Establishment and Quality Evaluation of Human iPS Cells Using an Improved Stealth RNA Vector

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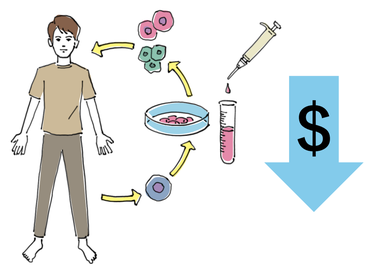
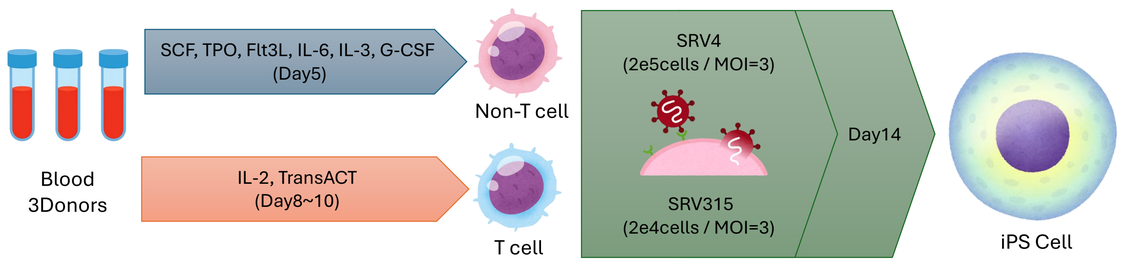
Introduction

To achieve cost reduction in autologous iPSCs generation within “my iPS®” cell project, we evaluated a high-efficiency and rapid manufacturing process using a newly developed Stealth RNA Vector (SRV).



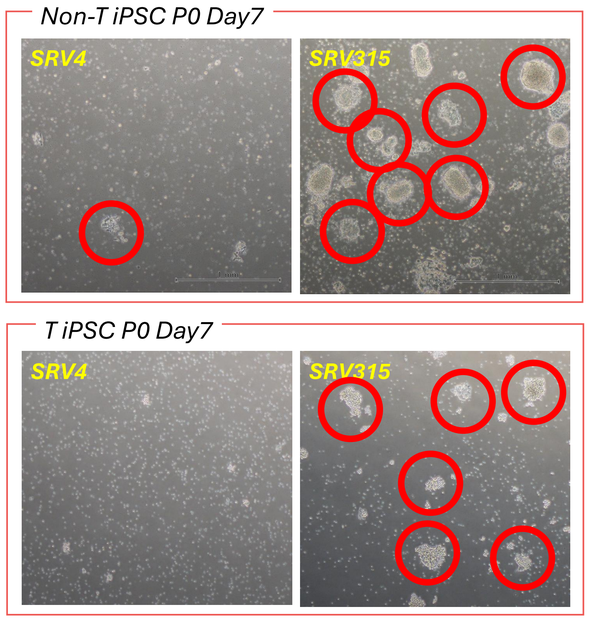
Materials & methods

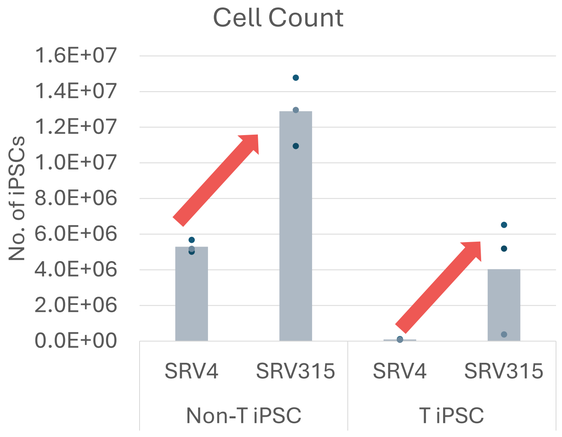
We developed a novel SRV vector, SRV315, and evaluated its efficiency and safety compared to the conventional SRV™ iPSC-4 Vector (SRV4). Unlike SRV 4, SRV 315 specifically reacts to the reagent A.



