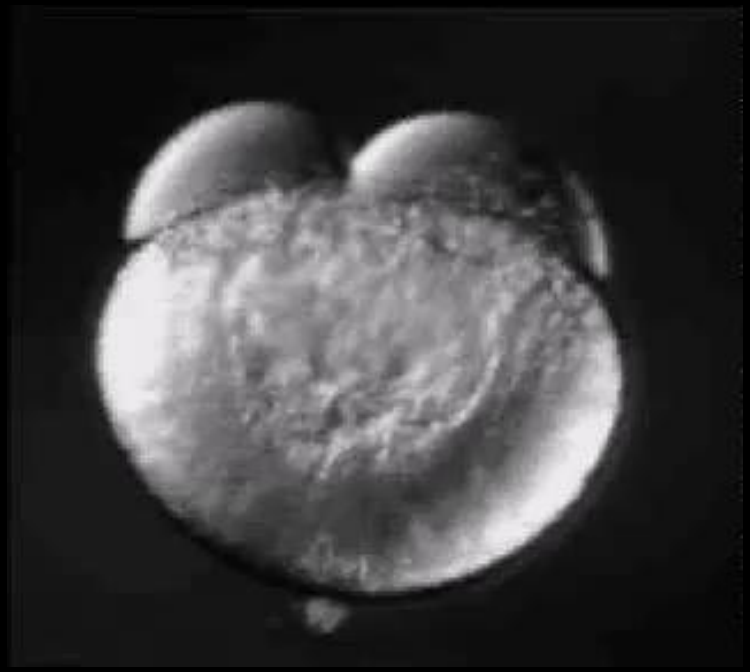
Manipulación génica del desarrollo neural en pez cebra



Curso Biología del Desarrollo
2022 - Módulo 7
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FACULTAD DE CIENCIAS
UDELAR | fcien.edu.uy

UNIVERSIDAD DE LA REPÚBLICA
URUGUAY

El pez cebra como sistema experimental

1960 — Late 1960s Streisinger obtains zebrafish from commercial suppliers.

1970 — Mid-1970s Homozygous diploid embryos derived only from the maternal genome are used to reveal recessive mutations present in the germ line of a female. Gynogenetic procedures are developed for mapping and gene-linkage studies.

1972 — Streisinger produces haploid embryos from eggs activated with ultraviolet-irradiated, genetically impotent sperm.

1980 — Mid-1980s Establishment of a research community focused on developmental and genetic studies with the zebrafish. Cell-lineage studies in the early embryo, visualization of neurite outgrowth in a living zebrafish embryo, and mutagenesis and mapping regimes are reported.

1981 — The method for producing clonal lines of homozygous zebrafish is published. Gynogenetic procedures and their potential applications are described.

1988 — The first description of an induced embryonic-lethal mutation in the zebrafish is published.

1990 — The first conference on zebrafish is convened in Eugene, Oregon. The fate map of the zebrafish gastrula reveals that organization of the early zebrafish embryo is similar to that of other vertebrates. Cell transplantation to generate genetically mosaic embryos is used to test autonomy of gene function.

1993 — 1993 Systematic large-scale screens for embryonic-lethal mutations begin in Tübingen, Germany, and Boston, USA.

1994 — The First Cold Spring Harbor Conference on Zebrafish Genetics and Development is held. Three hundred and fifty international researchers attend — the zebrafish is no longer just a promising model.

no tail is the first mutation to be identified molecularly, using a candidate-gene approach.

1996 — The results of the 'Big Screen' are published in a single issue of *Development*, volume 123.

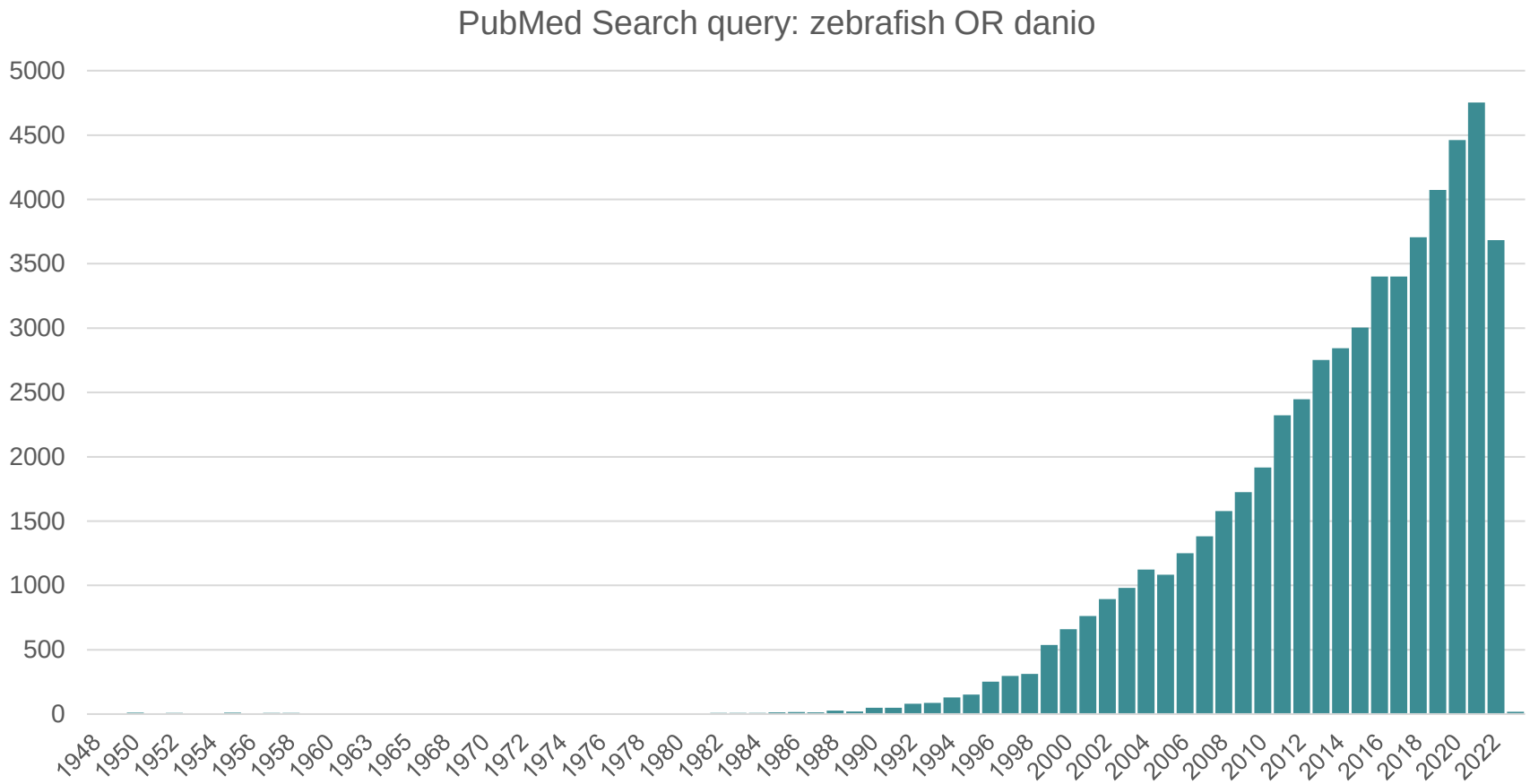
1997 — Mid-to-late 1990s Development of linkage map and genomic resources. Insertional mutagenesis is established and large-scale screens for insertional mutants begin.

1998 — Late 1990s to early 2000s Mutations are cloned and several genes that affect common processes are woven into molecular pathways. The Trans-NIH Zebrafish Initiative is launched. Establishment of a centralized, web-based database, ZFIN, and a stock centre, ZIRC.

one-eyed pinhead is the first mutation to be positionally cloned.

