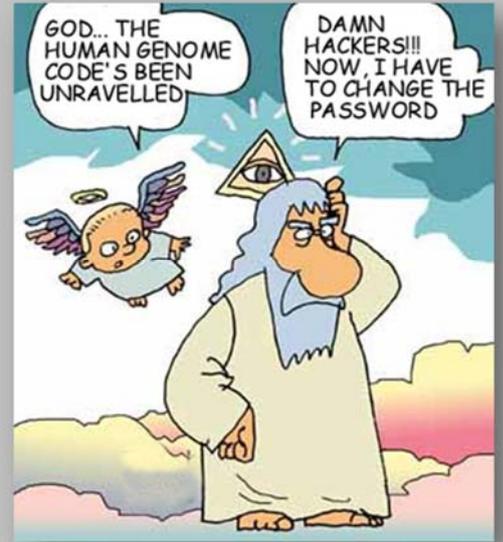
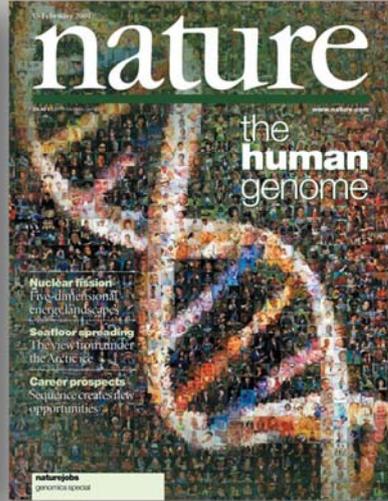
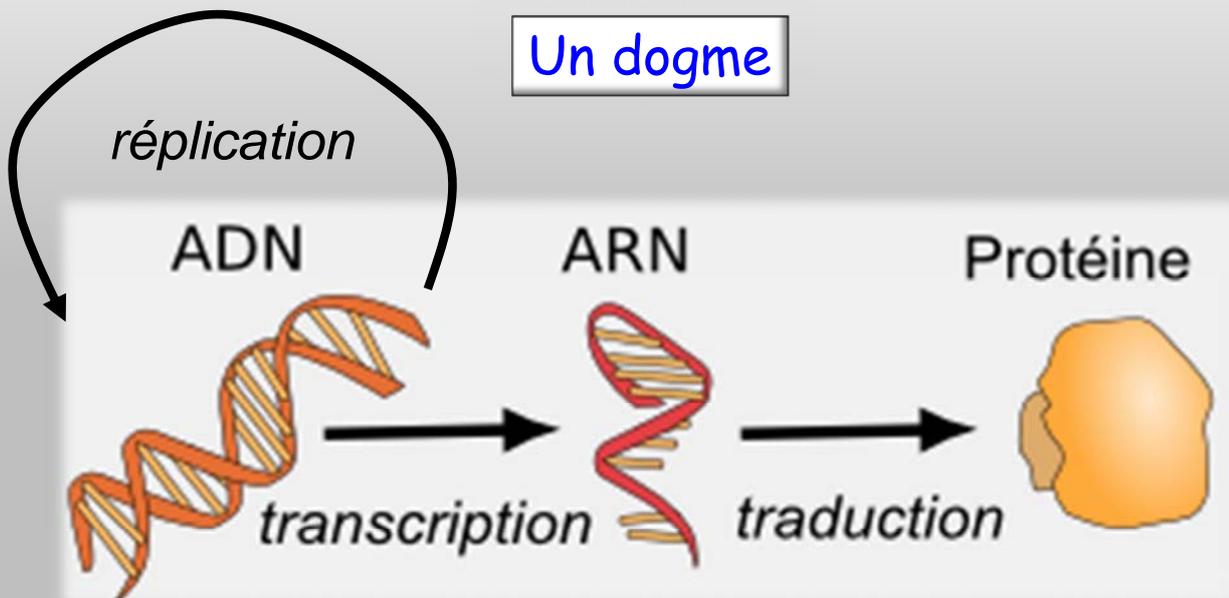


Je clone, tu clones, nous "OGMons" !

