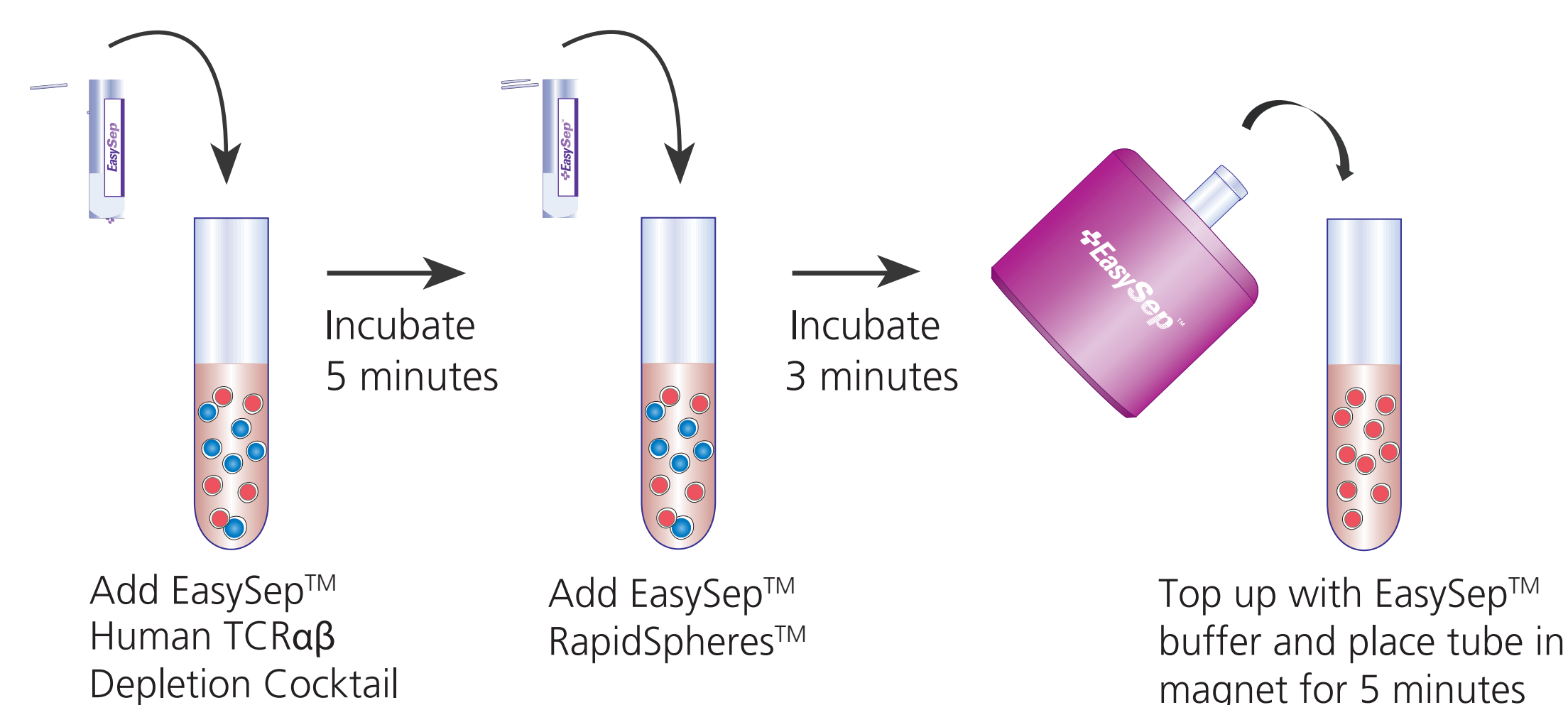
## METHODS



**FIGURE 1. A General Workflow of Human Primary T Cell Isolation, Activation, and Expansion.** Purified T cells were seeded at 1 x 10<sup>6</sup> cells/mL and stimulated with ImmunoCult™ Human CD3/CD28/CD2 or CD3/CD28 T Cell Activator (Catalog #10970 or 10971, respectively). Fresh culture medium was added to the cultures every 2 - 3 days until day 10 of culture. Cell counts and viability assessments were performed during the course of the expansion. Fold expansion during culture was analyzed relative to the initial cell seeding number.