2000 — The beginning of whole-genome sequencing

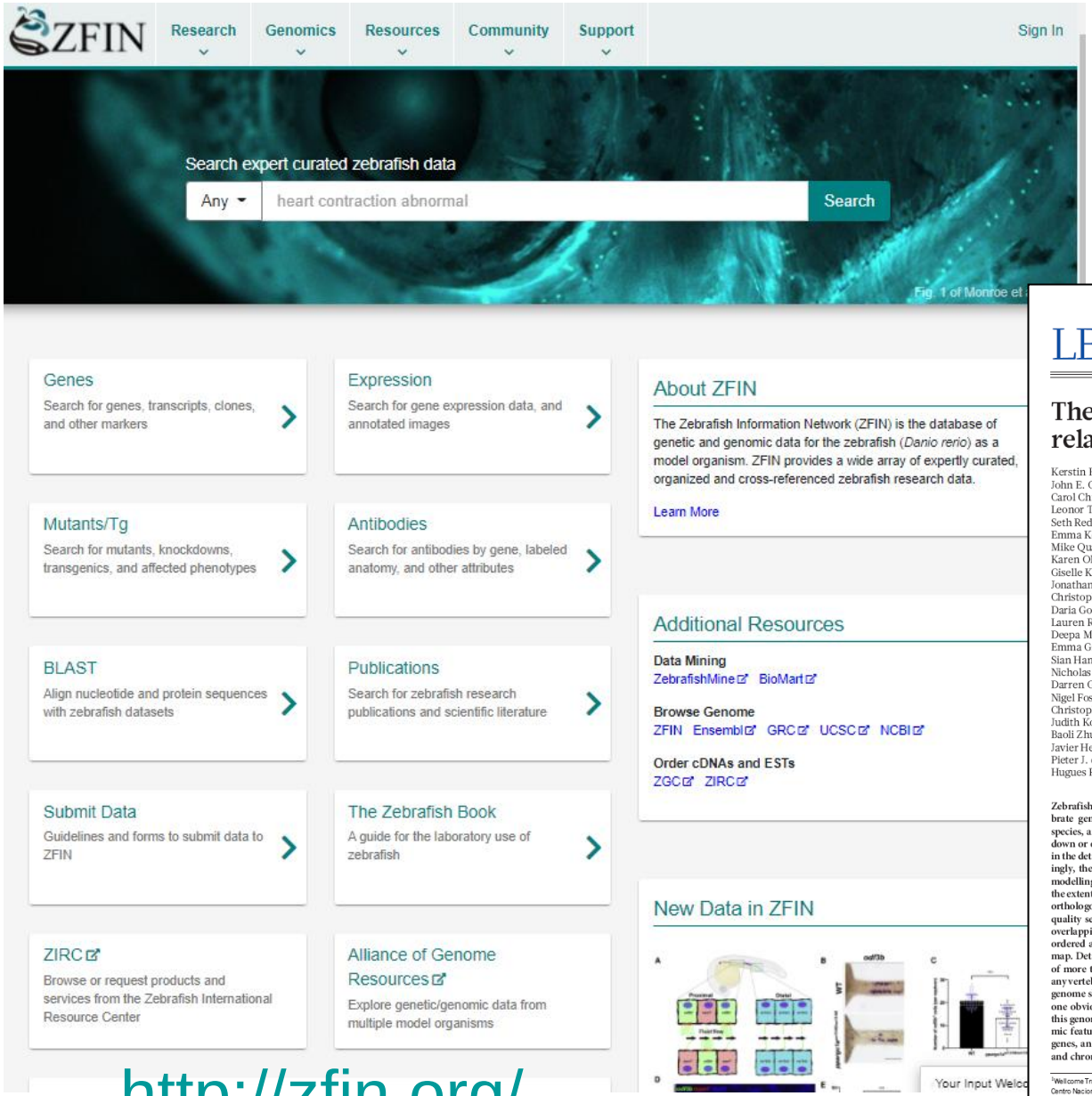
El pez cebra como sistema experimental



Algunas aplicaciones

- Desarrollo embrionario y regeneración
- Genética clásica y genómica molecular
- Biología celular *in vivo*
- Cáncer
- Screenings farmacológicos
- Ecotoxicología

Recursos públicos, genoma completo



http://zfin.org/

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LETTER

OPEN

doi:10.1038/nature12111

The zebrafish reference genome sequence and its relationship to the human genome

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Zebrafish have become a popular organism for the study of vertebrate gene function[1,2]. The virtually transparent embryos of this species, and the ability to accelerate genetic studies by gene knockdown or overexpression, have led to the widespread use of zebrafish in the detailed investigation of vertebrate gene function and increasingly, the study of human genetic disease[3–5]. However, for effective modelling of human genetic disease it is important to understand the extent to which zebrafish genes and gene structures are related to orthologous human genes. To examine this, we generated a high-quality sequence assembly of the zebrafish genome, made up of an overlapping set of completely sequenced large-insert clones that were ordered and oriented using a high-resolution high-density meiotic map. Detailed automatic and manual annotation provides evidence of more than 26,000 protein-coding genes[6], the largest gene set of any vertebrate so far sequenced. Comparison to the human reference genome shows that approximately 70% of human genes have at least one obvious zebrafish orthologue. In addition, the high quality of this genome assembly provides a clearer understanding of key genomic features such as a unique repeat content, a scarcity of pseudogenes, an enrichment of zebrafish-specific genes on chromosome 4 and chromosomal regions that influence sex determination.

The zebrafish (*Danio rerio*) was first identified as a genetically tractable organism in the 1980s. The systematic application of genetic screens led to the phenotypic characterization of a large collection of mutations[1,2]. These mutations, when driven to homozygosity, can produce defects in a variety of organ systems with pathologies similar to human disease. Such investigations have also contributed notably to our understanding of basic vertebrate biology and vertebrate development. In addition to enabling the systematic definition of a large range of early developmental phenotypes, screens in zebrafish have contributed more generally to our understanding of the factors controlling the specification of cell types, organ systems and body axes of vertebrates[7–9].

Although its contributions have already been substantial, zebrafish research holds further promise to enhance our understanding of the detailed roles of specific genes in human diseases, both rare and common. Increasingly, zebrafish experiments are included in studies of human genetic disease, often providing independent verification of the activity of a gene implicated in a human disease[3,3,10]. Essential to this enterprise is a high-quality genome sequence and complete annotation of zebrafish protein-coding genes with identification of their human orthologues.

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*These authors contributed equally to this work.

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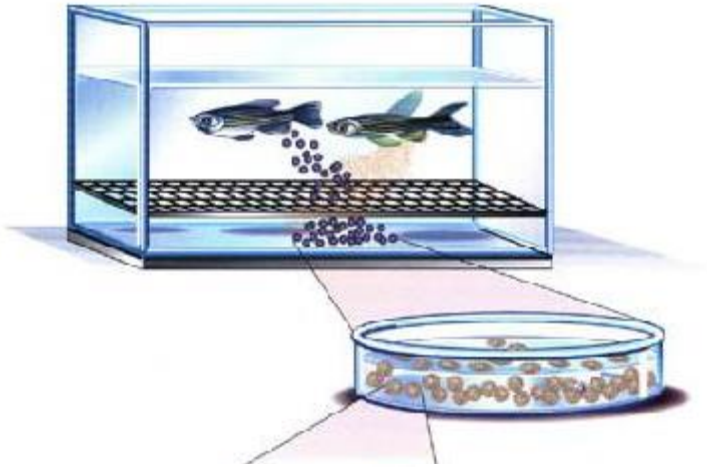
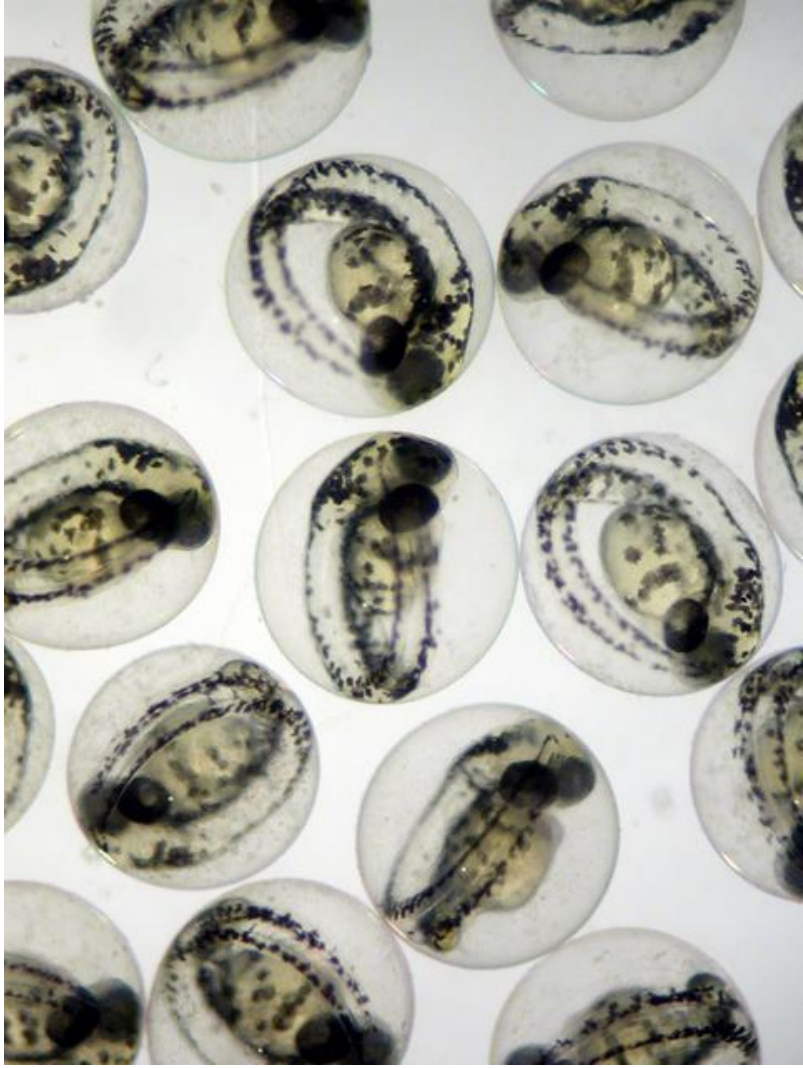
Sencilla manipulacion y mantenimiento

Agua:
200-800 μS/cm²
pH 6.9-7.5
26-28ºC

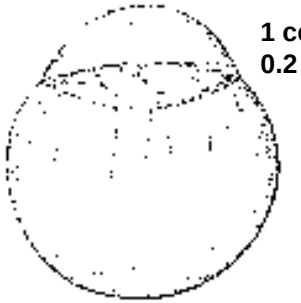
Tiempo de generacón: 3 meses
Sobrevida: 2.5 años