Pr Uriel Hazan
(uriel.hazan@ens-cachan.fr)



Un dogme



Histoire d'outils moléculaires

Les plasmides ?

4

Joshua Lederberg, 1952.

- **Plasmides conjugatifs : Facteur F, 1950, conjugaison, fertilité**
- **Plasmides de résistance : Facteurs R, 1959, Japon, 1^{er} patient résistant aux ab**

- Ce sont des ADN bactériens bicaténaires, circulaires et extrachromosomiques.
- Leur taille varie de 2 à 7 kb en général (jusqu'à 100 kb pour le facteur F)
- le nombre de copie par bactérie est élevé (de plusieurs dizaines à plusieurs centaines de copies)
- Capacité de répllication indépendamment de l'ADN chromosomique : Ce sont des réplicons autonomes
- Les plasmides sont des ADN CCC (Covalently Closed Circles) et donc surenroulés

➤ **Les plasmides de première génération** : Ce sont les premiers à avoir été utilisés en génie génétique. Ce sont des plasmides naturels ou non , après certaines modifications

- ✓ ColE1
- ✓ RSF 2124
- ✓ pSC 101

➤ **Les plasmides de deuxième génération** : ne sont pas des plasmides naturels mais résultent de plusieurs transformations : plasmides "*artificiels*".

La série la plus importante de ces plasmides est la **série pBR 312 à pBR 322**

Molec. gen. Genet. 142, 239—249 (1975)
 © by Springer-Verlag 1975

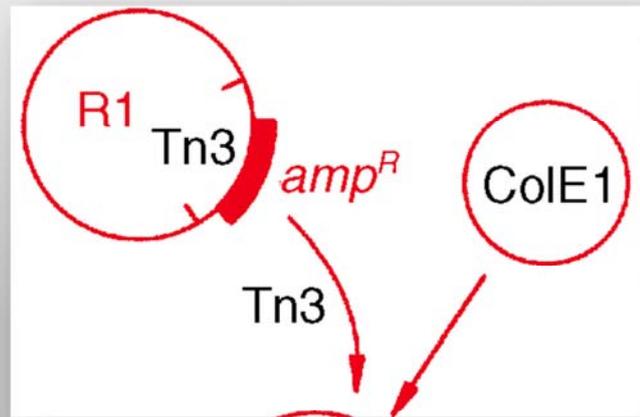
The Generation of a ColE1-Ap^r Cloning Vehicle which Allows Detection of Inserted DNA

Magdalene So, Ron Gill, and Stanley Falkow

Department of Microbiology, School of Medicine, University of Washington, Seattle, Washington 98195

Received September 22, 1975

Summary. A 3.2 Mdal sequence of DNA, TnA, which contains the ampicillin (Ap) resistance determinant has been translocated from an R plasmid to the plasmid ColE1. A total of 12 isolates were studied. There are at least 8 sites in ColE1 at which TnA has inserted. Insertion at five of these has resulted in a Col⁻ phenotype. One ColE1-Ap^r plasmid, RSF2124, was examined further and its replication properties are found to be similar to that of the parent plasmid. RSF2124 appears to be a useful plasmid vehicle for the molecular cloning of DNA from diverse prokaryotic sources: it codes for readily detectable Ap resistance and contains a single *EcoRI* site in a gene affecting colicin biosynthesis so that it is unable to produce colicin upon ligation to other DNA.



JOURNAL OF BACTERIOLOGY, Oct. 1977, p. 321-331
 Copyright © 1977 American Society for Microbiology

Vol. 132, No. 1
 Printed in U.S.A.

Isolation and Genetic Analysis of Deletion Mutants of Colicin E1 Plasmids Carrying a TnA Insertion

JOSEPH INSELBURG* AND PATRICIA WARE

Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755

Received for publication 22 April 1977

Deletions of colicin E1 (colE1) plasmid deoxyribonucleic acid (DNA) carrying the TnA transposon have been isolated. All except two were generated by nuclease digestion of plasmid DNA from its *EcoRI*-sensitive site. A plasmid containing about 16% of the ColE1 DNA (6.5×10^8 daltons) was generated that also contained the part of the TnA transposon conferring ampicillin resistance. The extents of different deletions were determined by analysis of restriction endonuclease fragments generated by the restriction endonucleases *Hae*II, *Bam*HI, and *Hinc*II.

