

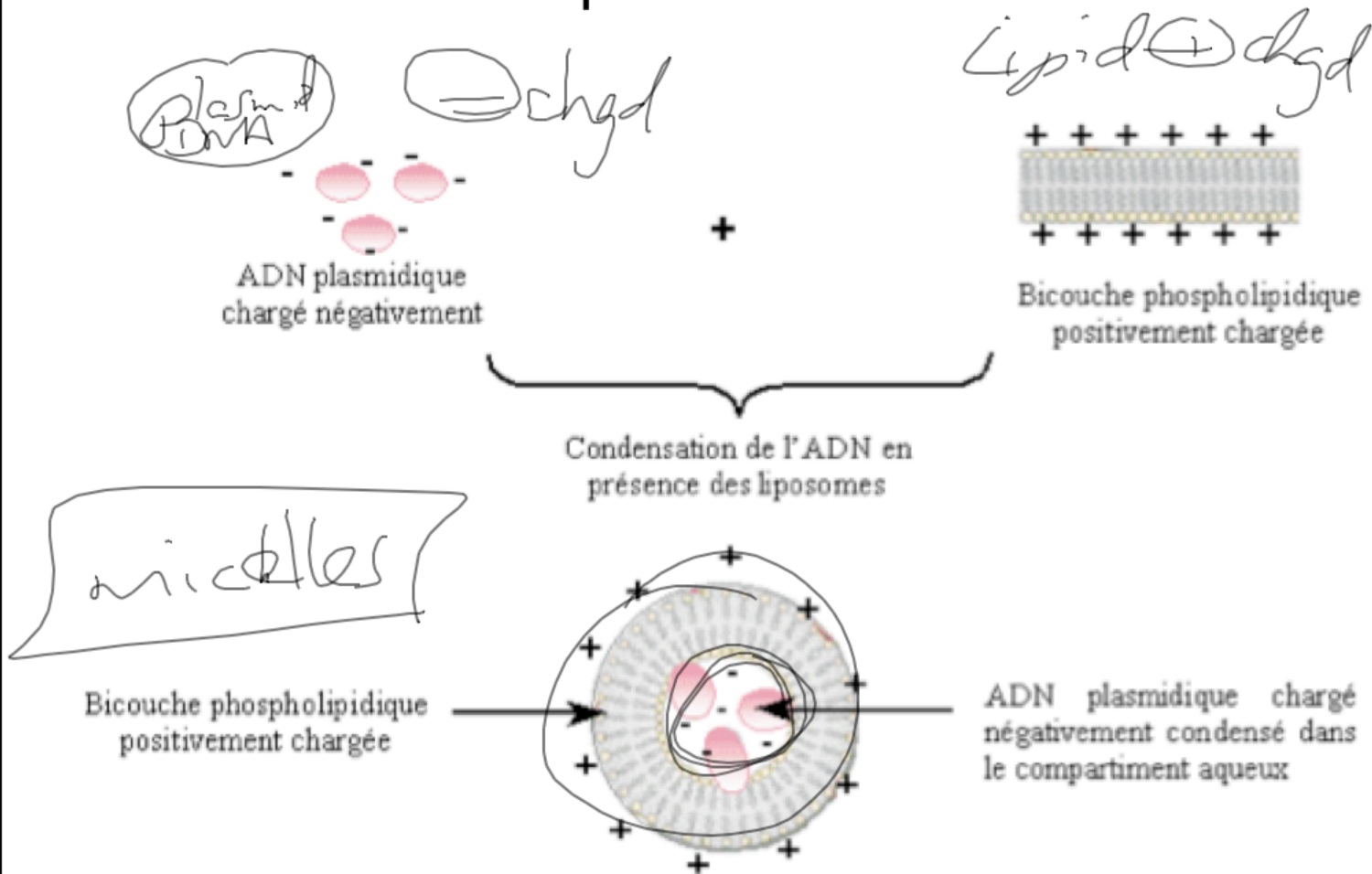
DNA Engineering

M1D7

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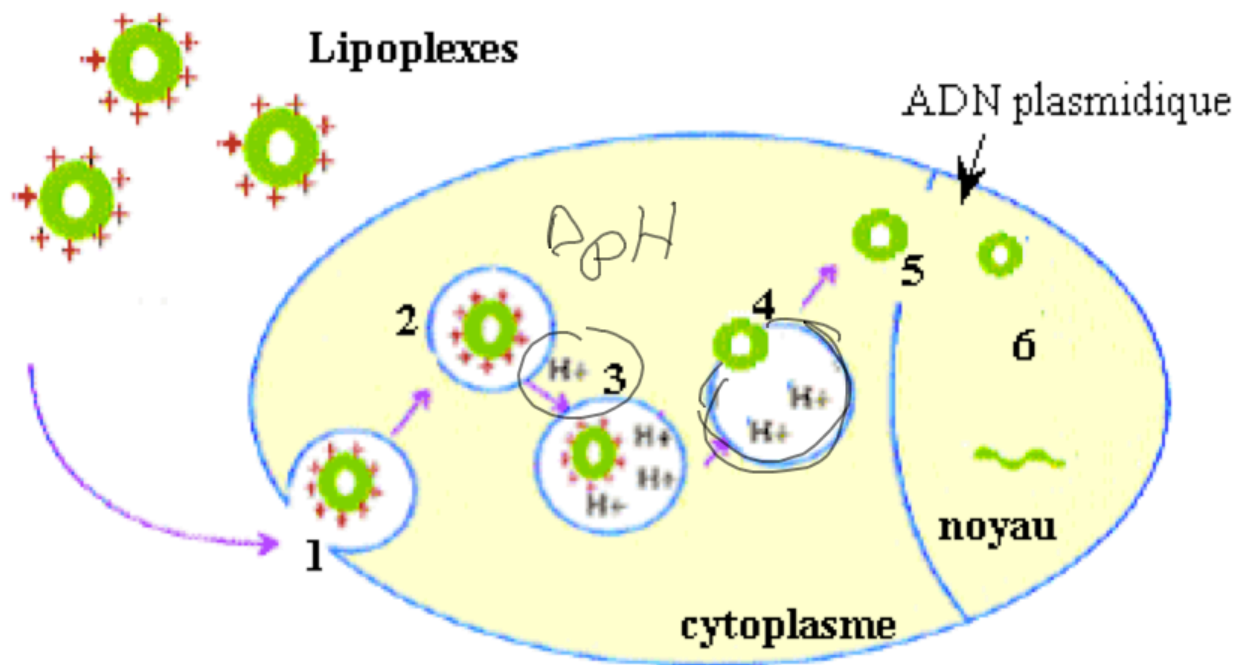
Lipofection



http://www.unilim.fr/theses/2003/sante/2003limo0100c/these_body.html



Lipofection



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Lipofection

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7413–7417, November 1987
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, μg	DEAE-dextran, mg/ml	Time, hr	DNA, μg	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
		0.50	5	25	1
CV-1	100		5	10	100
		0.25	5	10	9
COS-7	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 × 10 ⁵
L-M(TK ⁻)	pSV2neo	Calcium phosphate	3
		Lipid	45
ψ-2	pZIPSVX	Calcium phosphate	0.6
		Lipid	>49
ψ-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	pZIPSVX	Calcium phosphate	0.7
		Lipid	17
TA1	pMSGC5a	Calcium phosphate	1.3
		Lipid	19

Cells were transfected with 7 μg of the indicated plasmid with no carrier DNA, except for pSV2neo where 1 μg of plasmid and 10 μg 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

