TRANSFECTION OF HEK293T CELLS INTO 24-WELL PLATES

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BACKGROUND:

Transfection is the process by which nucleic acids are deliberately introduced into mammalian cells. In the Bowdish laboratory, the transfection of mouse or human Nod1 and Nod2 DNA into cell lines is commonly used as part of larger protocols.

NOTES:

☐ There exists many efficient ways to transfect cells; this is just one set of guidelines. Multiple transfection agents can be used, however this protocol is based on the use of polyethylenimine (PEI) with the HEK293T cell line. PEI is a very cost effective transfection factor which allows for external DNA to be endocytosed, and subsequently gain access to mammalian host DNA.

☐ The working solution of PEI is 1mg/1ml (1:1000). Aliquots of the working solution should be stored at -80°C until needed.

☐ Depending on the number of wells you wish to transfect, do the calculations in advance to allow for a more rapid procedure. *For example, if you are transfecting 2 24-well plates, multiply quantities per well by 48. You can then use a single tube for this volume, instead of 48 different tubes.*

☐ If attempting a stable transfection, the DNA of interest should be linearized prior to transfection. Furthermore, it is suggested that Superfect, instead of PEI, may increase efficiency of transfection when establishing stable lines.

☐ If stimulating transfected cells with DNA that results in expression of receptors on cell interior, add ligand prior to incubation of solution with PEI. This will allow ligand to be included in the liposome that is internalized by the cell

EQUIPMENT & MATERIALS:

- Level 2 Bio Hood, Tissue Culture Room (MDCL 4097)
- 37°C, 5% CO2 Incubator, Tissue Culture Room (MDCL 4097)
- Vortex, Tissue Culture Room (MDCL 4097)

- Serum Free Media heated to 37°C in water bath
- Complete Dulbecco's Modified Eagle Medium (DMEM) with 1% FBS, heated 37°C in water bath
- PEI working solution thawed on ice
- DNA of interest, empty vector & reporter DNA
- 24-well plate(s) with seeded cells (see below)

PROTOCOL:

Preparatory work:

- The day prior to transfection, plate $1x10^4$ cells per well into a 24-well plate in Complete DMEM. Depending on the cell line, the number of cells may differ
Note: cells should be seeded when approximately 60-70% in confluency
- Place serum free media and 1% FBS DMEM in the 37°C water bath, located in Tissue Culture room (MDCL 4097) **Note:** Wear laboratory coat and gloves upon entering Tissue Culture room

1. For each 24-well plate to be transfected, mix 25ul serum free media and 450ng of your DNA of interest into a polystyrene tube.
The amount of DNA to be used is dependent on the type of DNA. For transfections with hNod1/hNod2, 5ng of the total 450ng was either hNod1 or hNod2 and the remainder was an empty pcDNA plasmid.

2. If cell line does not consist of a reporter, add 50ng of reporter per well to solution.
A SEAP-NFkB reporter worked well for these transfections.

3. Add 3ul of PEI (per well). Immediately pulse vortex for 10 seconds.

4. Allow solutions to incubate at room temperature for 10 minutes. In the meantime, discard old media from 24-well plates.

5. After incubation time, 150ul per well of serum free media was added to the solution.
It might be easier to add the 150ul of media directly to the wells of the plate rather than to each solution.

6. Add each PEI solution in a drop-wise manner to the corresponding cells. Make sure that drops are distributed over entire well. Gently rock the plate to ensure even distribution. Do not swirl plate.

7. Incubate the plate(s) in the 37°C, CO2 Incubator. The duration required for transfection to occur depends on the cell line and the DNA being transfected.
For transfection of hNod1 or hNod2 DNA into HEK293T cells, it takes between 48-72 hours

8. After 3 hours of incubation, add 1mL of 1% FBS DMEM to each well.
If a SEAP-NFkB reporter was also transfected, you may add 1mL of HEK Blue Detection Media (Invivogen) instead of the 1% FBS DMEM. This will allow for real-time detection of SEAP expression.

CLEAN-UP:

☐ 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 or until solution is clear. 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.