Efficient Factorial Optimization of Transfection Conditions

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OVERVIEW

- Automated the creation of 96 factorial combinations of different transfection reagents, concentrations, and cell numbers to improve transfection efficiency
  - Fluorescent imaging assessed transfection efficiency and cytotoxicity
  - Significant differences seen across three cell lines
- Factorial approach quickly becomes time consuming and error prone
  - Combining Span-8 and multichannel selective tip pipetting eliminated the need for ~138 manual transfer steps
  - Consistent low volume transfers (5 µL) even with large capacity (1200 µL) head provides added flexibility

INTRODUCTION

Nucleic acid transfection is a procedure used in nearly any cellular laboratory and the abundance of commercially available reagents has made this a seemingly simple endeavor. However, whether one is transfecting plasmid DNA or inhibitory RNAs, different cell lines can have significant differences in the ease of transfection or cellular survival. Determining the optimal plating conditions for high transfection efficiency and low cytotoxicity can be highly involved. Differences can come from the transfection lipid chosen, the concentrations of the lipids and nucleic acids, and the number of cells plated.

Here we demonstrate how a Design of Experiment (DOE) approach can be quickly automated on the Biomek i7 Workstation (Figure 1) to identify optimal transfection conditions for a variety of cell lines.

MATERIALS AND METHODS

Cells and Reagents

Colorectal carcinoma (HCT116), pancreatic epithelioid carcinoma (PANC1) and renal cell adenocarcinoma (ACHN) cells were cultured in McCoy's 5A Modified Medium, Dulbecco's Modified Eagle's Medium, and Eagle's Minimum Essential Medium respectively, each supplemented with 10% fetal bovine serum (FBS). All cells were incubated within a humidified incubator at 37°C in 5% CO2. Transfection reagents tested include Lipofectamine RNAiMAX ("LF", Life Technologies) and DharmaFECT 1, 2, 3, and 4 ("DF1, DF2, DF3, DF4", GE Life Sciences). siGLO Green Transfection Indicator (GE Life Sciences), a FAM-labeled oligonucleotide, was used as a marker for successful siRNA transfection. Oligonucleotides and transfection reagents were diluted in Opti-MEM reduced-serum medium (Life Technologies). 24 hours following transfection, DRAQ7 (Beckman Coulter) was added to cells. After 30 minutes, media was replaced with FluoroBrite DMEM (Life Technologies) with 10% FBS to reduce background fluorescence in imaging experiments.

Automation

A Biomek i7 Automated Workstation with a 1200 µL-capacity 96-channel head and Span-8 pipettors (Figure 1) was utilized for all liquid transfer steps. The selective tip pipetting feature of the 96-channel head, with which any pattern of tips can be used, enabled the factorial combination of reagent additions across rows and columns. The HEPA-filtered enclosure maintained a sterile environment while the flexible deck configuration and integration capacity (i.e. incubators, analyzers) enable complete automation of cellular workflows.

Imaging Cytometry

The SpectraMax i3X MultiMode Detector with MiniMax 300 Imaging Cytometer was used to image cells at 4X magnification in brightfield, 541 nm, and 713 nm emission wavelengths. Cells were analyzed using SoftMax Pro 7 software. Cells in brightfield images were counted using StainFree Cell Detection Technology with the available CellsD setting, while 541nm positive cells and 713nm positive nuclei were identified based on size and intensity threshold. Total cells, transfected cells (siGLO positive), and dead cells (DRAQ7 positive) were counted from images in these wavelengths respectively. Positively stained cells were compared to total cell counts to determine transfection efficiency and toxicity for each condition. Brightfield cell counts were also compared to negative control wells that lacked transfection reagents.

AUTOMATED FACTORIAL TRANSFECTION OPTIMIZATION

Figure 1. Integrated Biomek i7 Automated Workstation. A Biomek i7 instrument with HEPA-filtered enclosure was directly integrated with a Cytomat 2C incubator (right) and SpectraMax i3X Multi-Mode Detection Platform with SpectraMax MiniMax 300 Imaging Cytometer (left).

