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Review

Tools for automated genome editing of stem cells

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Automated genome editing of stem cells represents a great advancement in the fields of disease modeling and regenerative medicine. This review evaluates the current tools and methodologies for implementing automated genome editing for induced pluripotent stem cells. The increasing demand for precise genome editing technologies, driven by the growing gene editing market, necessitates efficient and scalable solutions to overcome the complexities and costs associated with traditional editing methods. A comprehensive overview of various automation strategies is provided, focusing on key workflows that encompass stem cell expansion, genetic material delivery through viral vectors, lipid nanoparticles, and electroporation, as well as monoclonal expansion techniques. Advances in automation not only enhance editing efficiency and reduce labor intensity but also ensure quality and reproducibility in stem cell research. As the field progresses, the integration of artificial intelligence and the shift toward closed, good manufacturing practice-compliant systems are anticipated to further streamline automated genome editing processes.

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Introduction

Genome-edited stem cells transform disease modeling and the development of cell and gene therapies. Their ability to differentiate into specialized cells, combined with precise genome editing, enables 'disease-in-a-dish' models to study molecular disease mechanisms, test drug candidates, explore treatment options, and screen for target genes [1,2]. Furthermore, genome editing allows the development of effective cell and gene therapies, expanding the range of treatable diseases [3]. The gene editing market is expanding rapidly, with an estimated current approximated annual growth rate (CAGR) of 10% driven by increasing demand for cell and gene therapies [4]. However, genome editing of stem cells remains costly, and the associated manufacturing processes are complex. Therefore, there is a growing demand for automated approaches for genome editing of stem cells.

Stem cells are classified into pluripotent (PSCs) and multipotent types. Human PSCs include embryonic stem cells (ESCs) as well as induced pluripotent stem cells (iPSCs). IPSCs, first described by Shinya Yamanaka et al., are reprogrammed somatic cells. This allows researchers to obviate ethical concerns, as ESCs are derived from the inner cell mass of the blastocyst during early embryonic development [5,6]. PSCs, especially iPSCs, are central for disease modeling, drug testing, and regenerative therapies. Genome editing in hiPSCs enables the generation of isogenic iPSC pairs: With genome-edited hiPSCs and healthy control hiPSCs, it is possible to compare the cells in a genetically controlled background [7]. Multipotent stem cells include mesenchymal stem cells (MSCs) derived from bone marrow, adipose tissue, or synovium, and hematopoietic stem cells (HSCs) from blood or bone marrow [8,9]. Genome editing of MSCs is mainly explored to enhance therapeutic effects, while edited HSCs are applied in treating hematological diseases such as sickle cell anemia [10,11].

MSCs can also be derived from iPSCs, since they are a reliable and renewable source [12]. Additionally, genome editing of iPSCs involves distinct challenges and has the most promising applications [13]. This review focuses on automated solutions for genome editing of PSCs.

To fully exploit the potential of stem cells, precise and efficient genome editing tools are essential. Earlier genome modifications were random, for example, through radiation, while targeted editing began with the discovery of Zinc-finger-nucleases (ZFNs) in 1996 [14]. With the revolutionary advent of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated

(Cas) technologies, easy, low-cost, and precise gene modifications at defined loci were enabled [15]. Today, strategies applied in stem cell research include ZFNs, transcription activator-like effector nucleases, CRISPR/Cas, and newer approaches such as base and prime editing [16,17].

The process of genome editing of pluripotent stem cells typically includes four main steps: First, the isolated stem cells are expanded and maintained (i). Then, the *ex vivo* editing of the stem cells follows (ii). For that, the genome editing machinery or the genetic material must be delivered into the cells, which is possible via viral transduction, lipid nanoparticles (LNPs), or electroporation. For pluripotent stem cells, edited cells must be expanded from single cells to establish monoclonal lines (iii) [18,19]. The workflow is illustrated in Figure 1, which depicts the delivery approaches as well as the two techniques for monoclonal expansion discussed in this review, namely colony picking and single cell dispensing. Despite the clearly defined outline, the process remains complex, error-prone, and labor-intensive, hindering scale-up and widespread application. Recent automation advances range from semiautomated process steps to fully automated end-to-end solutions, improving efficiency while maintaining quality [18,19]. This review surveys advances in automation across the workflow of genome editing in pluripotent stem cells.

