

# DNA Engineering: M1D6 Lab Talk

20.109 (F12)

10.02.12

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# Quick review of TC practice



# Lipofection

*Proc. Natl. Acad. Sci. USA*  
Vol. 84, pp. 7413–7417, November 1987  
Biochemistry

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

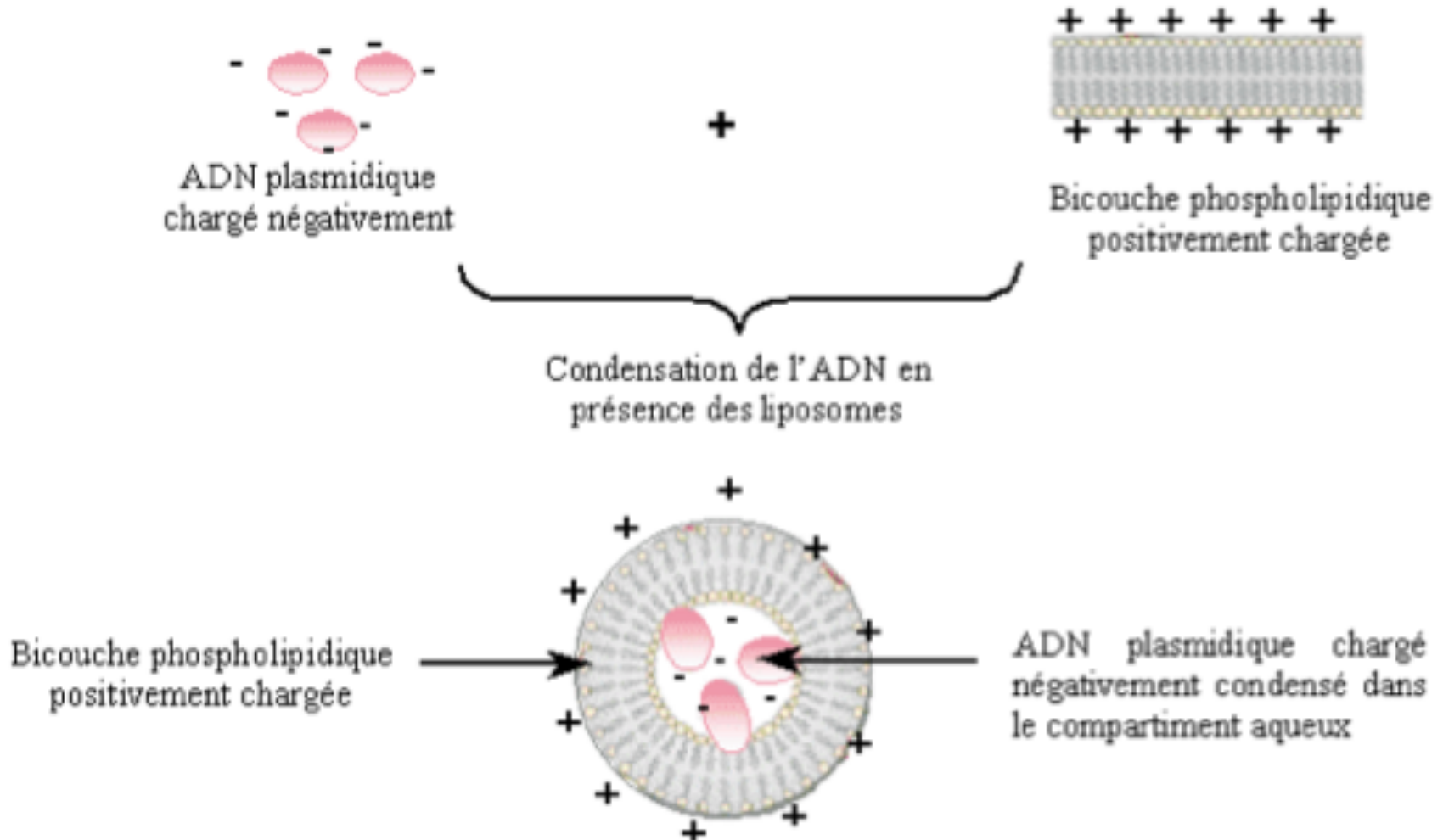
(liposomes/cationic lipid vesicles/gene transfer)

