Cellectricon

Cellaxess® HT cell-based assay and transfection lab

The Cellaxess® HT system is a new and revolutionary concept in cell-based assay technology; it is the only system on the market that permits electric-field manipulation of cells and tissue in a high-throughput format. Specifically, the system facilitates the transfer from cooperative cell lines to the use of primary cells in a wide range of screening applications such as RNA interference (RNAi) screening, intracellular target screening and electric-field stimulation assays.

The CellaxessHT system was originally developed in collaboration with leading pharmaceutical companies for genomic screening applications in which, for example, RNAi, a form of post-transcriptional gene silencing, provides a useful tool for generating gene knockout model systems and studying pathways and gene function in cell culture. The key to successful RNAi experiments is efficient delivery of substrates into cells. Traditional chemical methods such as lipid-mediated transfection are restricted mainly to immortalized cell lines, excluding analysis of interesting and biologically relevant cell types[1].

Electroporation works for transfection of a wide range of cell types, but conventional cuvette-based electroporation is limited to low throughput and to cells in suspension[2]. The CellaxessHT is a revolutionary technology that uses a capillary electroporation concept, meaning that a wide range of adherent primary cells and cell lines can be efficiently transfected in 384-well plates (**Fig. 1**). The CellaxessHT can be used for introduction of different types of molecules, ranging in size from target-designed dyes to oligonucleotides, siRNA and plasmids into relevant cell models such as differentiated neurons, macrophages and hepatocytes.

The key component in the CellaxessHT system (**Fig. 2a**) is the electroporation module with its 96 capillary electrodes (**Fig. 2b**). Each tip is composed of a non-conducting capillary equipped with an internal metal electrode and a ground electrode located on the outside of the capillary. The capillary electrode is positioned in close proximity to the cells, creating a virtual cuvette in each well (**Fig. 2c**). Liquid is aspirated to generate contact between the electrodes, and an electrical potential is applied. A focused electric field is created under the

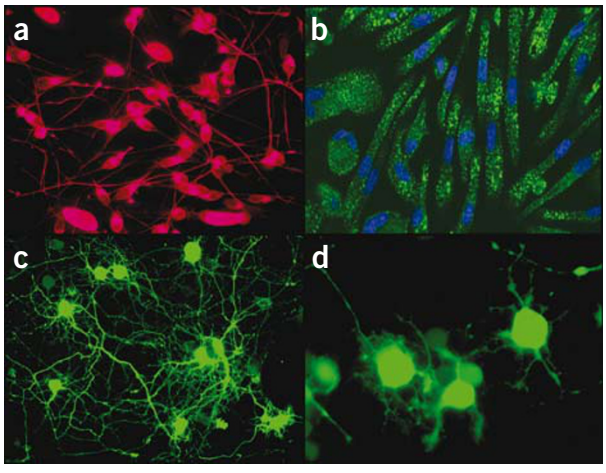

Figure 1 | With the CellaxessHT, a variety of substrates can be delivered to primary cells and cell lines. (**a**) Differentiated SH-SY5Y cells. (**b**) Primary human macrophages. (**c**) Primary hippocampal neurons from rat. (**d**) Differentiated Neuro2a cells.

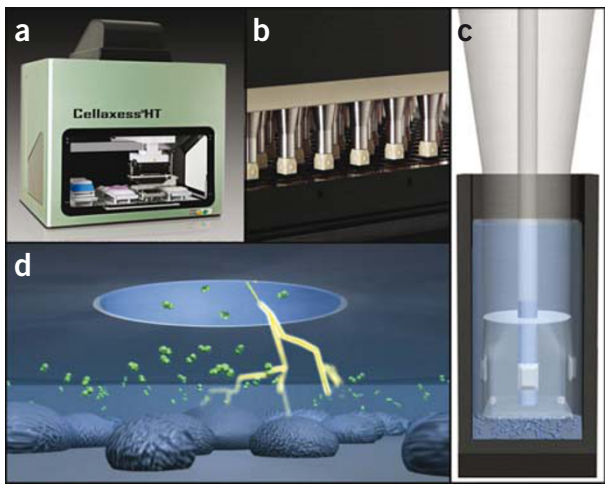

Figure 2 | Overview of the CellaxessHT platform. (**a**) The system in stand-alone configuration. (**b**) A close-up image of the capillary electrodes as they approach the CellaxessHT 384-well plate. (**c**) Electrical contact between the inner and outer electrodes is created when liquid is aspirated into the capillary. (**d**) A focused electrical field is formed and cells are electroporated.

