Implementing Custom Particle Swarm-Differential Evolution Optimization (PSODE) for GFP Plasmid DNA Transfection using Flowfect® Technology

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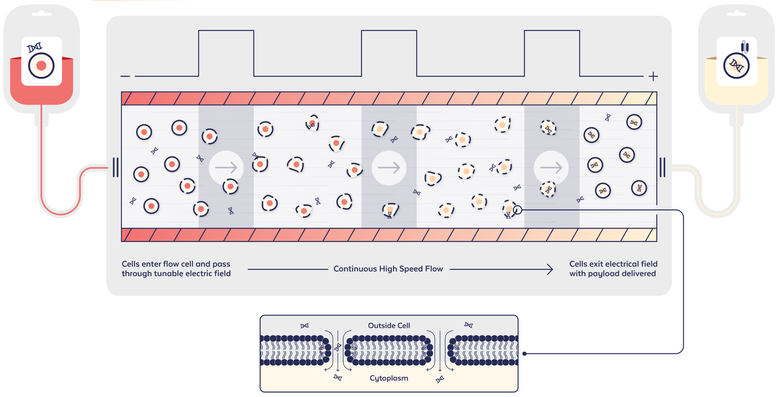
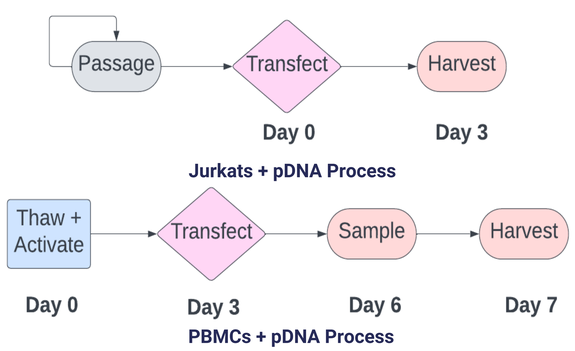
ABSTRACT

Transfection process optimization is imperative to successful cellular drug product manufacturing, with an optimized buffer being a key component. 22 buffer additives were evaluated to improve delivery of GFP plasmid DNA (pDNA) into Peripheral Blood Mononuclear Cells (PBMCs). Traditional DOE methods with 22 additives (i.e., 3k factorial design) results in a high-dimension space requiring >300E6 experimental iterations to characterize. Leveraging artificial intelligence and machine learning on the Flowfect® high throughput system, we implemented a hybrid particle swarm optimization and differential evolution (PSODE) algorithm to simultaneously screen and optimize buffer additives. PSODE identified optimal formulations in Jurkats after 5 iterations, demonstrating a 3-fold increase in transfection efficiency and viability over base buffer. Formulations were then tested in PBMCs, resulting in >75% viability and >50% efficiency. An advantage of the Flowfect® technology is the ability to modulate energy delivered during transfection via various electrical field and fluid flow parameters. Follow-up transfections with multiple Flowfect® profiles identified synergistic buffer and profile effects, demonstrating the value of tunable instrument settings.

TECHNOLOGY

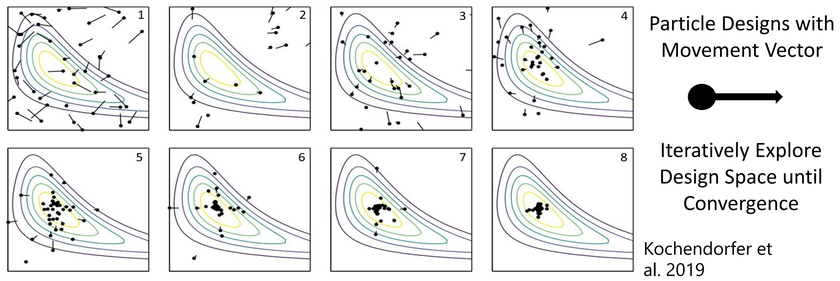


METHODS

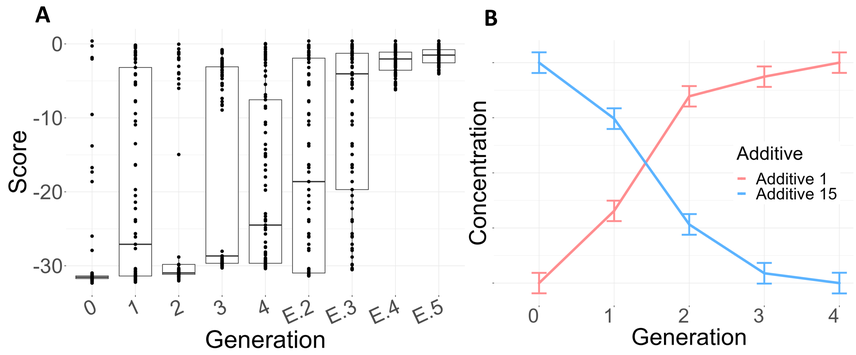


Experimental transfection and culture process for pDNA delivery. Jurkats were passaged every 1 to 3 days to ensure density did not exceed 2.5E6 live cells/mL (Passage 10-20). PBMC studies were conducted on Day-3 activated PBMCs.