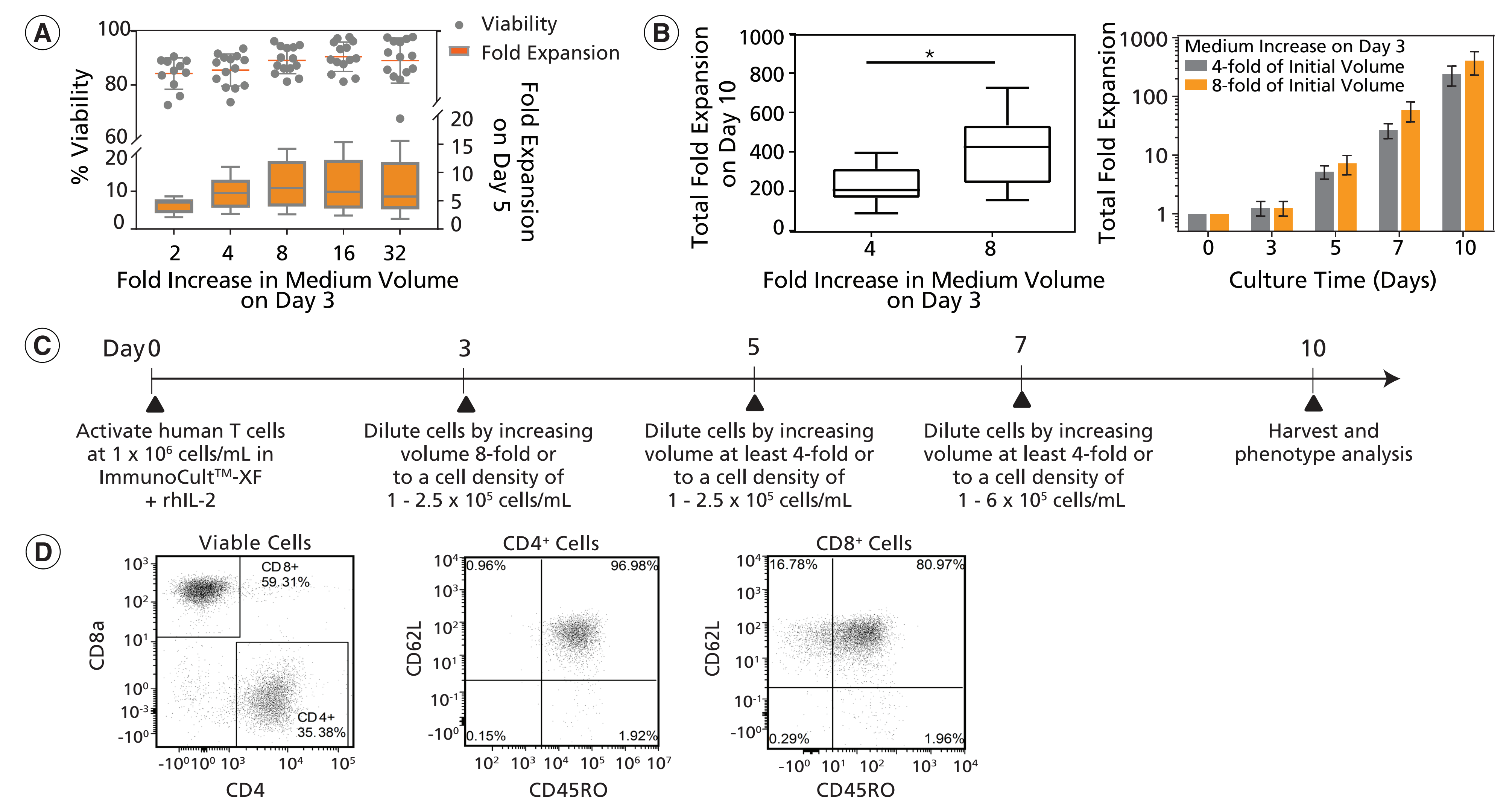


**FIGURE 2. Experimental Workflow of Human Primary T Cell Gene Editing.** Cryopreserved T cells were stimulated with T cell activation reagents for 1, 2, or 3 days in ImmunoCult™-XF T Cell Expansion Medium supplemented with recombinant human (rh) IL-2. **(A)** Activated cells were transduced with lentiviral vectors encoding GFP or **(B)** The ArciTect™ CRISPR-Cas9 RNP complex was delivered into