Efficient KO of PD1 in primary T cells using a new non-integrative lentiviral particle expressing CRISPR/Cas9 system

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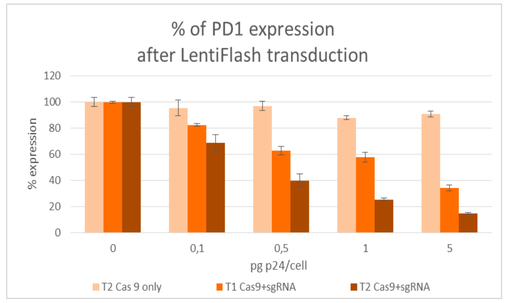
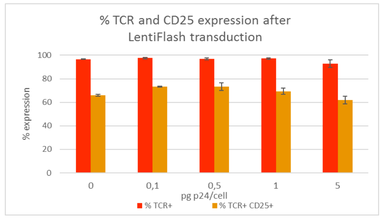
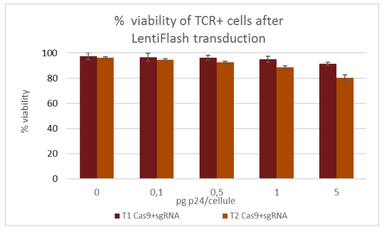
#458

A CRISPR approach using LentiFlash™ RNA delivery leads to a highly efficient and dose-dependent knock-out in activated human T lymphocytes, without genomic integration.

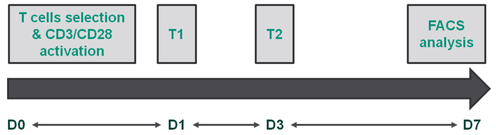
HIGHLY EFFICIENT DISRUPTION OF PD-1 IN HUMAN PRIMARY T-CELLS







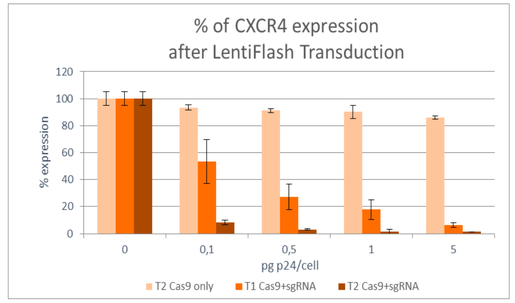
Materials & Methods

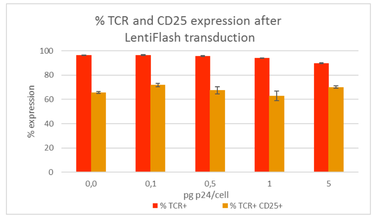


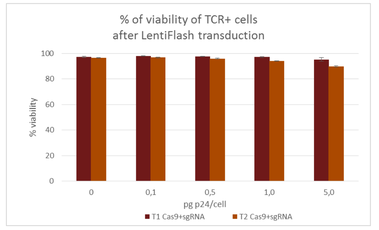
- ✓ Activated T cells were transduced by a range of LentiFlash vector (from 0 to 5 pg of p24/cell) expressing Cas9 and a sgRNA targeted eitheir the human PD-1 or CXCR4 gene.
- ✓ Transduction was performed once (T1) or twice (T2).
- ✓ Lentiflash™ delivering Cas9 is used as control of PD1 or CXCR4 editing.
- ✓ PD1 and CXCR4 expression is analyzed by FACS 4 days after the 2nd transduction, as well as their viability and expression of TCR and CD25.

Human T lymphocytes are efficiently transduced using highly purified and concentrated LentiFlash™ vectors without affecting viability and proliferation, and preserving the original cell phenotype.

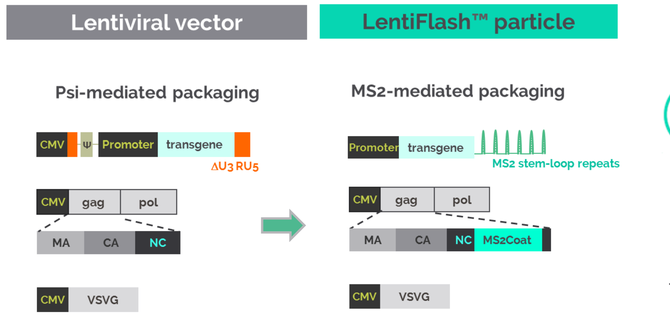
HIGHLY EFFICIENT DISRUPTION OF CXCR4 IN HUMAN PRIMARY T-CELLS

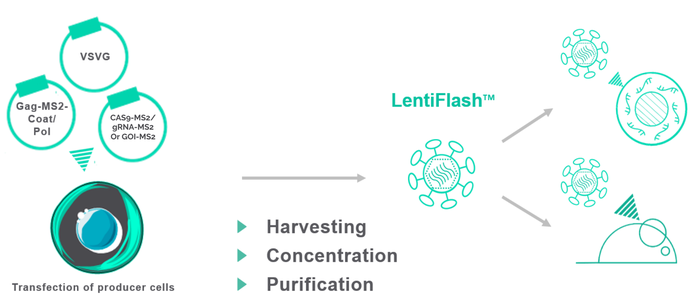






LENTIFLASH™: A NEW TOOL FOR TRANSIENT AND SAFE RNA DELIVERY



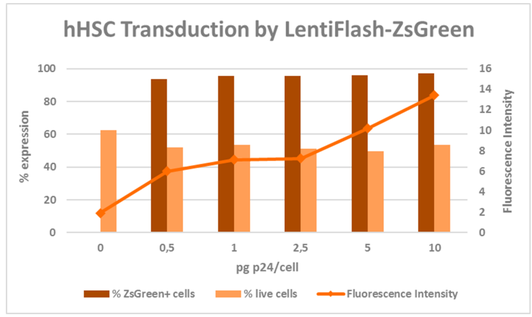


LentiFlash™ is a chimeric MS2-based lentiviral particle allowing to package different RNA molecules devoid of viral sequences, an important safety consideration for human use.

LentiFlash™ particles lead to safe RNA delivery through common properties with lentiviral vectors : same production platform & same transduction method

LENTIFLASH™ : A NEW TOOL FOR EFFICIENT hHSC TRANSDUCTION

- Using LentiFlash™, you can manage the dose effect, therefore the editing efficiency
- Transient expression of Cas9/gRNA or any transgene
- No delivered viral sequences
- No DNA integration
- No impact on viability nor cell phenotype



PoC: LentiFlash™ allows to achieve more than 90% of transduction efficiency after only one transduction of human Hematopoeitic Stem Cells, without changing cell viability, whatever the dose.