PHILIP L. FELGNER\*<sup>†</sup>, THOMAS R. GADEK\*, MARILYN HOLM\*, RICHARD ROMAN\*, HARDY W. CHAN\*,  
MICHAEL WENZ<sup>§¶</sup>, JEFFREY P. NORTHROP<sup>¶</sup>, GORDON M. RINGOLD<sup>§¶</sup>, AND MARK DANIELSEN<sup>¶</sup>

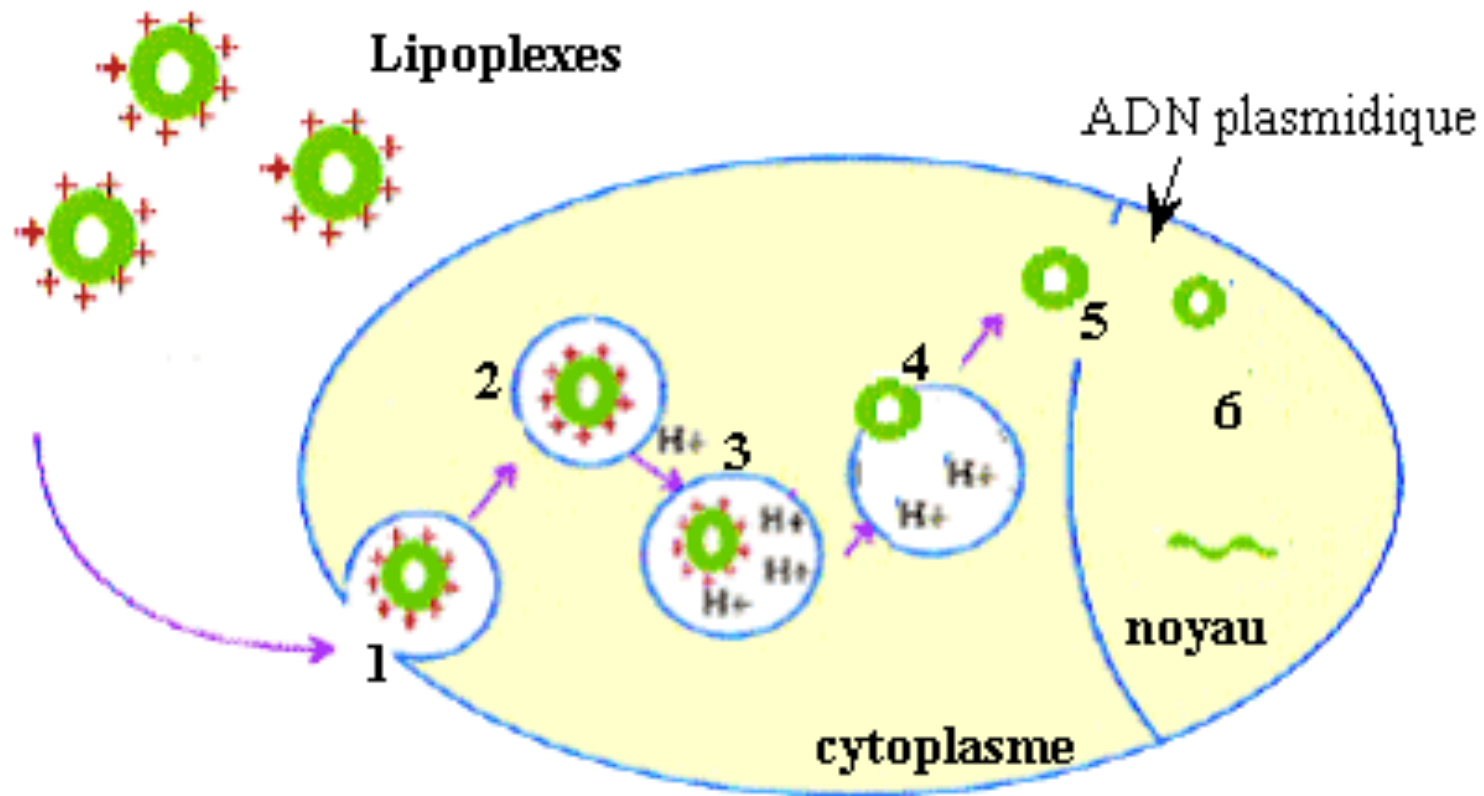
\*Institute of Bio-Organic Chemistry and <sup>§</sup>Cancer and Developmental Biology, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94303; and <sup>†</sup>Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