Cientos de huevos,
embriones transparentes



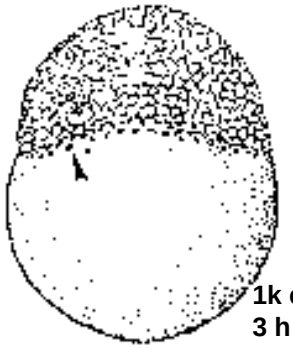


Eclosión: 48 horas
Vida libre: 5 días



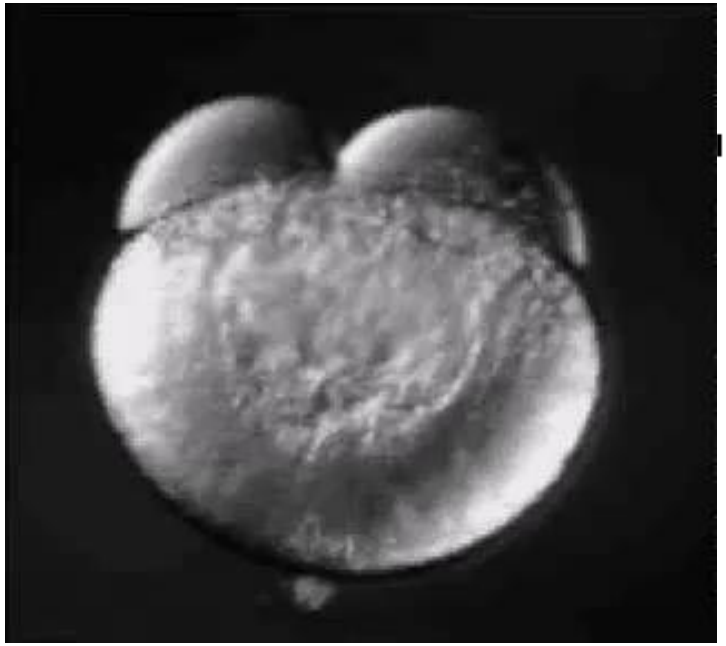
1 célula
0.2 h

64 células
2 h

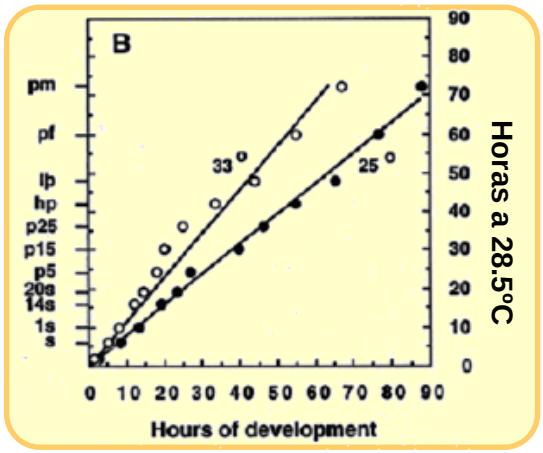


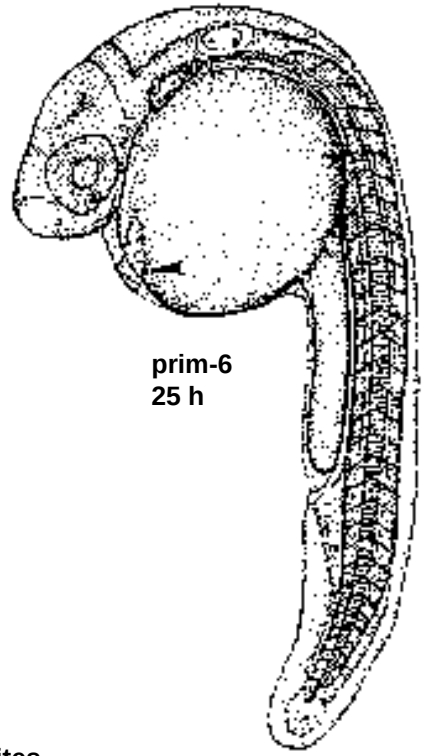
1k células
3 h

Desarrollo rápido, completamente accesible

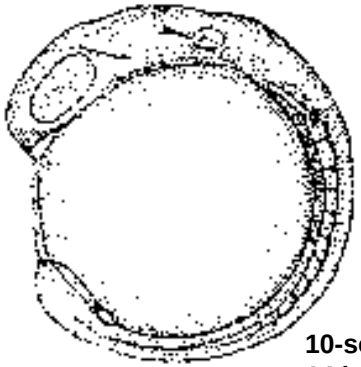


https://youtu.be/PGxkBluFiyA

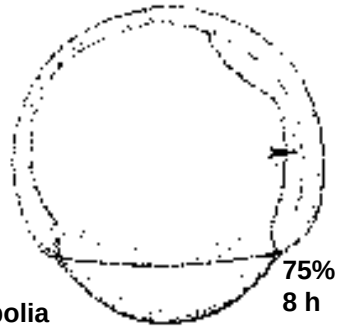




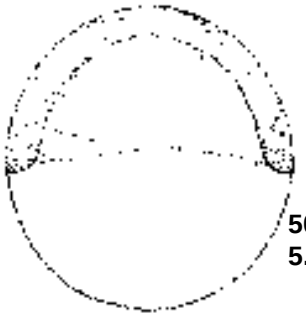
prim-6