activated T cells to knock out the TCR alpha constant (TRAC) locus.

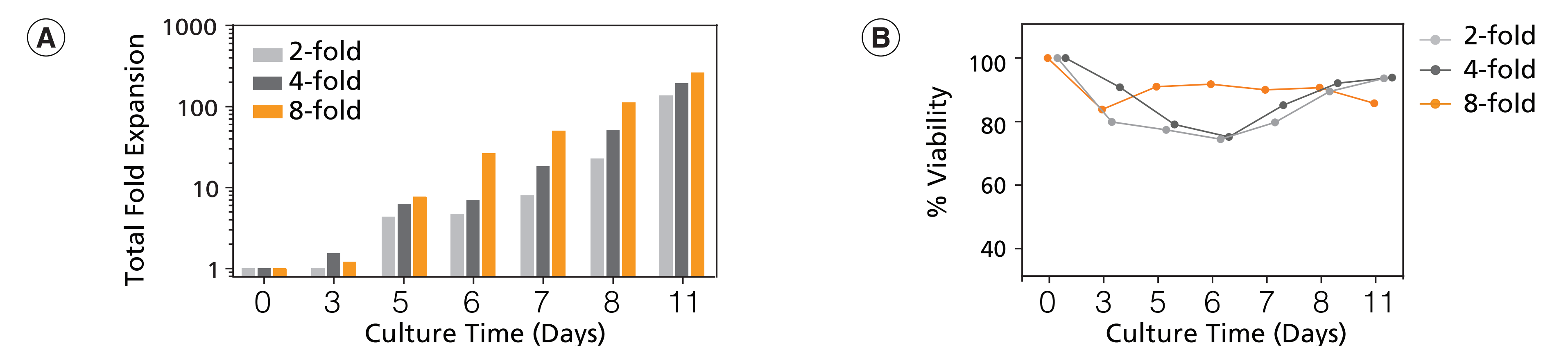


**FIGURE 3. EasySep™ Human TCRαβ Depletion Protocol.** Gene-edited TCRαβ KO T cells were expanded for 7 - 10 days, harvested, and resuspended in EasySep™ Buffer at 5 x 10<sup>7</sup> cells/mL. Following a 13-minute EasySep™ TCRαβ depletion protocol, flow cytometry was performed to assess the depletion of TCRαβ<sup>+</sup> cells.