Different automation advances in the steps of genome editing of stem cells

Stem cell expansion and maintenance

The first step in generating genetically engineered stem cells is expansion, which involves medium exchange, careful passaging, and, in the case of pluripotent stem cells, strict control of pluripotency and genomic stability. While many automated expansion solutions exist, most of them are used for immune cells, such as T cells, and are often adaptable to MSCs or HSCs [20,21]. Pluripotent stem cells remain more technically demanding, and systems specifically for iPSCs range from proof-of-concept prototypes to validated commercial platforms. A detailed comparison of automated expansion platforms for stem cells has been provided elsewhere [22]. Automated expansion systems that include genome editing will be introduced later.

Introduction of genetic material

The delivery of genetic material or genome editing machinery, such as CRISPR/Cas components, into stem cells is currently achieved through three main approaches (Figure 1): viral vectors, LNPs, and electroporation [23].

Every system offers distinct advantages and limitations. Viral delivery exploits the naturally evolved ability of viruses to deliver genetic material into host cells, making them highly efficient. Depending on the vector, they enable transient expression (e.g. adeno-associated virus (AAV), adenovirus) and long-term integration (e.g. Lentivirus). On the other hand, limitations exist, such as the limited cargo capacity (ca. 5 kb in AAV, up to 9 kb in Lentivirus), risk of insertional mutagenesis from integrating vectors, and the potential to trigger immunogenic responses [24,25].

LNPs circumvent some of these risks, as they are generally less immunogenic and have a higher cargo capacity. LNPs can deliver mRNA, RNPs, or plasmid DNA, enabling transient expression or editing. However, unlike viral vectors, they do not integrate DNA and therefore do not confer stable expression. They are scalable and relatively easy to manufacture. Unless engineered with specific targeting ligands, LNPs are typically taken up by endocytosis without strong cell-type specificity, resulting in less controlled targeting. Particular formulations can still induce inflammatory responses in host cells, and their transfection efficiency remains lower compared to viral vectors [26,27]. The formulation of LNPs is possible in an automated manner, using microfluidic systems, as in the NanoAssemblr and newer systems [28].