25 h



10-somites
14 h

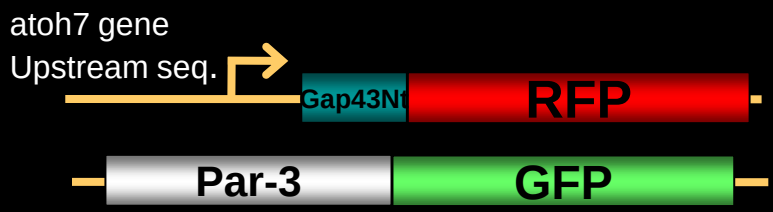


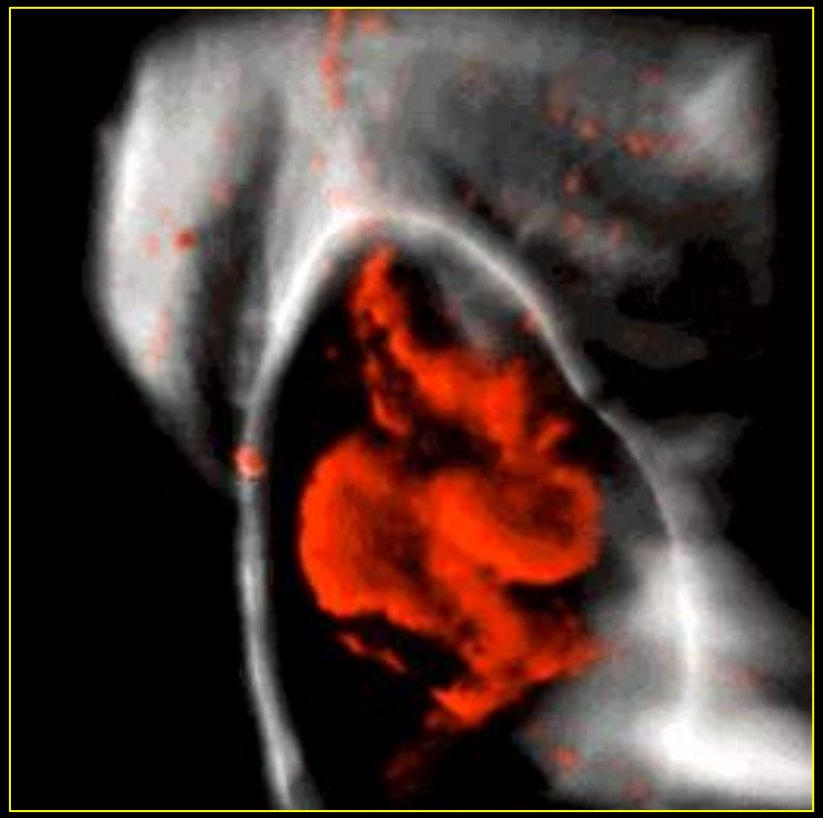
75% epibolia
8 h



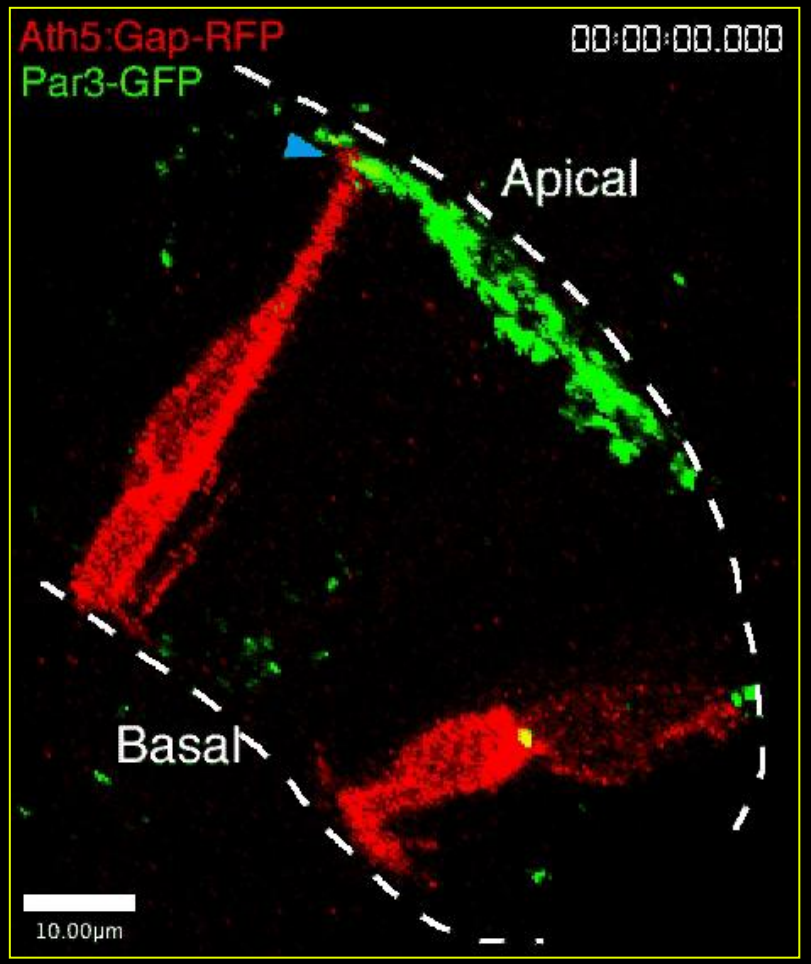
50% epibolia
5.3 h

Microscopía in vivo





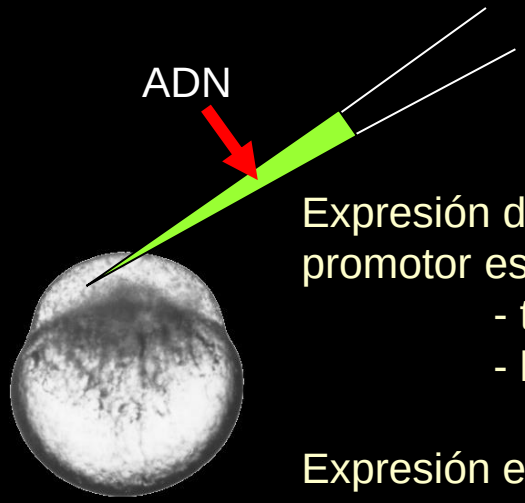
Liebling et al., 2005



https://youtu.be/-0ntEAml9ag

Zolessi et al., 2006

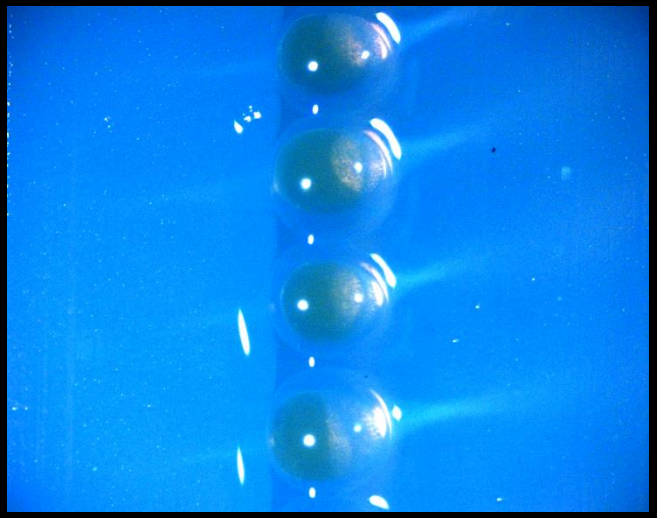
Expresión transitoria de proteínas exógenas

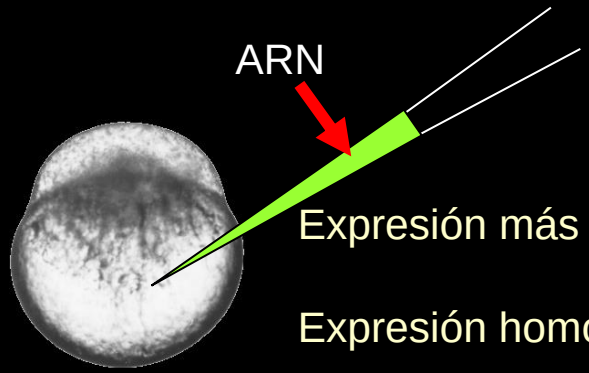


Expresión de genes regulada por promotor específico:

- tiempo
- lugar

Expresión en mosaico

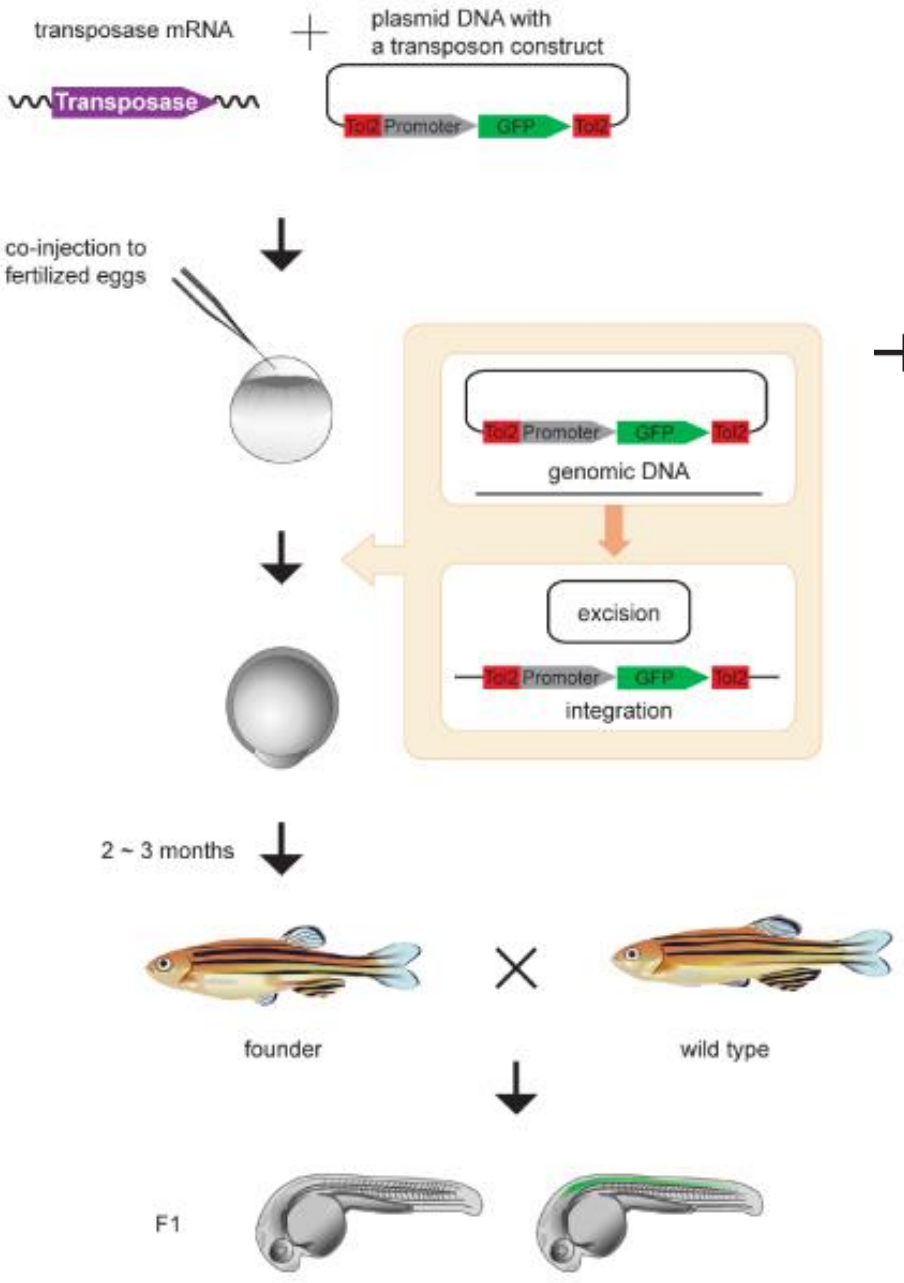


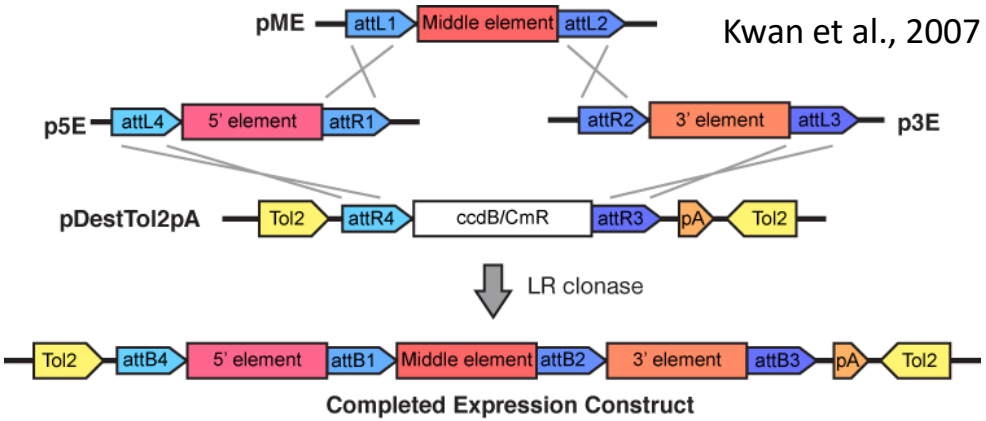


Expresión más rápida

Expresión homogénea

Transgénesis mediada por transposición: Tol2

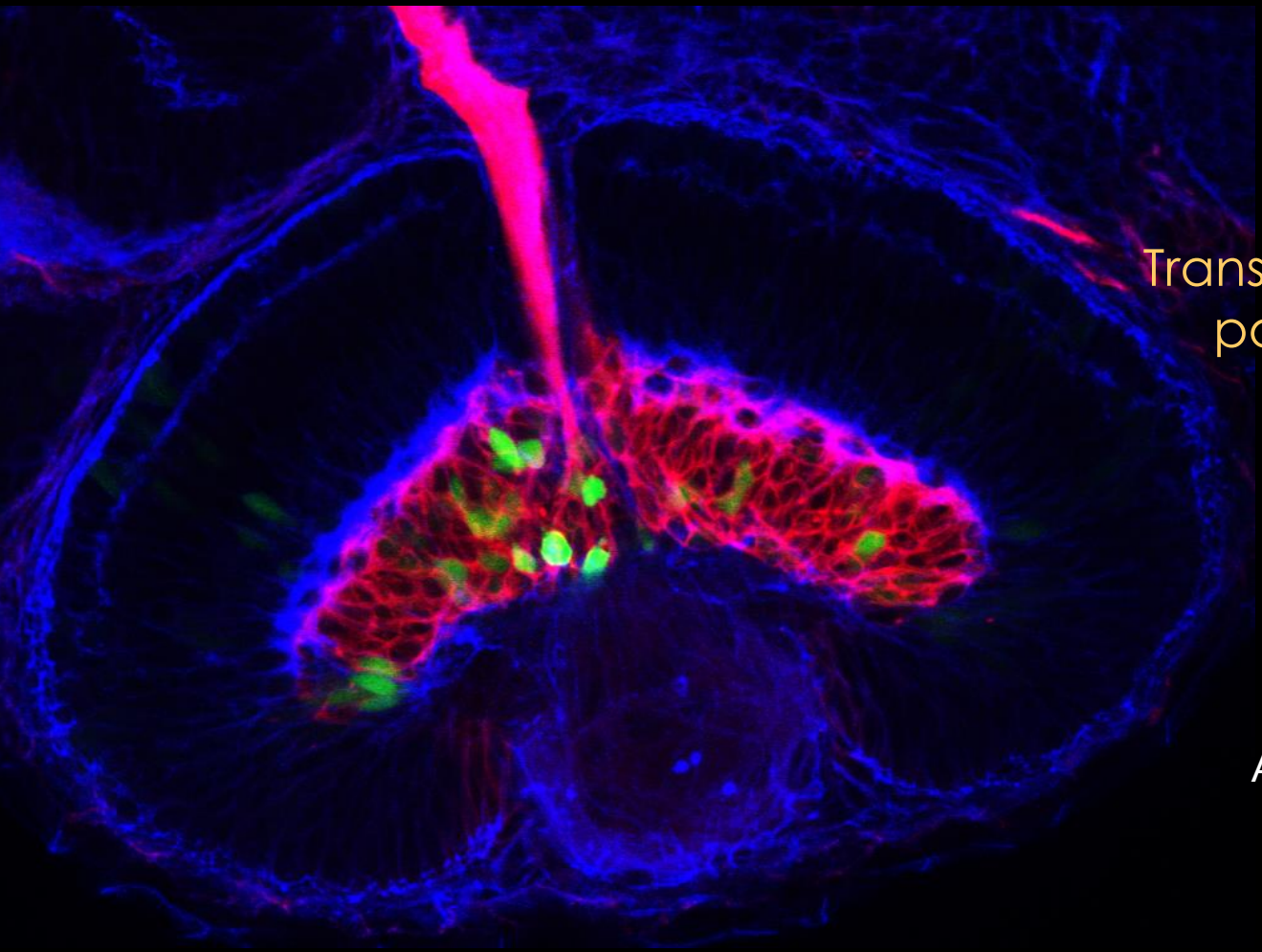




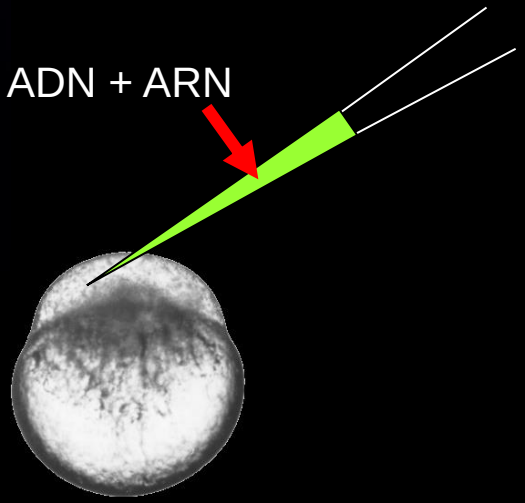
Kwan et al., 2007

http://tol2kit.genetics.utah.edu/

#	name	insert	size (bp)	made by	ver	MTA?
5' entry clones, attL4-R1 (kan resistant)						
299	p5E-*bactin2*	5.3 kb beta-actin promoter (ubiquitous)	7950	KMK	1.0	
380	p5E-*h2afx*	1 kb histone2A-X promoter (quasi-ubiquitous)	3604	KMK	1.0	
382	p5E-CMV/SP6	1 kb CMV/SP6 cassette from pCS2+	3704	KMK	1.0	
222	p5E-*hsp70l*	1.5 kb hsp70l promoter for heat-shock induction	4163	BM	1.0	
327	p5E-UAS	10x UAS element and basal promoter for Gal4 response	3127	DSC	1.0	
228	p5E-MCS	multiple-cloning site from pBluescript	2810	BM	1.0	
381	p5E-Fse-Asc	restriction sites for 8-cutters FseI and AscI	2663	EF	1.0	
middle entry clones, attL1-L2 (kan resistant)						
383	pME-EGFP	EGFP	3327	KMK	1.0	
384	pME-EGFPCAAX	membrane-localized (prenylated) EGFP; fused to 21 aa of H-ras	3345	KMK	1.0	
385	pME-nlsEGFP	nuclear-localized EGFP	3342	KMK	1.0	
386	pME-mCherry	monomeric red fluorophore mCherry	3261	KMK	1.0	
232	pME-mCherryCAAX H80D**	prenylated mCherry (deleterious H80D mutation; superseded by 450)	3321	KMK	1.0	
233	pME-nlsmCherry	nuclear-localized mCherry	3288	KMK	1.0	
234	pME-H2AmCherry	mCherry fused to zebrafish histone H2A.F/Z	3651	KMK	1.0	
387	pME-Gal4VP16	Gal4 DNA binding domain fused to VP16 transactivation domain	3204	EF	1.0	
237	pME-MCS	multiple-cloning site from pBluescript	2765	BM	1.1	
450	pME-mCherryCAAX**	prenylated mCherry (some preps contam w/ H80D, superseded by #550)	3321	KMK/SYC	1.2	
455	pME-EGFP no stop	EGFP, no stop (to make N-terminal fusions)	3324	KMK	1.2	
456	pME-mCherry no stop	mCherry, no stop (to make N-terminal fusions)	3258	KMK	1.2	
550	pME-mCherryCAAX	prenylated mCherry	3321	KMK/SYC	1.2	
3' entry clones, attR2-L3 (kan resistant)						
302	p3E-polyA	SV40 late polyA signal	2838	KMK	1.0	
229	p3E-MTpA	6x myc tag for C-terminal fusions, plus SV40 late polyA	3151	BM	1.0	
366	p3E-EGFPpA	EGFP for C-terminal fusions, plus SV40 late polyA	3634	MEH	1.0	
388	p3E-mCherrypA	mCherry for C-terminal fusions, plus SV40 late polyA	3586	MEH	1.0	
389	p3E-IRES-EGFPpA	IRES driving EGFP plus SV40 late polyA	4219	KMK	1.0	
390	p3E-IRES-EGFPCAAXpA	IRES driving prenylated EGFP plus SV40 late polyA	4250	KMK	1.0	
391	p3E-IRES-nlsEGFPpA	IRES driving nuclear EGFP plus SV40 late polyA	4248	KMK	1.0	
destination vectors, attR4-R3 (amp/chlor resistant; grow in ccdB tolerant cells)						



