Comparing the Infectious Efficiency of Different Transfection Methods

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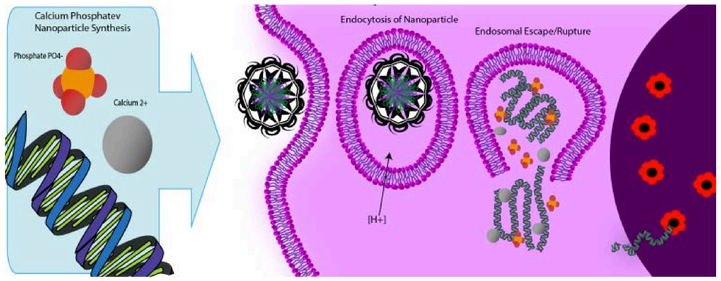
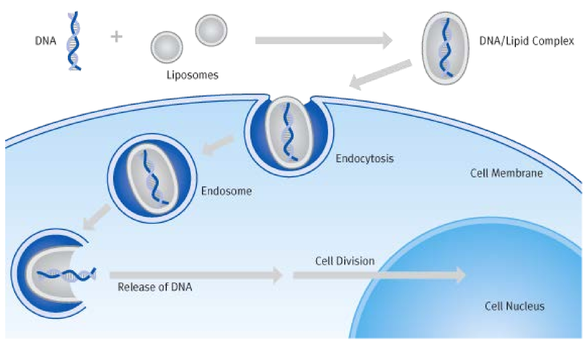
The Problem

The infections of viral particles into cells are resulting in astounding discoveries in the scientific world. These infections can induce the cell into acting a certain way such as knock down and overexpressing target sequences in which the scientist infecting the cell desires. However, a lot of problems may occur during infection. Infectious failure is a large problem which scientists in a variety of fields cannot avoid.

There exists many methods which scientists often use to infect various viral particles into cells. To obtain viral particles, the traditional method used is the Calcium Phosphate method, where the permeability of the membrane is increased to allow for plasmid to enter. Once the plasmid enters, it is combined with various substances to eject viral particles. In contrast, a slightly newer method has enjoyed favorable results worldwide, called Lipofection. Lipofection is a more expensive method where the cell swallows the plasmid. The lipid carrying the plasmid DNA has a positive charge and it binds to the negatively charged membrane, allowing the cell to swallow the lipid. The problem exists in comparing the efficiency of the two methods.

CaP and Lipofection

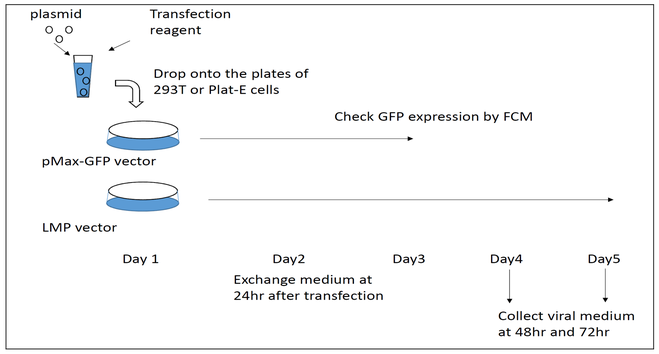




293T and Plat-E cells were used for comparing the infectious efficiency. Both types of cells are established from the fetal kidneys of mice. For 293T cells, the CaP method is known to be the very efficient. So far, we tried to employ CaP method for transfecting into Plat-E cells. However, compared to 293T CaP method, infectious efficiency by using Plat-E cells CaP was less sufficient. Therefore, we would like to scrutinize other optimal transfection methods for Plat-E cells.

The Plat-E cells contain a viral packaging sequence so the sequence is only injected into 293T cells through the pCL vector. The sequence, along with the plasmid, combine with a variety of substances(depending on the method) and eject viral particles.

Transfection

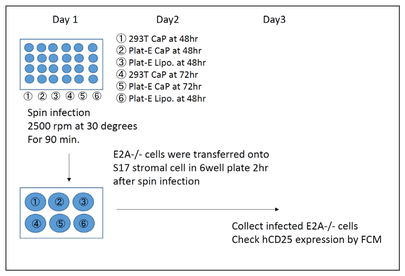


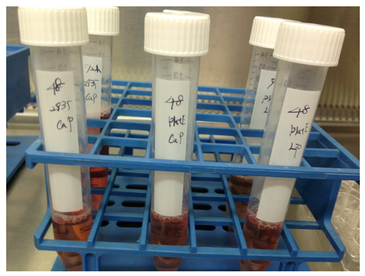
In the CaP method, CaCl, plasmids (5mg of GFP vector containing GFP sequence or 21mg of LMP vector containing hCD25 sequence with or without 10ug of pCL vector), and 2xHBS(containing phosphate) were mixed together. In the lipofection method, the plasmids were mixed with a lipofection reagent (X-treme gene 9 transfection reagent).