Result 1

SRV315 achieved significantly higher reprogramming efficiency and shortened the time to colony formation compared with SRV4.

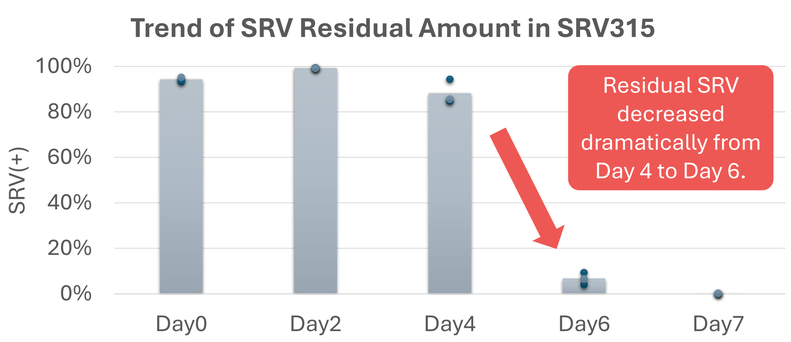




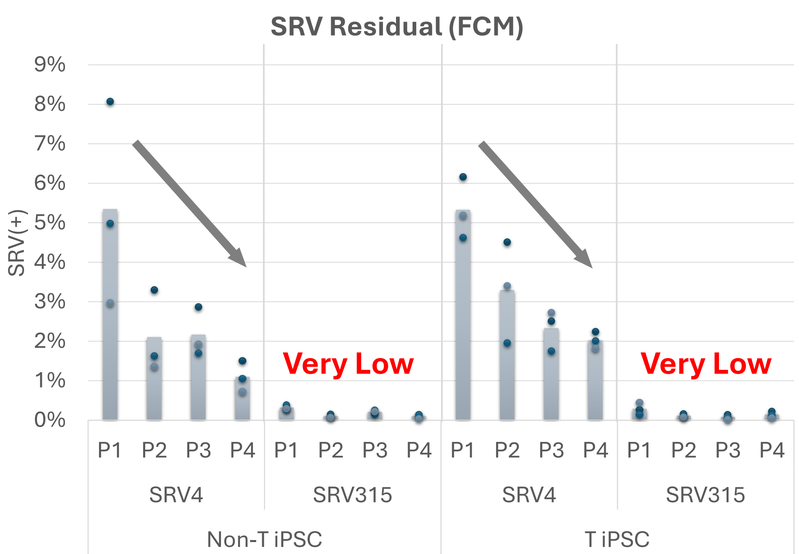
Non-T cells (2.0+E05) and T cells (2.0+E04) were infected with SRV at MOI = 3.
↑ The graphs show the number of recovered iPSCs at Day 14, with SRV315 yielding higher cell numbers compared to SRV4.
← The photos show the morphology at Day 7 post-induction, where SRV315 forms colonies more rapidly than SRV4.

Result 2

SRV315 was rapidly removed by adding Reagent A after reprogramming.



SRV315 sensitivity to Reagent A was confirmed by flow cytometry (FCM) . Cells 9 days post-infection were cultured with Reagent A, changed every other day. SRV levels dropped below 0.2% by Day 7 after Reagent A addition.

