### A Novel, Multifactorial Approach for hiPSC Differentiation and Reprogramming Using an Automated Cell Culture System


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### Introduction

A major challenge in the stem cell field is to define the optimal condition for cell expansion, differentiation and reprogramming. Because multiple intracellular and extracellular signaling pathways are involved in each cellular process, a combinatorial approach to screen multiple factors is highly desirable. To facilitate the exploratory processes, we have developed Callisto, an automated cell culture system for cell manipulation and environmental control. The system consists of an integrated fluidic circuit (IFC), an electropneumatic controller instrument, experimental designer software and automated run-time control software. Each IFC has 32 culture microchambers and 16 reagent inlets. Each microchamber can be dosed separately with different combinations and ratios of the 16 reagents at various predefined time points. Callisto enables long-term cell culture (more than three weeks) with three-day hands-off operation. Previously using this system we have developed a novel nanointegrating method for direct conversion of human BJ fibroblasts to neurons, and also demonstrated the reprogramming of human fibroblasts into human induced pluripotent cells (hiPSCs). Here we demonstrate an efficient transfection protocol of siRNAs and mRNAs in fibroblasts and hiPSCs. We have also demonstrated using lentivirus and retrovirus to differentiate hiPSCs to neurons and reprogramming human fibroblasts to hiPSCs. In summary, the automated microfluidic platform employs precise control of the microenvironment of cells, facilitates studies of multifactorial combinations, and enables development of robust, reproducible and chemically defined cell culture and manipulation.

### Results

#### Figure 1. Callisto system components

The major components of the Callisto system include: (A) an IFC to provide fluid paths and cell culture microchambers for cell seeding and treatment, (B) an instrument to provide thermal, pneumatic and environmental control (gas/humidity) for the IFC to enable long-term cell culture and dosing, (C) software to design, monitor and record experiments, and (D) a reagent kit to support cell loading, live harvest and lyse and harvest.

#### Figure 2. Principle of the cell culture IFC

The IFC features 32 culture microchambers, which can be individually treated with any combination of 16 input factors—simultaneously or on different schedules. The multiplexer, a fully programmable microfluidic delivery system, can input cells, media and reagents to individual culture chambers (1 mm² footprint, 100 nl volume) which can output into separate outlets supernatants, cells or lysates.

#### Figure 3. Callisto general workflow

The Callisto system allows easy setup for combinatorial dosing as demonstrated by delivery of two different fluorescent dyes at various ratios into individual chambers. Fluorescence intensities were represented in pseudocolor in (A) (scale bar = 490 µm). Using this system, we demonstrated a simple way to perform dose response. Examples shown here are mGFP mRNA transfection in human BJ fibroblasts cultured on IFC (scale bar = 30 µm) (B) and knockdown of Oct4 with siRNA transfection in hiPSCs (scale bar = 240 µm) (C). Immunostaining with DAPI antibody revealed significant decrease in Oct4-positive cells after knockdown with Oct4 siRNA. Cells were lysed and harvested on IFC and lysates were used for gene expression analysis through qPCR. Gene expression data correlated with immunostaining (D).

### Conclusion

- The Callisto system enables long-term culturing of different cell types and automated dosing of cells with combinations of miRNAs, mRNAs and small molecules at predefined times.
- We have developed a streamlined workflow to characterize cells by immunostaining and by single- or bulk-cell genomic analysis.
- The flexibility of the Callisto system supports complex and time-consuming applications including cell maintenance, RNA transfection, reprogramming and differentiation.

### References


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