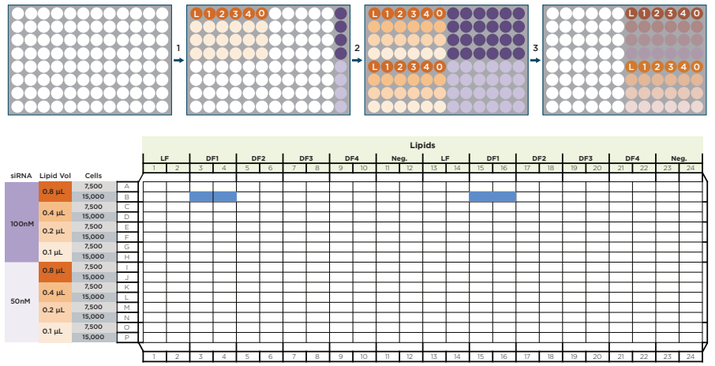

Figure 2. Automated transfection optimization workflow. A) Automated transfers on the Biomek i7 instrument. 1) Transfection lipids (Lipofectamine RNAiMAX (L), DharmaFECT 1-4, and negative control (0)), Opti-MEM media (light orange), and two siGLO Green concentrations (purple) were added to a 96-well plate. 2) Lipids were serially diluted down 4 rows and replicate stamped. siGLO was replicate stamped across 5 additional columns. 3) 48 lipid dilution wells were combined with the 48 siGLO wells. B) The 48 conditions were stamped into a 384-well plate and cells were added at 7,500 or 15,000 cells/well. The blue wells in the plate map represent the quadruplicate values of 15,000 cells transfected with 100 nM siGLO in the presence of 0.8 µL DharmaFECT 1.

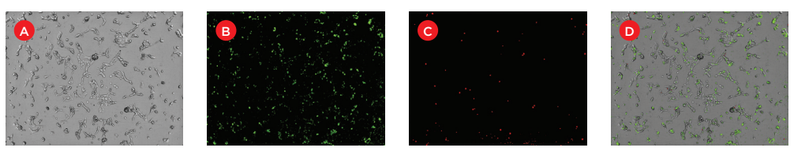

Figure 3. Measuring transfection efficiency and cytotoxicity. 24 hours after transfection with FAM-labeled siRNA oligonucleotides (siGLO) ACHN cells were stained with DRAQ7 to identify cytotoxic cells, and imaged with the SpectraMax MiniMax cytometer. A) Brightfield image utilized for total cell counts. B) 541 nm image utilized for transfected cell counts. C) 713 nm image utilized for dead cell count. D) Overlay of all three images.