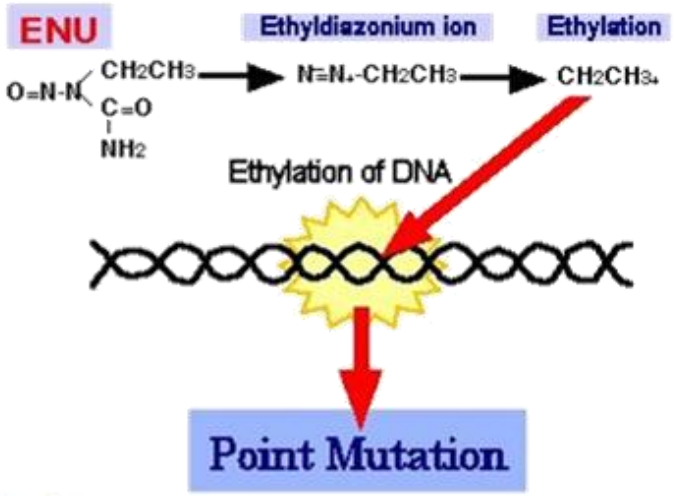
Transgénesis mediada por transposición

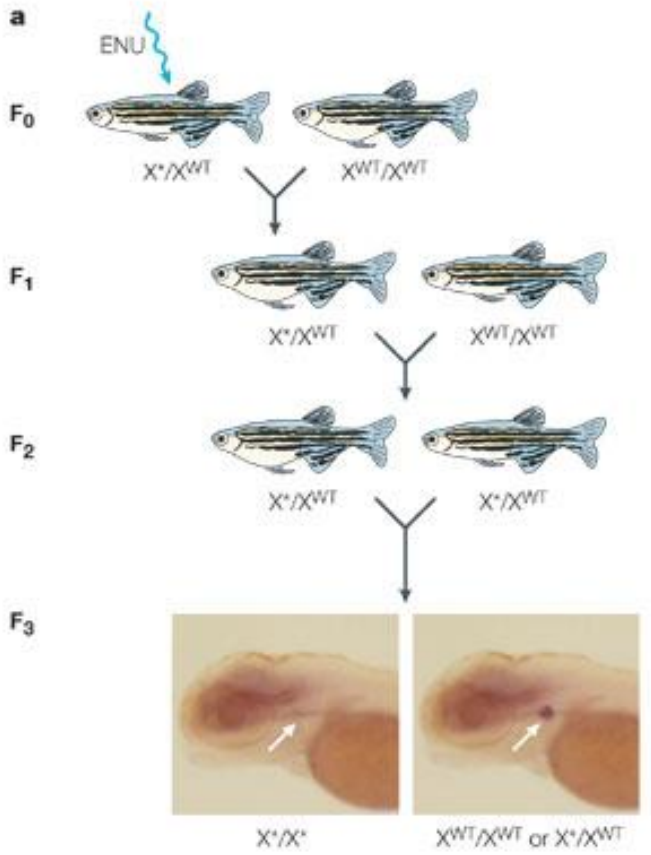


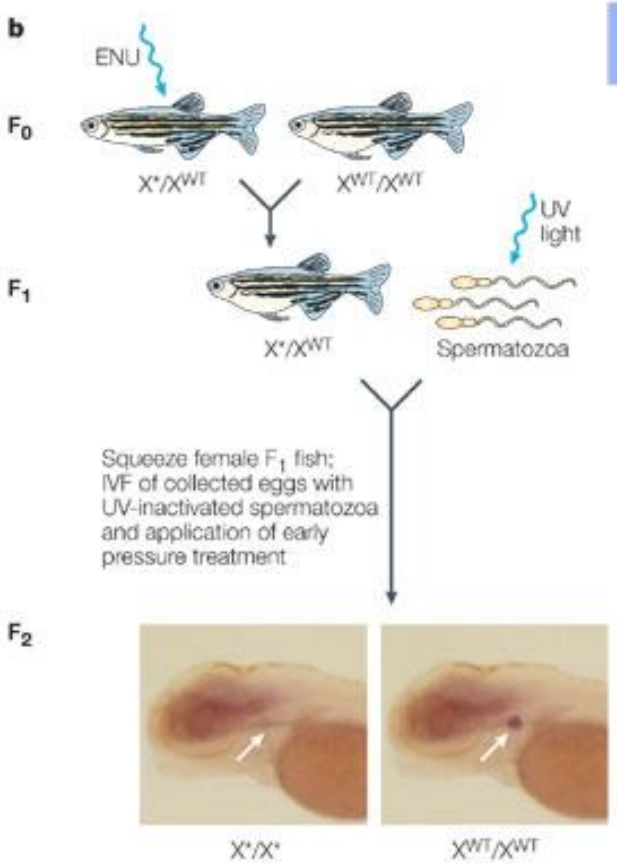
"Atoh7:NLS-GFP"

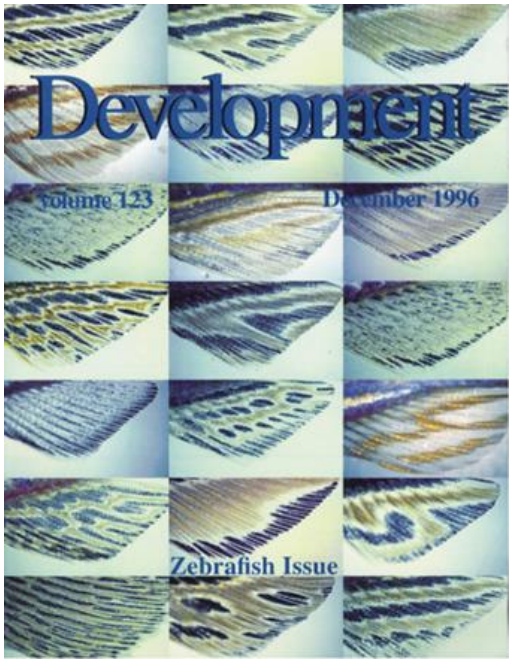
Mutagénesis química ("genética directa")

ENU = N-etil N-nitrosourea









Oligómeros de Morfolino

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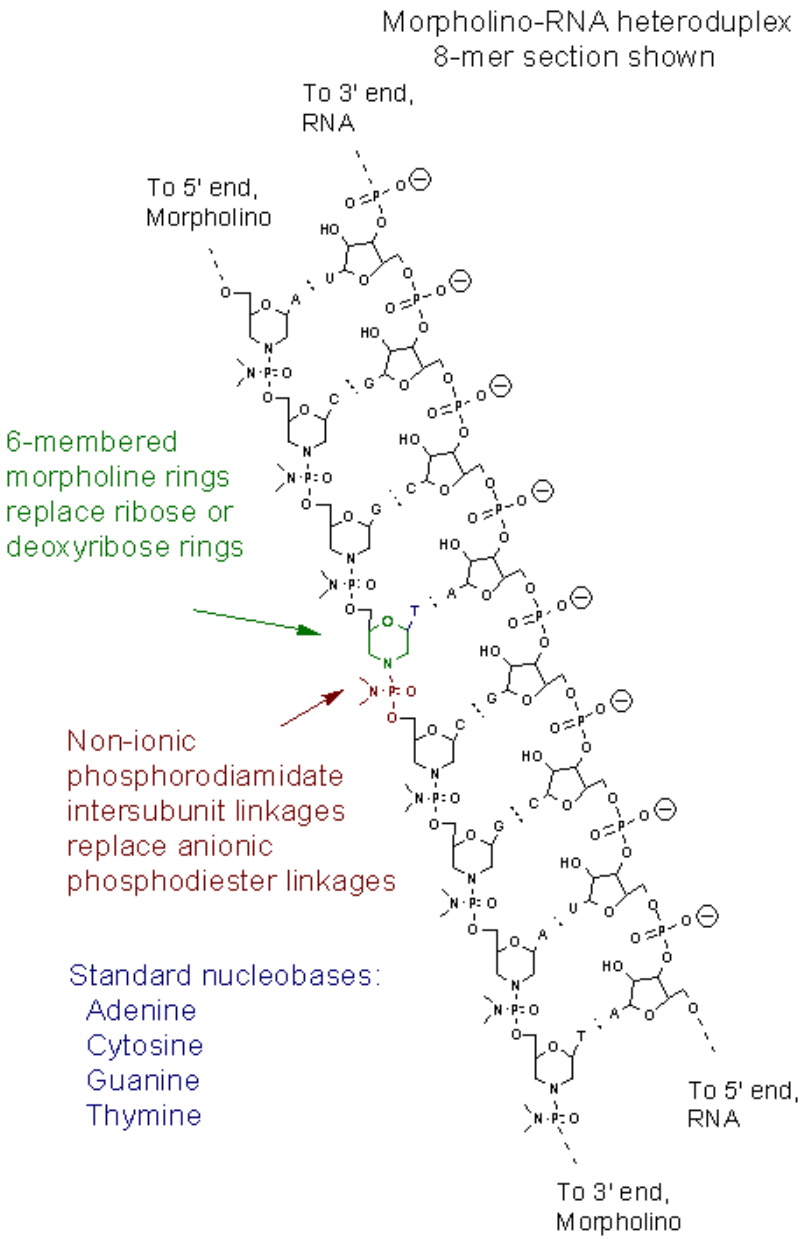
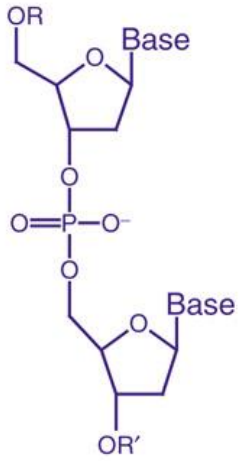
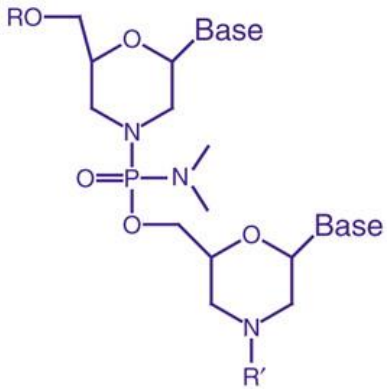
Comprehensive Service

