PEI TRANSFECTION

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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 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 viscous, 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.

EQUIPMENT AND MATERIALS

- Materials:
    - DMEM, both completed with 10% FBS and 2mM L-glutamine and incomplete
    - PBS
    - PEI (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.
    - Warm PBS, and complete and incomplete media to 37°C.

1. Mix DNA to be transfected in incomplete media at a ratio of 1ug DNA in 100ul media.
    a. For transfection in a 6-well plate, use 1ug of DNA. For a 10cm dish, use 5ug.
    b. If total DNA does not add up, add the difference in empty vector DNA.
2. Add 12ul of PEI per 100ul of incomplete DMEM. Immediately pulse vortex for 15 seconds.
3. Incubate the transfection mix at room temperature for 10 min.
4. Add 600ul of incomplete DMEM per 100ul of transfection mix.
5. Add the total solution to the cells drop-wise. Gently rock the plate to ensure even distribution. Do not swirl.
6. Incubate at 37°C for 3h. Add 2ml complete DMEM.
7. Incubate cells for up to 48h at 37°C.

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)				
		1	**1.5**	**2**	**4**	**0 ctrl**
PEI (ul)	**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

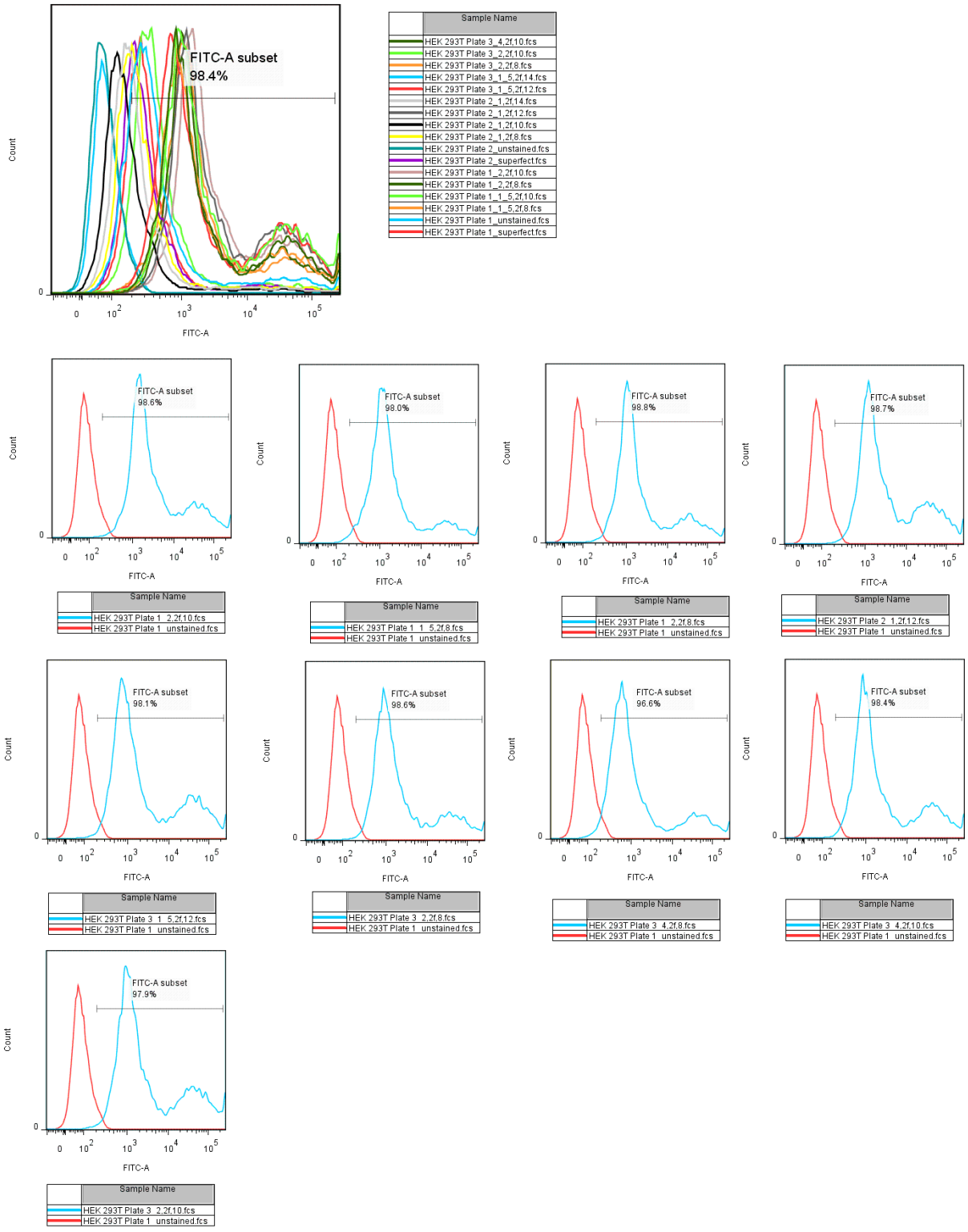

Figure 1: Results of flow cytometry analysis of the transfection efficiency of polyethylenimine (PEI) in HEK293T cells. Transfection efficiency was evaluated using various concentrations of PEI and DNA. Transfection efficiency was measured using GFP expression of the transfected GFP-N1 plasmid.