Le 1^{er} OGM célèbre : le plasmide pBR322

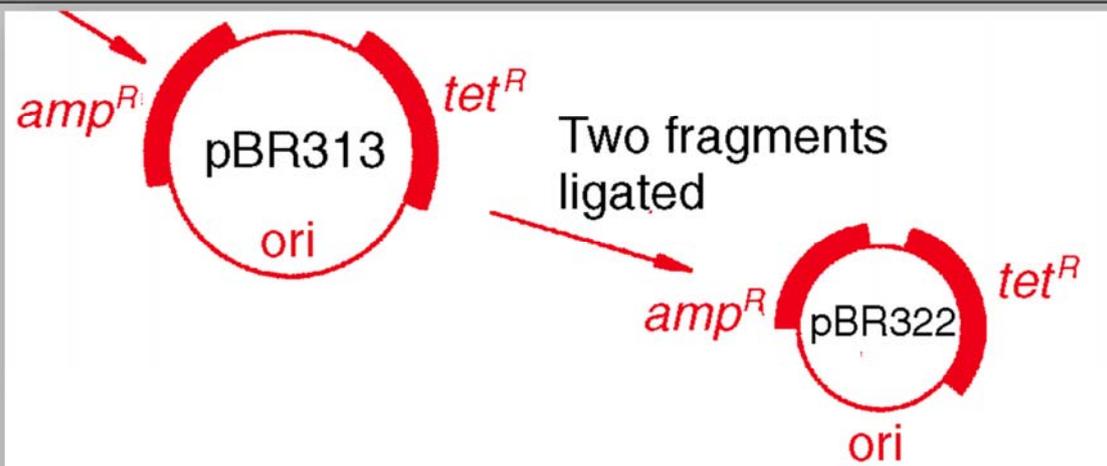
Gene, 1977;2(2):95-113.

Construction and characterization of new cloning vehicles. II. A multipurpose cloning system.

Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Heyneker HL, Bover HW, Crosa JH, Falkow S.

Abstract

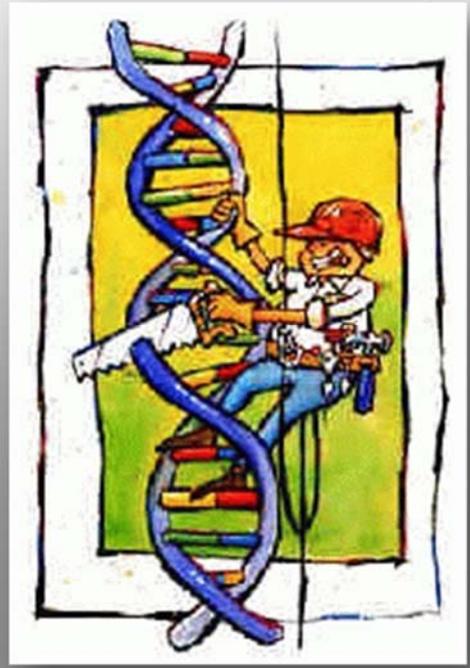
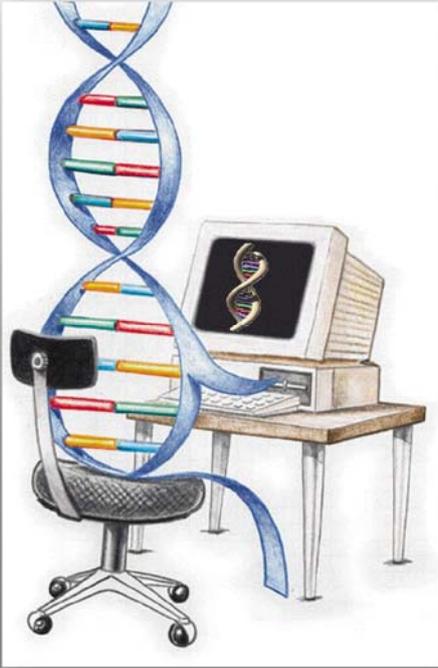
In vitro recombination techniques were used to construct a new cloning vehicle, pBR322. This plasmid, derived from pBR313, is a relaxed replicating plasmid, does not produce and is sensitive to colicin E1, and carries resistance genes to the antibiotics ampicillin (Ap) and tetracycline (Tc). The antibiotic-resistant genes on pBR322 are not transposable. The vector pBR322 was constructed in order to have a plasmid with a single *Pst*I site, located in the ampicillin-resistant gene (*Apr*), in addition to four unique restriction sites, *Eco*RI, *Hind*III, *Bam*HI and *Sal*I. Survival of *Escherichia coli* strain X1776 containing pBR313 and pBR322 as a function of thymine and diaminopimelic acid (DAP) starvation and sensitivity to bile salts was found to be equivalent to the non-plasmid containing strain. Conjugal transfer of these plasmids in bi- and triparental matings were significantly reduced or undetectable relative to the plasmid ColE1.