RESULTS

A

% Transfected							% Dead							% Control							Transfection % CV						
siRNA	Lipid Vol	Cell #	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con
100 nM	0.8 µl	15000	32.6%	62.6%	68.8%	66.7%	66.6%	0.0%	0.8%	8.1%	8.8%	8.6%	6.6%	0.2%	89.0%	62.2%	62.0%	58.5%	58.6%	99.1%	8.0%	9.3%	12.9%	11.3%	15.4%	165.5%
		7500	41.2%	100.3%	94.2%	108.2%	93.9%	0.2%	2.5%	11.6%	15.2%	13.3%	13.3%	0.2%	72.7%	38.5%	53.8%	45.6%	44.5%	102.8%	3.4%	7.1%	7.7%	13.1%	14.7%	113.2%
	0.4 µl	15000	3.6%	42.3%	36.0%	16.4%	40.3%	0.0%	0.2%	0.7%	1.7%	0.6%	0.7%	0.2%	102.6%	111.8%	103.6%	103.3%	107.2%	102.7%	111.2%	3.7%	12.3%	10.9%	3.4%	200.0%
		7500	14.9%	59.1%	51.1%	24.1%	54.1%	0.0%	0.4%	1.7%	2.0%	0.7%	1.2%	0.2%	96.9%	75.1%	88.3%	107.2%	96.5%	103.8%	54.3%	3.1%	7.3%	6.5%	7.6%	115.8%
	0.2 µl	15000	0.5%	2.6%	11.5%	5.1%	4.0%	0.0%	0.2%	0.2%	0.6%	0.4%	0.3%	0.3%	101.1%	107.3%	108.0%	107.2%	106.5%	100.0%	26.2%	16.0%	7.1%	8.4%	29.6%	-
		7500	1.1%	10.7%	21.2%	8.1%	16.1%	0.0%	0.2%	0.3%	0.7%	0.4%	0.2%	0.2%	101.9%	107.3%	102.0%	109.0%	109.2%	105.1%	72.1%	30.4%	7.4%	26.0%	10.2%	128.3%
	0.1 µl	15000	0.5%	0.3%	5.0%	2.5%	0.7%	0.0%	0.2%	0.3%	0.4%	0.4%	0.2%	0.2%	105.2%	112.5%	107.9%	110.8%	111.2%	99.3%	22.7%	41.4%	9.0%	10.2%	34.7%	200.0%
		7500	0.5%	0.9%	8.4%	4.2%	1.6%	0.0%	0.3%	0.2%	0.3%	0.3%	0.1%	0.2%	98.6%	105.2%	108.1%	110.9%	106.4%	104.9%	32.8%	88.9%	21.3%	6.9%	12.8%	200.0%
50 nM	0.8 µl	15000	6.9%	40.9%	36.8%	39.2%	25.4%	0.0%	0.5%	1.7%	5.3%	2.2%	0.9%	0.3%	118.5%	117.5%	116.3%	115.0%	122.0%	104.5%	47.4%	4.2%	16.5%	9.1%	4.4%	-
		7500	26.6%	55.5%	56.4%	57.4%	40.4%	0.0%	1.6%	3.6%	8.0%	3.0%	1.9%	0.2%	76.6%	88.7%	80.6%	79.0%	102.7%	98.1%	20.0%	9.0%	6.0%	5.7%	15.8%	-
	0.4 µl	15000	1.3%	35.5%	36.4%	20.4%	32.7%	0.0%	0.3%	0.4%	1.7%	0.5%	0.5%	0.2%	105.4%	113.2%	103.3%	107.5%	115.9%	97.4%	36.9%	10.6%	3.6%	18.9%	8.1%	200.0%
		7500	8.5%	54.0%	49.6%	31.7%	50.9%	0.0%	0.5%	1.3%	2.0%	0.6%	1.0%	0.1%	101.1%	79.8%	103.5%	103.0%	98.2%	103.2%	82.7%	3.7%	8.0%	4.2%	3.1%	-
	0.2 µl	15000	0.7%	7.2%	15.5%	7.4%	12.5%	0.0%	0.3%	0.3%	0.7%	0.3%	0.3%	0.3%	108.0%	110.2%	109.8%	107.3%	114.4%	98.5%	20.2%	15.4%	10.4%	15.5%	31.8%	200.0%
		7500	8.6%	43.6%	36.6%	11.4%	43.9%	0.0%	0.4%	0.4%	0.8%	0.4%	0.4%	0.2%	91.0%	85.4%	84.8%	92.0%	87.7%	90.0%	65.2%	24.4%	7.3%	21.4%	12.3%	200.0%
	0.1 µl	15000	0.5%	0.7%	7.2%	2.8%	1.1%	0.0%	0.2%	0.3%	0.3%	0.5%	0.3%	0.2%	98.1%	105.7%	99.5%	100.2%	93.0%	97.5%	36.2%	17.6%	9.4%	32.2%	36.9%	124.1%
		7500	2.3%	14.7%	14.6%	5.3%	7.8%	0.0%	0.3%	0.2%	0.5%	0.3%	0.2%	0.3%	86.9%	81.7%	76.1%	78.0%	86.2%	92.1%	101.1%	29.1%	12.2%	17.4%	44.8%	200.0%