*Communicated by Avram Goldstein, June 24, 1987 (received for review April 23, 1987)*

# Lipofection



# Lipofection



# 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
		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

# of publications citing Lipofectamine  
2000 transfection reagent

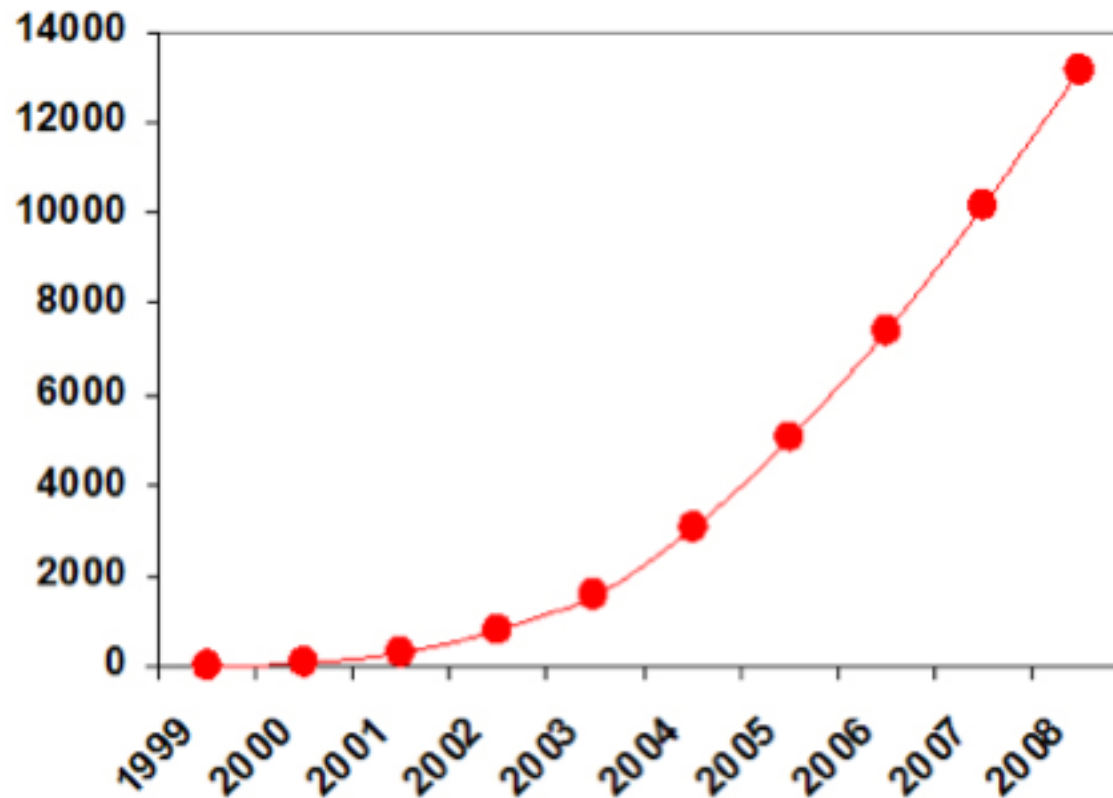
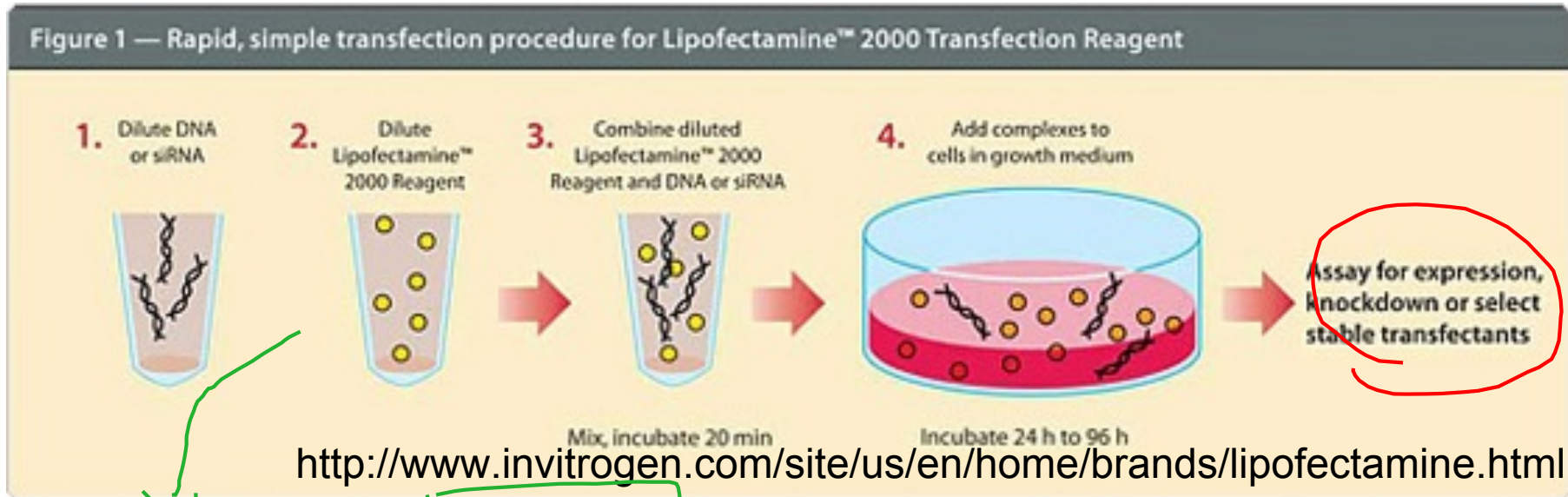