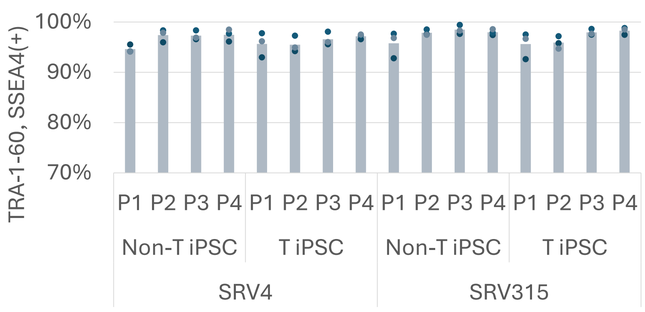


Cells at Day 14 post-SRV infection were tested by FCM.
Only Passage 1 (P1) was cultured with Reagent A, changed every other day; later passages were without Reagent A. Passages occurred every 7 days, and SRV levels were measured at each passage. SRV4 was naturally eliminated, with no difference in persistence between non-T and T iPSCs.

Result 3

The quality of the iPSC produced using SRV4 and SRV315 was comparable.

◆Pluripotency marker expression



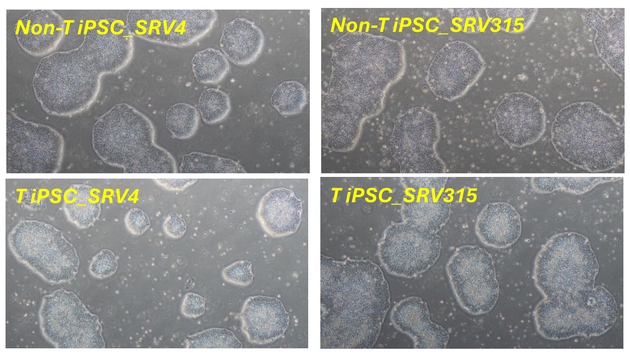
The undifferentiated potential of cells at Day 7 post-establishment for each condition (P1–P4) was measured by FCM. High undifferentiated efficiency was demonstrated under all conditions.

◆Karyotype stability

Donor	SRV4		SRV315	
	Non-T iPSC	T iPSC	Non-T iPSC	T iPSC
A	46,XX	46,XX	46,XX	46,XX
B	46,XY	46,XY	46,XY	46,XY
C	46,XY,+1,der(1;4)(q10;q10)[4] 46,XY[16]	46,XY	46,XY	46,XY

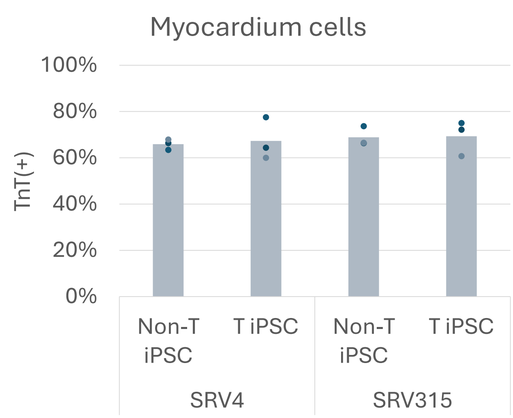
Results of G-banding analysis on P3 post-establishment were presented. A karyotype abnormality was confirmed in one sample.

◆Morphological evaluation



The cell morphology at P4 day 7 is shown.
In all samples, no visible morphological differences were observed from P1 onwards.

◆Differentiation potential



Following establishment, the mean values for differentiation induction at P1–4 with N=2 are shown. A similar induction efficiency was obtained under all cell conditions.

Discussion

◆Conclusion

	SRV315 achieves superior reprogramming performance through optimized SRV genome design and a robust manufacturing system.
	SRV315 provides a **high-efficiency, non-integrating tool** for rapid human iPSC generation.
	SRV315 is promising for **regenerative medicine and disease modeling**.
	SRV315 enables **cost-effective, automated production** of autologous iPSCs using closed systems, because SRV clears rapidly.

◆Future Directions

	Implement closed-system platforms for large-scale, automated iPSC production.
	Expand use in regenerative medicine, cell therapy, and disease modeling.
	Develop protocols for diverse starting materials such as blood and immune cells.
	Reduce manufacturing costs and improve accessibility through GMP-compliant processes.

Acknowledgements

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