

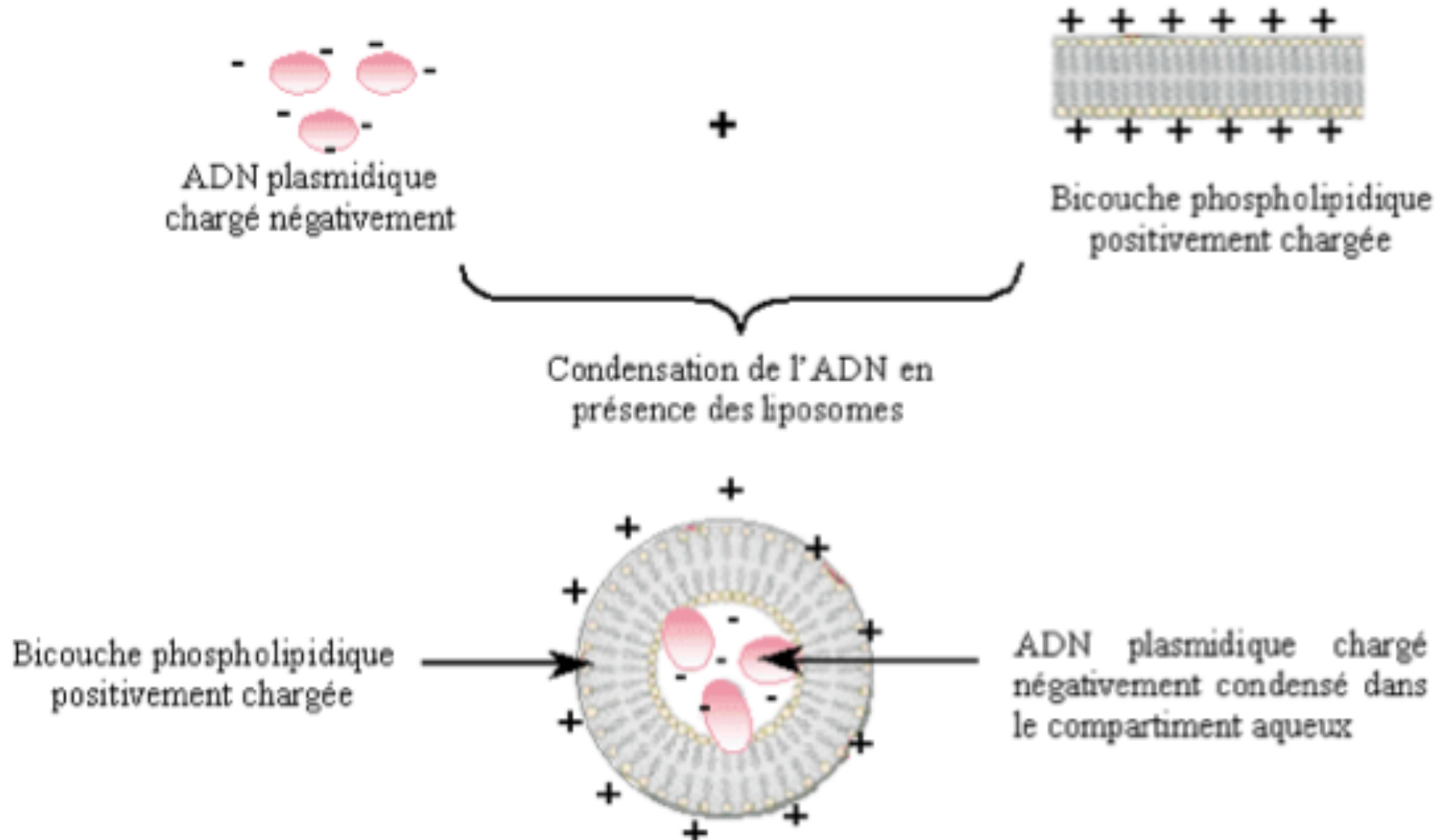
# DNA Engineering: M1D6 Lab Talk

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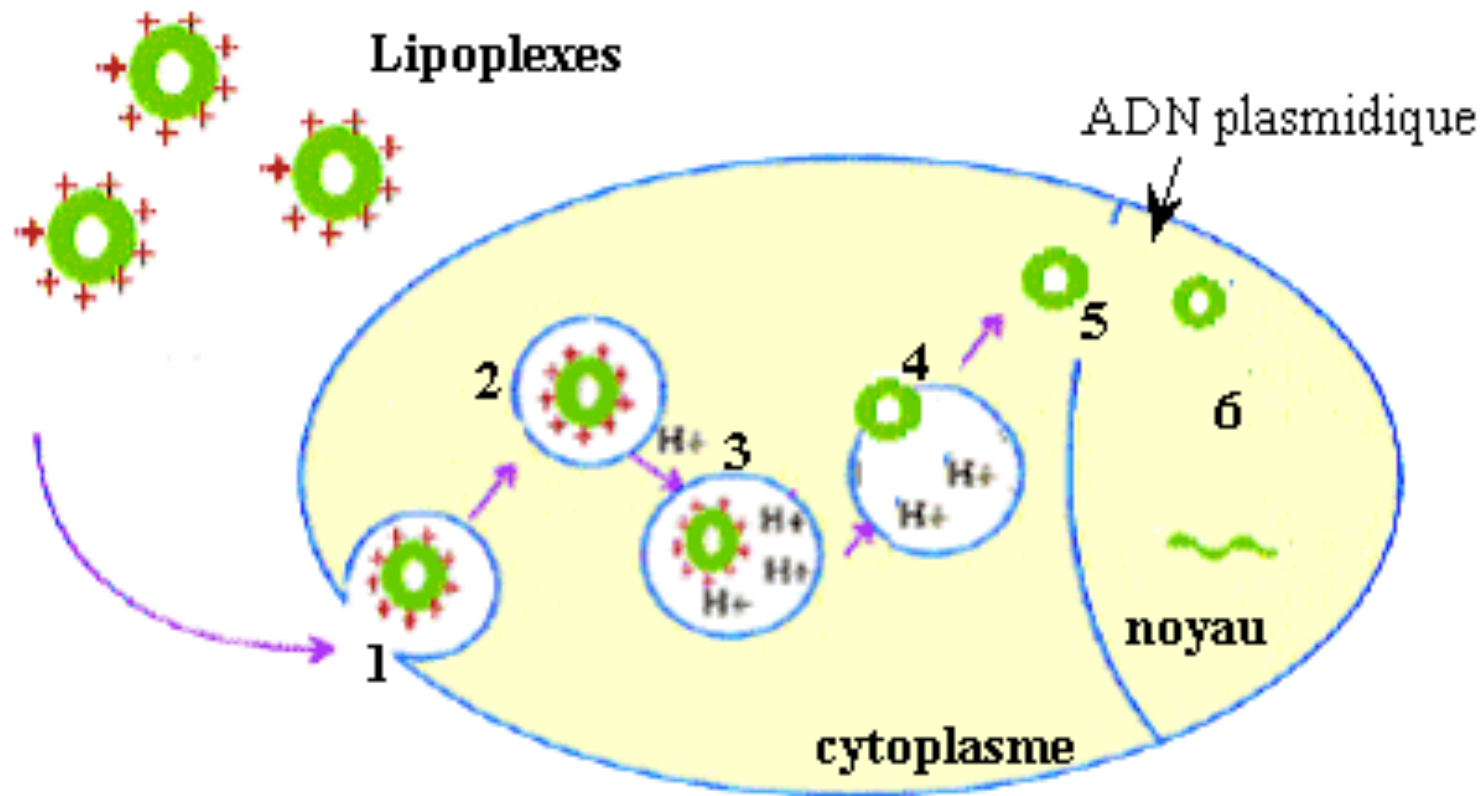
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# Lipofection



[http://www.unilim.fr/theses/2003/sante/2003limo0100c/these\\_body.html](http://www.unilim.fr/theses/2003/sante/2003limo0100c/these_body.html)

# Lipofection



# Lipofection

*Proc. Natl. Acad. Sci. USA*  
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Biochemistry

## **Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure**

(liposomes/cationic lipid vesicles/gene transfer)

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# Lipofection

Table 1. Transient transfections: Lipid compared with DEAE-dextran

| Cell line | Lipid, $\mu\text{g}$ | DEAE-dextran, mg/ml | Time, hr | DNA, $\mu\text{g}$ | CAT specific activity, % maximal |
|-----------|----------------------|---------------------|----------|--------------------|----------------------------------|
| JZ.1      | 100                  |                     | 3        | 5                  | 19                               |
|           | 100                  |                     | 3        | 25                 | 100                              |
|           | 150                  |                     | 3        | 25                 | 80                               |
|           |                      | 0.25                | 5        | 5                  | <1                               |
|           |                      | 0.25                | 5        | 25                 | 1                                |
|           |                      | 0.25                | 16       | 25                 | <1                               |
| CV-1      | 100                  | 0.50                | 5        | 25                 | 1                                |
|           | 100                  |                     | 5        | 10                 | 100                              |
| COS-7     | 100                  | 0.25                | 5        | 10                 | 9                                |
|           | 100                  |                     | 5        | 1                  | 100                              |
|           |                      | 0.25                | 5        | 1                  | 7                                |
|           |                      | 0.25                | 5        | 10                 | 16                               |
|           |                      | 0.50                | 5        | 1                  | 7                                |
|           | 0.50                 | 5                   | 10       | 11                 |                                  |

Transfections were carried out as described in *Methods*; lipid corresponds to PtdEtn/DOTMA (1:1). Each cell line represents an independent experiment, and in each case the transfection that yielded the highest level of CAT activity was set at 100%. All transfections were performed in duplicate, and CAT assays from each were performed in duplicate.