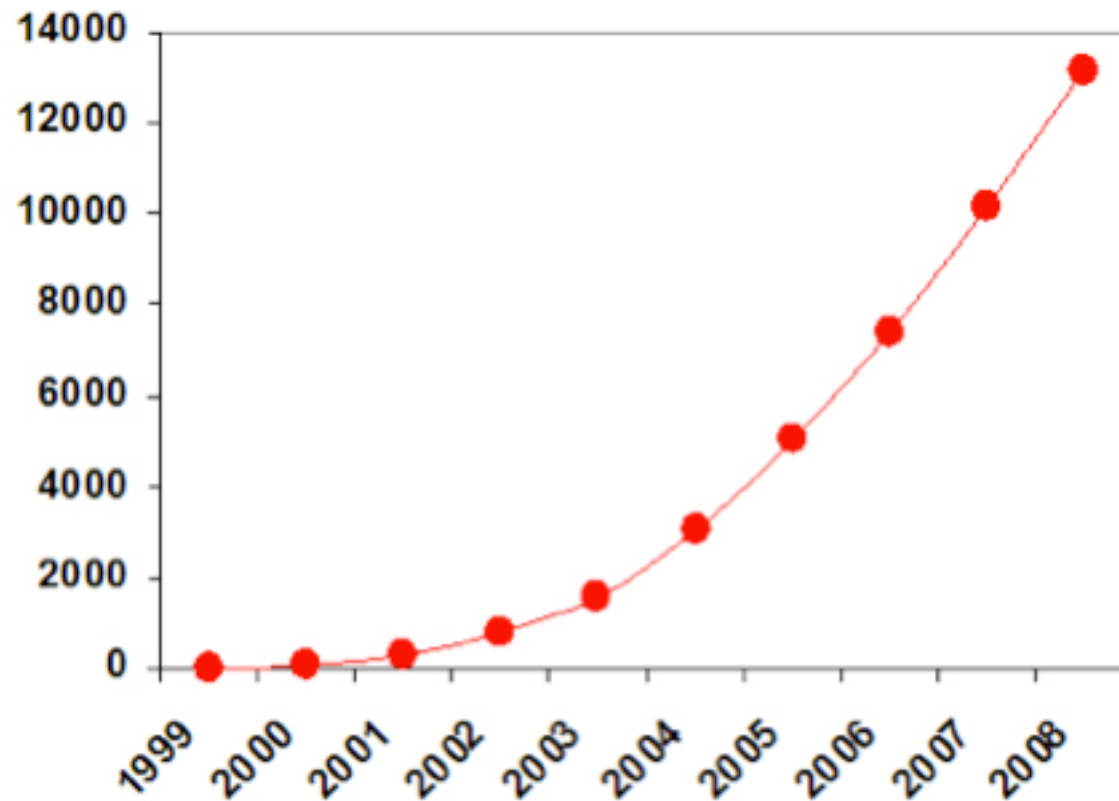


Figure 2. Number of publications citing the use of Lipofectamine™ 2000 transfection reagent since 1999.

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

Your experiment and your expectations

Dilute Lipofectamine
 (x16.5)

2.5 μ l \rightarrow 50 μ l
 Find OptiMEM

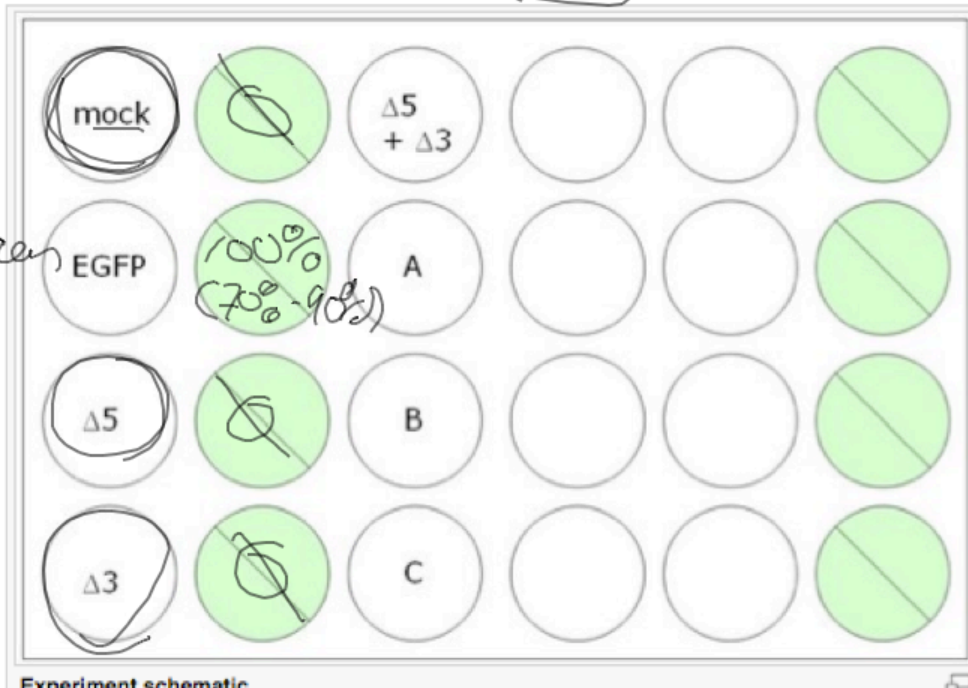
Dilute and mix DNA
 (x1 or x3)