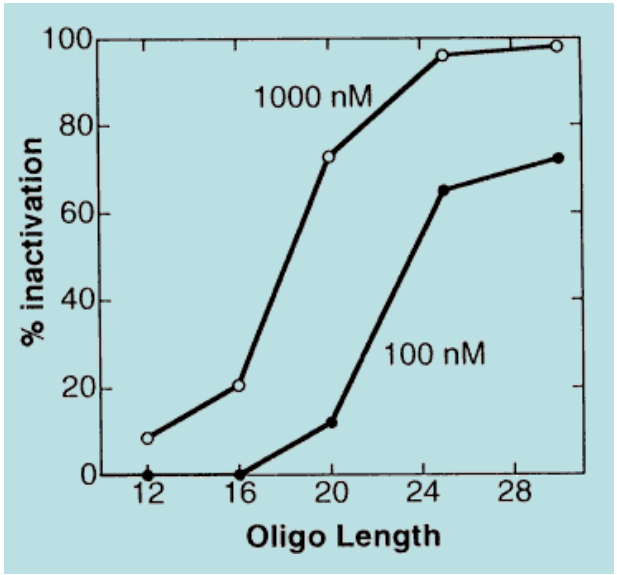




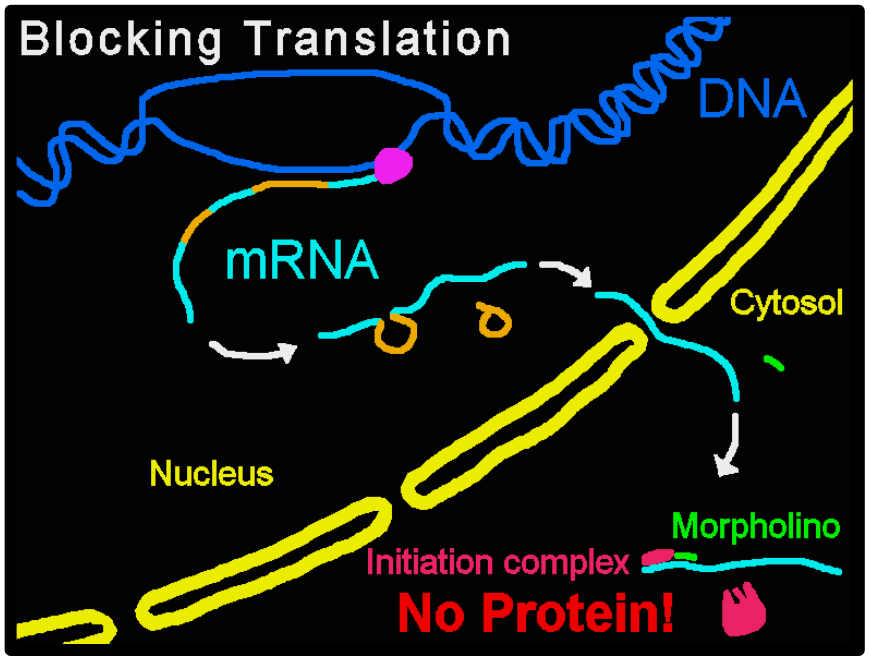
Phosphodiester
DNA

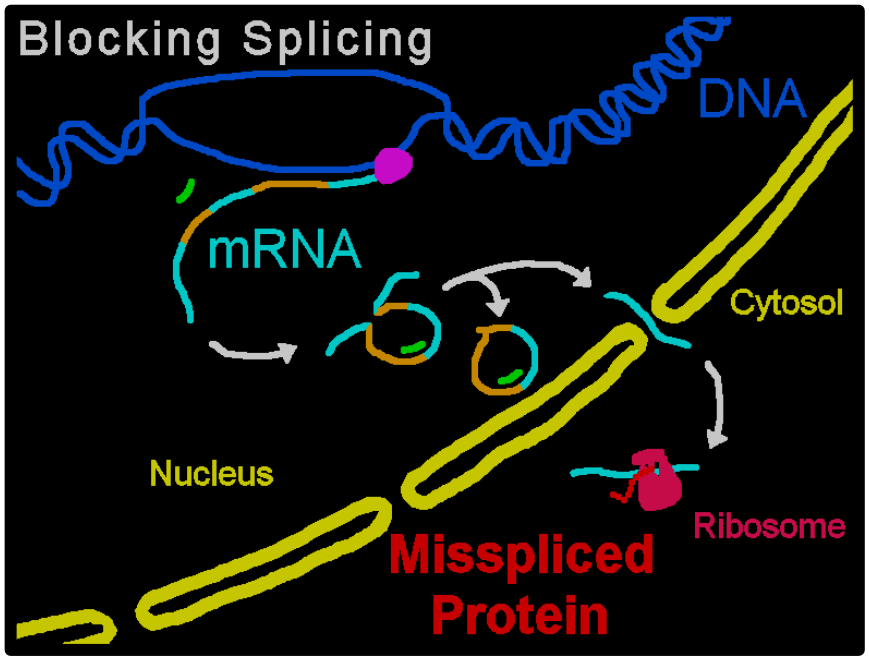


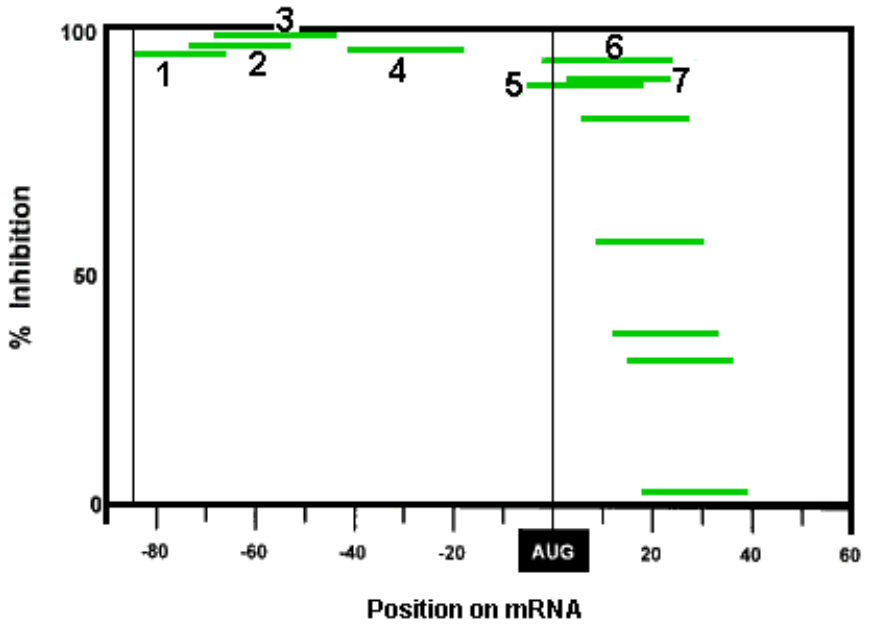
Morpholino

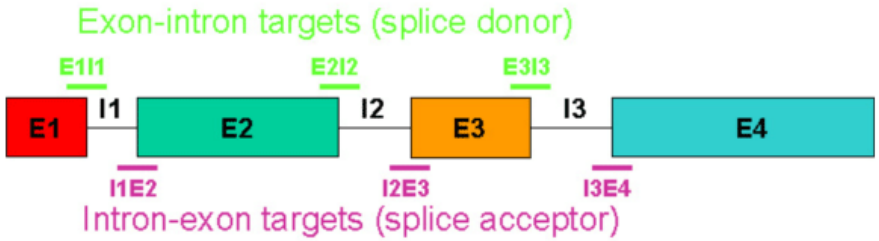


Oligómeros de Morfolino

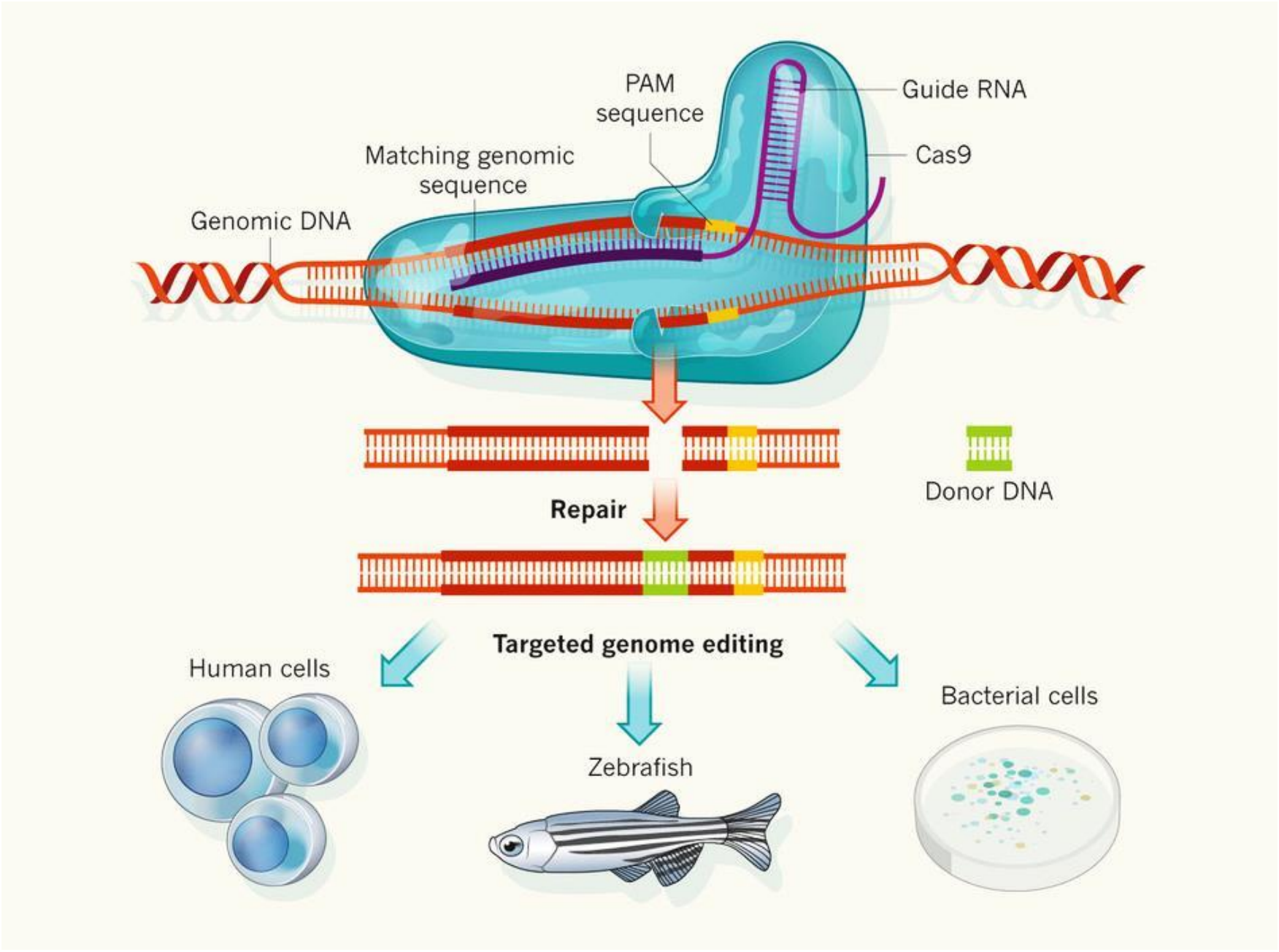




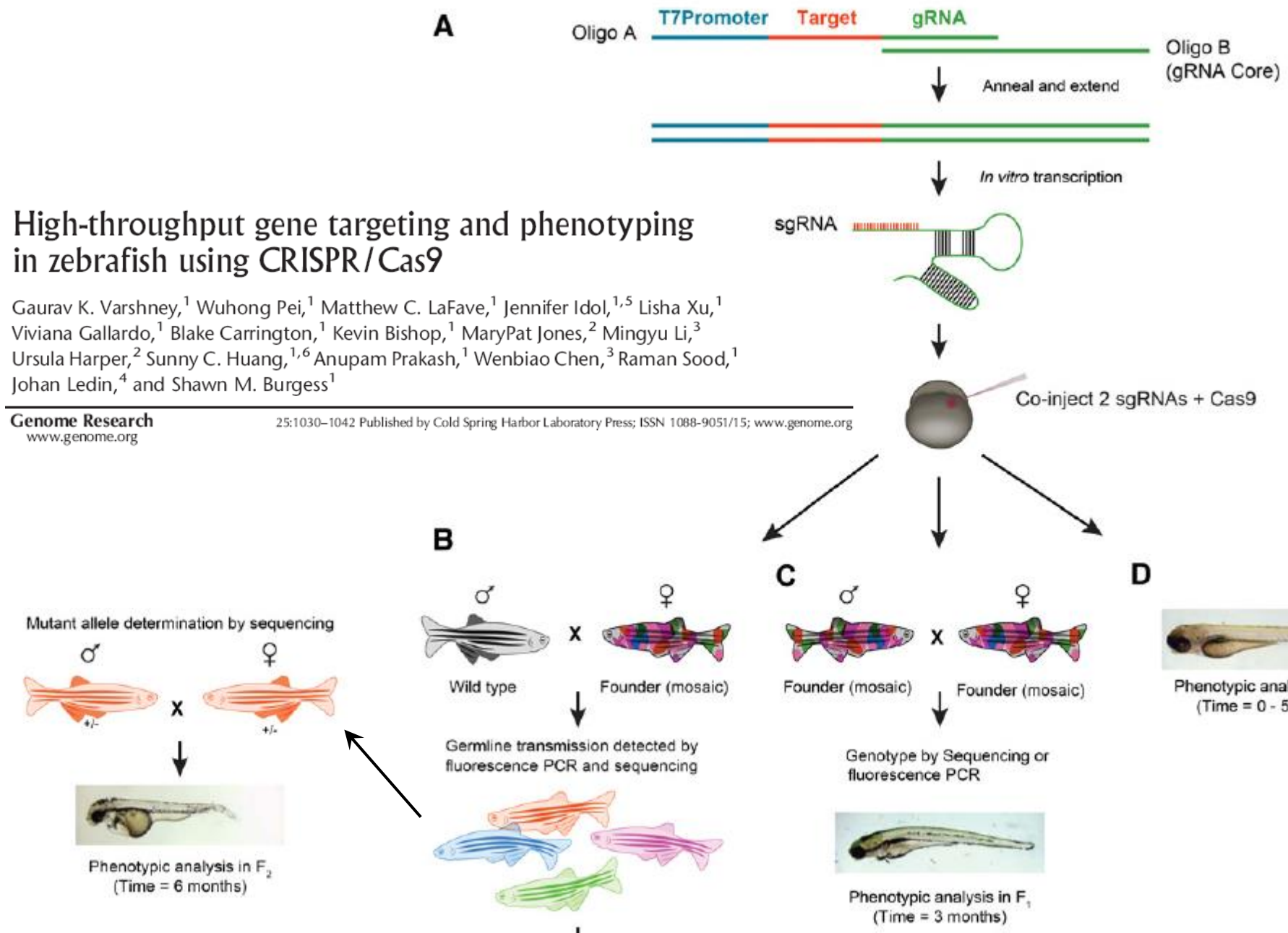




CRISPR


PAM sequence
Guide RNA
Matching genomic sequence
Cas9
Genomic DNA
Donor DNA
Repair
Targeted genome editing
Human cells
Zebrafish
Bacterial cells

Aplicaciones de CRISPR en zebrafish: generación de mutantes



High-throughput gene targeting and phenotyping in zebrafish using CRISPR/Cas9

Gaurav K. Varshney,[1] Wuhong Pei,[1] Matthew C. LaFave,[1] Jennifer Idol,[1,5] Lisha Xu,[1] Viviana Gallardo,[1] Blake Carrington,[1] Kevin