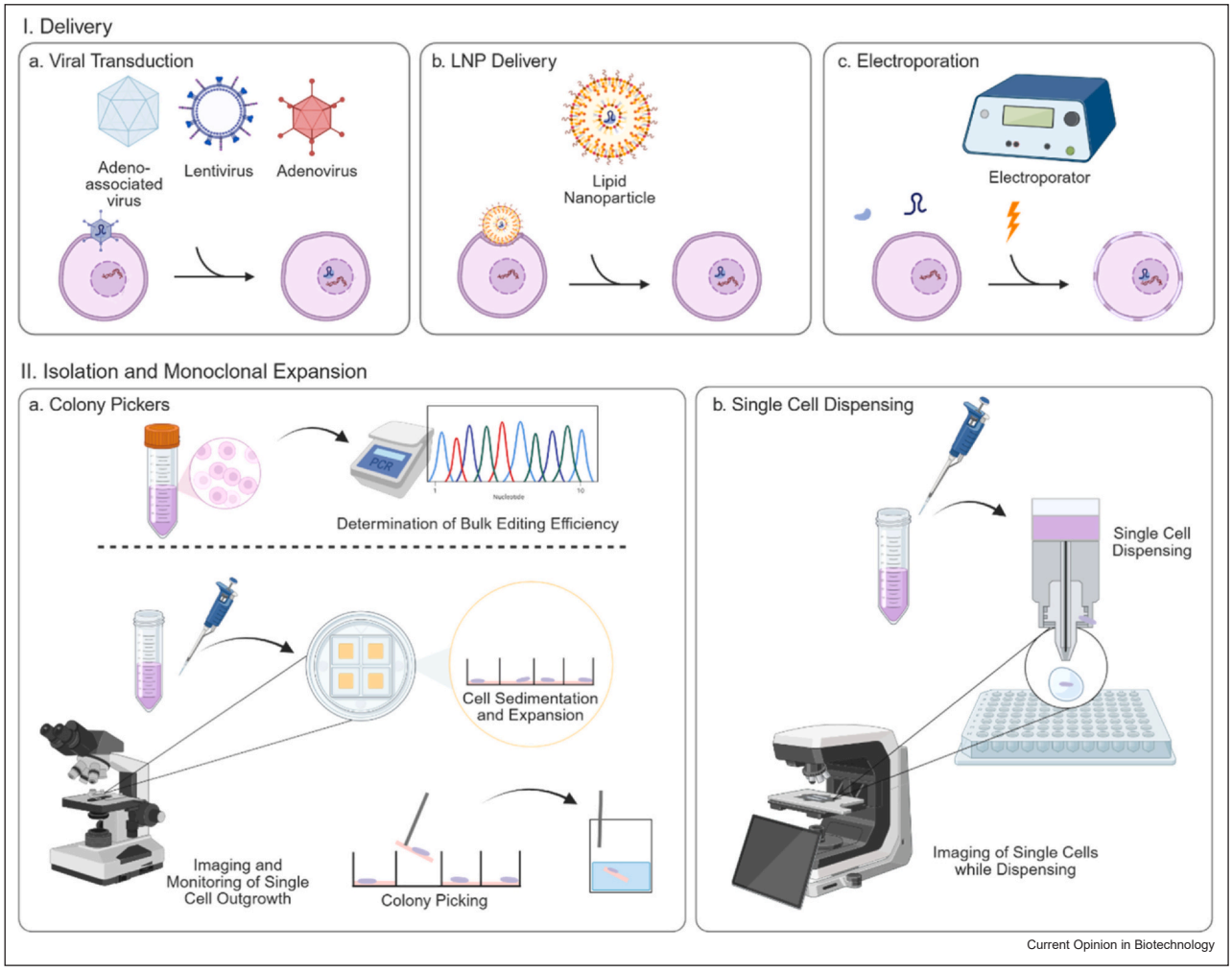
Electroporation is based on the destabilization of cell membranes caused by an electric field and therefore relies on an extra device [29]. It is currently the gold standard for CRISPR/Cas delivery into stem cells, since it supports the delivery of large cargos and achieves high editing efficiencies [30]. Nevertheless, it is associated with significant cytotoxicity and introduces genetic material through a combination of electrophoresis during the pulse and diffusion through transient pores, in a largely untargeted manner [29]. While viral vectors and LNPs are suitable for *in vivo* delivery [31], electroporation is restricted to *ex vivo* applications, as it relies on an additional device.

Several automated systems for electroporation exist. Their capacity, scale-up potential, consumable format, and proof of concept studies for the transfection of iPSCs are summarized in Table 1.

Isolation and monoclonal expansion

For several research applications, monoclonal colonies are required, so colony growth must derive from single cells. Two different strategies exist: prospective dispensers isolate single cells at the time of dispensing and document clonality immediately, while retrospective pickers monitor cell growth and later pick validated colonies (Figure 1). Retrospective systems typically generate thousands of colonies, making it impractical to expand all of them. Therefore, bulk quality control (QC) tools such as PCR and Sanger sequencing are applied beforehand to estimate how many colonies need to be picked [32]. In

Figure 1



Schematic outline of genome editing in stem cells. (I) Delivery can be achieved by viral transduction, LNPs, or electroporation. (II) Monoclonal expansion follows, using either colony picking systems (retrospective isolation after outgrowth on grids or rafts, validation of monoclonality is performed by imaging before colonies of interest are expanded) or single-cell dispensers (prospective seeding into plates, validation through imaging).

prospective dispensing workflows, the number of clones is predefined by the plate format. It is more limited, so QC is less critical for planning but still required later to confirm the genotype of expanded colonies.