A quoi ça sert ??

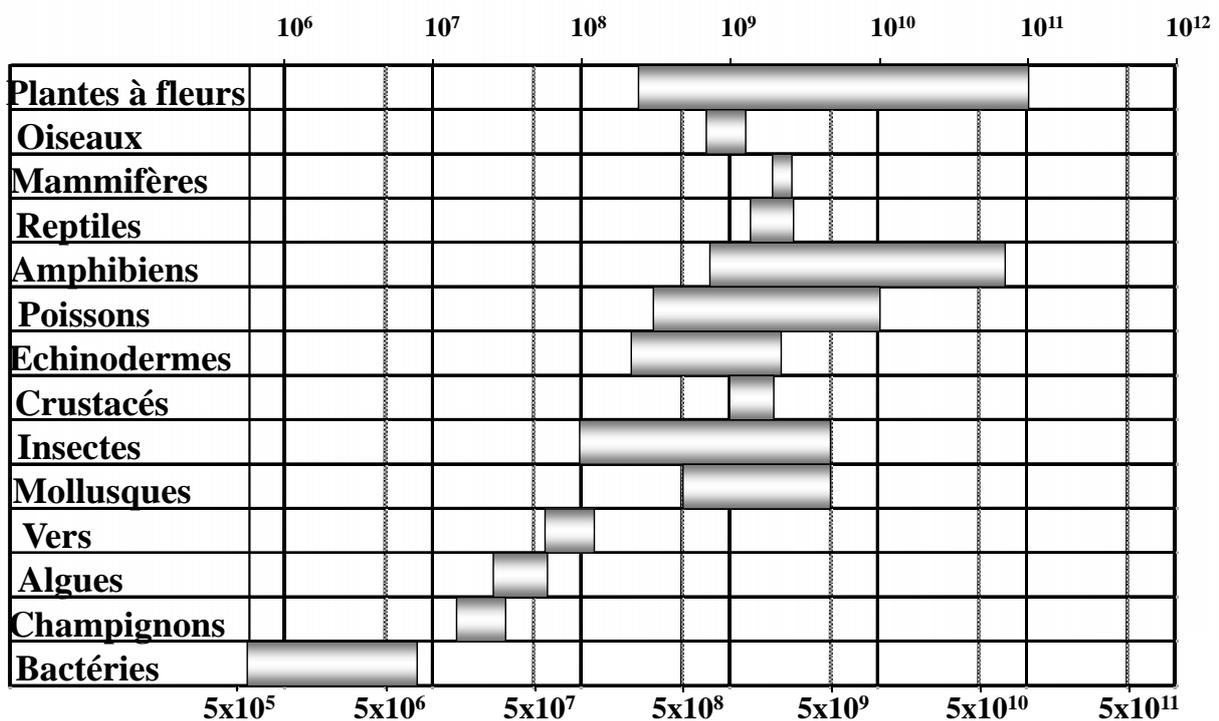
Transporter, analyser, manipuler

- les gènes
- Les génomes



Taille du génome des êtres vivants

8



Séquençage des génomes...