B

% Transfected							% Dead							% Control							Transfection % CV						
siRNA	Lipid Vol	Cell #	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con
100 nM	0.8 µl	15000	4.5%	26.5%	35.3%	36.1%	27.2%	0.0%	0.5%	1.5%	2.2%	4.6%	1.8%	0.5%	92.0%	102.0%	91.6%	94.1%	102.1%	90.8%	40.1%	9.5%	7.7%	12.0%	10.4%	119.0%
		7500	14.1%	32.4%	43.1%	40.6%	41.9%	0.1%	0.8%	4.0%	3.3%	5.4%	2.7%	0.6%	87.4%	102.5%	93.6%	101.7%	82.8%	102.2%	15.9%	30.2%	23.6%	7.1%	13.7%	102.3%
	0.4 µl	15000	2.3%	9.3%	1.2%	11.2%	0.0%	0.0%	0.6%	0.5%	0.8%	0.8%	0.7%	0.9%	116.5%	128.1%	129.9%	117.9%	120.4%	101.4%	57.9%	53.6%	9.2%	76.9%	34.3%	200.0%
		7500	2.4%	13.2%	21.2%	4.8%	26.4%	0.0%	0.8%	0.9%	1.1%	1.1%	1.0%	0.9%	101.1%	119.4%	114.4%	127.8%	111.0%	110.9%	36.5%	28.6%	12.2%	33.1%	12.4%	117.6%
	0.2 µl	15000	0.2%	0.1%	2.6%	0.3%	0.4%	0.0%	0.7%	0.6%	0.7%	0.6%	0.6%	0.5%	114.0%	127.7%	126.0%	120.5%	122.5%	107.2%	30.0%	126.5%	14.8%	32.5%	85.1%	200.0%
		7500	0.2%	0.1%	6.3%	1.3%	2.2%	0.0%	0.8%	0.9%	1.1%	0.7%	0.7%	0.7%	114.0%	121.3%	115.8%	113.8%	116.2%	105.8%	30.5%	87.8%	15.6%	25.9%	31.3%	-
	0.1 µl	15000	0.0%	0.0%	1.1%	0.2%	0.0%	0.0%	0.7%	0.6%	1.0%	0.7%	0.5%	0.7%	114.0%	127.0%	109.0%	120.6%	122.3%	115.7%	124.0%	115.7%	37.9%	82.0%	81.0%	200.0%
		7500	0.1%	0.0%	2.4%	0.4%	0.1%	0.1%	0.7%	0.8%	0.8%	1.0%	1.1%	0.9%	101.6%	117.2%	109.3%	112.7%	108.8%	102.0%	69.2%	115.5%	12.5%	52.2%	67.1%	117.3%
50 nM	0.8 µl	15000	0.2%	10.6%	7.5%	11.7%	4.1%	0.0%	0.6%	0.7%	1.4%	0.9%	0.6%	0.6%	128.6%	124.6%	131.6%	128.3%	119.6%	100.8%	96.5%	20.5%	25.7%	13.0%	33.6%	-
		7500	0.3%	24.3%	18.6%	18.1%	14.2%	0.0%	1.3%	1.1%	3.0%	1.2%	1.0%	0.6%	117.3%	103.2%	105.8%	93.0%	111.4%	99.9%	42.3%	16.6%	16.8%	13.0%	16.8%	-
	0.4 µl	15000	0.1%	3.4%	9.8%	3.1%	5.2%	0.0%	0.5%	0.6%	0.8%	0.6%	0.6%	0.9%	126.4%	115.2%	119.4%	118.3%	125.8%	100.7%	57.5%	51.5%	26.2%	37.2%	54.2%	200.0%
		7500	0.1%	19.1%	23.6%	8.4%	23.0%	0.0%	1.4%	1.2%	1.5%	1.1%	1.1%	0.9%	112.5%	104.1%	96.2%	102.9%	100.6%	99.7%	38.6%	15.2%	15.1%	25.8%	11.7%	-
	0.2 µl	15000	0.1%	0.0%	2.8%	0.4%	0.3%	0.0%	0.7%	0.6%	0.6%	0.7%	0.7%	0.7%	104.0%	118.2%	128.2%	118.5%	114.3%	90.6%	31.5%	114.4%	15.2%	59.7%	45.5%	200.0%
		7500	0.4%	1.5%	6.8%	1.0%	5.0%	0.0%	1.1%	0.7%	0.8%	1.0%	0.8%	0.7%	104.5%	117.1%	110.6%	110.5%	99.4%	97.1%	64.6%	70.9%	18.2%	47.5%	42.0%	-
	0.1 µl	15000	0.1%	0.0%	1.3%	0.2%	0.0%	0.1%	0.6%	0.7%	0.7%	0.8%	0.5%	0.6%	108.3%	103.7%	102.6%	109.5%	106.7%	92.9%	127.1%	141.1%	23.8%	20.8%	-	112.7%
		7500	0.3%	0.2%	3.5%	0.5%	0.4%	0.0%	1.0%	1.6%	0.9%	0.8%	0.7%	0.9%	88.3%	90.8%	92.6%	78.3%	85.5%	82.3%	42.4%	81.0%	49.4%	13.4%	151.6%	200.0%