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capillary, which causes the cells to be electroporated (**Fig. 2d**). The tip module moves in quadrants and electroporates in the 384-well format. Because cells are transfected *in situ*, no trypsinization step is required, saving time and resulting in very low cell consumption. The focused electrical field minimizes electrochemical toxicity and joule heating, and differentiated cells can be transfected in any developmental state with retained morphology and excellent viability.

When optimizing CellaxessHT transfection, 16 different pulse protocols can be applied in a single 384-well plate (24 wells per condition), making the optimization procedure very time effective. The key parameters that influence electroporation efficiency are pulse length, number of pulses, voltage and interval between pulses. The CellaxessHT electroporation protocols are cell specific but not substrate specific, making it possible to co-transfect with, for example, plasmids and siRNA, or to move from one substrate to another, without re-optimization. Transfection efficiencies are largely independent of cell density, making it possible to further reduce the number of cells needed for large-scale experiments. This is important to take into account when screening a large number of genes in cell types that are challenging or expensive to prepare. In comparison to electroporation of single samples, multiple transfections at a time significantly reduce handling time and variability, and transfection efficiencies are robust across the 384-well plate as well as between plates (**Fig. 3**). The CellaxessHT instrument not only enables a unique form of electroporation but also caters for all liquid-handling steps required for transfection, including removal of cell-culture medium, addition of electroporation buffer and substrates, and addition of medium after electroporation. The throughput of the instrument is in the range of 20,000–50,000 wells per working day, depending on the level of automation that supports the instrument.

Biological variance is present in all research conducted in living systems, and when examining every gene in the genome, the total variance of the readout of interest can be massive[3]. This can be minimized by using biologically relevant models and complex readouts for RNAi screens. The customized CellaxessHT plates are fully compatible with most high-content analysis platforms, making it possible to obtain multifaceted high-content readouts. Primary cells are desired for gene-silencing experiments because they better model the *in vivo* cells than immortalized cell lines. The fact that the CellaxessHT delivers genetic material *in situ* to as many as 384 samples at a time permits high-throughput and high-quality screening also for hard-to-transfect primary cells. With the CellaxessHT, gene function can be studied on a genome-wide scale in cells previously inaccessible to genetic manipulation.

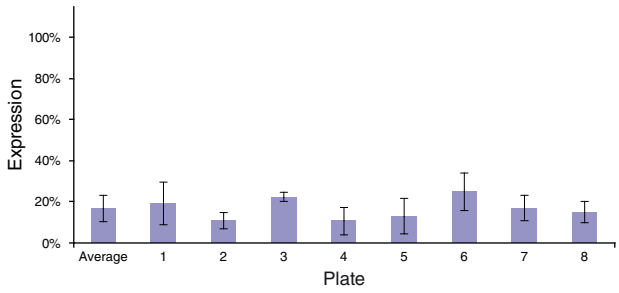

Figure 3 | Eight plates of Neuro2a cells were run by different operators and analyzed over a 1-month period, showing low plate-to-plate variation in knockdown efficiency of GSK-3β compared to non-targeting siRNA.

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2. Ovcharenko, D.R. *et al.* High-througput RNAi screening in vitro: from cell lines to primary cells. *RNA* **11**, 985–993 (2005).
3. Bushman, F.D. *et al.* Host cell factors in HIV replication: meta-analysis of genome-wide studies. *PLoS Pathog.* **5**, 1–12 (2009).