Sonoporation: A Novel Method for Mammalian Cell Transfection

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Introduction
- We present a novel transfection method called sonoporation, which introduces genetic material and proteins into mammalian cells using the acoustic excitation of microbubbles.
- Antibody-coated microbubbles are combined with the transfection material (nucleic acids, proteins, or small molecules) and bound to cells. The cells are irradiated with ultrasonic energy, oscillating the microbubbles to create transient pores in the cell membrane.
- We demonstrate proof of concept of this novel transfection method using both plasmid DNA and CRISPR ribonucleoprotein complexes (RNPs).
- Plasmid DNA transfection at low efficiencies with almost no cell death. This is highly advantageous for cell lines that are normally difficult to transfect.
- CRISPR RNPs are transfectable at higher efficiencies, and they maintain their ability to edit the genome.
- All experiments were performed in a 384-well microplate and can adapt to high-throughput screening (HTS) modalities.

Background
Transfection refers to the incorporation of foreign material into host cells. Traditional biological, chemical, and physical transfection methods have challenges, including low transfection efficiency rates, high cell toxicity, and inadequate scalability.

Material & Methods
Sonoporation Workflow

1. Bind antibodies and transfection material (nucleic acids, proteins, or small molecules) to microbubbles. Add complex to cells.
2. Flip plate upside down and incubate for 5 minutes to allow binding reaction of microbubbles and cells.
3. Turn plate upright and use prototype Echo® acoustic instrument with components suitable for sonoporation to quickly pulse the well with ultrasound. Acoustic energy causes bubbles to resonate.
4. Incubate plate 24-48 hours, then analyze the cells for desired phenotype.

Results: Plasmids
Sonoporation of Plasmid DNA:
A reporter plasmid expressing GFP was transfected into HEK-293 cells. After incubating for 48 hours, the cells were analyzed by FACs.

Results: CRISPR
Sonoporation of CRISPR RNPs:
CRISPR RNPs for HPRT were transfected in HEK293 cells. After incubating for 48 hours, the cells were analyzed by T7E1 CRISPR mutation detection assay.

Discussion
- We thank our collaborators at Targeson and Intellicyt.
- We demonstrate proof of concept of this novel transfection method using both plasmid DNA and CRISPR ribonucleoprotein complexes (RNPs).
- CRISPR Results Summary
  - Uptake of the RNPs by the cells transfected by sonoporation is about 4-fold higher than the background.
  - Compared to lipid mediated transfection, the sonoporated cells have a lower transfection efficiency.
  - No FACS data for cell death using these two methods.
  - Assay optimization needed for specific cell lines and workflows.

Summary
- We present a novel acoustic method for transfection cells called sonoporation.
- Sonoporation uses an acoustic radiation generator to generate or direct acoustic radiation that excite functionalized microbubbles during cellular transfection.
- We demonstrate proof of concept of this novel transfection method using both plasmid DNA and CRISPR ribonucleoprotein complexes (RNPs).
- All experiments were performed in a 384-well plate and can be adapted to high-throughput screening (HTS) modalities.

Acknowledgements
- We thank the management team and scientific collaborators of Labcyte Inc.
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Properties of Microbubbles
- Gas core
- Biotinylated Lipid
- Streptavidin
- Hydrogel
- PEG

Reagents and Consumables Used
<table>
<thead>
<tr>
<th>Description</th>
<th>Manufacturer</th>
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<tr>
<td>Echo® qualified 384-well polypropylene microplate</td>
<td>Labcyte Inc.</td>
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<tr>
<td>HEK-293 cell line</td>
<td>ATCC</td>
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<td>Targeted mRNA/microbubbles</td>
<td>Targxon</td>
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<td>Antibodies</td>
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<td>HNF™-GFP reporter plasmid</td>
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<td>MultiColor™ membrane integrity FL3/BL4 screening kit</td>
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<td>Kapa HiFi™ HotStart DNA Polymerase</td>
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<tr>
<td>CRISPR Discovery Gey Kit</td>
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Results: CRISPR
CRISPR RNPs for HPRT were transfected in HEK293 cells. After incubating for 48 hours, the cells were analyzed by T7E1 CRISPR mutation detection assay.

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