

SoluPrime: Priming Cells for Higher Knock-In Efficiency with Electroporation Delivery

Introduction

Non-viral methods for introducing genetic material into primary human T cells are becoming increasingly popular, particularly in the context of advanced cell engineering applications. Despite its widespread use, methods like electroporation used in achieving DNA knock-in, in primary human T cells, still face challenges. T cells are highly sensitive, and the intense electrical pulses required for DNA delivery often cause significant toxicity, reducing cell viability and performance. Even with optimized conditions, DNA uptake and integration rates are typically low, making it difficult to produce sufficient edited cells. These challenges highlight the need for solutions that improve the efficiency of electroporation while preserving cell health—critical for scalable, reliable T cell workflows.

Key Benefits of SoluPrime

	Higher Knock-In Efficiency Improved integrated knock-in for more efficient genome editing results		Increased Yield Greater yield of edited T cells while maintaining viability
	Ease of Use Simple protocol that integrates seamlessly into electroporation processes		Workflow Compatibility Incorporates into standard T cell engineering processes
	Scalable Solution Supports advanced immunotherapy applications		GMP Ready DMF Filed with FDA.

Methods

In the study described, we set out to show that SoluPrime could enhance the knock-in efficiency of ssDNA and nanoplasmid constructs, expressing EGFP and CD19 CAR, respectively, using electroporation. The HDR donor template is targeted for insertion into the TRAC locus and expression of the constructs are placed under the endogenous TCR promoter.

After 3 days of activation (TransAct; Miltenyi), 10e6 T cells, from 5 different healthy donors, were transferred to a T25 flask. SoluPrime was added by pipette, gradually, whilst swirling the flask, so that the final concentration was 5% v/v. The cells were returned to the incubator and left for 30min prior to preparation for electroporation.

2e6 cells (with and without SoluPrime pretreatment) were centrifuged at 350 RCF for 5min, the media was removed and 200µL PBS added. The cells were centrifuged at 200 RCF for 5min and the supernatant removed. The cells were resuspended in P3 buffer containing the cargo (Cas9 RNP (TRAC; IDT) + ssDNA_TRAC_EGFP_CTS (Genscript) or nanoplasmid CD19 CAR (Aldevron)) and electroporated using pulse code EO115. Electroporation was carried out on the Nucleofector 4D (Lonza) using the Primary Cell 4D-Nucleofector® X Kit. Cells were immediately transferred to warm media and 1e6 cells were added to a well of 24-well GRex plate (Wilson-Wolf). Cells were allowed to incubate for 7 days in culture before being assessed for integrated EGFP or CD19 CAR expression by flow cytometry.