Figure 2. Number of publications citing the use of Lipofectamine™ 2000 transfection reagent since 1999.

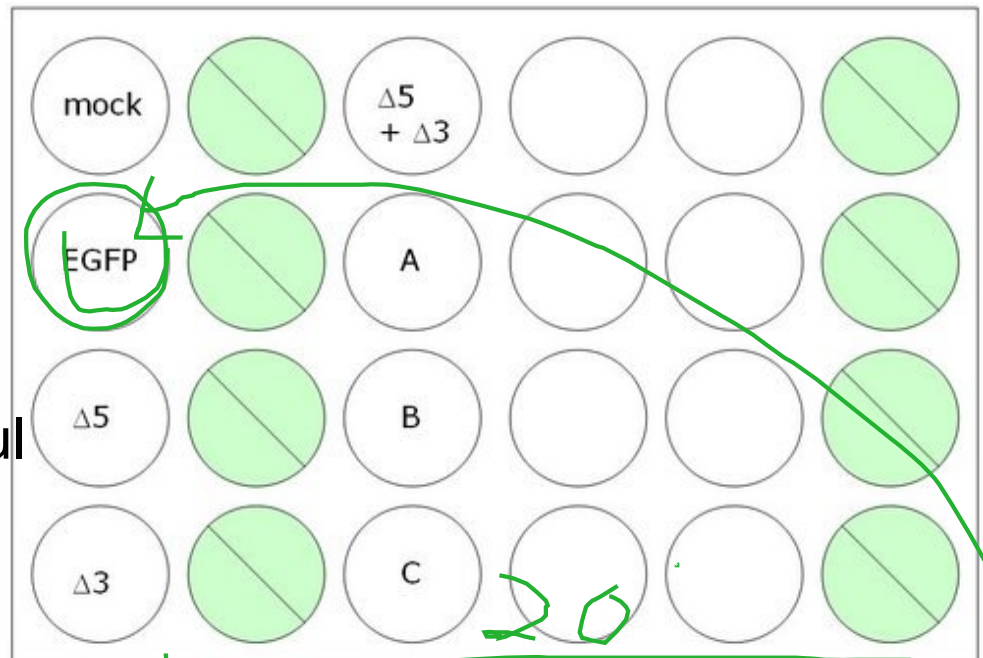
<http://www.invitrogen.com/site/us/en/home/brands/lipofectamine.html>