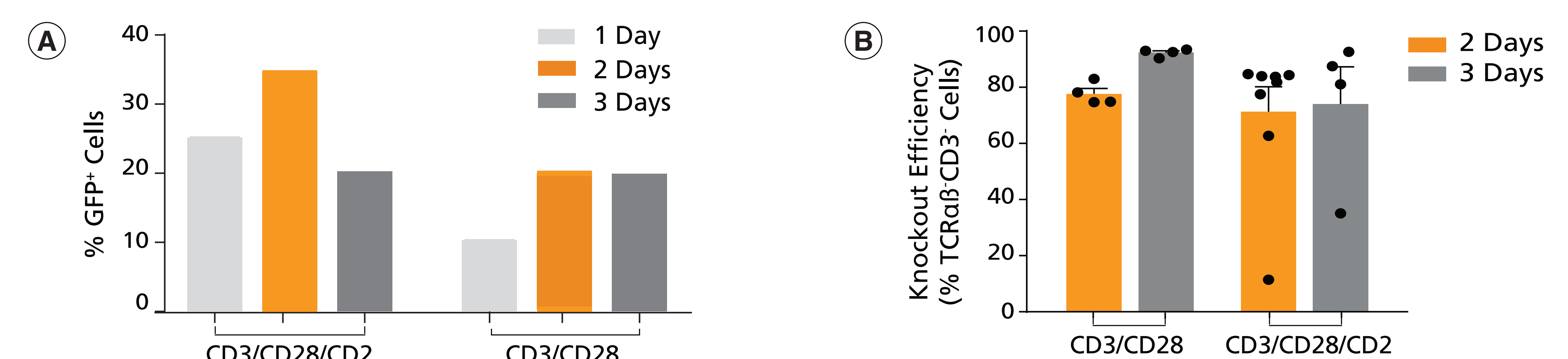
## RESULTS



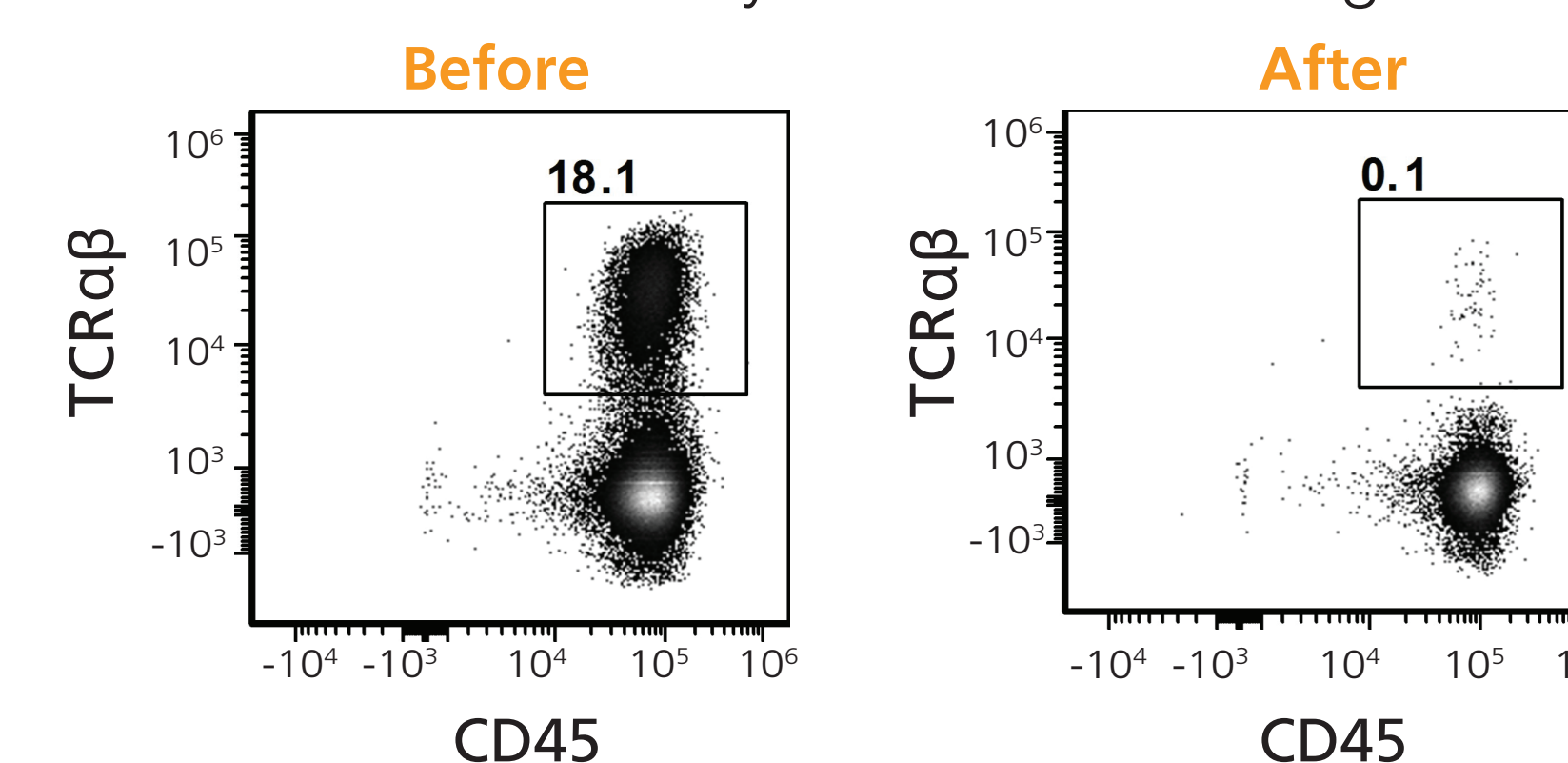
**FIGURE 4. Diluting T Cells To A Lower Cell Density Three Days Following Initial T Cell Activation Improved T Cell Growth and Viability.** T cells were stimulated on day 0 with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator. **(A)** An 8-fold volume increase on day 3 resulted in better viability and a higher fold expansion at day 5. **(B)** Increasing the culture volume by 8-fold on day 3 followed by a further volume increase by 4-fold on days 5 and 7 until day 10 of culture resulted in an overall 405 ± 174 total fold expansion (mean ± SD, n = 14). **(C)** The recommended human T cell expansion protocol with ImmunoCult™ reagents in static culture. **(D)** Representative FACS plots of expanded T cells using ImmunoCult™ products after 10 days of culture.