C

% Transfected							% Dead							% Control							Transfection % CV						
siRNA	Lipid Vol	Cell #	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con	LF	DF1	DF2	DF3	DF4	Con
100 nM	0.8 µl	15000	32.1%	63.9%	62.9%	55.4%	59.2%	0.0%	5.7%	14.4%	18.6%	10.3%	13.0%	3.3%	86.0%	56.2%	55.9%	67.7%	62.5%	89.2%	12.5%	13.4%	11.3%	4.2%	9.4%	96.7%
		7500	29.2%	61.4%	72.9%	55.4%	55.5%	0.1%	4.8%	13.8%	23.0%	12.7%	12.4%	4.0%	91.1%	78.5%	55.7%	77.1%	41.4%	97.9%	4.6%	11.8%	12.1%	8.1%	2.9%	55.3%
	0.4 µl	15000	2.1%	39.7%	42.5%	21.0%	45.7%	0.0%	4.8%	6.1%	7.4%	5.1%	6.4%	3.3%	104.1%	98.1%	97.5%	96.1%	100.0%	98.1%	31.0%	26.6%	9.2%	9.3%	7.7%	78.3%
		7500	7.7%	47.2%	44.4%	27.5%	49.4%	0.0%	4.2%	5.1%	7.7%	4.8%	5.7%	4.0%	85.5%	109.1%	88.4%	103.0%	101.0%	100.0%	47.1%	10.8%	6.8%	7.7%	14.4%	155.6%
	0.2 µl	15000	0.7%	3.5%	11.1%	3.8%	2.7%	0.1%	4.6%	6.5%	4.6%	5.1%	6.1%	4.2%	102.6%	97.8%	104.7%	100.6%	99.0%	99.4%	35.5%	27.2%	15.0%	11.3%	11.1%	27.5%
		7500	0.6%	15.1%	14.7%	7.4%	4.5%	0.1%	4.4%	5.8%	5.1%	5.6%	4.5%	3.5%	96.1%	89.9%	99.5%	87.7%	105.7%	104.3%	33.8%	47.3%	5.5%	23.1%	69.0%	36.8%
	0.1 µl	15000	0.4%	0.3%	5.8%	1.9%	1.1%	0.0%	4.0%	4.7%	5.2%	4.1%	5.6%	4.3%	96.6%	102.6%	102.8%	95.5%	96.9%	102.8%	13.2%	49.2%	12.0%	11.7%	58.5%	46.7%
		7500	0.5%	0.4%	7.0%	2.7%	1.1%	0.1%	4.2%	3.4%	5.1%	3.9%	3.5%	3.1%	98.3%	103.8%	92.6%	90.4%	88.0%	97.9%	28.5%	45.6%	19.2%	10.2%	36.6%	103.9%
50 nM	0.8 µl	15000	7.2%	46.9%	42.6%	45.8%	44.0%	0.0%	6.2%	6.2%	11.4%	8.6%	4.4%	3.5%	100.0%	103.5%	95.0%	87.6%	100.4%	101.5%	26.8%	8.3%	7.3%	9.2%	11.6%	-
		7500	14.6%	49.3%	49.0%	49.1%	45.1%	0.0%	4.7%	6.1%	16.2%	8.4%	7.7%	4.2%	89.7%	85.4%	64.0%	65.8%	85.9%	86.8%	28.7%	5.8%	5.9%	4.4%	5.6%	200.0%
	0.4 µl	15000	1.4%	44.4%	39.4%	24.7%	32.3%	0.0%	5.0%	4.1%	6.3%	5.4%	4.1%	4.0%	94.2%	108.8%	101.5%	102.4%	105.3%	97.5%	28.0%	8.7%	9.6%	7.7%	15.6%	79.3%
		7500	4.8%	44.9%	37.6%	26.9%	37.5%	0.0%	4.5%	3.9%	5.4%	5.0%	3.5%	3.6%	87.7%	111.1%	103.4%	101.6%	103.0%	99.4%	60.8%	14.4%	5.5%	13.1%	12.0%	88.7%
	0.2 µl	15000	0.7%	5.9%	18.9%	6.1%	2.8%	0.0%	4.7%	4.9%	4.5%	5.8%	6.6%	4.6%	100.9%	101.3%	99.2%	100.2%	95.9%	101.7%	12.3%	50.2%	16.6%	11.3%	20.7%	200.0%
		7500	0.7%	20.0%	19.1%	10.1%	6.6%	0.0%	5.5%	5.4%	5.4%	4.7%	4.7%	3.8%	99.4%	95.5%	87.9%	87.8%	96.3%	105.8%	26.3%	31.7%	15.1%	28.4%	21.9%	200.0%
	0.1 µl	15000	0.7%	0.3%	6.2%	2.4%	0.6%	0.0%	4.9%	3.8%	4.0%	4.0%	5.7%	3.5%	111.9%	110.1%	107.0%	102.7%	110.4%	109.8%	12.3%	55.0%	12.7%	10.0%	34.8%	131.8%
		7500	0.4%	1.0%	6.2%	2.1%	0.9%	0.0%	4.1%	3.9%	4.7%	4.1%	3.8%	2.7%	115.2%	114.4%	108.4%	105.6%	104.9%	108.0%	62.4%	23.3%	3.6%	23.1%	33.2%	128.8%