# Lipofection-- workflow



Lipofectamine  
16.5 rxns total

DNA  
0.1ug/rxn



Stock = 0.05 ug/ul

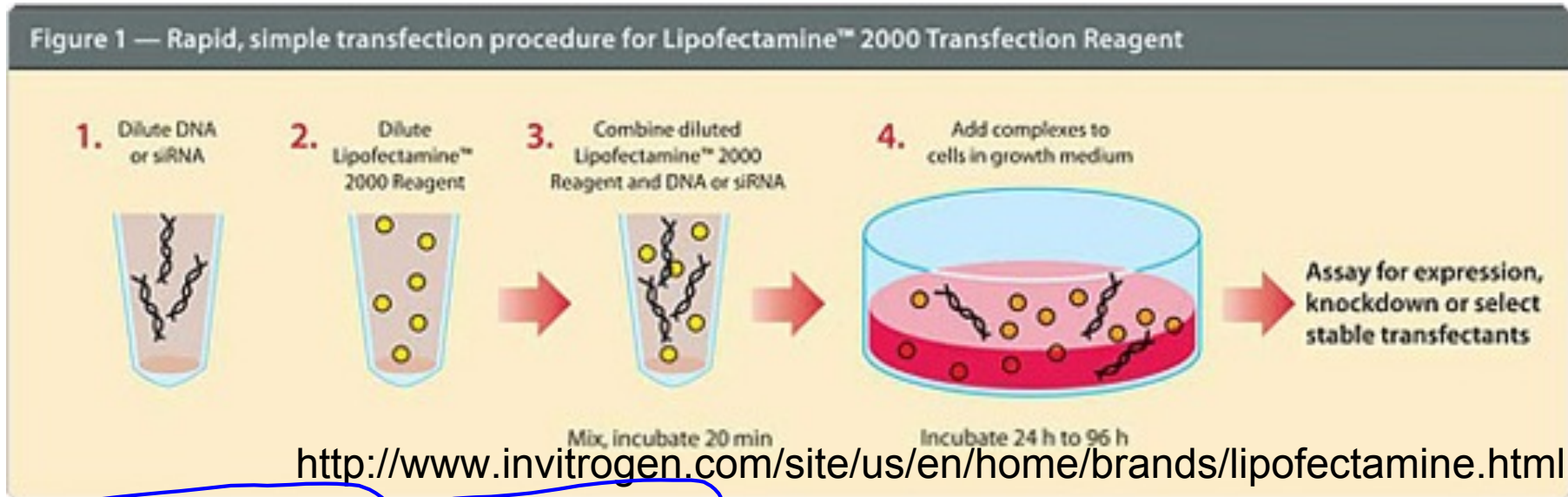
5-30  
2.5ul  
47.5ul Opt-M  
50ul  
x 16.5

2ul DNA  
48ul Opt-M  
50ul Lipofectamine

20  
50ul DNA + 50ul L

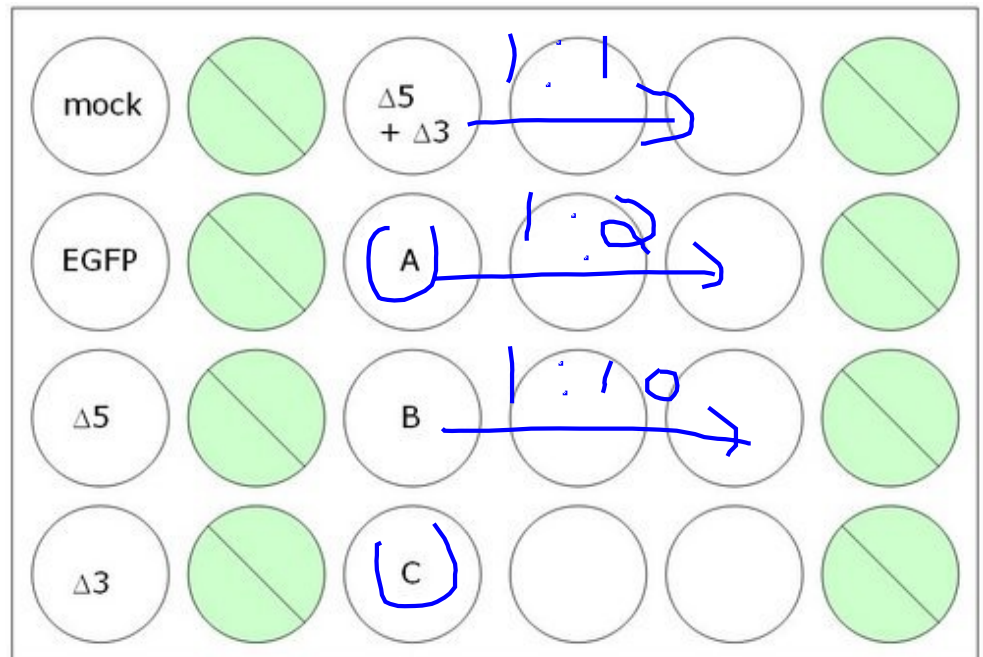
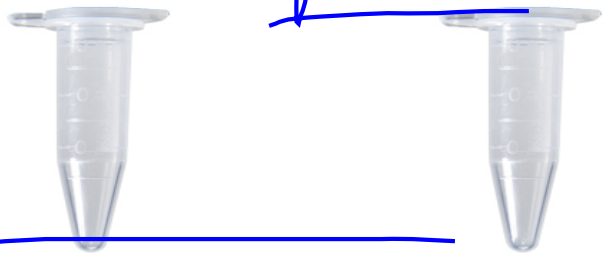