**FIGURE 5. Large-scale T Cell Expansion in the Xuri™ W25 Cell Expansion System Benefited from Early Cell Dilution for T Cell Expansion.** T cells were stimulated on day 0 with ImmunoCult™ Human CD3/CD28 T Cell Activator in culture flasks. On day 3 of culture, fresh medium was added to increase the total culture volume by 2-, 4-, and 8-fold. **(A)** The highest fold expansion (263) was observed with the 8-fold volume increase on day 3 following 11 days of culture as outlined in Figure 1. **(B)** Viability was similar across the different cell dilutions.



**FIGURE 6. ImmunoCult™ Activation Kinetics for Optimal Lentiviral Transduction and CRISPR/Cas9-Mediated TRAC Knockout of Human T Cells.** **(A)** Activation with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator for 2 days resulted in the highest percentage of GFP-positive cells. **(B)** Activation with ImmunoCult™ Human CD3/CD28 T Cell Activator for 3 days resulted in the highest TRAC knockout efficiency.



**FIGURE 7. Residual TCRαβ<sup>+</sup> Cells can be Removed From Expanded TRAC KO Cell Populations Using EasySep™ Human TCRαβ Depletion Kit.** Representative data plots are shown for the frequency of TCRαβ cells in the expanded TRAC KO cells before and after EasySep™ depletion.

## Summary

- An optimized T cell expansion protocol using ImmunoCult™ reagents has been developed for robust growth of viable T cells
- Expanded T cells using ImmunoCult™ reagents have a less-differentiated phenotype (CD45RO<sup>+</sup>CD62L<sup>+</sup>)
- ImmunoCult™-activated human T cells can be genetically modified with high editing efficiency
- A combination of ImmunoCult™, ArciTect™ CRISPR-Cas9 system, and an EasySep™ cell isolation/depletion strategy provides a complete workflow for the production of TCRαβ KO cells from leukapheresis samples