

Lab on a Chip

Supplementary Information

Title: Sequential Intracellular Delivery of Genetic Coding Molecules Using an Acoustic Electric Microfluidic Platform

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Table S1 Components of a MIFlowCyt-compliant experiment description.

Experiment Overview	Purpose/Goal/Hypothesis Quantify number of transfected HeLa cells by plasmid and Cas9 RNP
	Experiment Variables HeLa cells were transfected with GFP-expressing plasmid DNA (0.5 µg, 1 µg, and 1.5 µg) and Cas9 RNP (1:1, 1:3) on D0. Plasmid transfection efficiency analyzed on Day 2 and Cas9 KO analyzed on Day 7
	Conclusions HeLa cells were successfully transfected in all conditions
	Quality Control Untransfected cells were used to provide negative plasmid control and unstained cells were used to provide negative staining control
Flow Sample (Specimen)	Material Cancer cell line
	Source/Organism/Location HeLa cells
	Treatment Plasmid: cells were washed with PBS Cas9 RNP: cells were stained with a PE-conjugated CD47 antibody
	Reagent/Analyte/Detector/Reporter Plasmid: GFP Cas9 RNP: PE
Data Analysis	List-Mode Data FCS data files can be obtained by contacting Michelle Zhang after this work has been published
	Compensation N/A, only single channel
	Gating Same gating strategy has been used for all data files
	Descriptive Statistics % fluorescence
Instrument Details	Instrument Identification NovoCyt 3000
	Fluidics Configuration Instrument has not been altered
	Optical Configuration Instrument has not been altered
	Electronic Configuration Instrument has not been altered

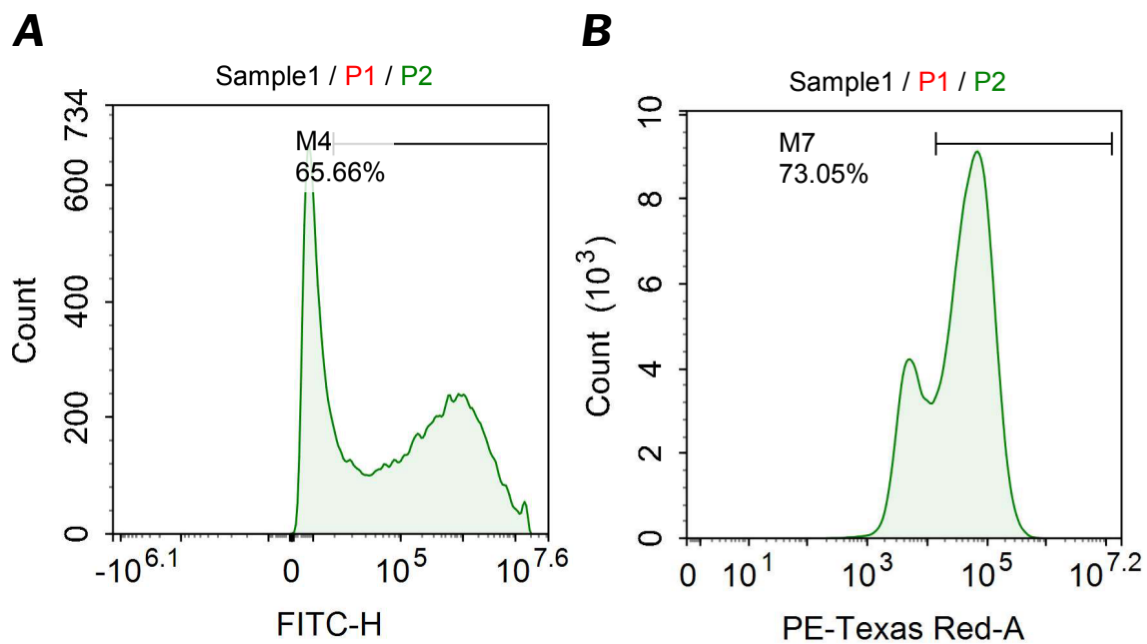


Figure S1 Flow cytometry gating strategy; A) Transfection efficiency of ~3.5kbp GFP-expressing plasmid DNA, B) Percentage of cells in the population that did not express fluorescence upon CD47 antibody staining.

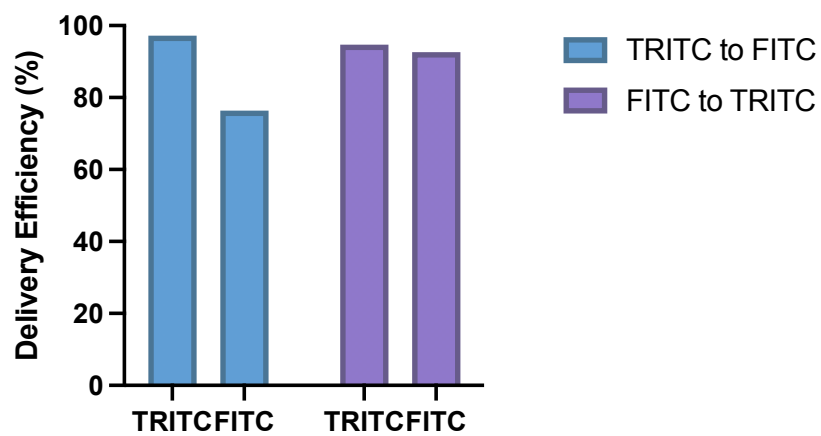


Figure S2 Sequential delivery efficiency based on the order of delivery of 150 kDa TRITC and 500 kDa FITC dextran molecules.

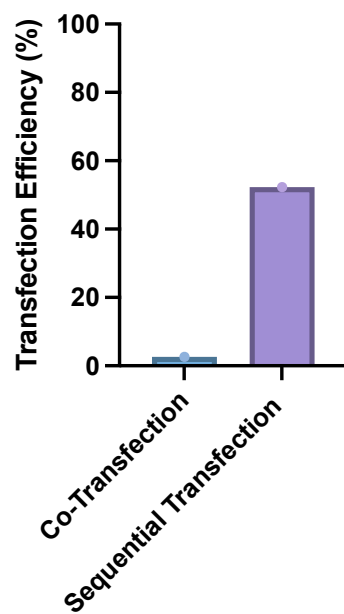


Figure S3 Plasmid transfection efficiency with co-transfection strategy compared to sequential transfection of plasmid DNA and Cas9 RNP using the AESOP platform.