0.1 μ g
 Stock = 0.05 μ g
 50 μ l
 OptiMEM

50:50
 mix (1:1) and incubate

495

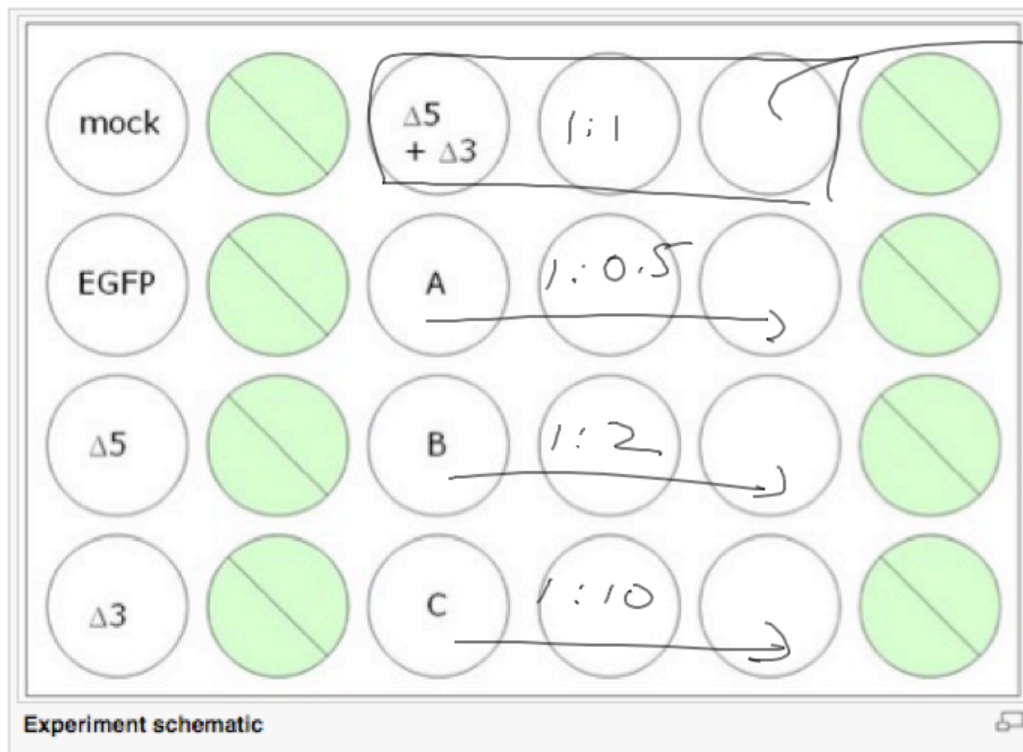
transfection can't be



Experiment schematic

Your experiment and your expectations

What could you do to increase homologous recombination of $\Delta 5$ and $\Delta 3$?



HOR
mutator ~~data~~
DSB → UV
OR
Amnt of Data

This week in lab...

① Lipofectin / STATs / paper
discuss

② Come to lab in groups
↳ FACS

Tuesday of next week: "memo" due...

weekend office hours?

See 3-5

then on to Mod 2!

