Application Protocol

mRNA Transfection of Somatic Cells

Introduction

This Application Protocol describes the method of transfecting adherent somatic cells, plated on two wells of a 24-well plate, with an mRNA cocktail.

Required Consumables

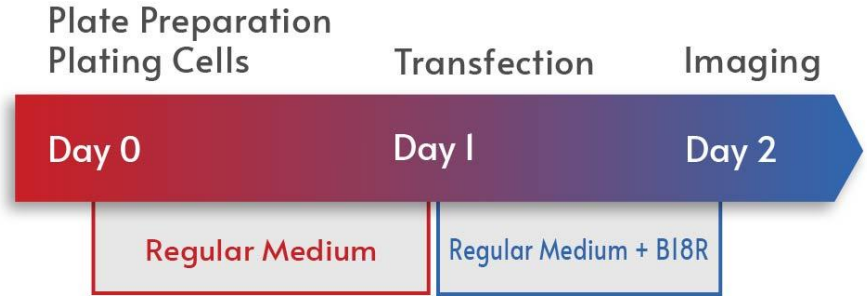
Item	Vendor	Catalog Number
24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
Lipofectamine MessengerMAX	ThermoFisher	LMRNA001
Opti-MEM I Reduced Serum Medium	ThermoFisher	31985062
Cell dissociation reagent (e.g., TrypLE Select Enzyme (1X))*	ThermoFisher	12563011
0.02% EDTA in DPBS*	Sigma-Aldrich	E8008-100ML
B18R recombinant protein	STEMCELL Technologies Inc.	78075
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
mRNA of choice (e.g.: EGFP)	varies	varies

*Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Technical Support

For technical support, please contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

Workflow



Day 0

1-4 hours

Plate preparation

If the somatic cells of interest require special coating conditions for their adhesion, prepare wells for plating them. Otherwise regular tissue culture-treated plasticware supports cell adhesion and no additional coating is needed.

Passaging

1. Dissociate the cultured somatic cells (e.g., human dermal fibroblasts from neonates or HEK293 cells) using a cell dissociation reagent such as TrypLE Select (1x).
2. Neutralize the cell dissociation reagent using the same volume of 10% FBS or by dilution.
3. Harvest cells and plate 1x 10^4 ~3 x 10^5 cells per well of a 24-well plate.
   Note: A lower plating density is recommended for cells that double once a day if cells are kept several days without passaging.

Day 1

< 5 hours

Medium Change

1. Warm medium for 20-30 minutes at room temperature.
2. Transfer 3.5 ml of the medium to a tube and add B18R to a concentration of 200 ng/ml.
3. Aspirate the old medium from the wells and add 500 µl medium with 200 ng/ml B18R
4. Incubate the culture at 37°C, 5% CO_2 for a minimum of 2 hours

Transfection

1. Thaw a vial of mRNA on ice for 30 minutes and warm Opti-MEM and medium at room temperature for 20-30 minutes.
2. Prepare transfection complex by the following steps:
   - Prepare two 1.5 ml tubes with 100 µl Opti-MEM each. Label one tube “Mix 1” and the other tube “Mix 2”.
   - Add 4 µl Lipofectamine MessengerMax (LMM) to the Mix 1 tube and mix by brief vortexing. Leave it at room temperature for 10 minutes (Mix 1).
   - **IMPORTANT!** Immediately before 10 minutes pass (i.e., around 8 minutes), add 2 µg mRNA to the other 1.5 ml tube with Opti-MEM (Mix 2). There is no need to mix it at this point.
   - 10 minutes after mixing LMM with Opti-MEM, add Mix 2 into Mix 1, and pipet up and down 8-10 times.
   - Leave at room temperature for 5 minutes and no longer.

Mix 1 Reagents	Volume
Opti-MEM	100 µl
LMM	4 µl

Mix 2 Reagents	Volume
Opti-MEM	100 µl
mRNA	2 µg

3. Add 1 ml Medium with 200 ng/ml B18R to the Mix (for a final volume of 1.2 ml) and pipet up and down 2-3 times to mix.
4. Aspirate the old medium out and add 600 µl of mixture to each well.
5. Incubate the culture plate at 37°C, 5% CO_2 for 2.5 hours.
6. Aspirate the old medium with RNA-LMM complex and add 500 µl of fresh medium with 200 ng/ml B18R.
7. Incubate the culture plate at 37°C, 5% CO_2 overnight.