There were three plates for transfection: 293T cells using the CaP method and Plat-E cells using the CaP and lipofection method respectively. Three more plates were made for those that used the LMP vector instead of the GFP vector. The GFP vector gave off fluorescent light indicating the success of transfection, whereas LMP indicates the success of infection.

Transfection was carried out and the medium was exchanged 24 hours(hr) later, the GFP expression checked at 24 hr after exchanging medium, and the viral particles collected at 48 hr and 72 hr after exchanging medium.

Spin Infection

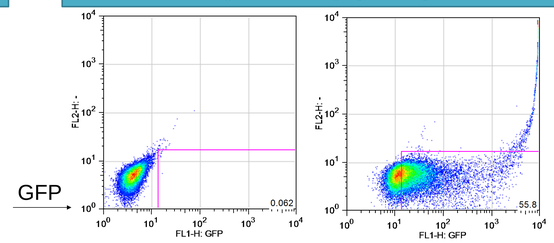




The purpose of spin infection is to infect the viral particles into the target E2A and EBF knockout cells, which were established from fetal mice liver. I mL of viral particles and 1 million target cells were mixed in a well in 24 well plate and spun at 30 degrees at 2500rpm for 90 minutes. The viral particles entered the cell, infecting them.

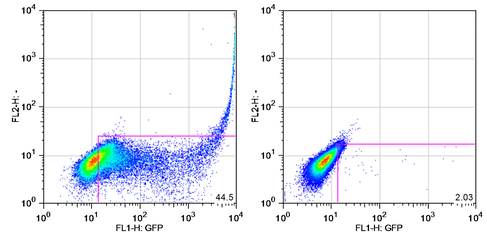
Spin infection was carried out in two different sets at 48 and 72 hours. There were six plates for viral particles of: 293T CaP, Plat-E CaP, and Plat-E Lipofection respectively. There were two pairs of each, one for each set. All of these cells contain the LMP vector, which contains the sequence for human CD25, an antigen which an antibody attaches to, giving off a fluorescent light for the indication of infection success.

Flow Cytometry (FCM) Results for Transfection

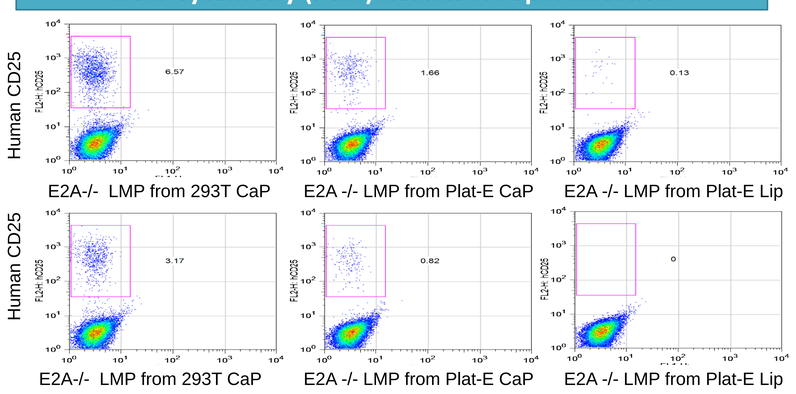


The gated population indicates that the cells successfully expressed the GFP protein after transfection.
The top left graph is the control, it is plain 293T cells without transfection. The top right graph represents the 293T cells that expressed GFP under the CaP method.

The bottom left graph represents the Plate-E cells that expressed GFP under the CaP method. The bottom right graph shows the Plate-E cells that expressed GFP under lipofection.



Flow Cytometry (FCM) Results for Spin Infection



The gated population represents the cells that successfully expressed human CD25 protein in murine cell line E2A-/- cells. The FCM in the top row shows the 293T cells and Plat-E cells used by CaP as well as the Plat-E cells with the lipofection. The bottom row shows the same cells cultured for 72 hours in contrast to 48 hours.

CONCLUSIONS AND FUTURE DIRECTIONS

- **The CaP method is much more efficient for transfection than lipofection is.**
- Instead of the separate independent experiments we did, combine them if given more time and use the same plasmid/viral particles for transformation, transfection, etc.
- Test another transfection method with CaP to see which one is better.
- Try to carry out transfection with other cells.
- Use other cells for infection.
- Use another antigen instead of human CD 25.
- Use another type of DNA fragment in the LMP vector instead of shRNA, see if the results stay constant.

References

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