# Lipofection-- workflow



Lipofectamine  
16.5 rxns total

DNA  
0.1ug each/rxn



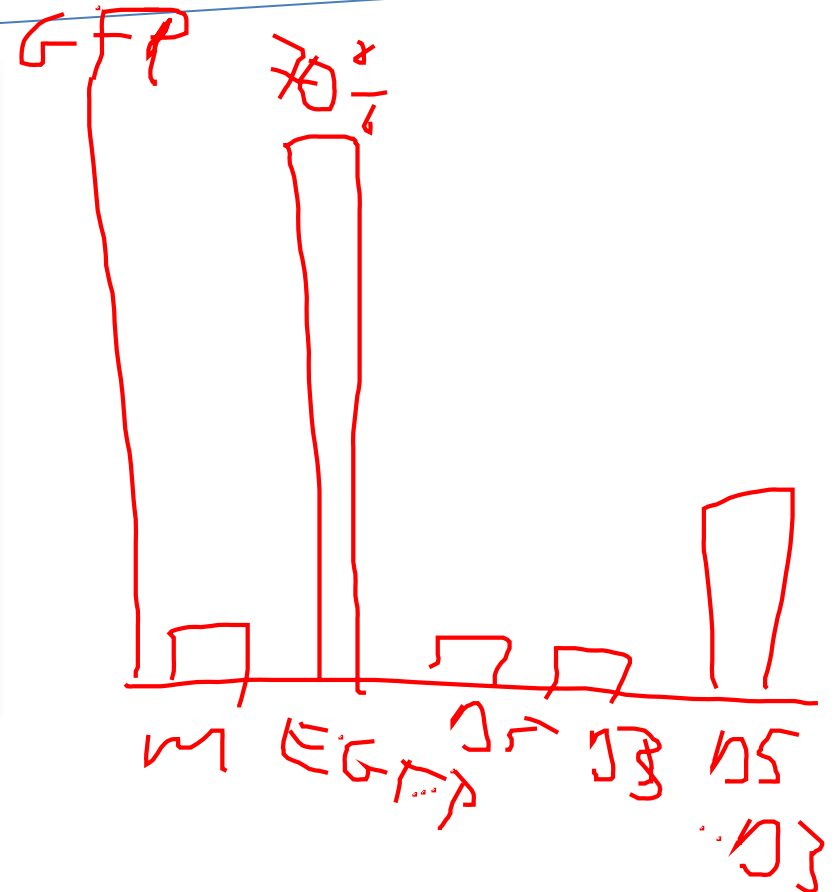
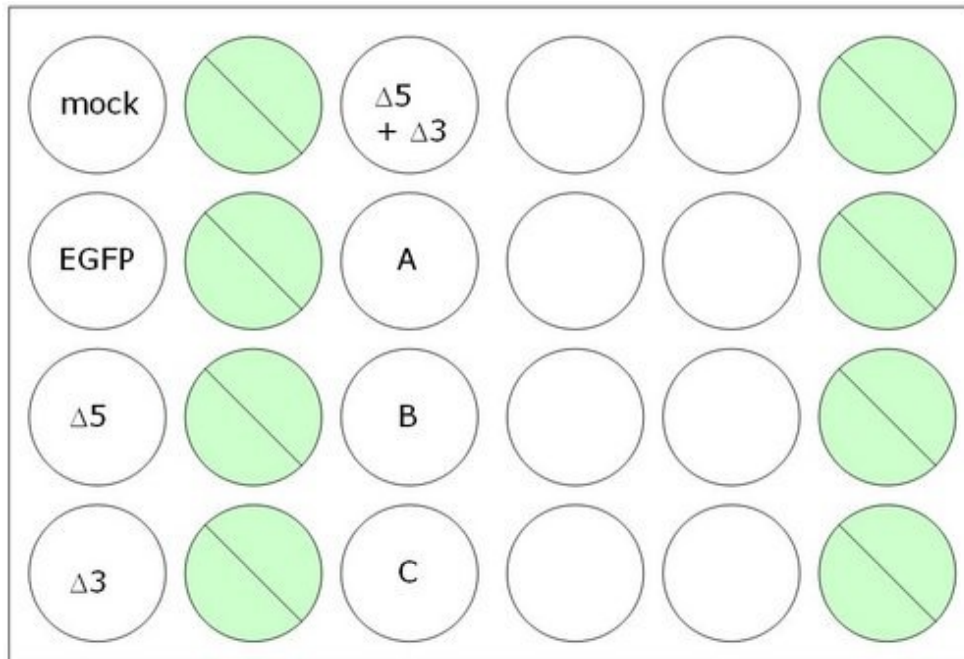
Δ5: 21/rxn  
 Δ3: 21/rxn  
 Optim: 46