2001
35 000 gènes

2004
20- 25 000 gènes
codant
des protéines
+ ... gènes des ncRNAs
NATURE | VOL 431 | 21 OCTOBER 2004

2006
21 561 gènes codant
des protéines
mais 69 185 gènes
prédits : ncRNAs
<http://www.ensembl.org>

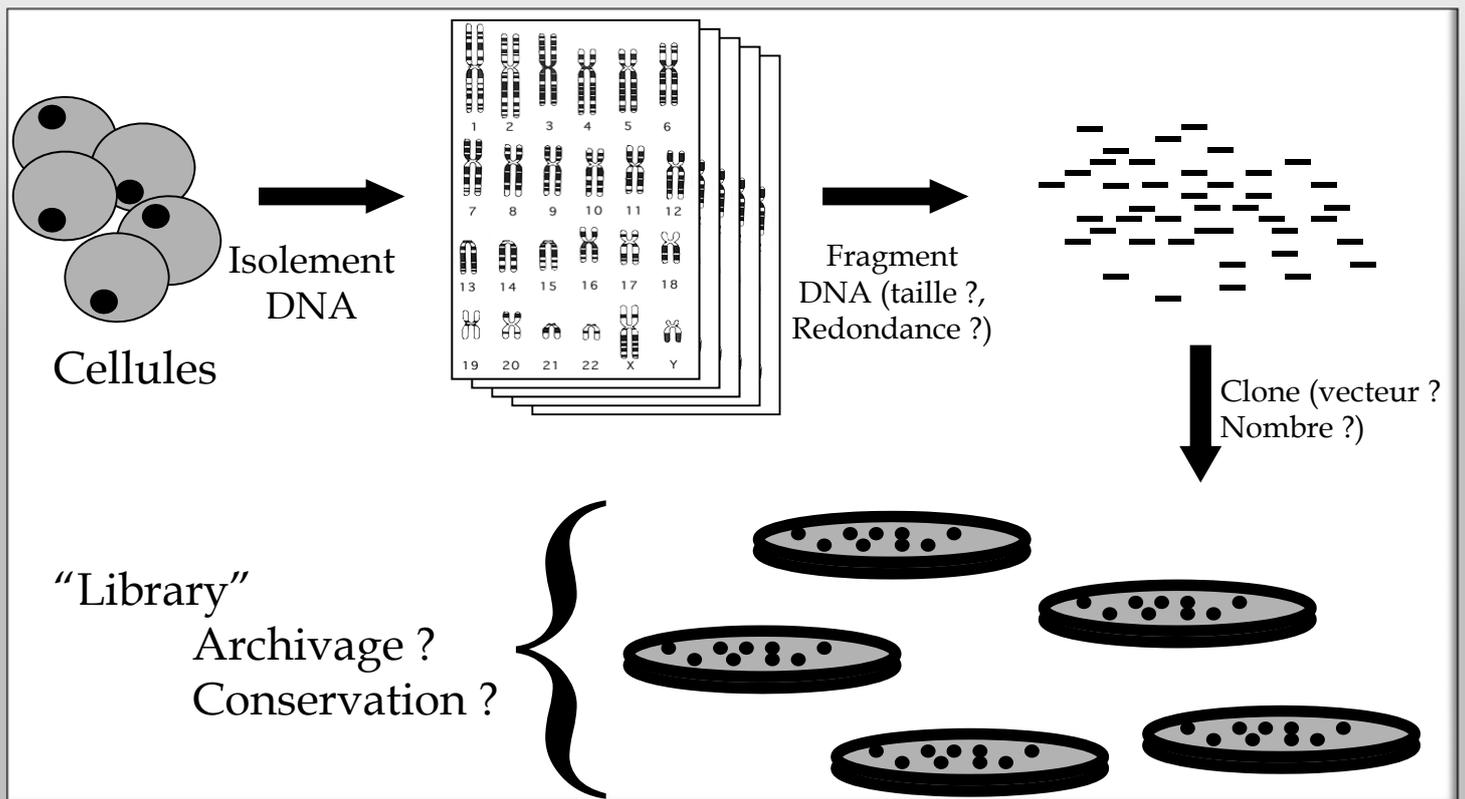
Le Monde du 13 Février 2001
13 601 gènes