In either case, the isolation and monoclonal expansion step remains one of the major bottlenecks, as iPSCs are sensitive to various environmental factors such as osmolarity, pH, nutrition supply, mechanical stress, and especially loss of cell–cell and cell-extracellular matrix interaction [33–35]. To improve survival and downstream processes, gentle single-cell isolation and proof of monoclonal colonies are necessary. Traditional methods, such as manual cell picking, limiting dilution, or fluorescence-activated cell sorting, are often inefficient or cannot guarantee monoclonality, which has led to the development of automated devices [36]. In Table 2, automated devices for monoclonal expansion are compared in terms of the method of isolation and ensuring monoclonality, as well as their applications to PSCs.

Discussion

The automated production of genome-edited pluripotent stem cells can be achieved through six distinct workflows, defined by the delivery method (viral vectors, LNPs, or electroporation) and method of monoclonal expansion (prospective single cell dispensing and retrospective colony picking). Their applicability depends on the type of genetic modification, the sensitivity of the cells, and the presence of markers to identify edited cells.

Prospective single-cell dispensers yield a limited number of colonies, restricted by the plate format,

Table 1

Overview of automated and commercially available electroporation systems applicable for genome editing in stem cells. Devices differ in scale-up potential, capacity, consumable formats, proof-of-concept data in PSCs, and potential integration into automated expansion systems.

Device/system	Scale-up	Cell capacity	Volume range	Consumable format	Proof of concept, PSC data	Integration in expansion platforms	References
Neon NxT (Thermo Fisher Scientific)	Transition to CTS Xenon without re-optimizing parameters	1×10^4 to 1×10^7 cells	10 µL or 100 µL	Disposable pipette tips	No PSCs data; Manufacturer data: 41% Knock in (KI) in T cells	Standalone research-scale solution	[37]
CTS Xenon (Thermo Fisher Scientific)	Designed for clinical scale	2.5×10^9 cells	1 mL cuvette or 5–25 mL cartridge (sequential)	Cuvette or cartridge	Manufacturer data: 45% KI in CAR-T cells; ca. 15% KI in CAR-iPSCs	Can be integrated into Gibco CTS Rotea Counterflow Centrifugation System	[38,39]
CliniMACS Electroporator	Integrated into CliniMACS Prodigy workflow for large-scale production	Not specified, suitable for large-scale cell generation		Integrated cuvette connected with the single-use tubing set	No PSCs data; Independent data: Applied to HSCs, CAR-T cells, and primary T cells	Fully integrated in the CliniMACS Prodigy	[40–42]
ExPERT GTx (MaxCyte)	Flexible, small to large volumes	$0.5–2\times10^8$ cells	20 µL to 100 mL	Suspended in a cartridge (static electroporation) or pumped through a tunnel (flow electroporation)	Used in FDA-approved Casgevy; 40% KI for loxP insertions in iPSCs; >80% KI TRAC gene with ZFNs in iPSCs	Standalone solution	[43–46]
4D Nucleofector System (Lonza)	Nucleofector requires different units for scale-up	1×10^7 to 1×10^9 cells (LV unit)	1 mL cuvette or 20 mL cartridge	Differs pro unit: plates, cuvettes, cartridges, and bags	Widely used, independent data: Up to 98% KI in iPSCs	Can be integrated into the Cocoon Platform (Lonza), or research-stage StemCellFactory	[47–52]

Table 2

Overview of automated systems for single-cell isolation and monoclonal expansion. Systems differ in their dispensing or picking principle, proof of monoclonality, consumables, and degree of validation in pluripotent stem cells.