Day 2

< 1 hour

Assay or Continuous Culture

- On Day 2 cultures are ready for imaging.
- Cells can be kept in culture under regular culture conditions hereafter.

Appendix

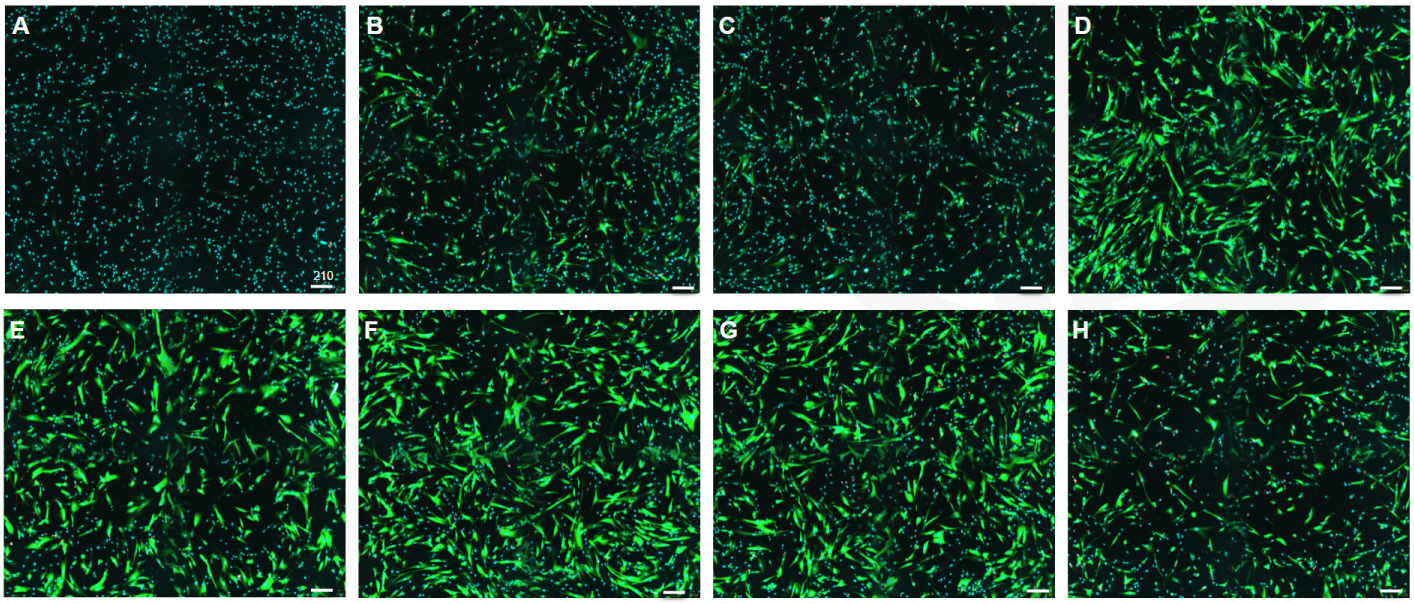


Figure 1. Fluorescence images of human neonatal dermal fibroblasts (HDFn) transfected with a variety of EGFP mRNAs (scale bars = 210 μm). EGFP mRNAs were transcribed *in vitro* using the following modified nucleotides in combination with unmodified ones: 5MeCTP and PseudoUTP (A & B), 5MeCTP and N1-Me-PseudoUTP (C), 5MeCTP and 5MoUTP (D), PseudoUTP (F), N1-Me-PseudoUTP (G) and 5MoUTP (H), and unmodified nucleotides only (E). All EGFP mRNAs were capped enzymatically, except for the one transfected in A that was co-transcriptionally capped using the anti-reverse cap analogue. 50,000 cells were plated in each well of a 96-well plate and transfected as instructed in this Application Protocol although without B18R treatment. Nuclei were counterstained with Hoechst 33342.