Figure 4. Integrated Biomek i7 instrument with HEPA-filtered enclosure was directly integrated with a Cytomat 2C incubator (right) and SpectraMax i3X Multi-Mode Detection Platform with SpectraMax MiniMax 300 Imaging Cytometer (left).

Table 1. Results with optimal transfection conditions for each cell line.

Cell Line	Lipid and Volume	siRNA	Cells plated	% Tf (541/BF)	% Dead (713/BF)	% Control (BF/BF)	Tf% CV	Equivalent Alternative
HCT116	0.4 µL DF4	100 nM	7500	54.2%	1.2%	96.5%	7.5%	0.4 µL DF2 50 nM siRNA
ACHN	0.8 µL DF3	100 nM	7500	46.6%	3.4%	101.7%	7.8%	
PANC1	0.8 µL DF1	50 nM	7500	49.3%	6.3%	85.6%	5.8%	0.8 µL DF4

CONCLUSION

- Different optimal transfection conditions were identified across cancer lines, illustrating the value of optimization.
  - ACHN cells require and tolerate higher concentrations of reagents than PANC1 and HCT116.
- Automation of this approach replaced ~138 manual transfers with single and multichannel pipettes and was completed in less than 20 minutes.
  - Approach can be applied to different transfection constructs and readouts.
- The flexibility of the Biomek i7 Automated Workstation, with its large deck capacity, multichannel and span pipetting features, and ease of integrations, means the same system can be used to apply these optimized conditions to large siRNA screens.
  - These screens can benefit from the 1200 µL multichannel head, which still has the ability to precisely transfer low volumes used in the 384-well assay described here.

*Data obtained during development
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