Device/system	Dispensing/picking	Proof of monoclonality	Consumables	Application to PSC	Notes	References
cellenONE HT (Cellenion)	Piezo-acoustic dispensing with glass capillaries	Prospective: brightfield and fluorescence; algorithm-based	Dispensing and expansion in 96-, 384-, 1536-well plates	Manufacturer claims only; Earlier versions of each device have been tested independently with iPSCs	Temperature control for viability	[36,53]
up.sight (Cytena)	Inkjet-like droplet dispensing with pneumatic shutter	Prospective: two-factor: droplet imaging + 3D-well or bottom imaging, algorithm based	Dispensing and expansion in 96- and 384-well plates		Suited for high-throughput assays	[36,54]
Cloning platform XT (iota Sciences)	Oil-based grid isolates cells, and the picking and transfer of single cells follows	Prospective: Brightfield and fluorescence, algorithm-based	Separation: 256 chambers on 60-mm polystyrene dishes (grid); expansion in 96-well plates		Requires picking a step of single cells from a grid	[36,55]
LIFToscope (Fraunhofer)	Laser-induced forward transfer (LIFT) with AI-based imaging	Prospective: High-speed camera + AI segmentation	Dispensing and expansion in Microtiter plates	No PSC data yet	Contactless transfer; research stage prototype	[56]
CellRaft Air (Cell Microsystems)	Sedimentation into Cell Rafts in Arrays, magnetic raft retrieval of colonies	Retrospective time-lapse imaging (brightfield/ fluorescence) of clonal outgrowth	Expansion in Cell Raft Arrays (up to 40k CellRafts Per Array), transfer to 96-well plate	Manufacturer claims only: 25× more efficient for iPSCs than limiting dilution	Shared medium improves iPSC survival during outgrowth	[57]
CellCelector Flex (Sartorius)	Sedimentation into nano wells, robotic glass capillary aspiration of colonies	Retrospective: growth record imaging (brightfield, phase contrast, fluorescence)	Expansion in nano wells (up to 2000 cells per plate), transfer into standard culture plates	Manufacturer claims only: Tested with CRISPR-edited iPSCs + independent validation	Shared medium improves iPSC survival during outgrowth; three interchangeable picking modules; Integrated in StemCellFactory	[32,51,58]

AI, Artificial Intelligence.

making it appropriate for high-efficiency edits (e.g. knock-outs, base editing [59]) or when a marker such as green fluorescent protein enables identification in the moment of dispensing. Retrospective colony picking is feasible for low efficiency editing (e.g. large insertions, prime editing [17,59]) as far more colonies are generated to later identify rare correctly edited clones. Furthermore, cell viability is supported by growth in shared medium, making it practicable in combination with harsh delivery.

The use of viral vectors enables precise, efficient, and gentle delivery, but is restricted by cargo capacity. Therefore, this method is best suited for small donor templates or stable integration of limited length. Since delivery is gentle, prospective dispensing is suitable when edits are marker-based, and the editing is efficient. LNPs are comparably gentle but are less immunogenic, allowing the delivery of larger cargoes. Since their delivery efficiency is lower, retrospective pickers can be a better choice unless the editing event occurs frequently. Complex editing and the delivery of large constructs require electroporation; however, due to its cytotoxicity and the inherently low efficiency of extensive edits, retrospective picking is almost always necessary.

Outlook

The market for CRISPR/Cas technologies and iPSCs is experiencing significant growth, with a current approximated annual growth rate (CAGR) of 10% and 11%[4,60]. This trend indicates a strong focus on the development of automated genome editing in stem cells, primarily utilizing CRISPR/Cas technologies in iPSCs. As protocols become increasingly standardized, reproducibility across platforms will be enhanced, streamlining research and therapeutic applications. The adoption of LNP systems for delivery is expected to facilitate *in vivo* transfection, reducing reliance on *ex vivo* electroporation and mitigating viral risks. The integration of artificial intelligence, exemplified by the LIFToscope [56], is anticipated to minimize manual intervention, with deep-learning algorithms playing a crucial role in decision-making. Additionally, there is a shift toward closed, good manufacturing practice (GMP) compliant facilities. However, the complete integration of automated genome editing and clonal expansion within a single platform remains primarily in the research phase. This gap is likely to be addressed in the coming years. These advancements point toward a promising future for automated genome editing in stem cell research and therapy, emphasizing the importance of innovation and standardization in this evolving field.

CRediT authorship contribution statement

Bastian Nießing: Conceptualization, Investigation, Visualization, Writing – original draft. **Rebekka Wagner:** Investigation, Writing – original draft. **Laura Herbst:** Writing – review & editing. **Robert H Schmitt:** Project administration, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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