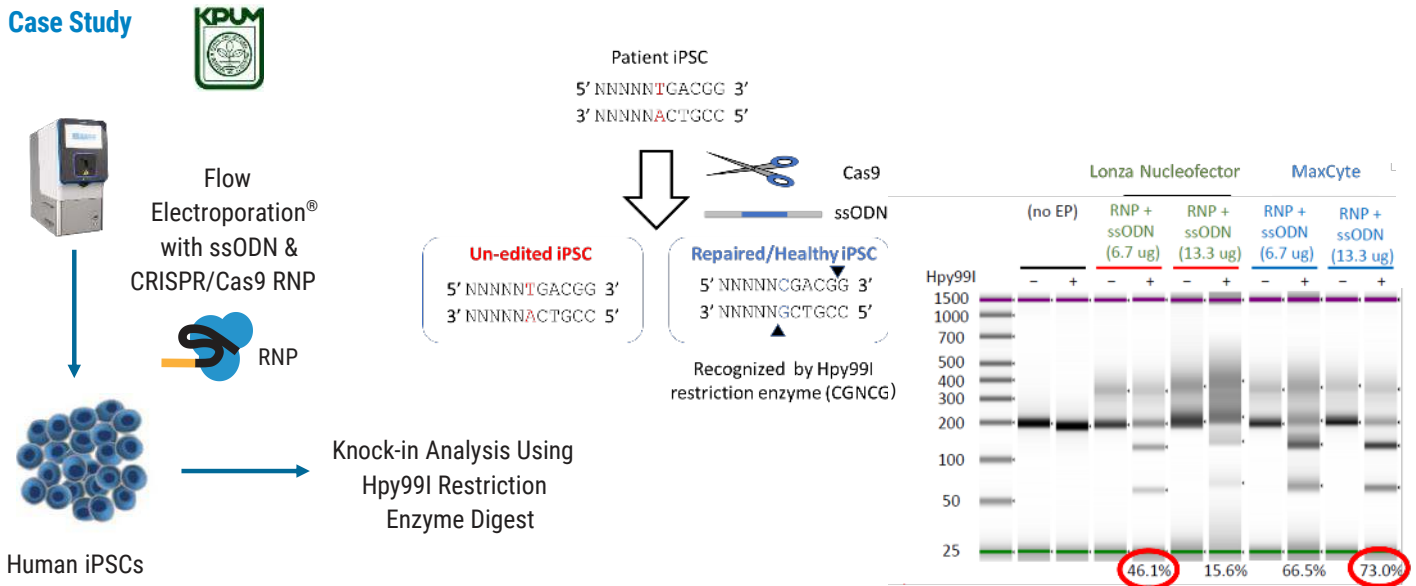


Abstract – Advancing Adoptive Cellular Therapies Using Non-viral-based Engineering

Excitement over recent promising breakthroughs in autologous cellular therapies has been tempered by the manufacturing costs associated with viral-based gene delivery methods and by safety concerns associated with random integration of viral vectors. As an alternative to viral-based gene delivery, we describe the use of the clinically validated and scalable MaxCyte Flow Electroporation® Technology for delivering ribonucleoprotein (RNP) and single-stranded oligonucleotide donor (ssODN) to induced pluripotent stem cells (iPSCs). Through a restriction fragment length polymorphism (RFLP) assay we show highly efficient gene editing following co-transfection of CRISPR/Cas9 and donor construct.

Case Study



CRISPR-Cas9 Engineering of Patient-derived iPSCs by ssODN Knock-in for Efficient and Scarless Genome Editing.

In order to generate an isogenic control for drug screening purposes, patient-derived induced pluripotent stem cells (iPSCs) were electroporated by MaxCyte or Lonza Nucleofector with CRISPR-Cas9 RNP and 100-bp ssODN to repair a premature stop codon from TGA->CGA. iPSCs were harvested three days after electroporation, genomic DNA was extracted, and the genomic region of interest was amplified by PCR. Successful knock-in was evaluated by cleavage of the PCR amplicon by Hpy99I restriction enzyme digestion. *Data from collaboration with CiRA (U. Kyoto).*

Summary

- Non-viral engineering enables rapid development of next-generation therapies with simplified, more cost-effective manufacturing.
- The high efficiency and low toxicity of MaxCyte Flow Electroporation® provides for high knock-in frequencies.
- MaxCyte Flow Electroporation Technology (co)delivers a diversity of payloads including mRNA, sgRNA, RNPs, and plasmid & minicircle DNA providing flexibility for sophisticated, non-viral engineering including:
 - transient mRNA expression
 - nuclease-mediated gene editing (CRISPR, TALEN, ZFN)
 - transposon insertion (Sleeping Beauty, piggyBac)
- MaxCyte clinical scalability and regulatory compliance provide for streamlined clinical translation of new therapies.