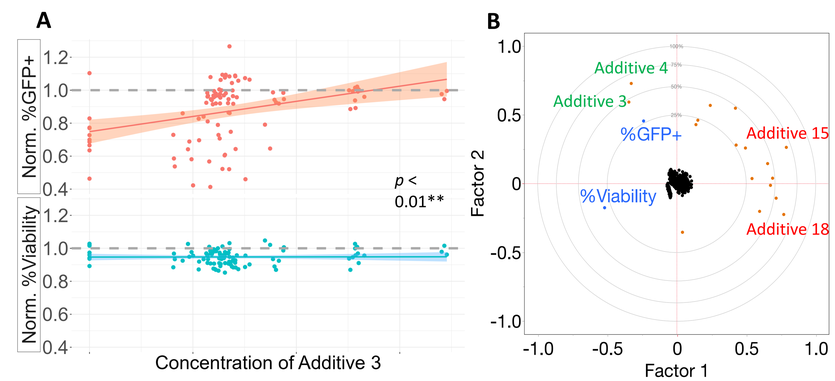
ANALYSIS



Particle swarm optimization mimics collective information sharing observed in nature. Particle designs and initial movement vectors are stochastically initialized. Per sequential generation, particles balance exploration and information exploitation to pick new directions, converging on optimized regions with respect to performance score metrics. In our application, particles are experimental designs of buffer additive concentrations, swarming to regions of optimized post-transfection viability and transfection efficiency.

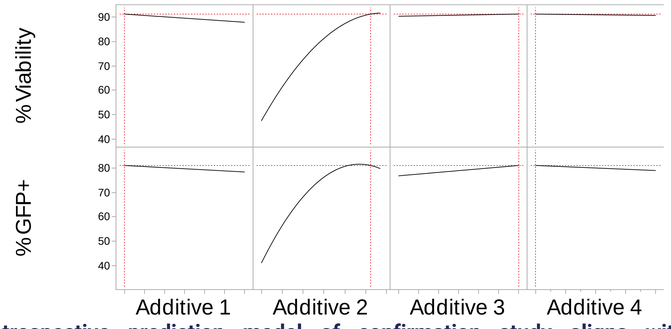


Algorithm converges on optimized buffer. (**A**) Iterative experimental populations (Generations 0-4) explore the design space with varying performance. Emergent populations (E.#) are highest performing particles from the previous two generations. (**B**) Mean concentration of beneficial (Additive 1) and detrimental (Additive 15) components converge over trial populations. Error Bars: 1 SE.

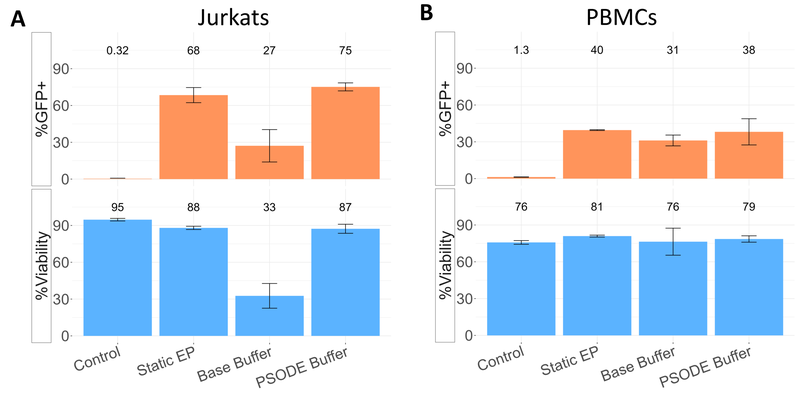


Additive concentrations trend and correlate with transfection efficiency. (**A**) Additive 3 has positive impact on harvest GFP expression (72hr post-transfection, p<0.01). Some formulations exhibit higher %GFP with increasing concentration. (**B**) Principal component analysis loadings plot identifies additives with positive (3,4) and negative impact (15,18) on %GFP+ and %viability, respectively. Shaded Regions: 95% CI.

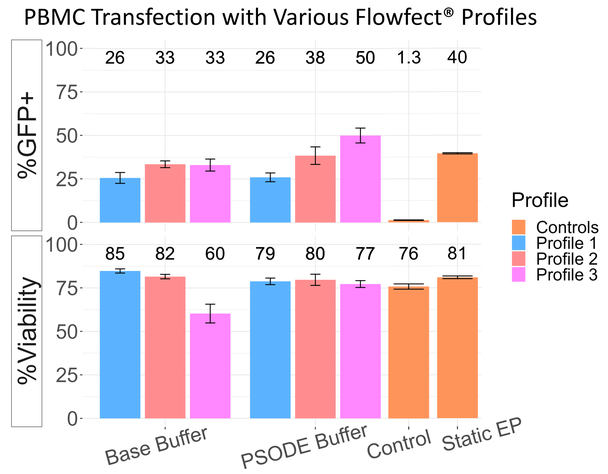
RESULTS



Retrospective prediction model of confirmation study aligns with PSODE performance. Additive 2 has the most pronounced impact on output metrics (p<0.01). Model fit used least squares regression and with maximized desirability.



PSODE buffer formulations improve performance over base formulation. (**A**) PSODE Buffers with additives outperform base formulation (Base Buffer) in Jurkats. (**B**) PSODE Buffer performance confirmed in Day 3 activated PBMCs. Error Bars: 1 SD.



Optimizing Flowfect® parameter profiles with new buffers. PSODE buffers were evaluated with new Flowfect® profiles modulating energy delivery. Improved transfection efficiencies with Profiles 2 and 3 indicate synergy between tunable Flowfect® instrument parameters and new buffer formulations (p<0.01). Error Bars: 1 SD.

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