# Lipofection

Table 2. Stable transfection: Lipid compared with calcium phosphate

| Cell line             | Plasmid  | Method            | Frequency<br>$\times 10^5$ |
|-----------------------|----------|-------------------|----------------------------|
| L-M(TK <sup>-</sup> ) | pSV2neo  | Calcium phosphate | 3                          |
|                       |          | Lipid             | 45                         |
| $\psi$ -2             | pZIPS VX | Calcium phosphate | 0.6                        |
|                       |          | Lipid             | >49                        |
| $\psi$ -2             | pZIPC5a  | Calcium phosphate | 1.8                        |
|                       |          | Lipid             | >68                        |
| MSN610.2              | pSV2neo  | Calcium phosphate | 1.3                        |
|                       |          | Lipid             | 8.2                        |
| TA1                   | pSV2neo  | Calcium phosphate | 2                          |
|                       |          | Lipid             | 14                         |
| TA1                   | pZIPS VX | Calcium phosphate | 0.7                        |
|                       |          | Lipid             | 17                         |
| TA1                   | pMSGC5a  | Calcium phosphate | 1.3                        |
|                       |          | Lipid             | 19                         |

Cells were transfected with 7  $\mu$ g of the indicated plasmid with no carrier DNA, except for pSV2neo where 1  $\mu$ g of plasmid and 10  $\mu$ g of carrier DNA were used. The transfection frequency is the number of drug-resistant colonies expressed as a fraction of the total number of cells plated.

# Lipofection

# of publications citing Lipofectamine  
2000 transfection reagent

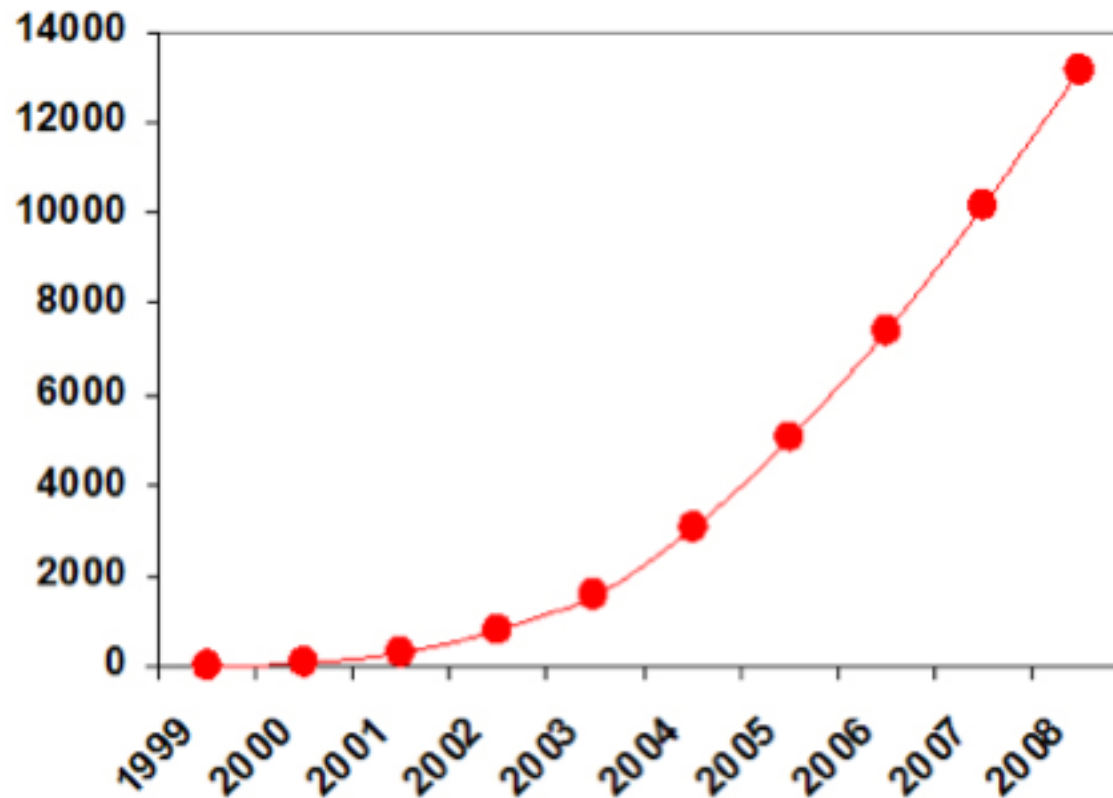


Figure 2. Number of publications citing the use of Lipofectamine™ 2000 transfection reagent since 1999.

<http://www.invitrogen.com/site/us/en/home/brands/lipofectamine.html>

# Your experiment and your expectations

Dilute Lipofectamine

(x16.5)

in *OptiMEM*

Dilute and mix DNA

(x1)

mix (1:1) and incubate



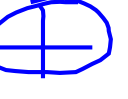



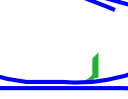

Mock

RT

GFP

$\Delta 5$

$\Delta 3$

|            |   |   |                             |
|------------|---|---|-----------------------------|
|            |    |    | $\Delta 5 + \Delta 3$       |
| GFP        |   |   | $\Delta 5 + \Delta 3 + Pme$ |
| $\Delta 5$ |  |  | $\Delta 5 + \Delta 3 + Bam$ |
| $\Delta 3$ |  |  |                             |



# Your experiment and your expectations

What could you do to change frequency of homologous recombination of delta5 and delta3?

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