Results

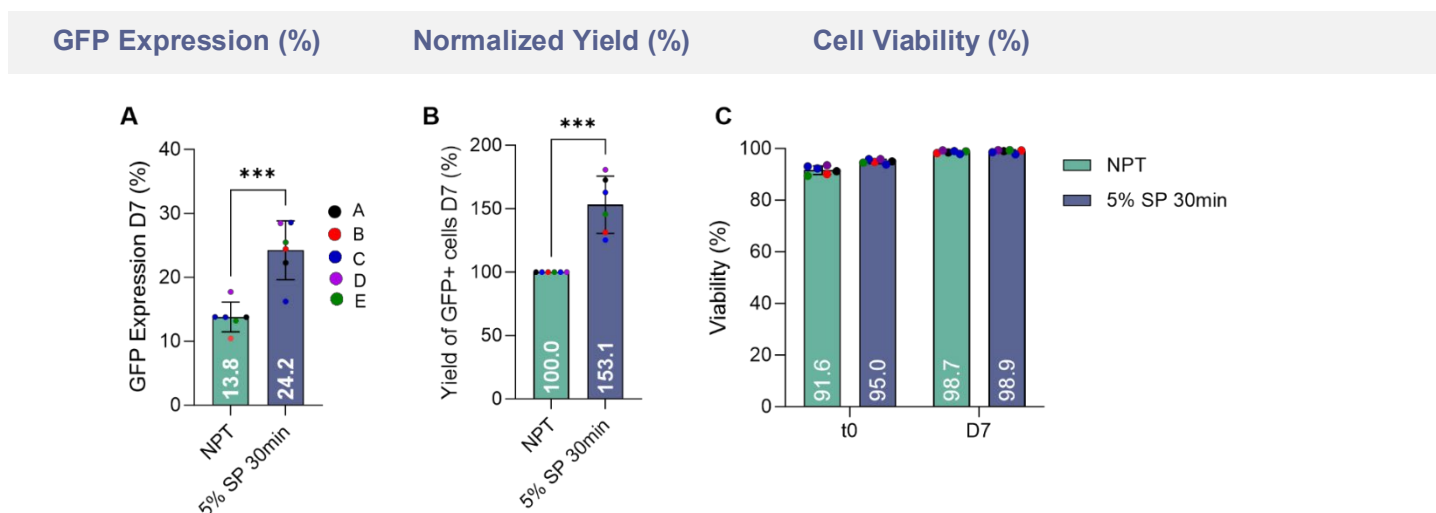


Figure 1 Activated T cells (72hr; 5 donors) were pretreated with SoluPrime (SP; 5% v/v) for 30min prior to electroporation. The knock-in cargo (Cas9 RNP (TRAC; 36pmol) + ssDNA_TRAC_EGFP_CTS (8pmol)) was delivered using pulse code EO115. Cells were cultured for 7 days post-electroporation. EGFP expression (A) was assessed by flow cytometry. Yield of EGFP positive cells (B; normalised to NPT) and viability (C) immediately post-electroporation (t0) and at 7 days (D7) is also shown. Data is depicted as the mean ± SD from 5 donors (2-4 technical replicates per donor). NPT = No Pretreatment. **p<0.01; ***p<0.001, ttest.

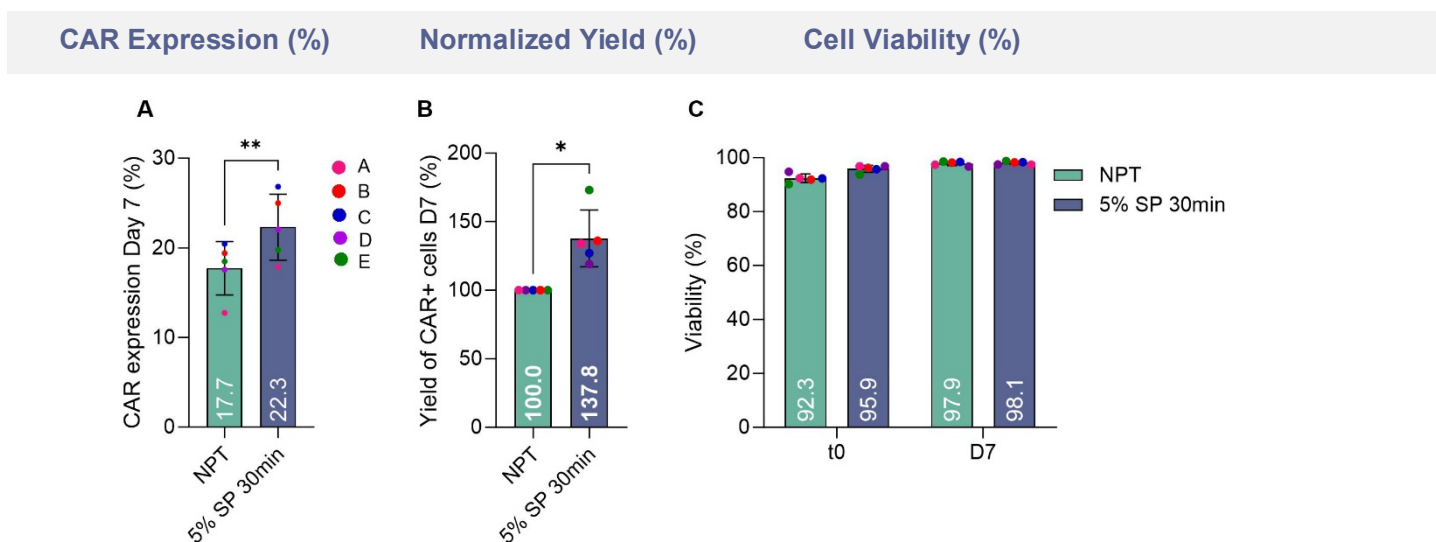


Figure 2 Activated T cells (72hr; 5 donors) were pretreated with SoluPrime (SP; 5% v/v) for 30min prior to electroporation. The knock-in cargo (Cas9 RNP (TRAC; 36pmol) + npCD19 CAR (8pmol)) was delivered using pulse code EO115. Cells were cultured for 7 days post-electroporation. CAR expression (A) was assessed by flow cytometry. Yield of CAR positive cells (B; normalised to NPT) and viability (C) immediately post-electroporation (t0) and at 7 days (D7) is also shown. Data is depicted as the mean ± SD from 5 donors (2 technical replicates per donor). NPT = No Pretreatment. *p<0.05; **p<0.01, ttest.

SoluPrime pretreatment (5% for 30min) increased the knock-in of both ssDNA GFP and nanoplasmid CD19 CAR, by 75% and 26% respectively, compared with the no pretreatment controls (NPT) when assessed 7 days post-electroporation. The yield of GFP- and CAR-expressing cells were also increased after 7 days in culture (53% and 38%, respectively). The expansion of the cells was unaffected by SoluPrime treatment. The viability of pretreated cells was unchanged from those that were untreated, either immediately post-electroporation or after 7 days of culture.

Conclusion

SoluPrime addresses the challenge of increasing knock-in editing of T cells. Its ease of use and seamless integration into existing T cell workflows eliminates the need for complex protocol changes, enabling higher knock-in rates and yields without compromising functionality. SoluPrime provides a practical, scalable solution for reliable genome editing in advanced therapy applications.