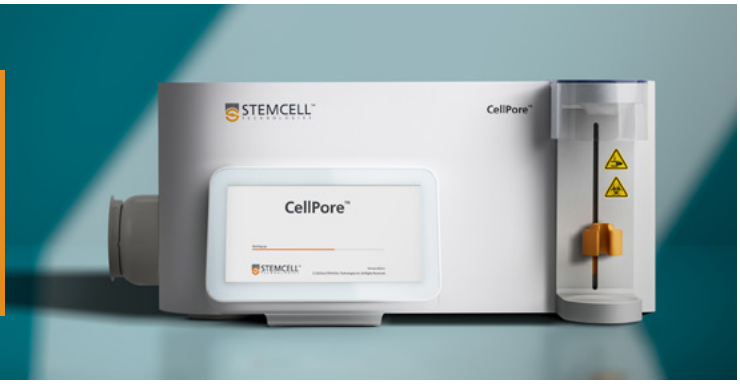
# EFFICIENT GENE EDITING OF CD34+ HSPCs

## With the CellPore™ Transfection System




CD34+ hematopoietic stem and progenitor cells (HSPCs) play a vital role in regenerating the hematopoietic system and offer immense potential for treating genetic blood disorders and immune diseases. Gene editing, especially using CRISPR-Cas9, opens the door to precise modifications. However, challenges like low editing efficiency, off-target effects, cell toxicity, poor delivery, and loss of stem cell properties can limit success.

The CellPore™ Transfection System addresses these challenges by enabling robust gene editing in primary human CD34+ HSPCs while preserving cell phenotype and function. This easy-to-use transfection system uses pressure to deliver a range of cargoes into hard-to-transfect cells with minimal cell perturbations. It enables superior viability and delivery compared to other transfection methods, requiring only minor protocol adjustments for seamless integration into your existing workflows.

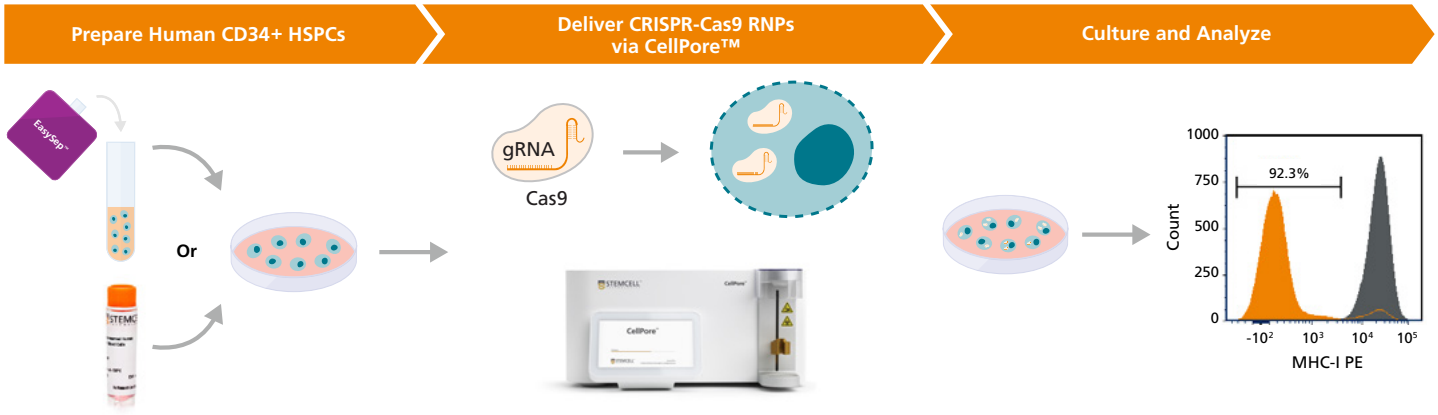



**Figure 1.** Experimental Workflow for Gene Editing of Human CD34+ HSPCs Using the CellPore™ Transfection System

Human CD34+ HSPCs are isolated from cord blood or mobilized peripheral blood using EasySep™ or sourced as cryopreserved cells. Initial culturing occurs for 4 - 24 hours in StemSpan™ SFEM II medium supplemented with CD34+ Expansion Supplement and optional addition of 1 µM UM729. CRISPR-Cas9 ribonucleoproteins (RNPs) are prepared by complexing Cas9 with sgRNAs targeting a specific gene. Cells are harvested and transfected with the CellPore™ Transfection System using CellPore™ Transfection Kit 300. Post-delivery, cells are returned to culture and editing efficiency is assessed via flow cytometry or T7 Endonuclease assays. Functionality of edited progenitors can be tested through in vitro CFU assays or lineage-specific differentiation using tailored StemSpan™ media and supplements.