Science
THE HUMAN GENOME

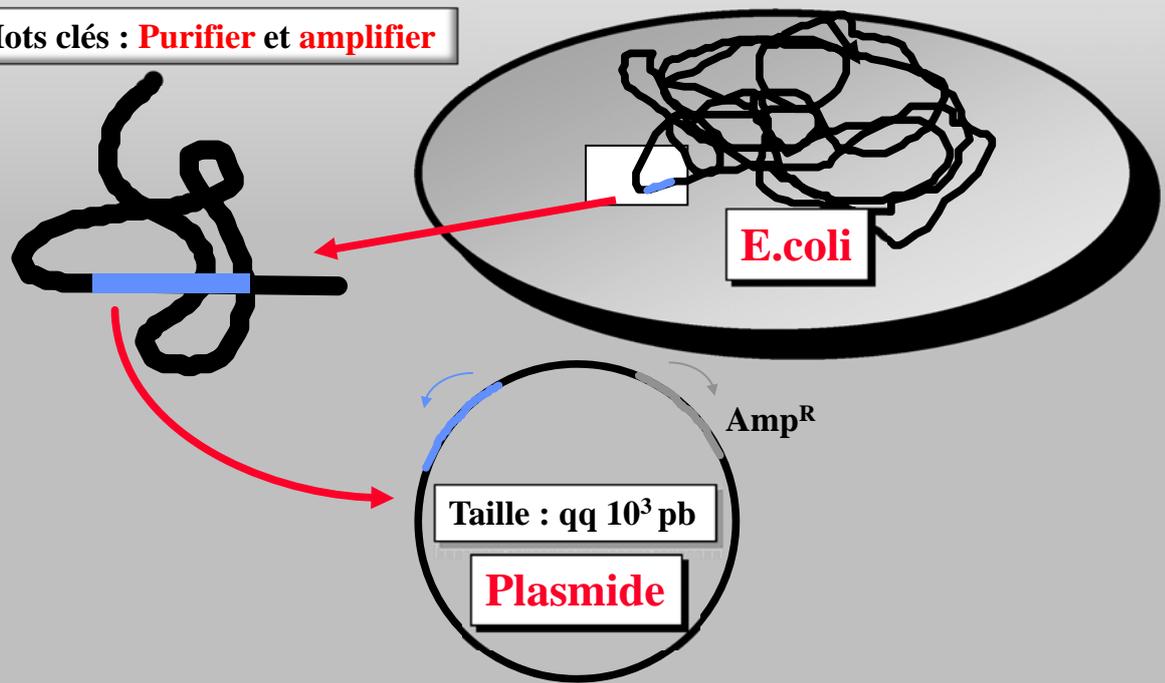
nature
the human genome

PANCHO

Une bibliothèque de gènes



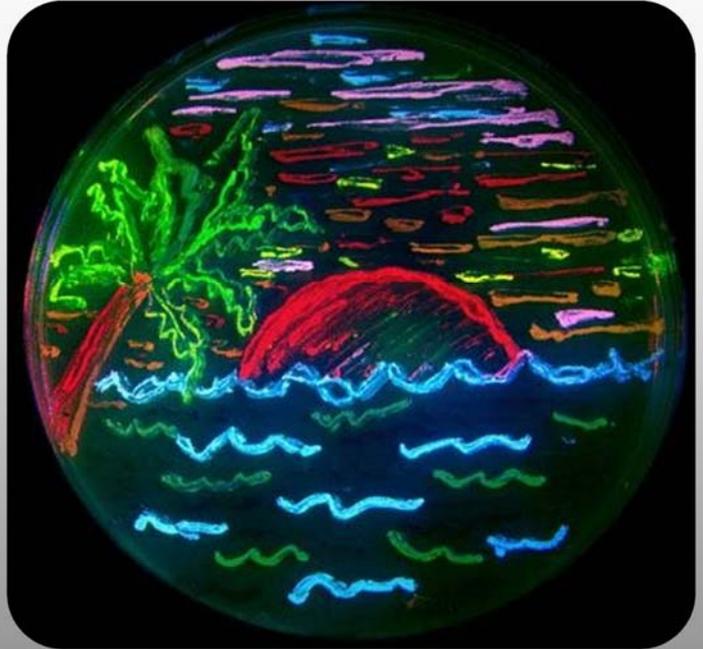
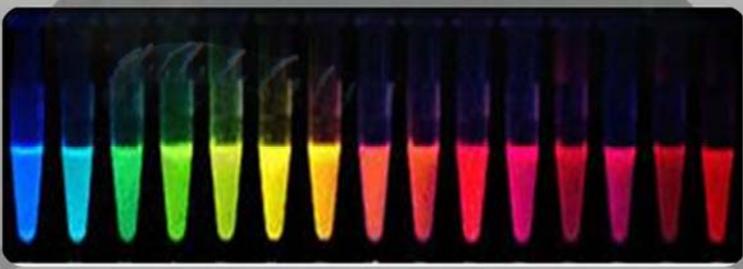
➤ Mots clés : **Purifier** et **amplifier**



Un exemple : croisement méduse/bactérie ??



Aequorea victoria



Roger Y. TSIEN

Un croisement méduse/souris ??

13



Un croisement méduse/mouche à vinaigre ??