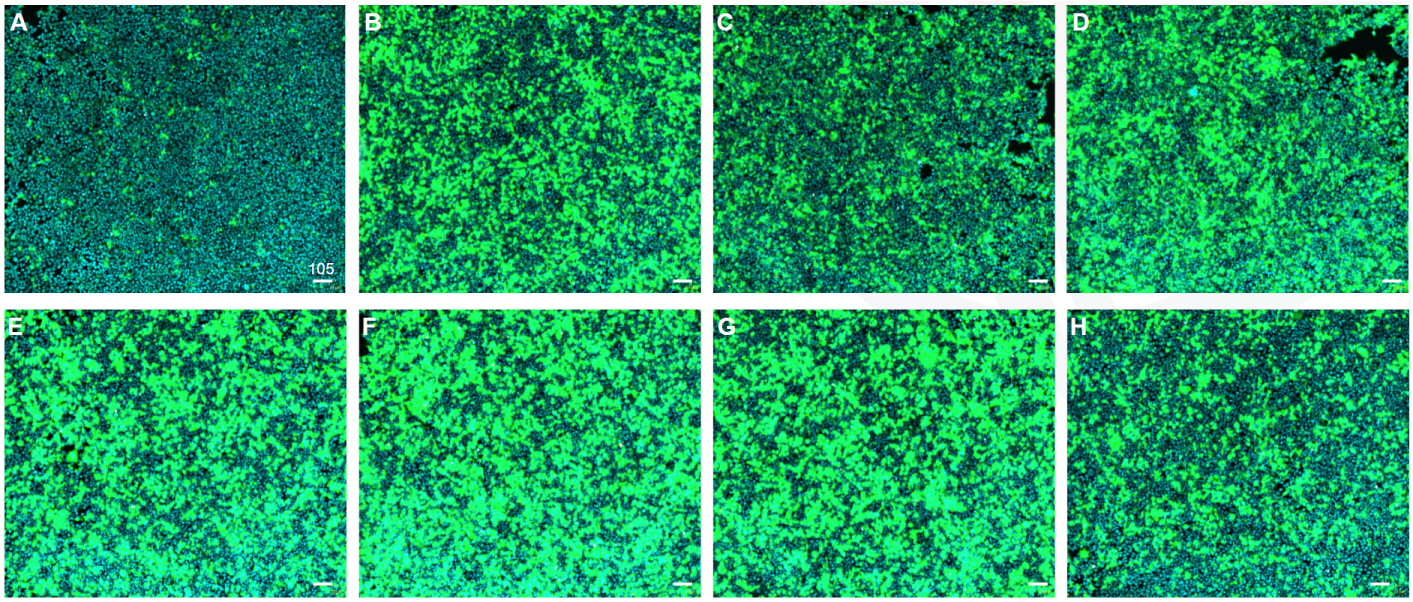


Figure 2. Fluorescence images of human embryonic kidney epithelial cells (HEK293T) transfected with a variety of EGFP mRNAs (scale bars = 105 μm). EGFP mRNAs were transcribed *in vitro* using the following modified nucleotides in combination with unmodified ones: 5MeCTP and PseudoUTP (A & B), 5MeCTP and N1-Me-PseudoUTP (C), 5MeCTP and 5MoUTP (D), PseudoUTP (F), N1-Me-PseudoUTP (G) and 5MoUTP (H), and unmodified nucleotides only (E). All EGFP mRNAs were capped enzymatically, except for the one transfected in A that was co-transcriptionally capped using the anti-reverse cap analogue. 50,000 cells were plated in each well of a 96-well plate and transfected as instructed in this Application Protocol although without B18R treatment. Nuclei were counterstained with Hoechst 33342.

