TRANSFECTION

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BACKGROUND

- Cell transfection is the act of deliberately introducing a novel nucleic acid into cells in a cell culture. In the Bowdish laboratory, the transfection of mouse or human MARCO DNA into cell lines is commonly used as part of larger protocols.

NOTES

- There are many different ways to transfect cells; this is just one set of guidelines. There are multiple transfection reagents that can be used; this protocol is based on the use of polyethylenimine (PEI) with the HEK 293T cell line. PEI is the basis of most commercially available transfection agents and alone acts as a very cost effective transfection vector. For a summary of transfection efficiency results with PEI at different concentrations and compared to other commercially available transfection agent, please see the Results section below.
- The **working** solution of PEI is 1ug/1ml (1:1000). PEI is amazingly viscious, however, so it may be easier to first make a 1:100 solution. Aliquots of the working solution should be stored at -80°C until needed. Our stock solutions are 1mg/ml.

EQUIPMENT

- Equipment:
  - Level 2 Bio Hood, Tissue Culture Room (MDCL 4097) (see Level 2 Bio Hood SOP)
  - 37°C, 5% CO_2 Incubator, Tissue Culture Room (MDCL 4097) (see Incubator SOP)
  - Centrifuge, Tissue Culture Room (MDCL 4097) (see Centrifuge SOP)
  - Vortex, Tissue Culture Room (MDCL 4097) (see Vortex SOP)
- Materials:
  - Cells in culture (in a T-series flask or cell culture dish)
  - PBS (stored at 4°C, Tissue Culture Room)
  - Incomplete DMEM "serum free media" (stored at 4°C, Tissue Culture Room)
  - Dulbecco's Modified Eagles Medium (DMEM) (stored at 4°C, Tissue Culture Room)
  - PEI stock solution (stored at -20°C, Darwin, Bowdish Laboratory) diluted from branched polyethylenimine – makes cells more porous (Sigma, Co#: 408727)

PROTOCOL

- Preparatory Work:
  - The day before transfection, plate 1 x 10^5 cells into 6-well plates in DMEM. The number of cells may differ depending on the cell line and plates used.

- In the Tissue Culture room, place the PBS, serum free media, and DMEM in the 37°C water bath approximately 30 minutes before the initiation of the protocol. Be sure to adorn your tissue culture specific laboratory coat and gloves upon entering the room.
- Sign out a Level 2 Bio Hood in the Tissue Culture room. Spray down all surfaces of the workspace with 70% ethanol solution. Set up your workspace with a clear biohazard bag, liquid waste container, and sharps container.
- Don't use small tips in DMEM (risks contamination). Instead, pour into a conical tube and take out of that.

1. For each well of a 6-well plate to be transfected, mix 100μL of Incomplete DMEM "serum free media" and 2ug of your DNA-of-interest in a polystyrene tube (falcon white top). Serum interferes with the formation of transfection complexes.
2. Add 12ul of PEI stock solution. Immediately pulse vortex 15 times for 1 second each.
3. Incubate the solution at room temperature for 10 min.
4. Add 600ul of Complete DMEM to the solution.
5. Remove old media from the 6-well plate and wash once with PBS.
   -Note: If transfecting a large number of wells, it is best to do it one plate at a time to prevent wells from drying out.
6. Add the total solution (~700ul) to the well dropwise (or just pour in). Distribute drops over the entire well. Gently rock the plate to ensure even distribution. Do not swirl plate.
7. In 2-3 hours, add approximately 2mL more complete DMEM.
8. Incubate cells for 24 or 48 hrs in the 37°C, CO_2 Incubator.

- Concluding Work:
  - Place all solid waste into the clear biohazard bag within the workspace. In the Level 2 Bio Hood, close the bag. While holding it closed, remove the bag from the workspace and tie closed with a twist tie.
  - Place all liquid waste into the liquid waste container. Add approximately 10% of the waste volume in bleach. Leave the solution within the Level 2 Bio Hood for 30 minutes. After this time, pour the solution down the sink while letting the water run for approximately 3 minutes.
  - Remove all other tools from the workspace. Spray down the workspace with 70% ethanol.

REFERENCE:

J Immunol. 2013 Jan 1;190(1):250-8. doi: 10.4049/jimmunol.1202113. Epub 2012 Nov 28. MARCO Is Required for TLR2- and Nod2-Mediated Responses to *Streptococcus pneumoniae* and Clearance of Pneumococcal Colonization in the Murine Nasopharynx. Dorrington MG, Roche AM, Chauvin SE, Tu Z, Mossman KL, Weiser JN, Bowdish DM.

http://www.jimmunol.org/content/190/1/250.long

RESULTS

Our laboratory has found transfection with polyethylenimine (PEI) to be very successful and cost effective. From the results displayed in Figure 1 and quantified in Table 1, we determined that the optimal concentrations were 10ul of PEI mixed with 2ug of DNA.