# Your experiment and your expectations

Dilute Lipofectamine  
(x16.5)

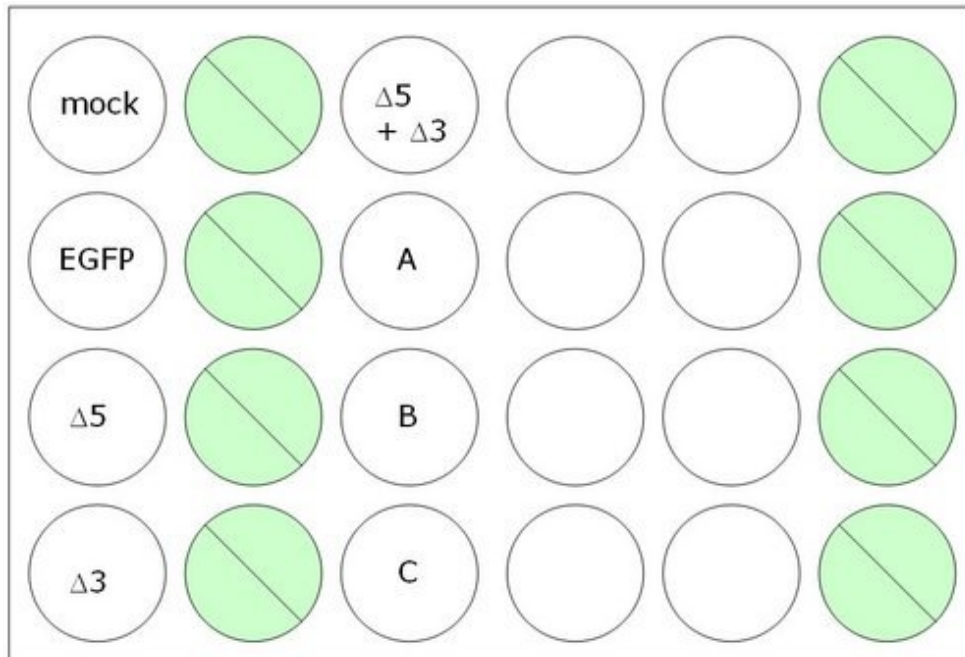
Dilute and mix DNA  
(x1)

mix (1:1) and incubate



# Your experiment and your expectations

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



## Finishing Module 1...

T Lipofect / Lab C + 2 Lab

R FACS

(F) Blog

next week: No lab or lecture Tuesday  
Office hours?  
FACS/ppt due 10.11.12  
Mod2 starts