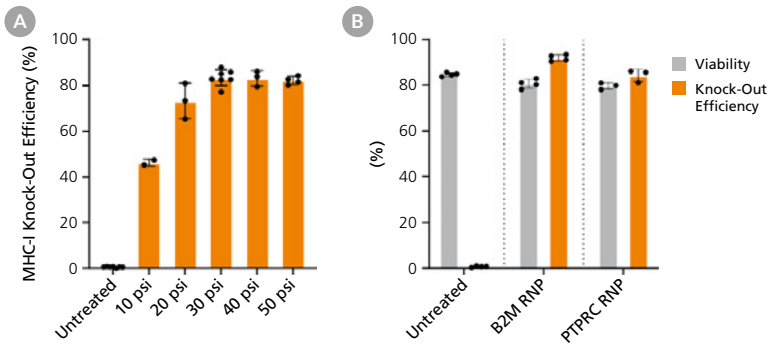



**Figure 2.** The CellPore™ Transfection System Enables Efficient Gene Knock-Out in CD34+ HSPCs

Cryopreserved cord blood-derived CD34+ HSPCs were thawed and cultured in StemSpan™ SFEM II medium supplemented with StemSpan™ CD34+ Expansion Supplement and 1 µM UM729 for 24 hours. 40 pmol of RNP complexes (Cas9:sgRNA 1:2.5) targeting the *B2M* or *PTPRC* gene were delivered to 5 x $10^4$ HSPCs in 80 µL reactions using the CellPore™ Transfection System and CellPore™ Transfection Kit 300. Flow cytometry was used to assess viability and *B2M* (surface MHC-I marker) or *PTPRC* (surface CD45 marker) knock-out efficiency four days post-transfection. (A) A pressure sweep was performed to identify the optimal delivery pressure for CD34+ HSPCs, which was determined to be 30 psi. (B) At 30 psi, average knock-out efficiency achieved for *B2M* was 91.8% ± 1.4 and for *PTPRC* was 84.2% ± 2.7, while viability was preserved. Data are presented as mean +/- SD (n = 2 - 7).

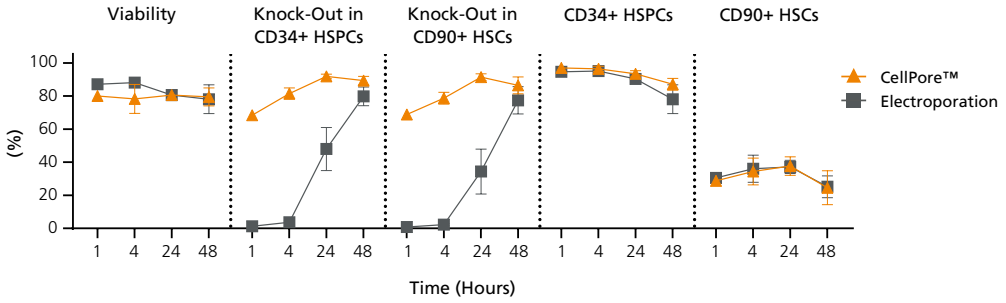



**Figure 3.** The CellPore™ Transfection System Enables Earlier Gene Editing of CD34+ HSPCs

Cas9 RNP complexes targeting *B2M* (40 pmol to 5 x $10^4$ cells in 80 µL, Cas9:sgRNA 1:2.5) were delivered to CD34+ HSPCs using the CellPore™ Transfection System at 30 psi or via an optimized electroporation protocol following pre-transfection culture durations of 1, 4, 24, or 48 hours. Four days post-transfection, viability, *B2M* gene knock-out efficiency, and CD45+CD34+ HSPC and primitive CD34+CD45RA-CD90+ HSC subset frequencies were assessed by flow cytometry. CellPore™ achieved ≥80% knock-out efficiency with just 4 hours of pre-transfection culture, whereas electroporation required 48 hours of culture to consistently reach high editing efficiency. This shortened CellPore™ workflow presents a significant advantage in maintaining high bulk CD34+ HSPC as well as primitive CD90+ HSC frequencies. Data are shown as mean ± SD (n = 1 - 6).

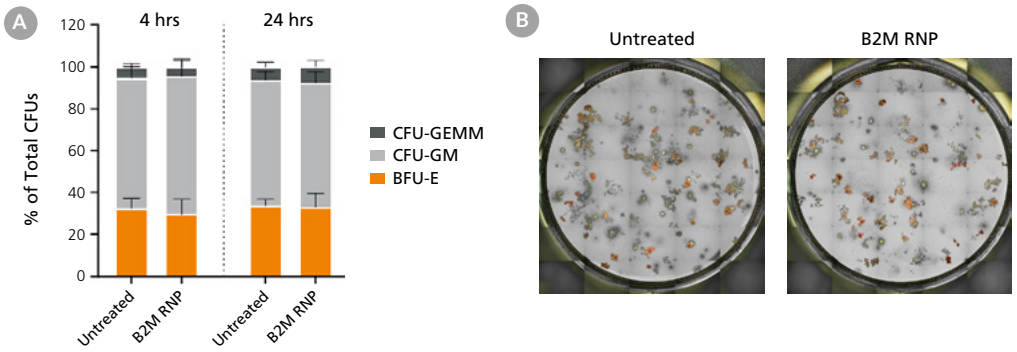



**Figure 4.** The CellPore™ Transfection System Preserves the Proliferation and Differentiation Potential in CD34+ HSPCs

CD34+ HSPCs were pre-cultured for either 4 or 24 hours before delivery of Cas9 RNP complexes targeting *B2M* (40 pmol to 5 x $10^4$ cells in 80 µL, Cas9:sgRNA 1:2.5) using the CellPore™ Transfection System at 30 psi. 24 hours after transfection, cells were plated in triplicate in MethoCult™ H4435 Enriched medium and cultured for 14 days (A) CFU colony sub-type distribution and (B) colony sizes remained comparable to untreated controls. Data are shown as mean ± SD (n = 3 - 4).

## Why Use CellPore™?

**HIGH PERFORMANCE.** Achieve high delivery and viability for your cells

**EASY TO USE.** Fine-tune a single parameter to identify optimal delivery and viability conditions

**GENTLE.** Deliver cargoes intracellularly without altering cell quality

**FAST.** Transfect up to 10 million cells per second

**VERSATILE.** Deliver a wide variety of cargoes to a broad range of cell types

**View Protocol**
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