Table 1: Quantification of flow cytometry results of polyethylenimine (PEI) transfection. Transfection efficiency was tested in HEK293T cells with various concentrations of PEI and DNA. Results were collected 24 hours post transfection using flow cytometry. Transfection efficiency was measured using GFP expression of the transfected GFP-N1 plasmid.

		DNA (ug)				
PEI (ul)		1	1.5	2	4	0 ctrl
	8	57	98	98.8/98.6	96.6	ND
	10	31	88.8	98.6/97.9	98.4	ND
	12	98.7	98.1	ND	ND	ND
	14	45.4	77.3	ND	ND	ND
	SF ctrl	ND	ND	76.3/67.3	ND	ND
	0 ctrl	ND	ND	ND	ND	5.2/3.53

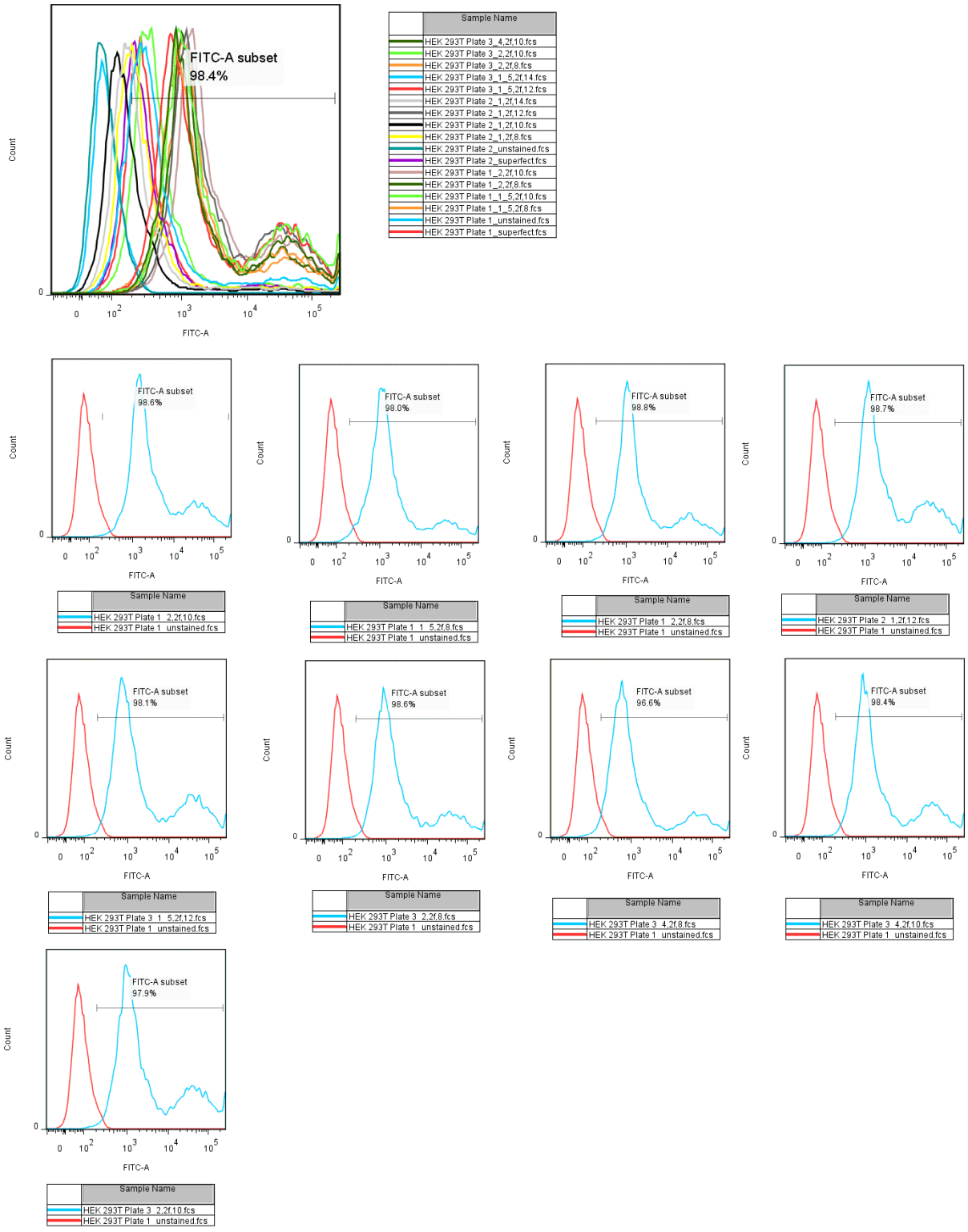

Figure 1: Results of flow cytometry analysis of the transfection efficiency of polyethylenimine (PEI) in HEK293T cells. Transfection efficiency was evaluated using various concentrations of PEI and DNA. Transfection efficiency was measured using GFP expression of the transfected GFP-N1 plasmid.