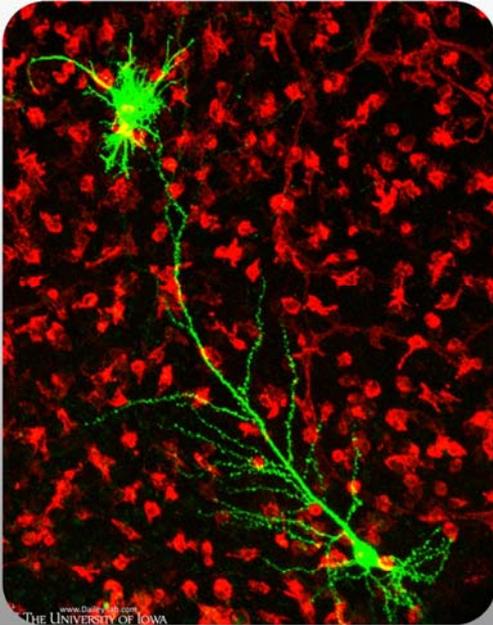
14



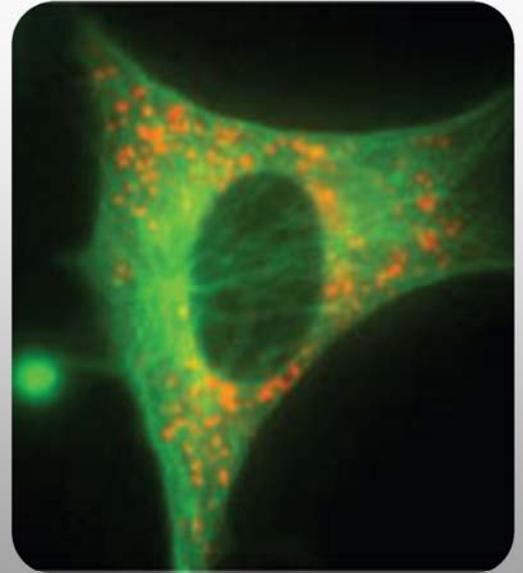
Localisation de la protéine "engrailed" chez la mouche *Drosophila melanogaster*.

Un croisement méduse/cellule ??

15



Un neurone GFP



Visualisation de la tubuline fusionnée à la GFP, dans une cellule de souris

Enfin, le mouton-méduse

16



Applications...

- Recherche fondamentale
- Diagnostics cliniques
- Vaccins
- Traitements