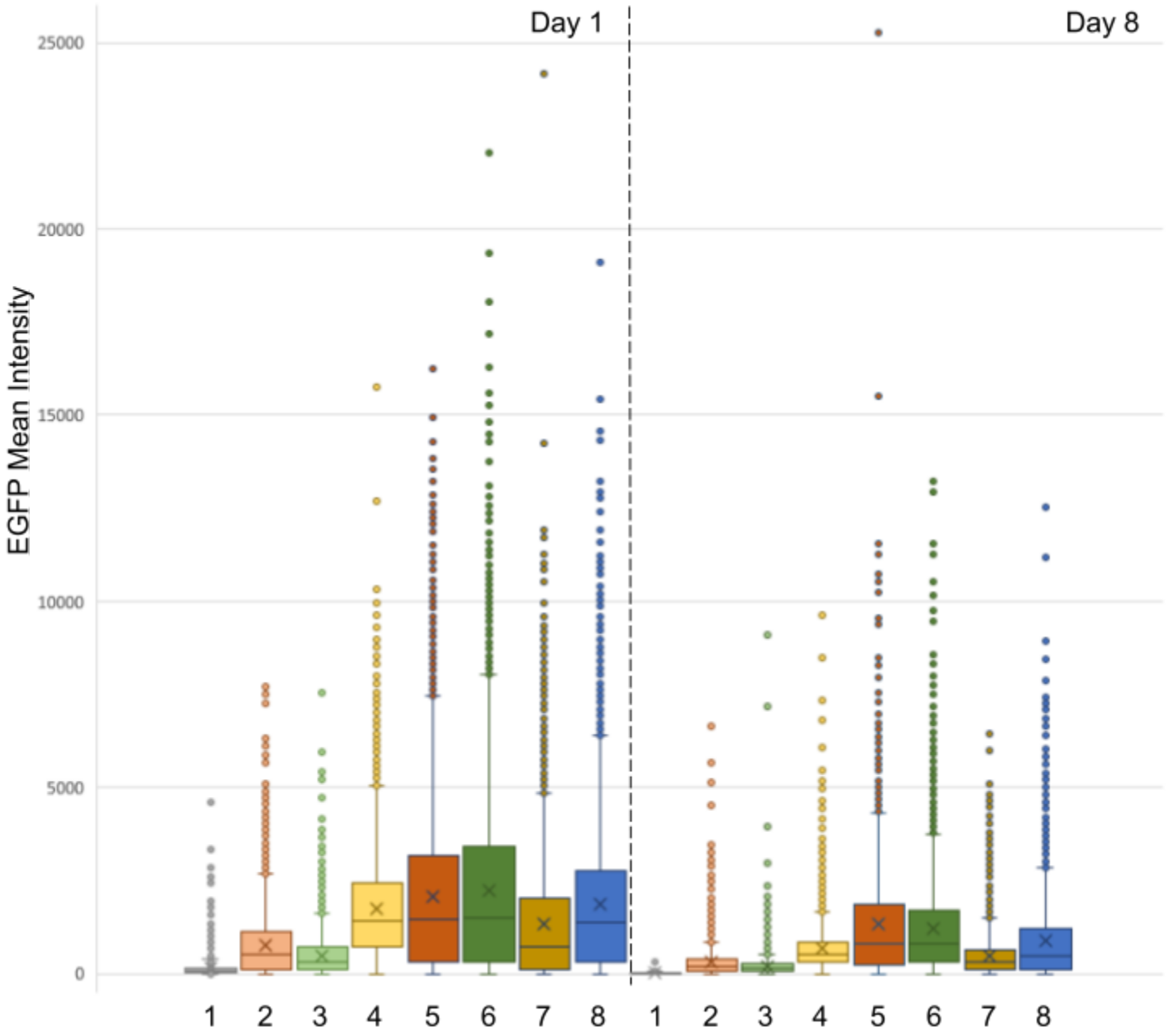


Figure 3. Box plots representing the mean intensities of EGFP fluorescence per cell as quantified using fluorescence images of HDFn transfected with a variety of EGFP mRNAs (Figure 1). Data were obtained using images taken 1 day (left) and 8 days (right) after transfection. Transfected EGFP mRNA contained the following modifications: 5MeCTP, PseudoUTP and ARCA (1), 5MeCTP, PseudoUTP and Cap 1 (2), 5MeCTP, N1-Me-PseudoUTP and Cap 1 (3), 5MeCTP, 5MoUTP and Cap 1 (4), PseudoUTP and Cap 1 (5), N1-Me-PseudoUTP and Cap 1 (6), 5MoUTP and Cap 1 (7), and unmodified nucleotides only and Cap 1 (8).