Bishop,[1] MaryPat Jones,[2] Mingyu Li,[3] Ursula Harper,[2] Sunny C. Huang,[1,6] Anupam Prakash,[1] Wenbiao Chen,[3] Raman Sood,[1] Johan Ledin,[4] and Shawn M. Burgess[1]

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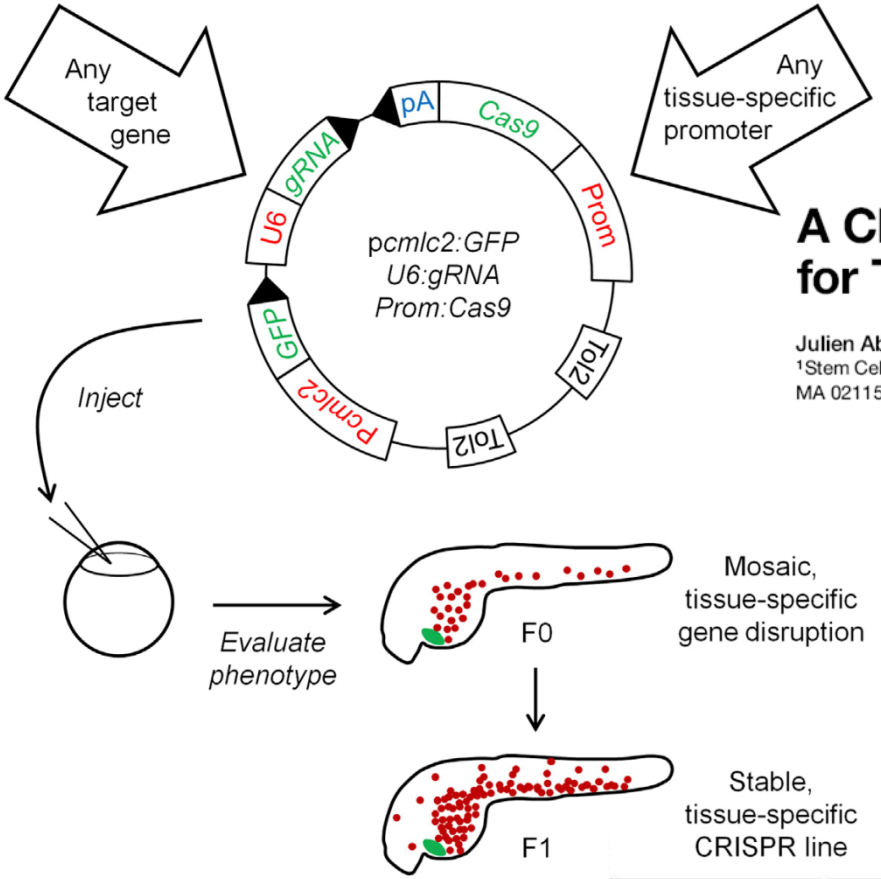
25:1030–1042 Published by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/15; www.genome.org

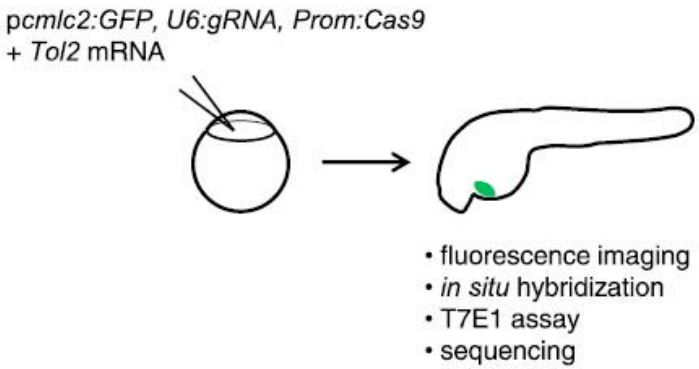
Aplicaciones de CRISPR en zebrafish: modificaciones condicionales

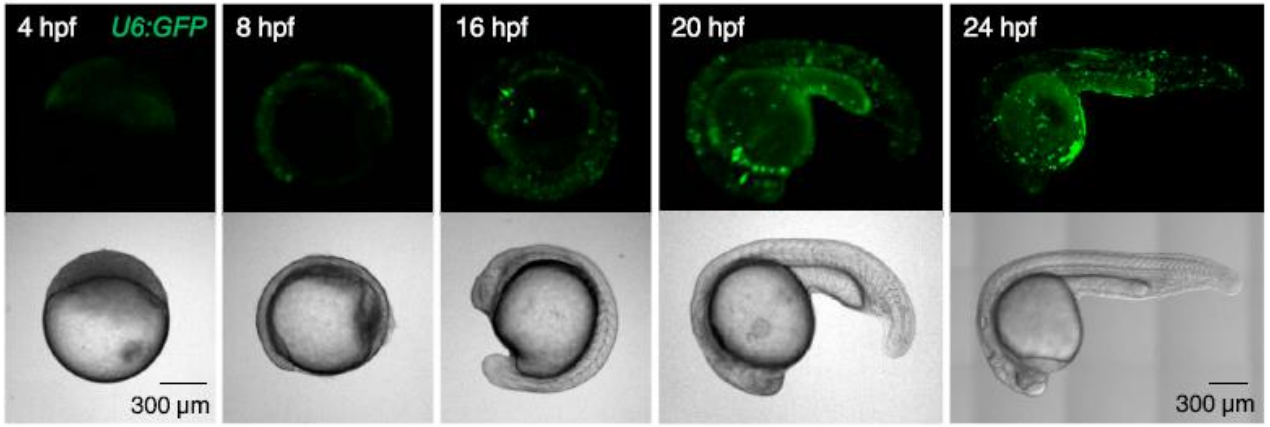


Developmental Cell 32, 756–764, March 23, 2015

A CRISPR/Cas9 Vector System for Tissue-Specific Gene Disruption in Zebrafish

Julien Ablain,[1] Ellen M. Durand,[1] Song Yang,[1] Yi Zhou,[1,2] and Leonard I. Zon[1,2,3,*]
[1]Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Boston, MA 02115, USA

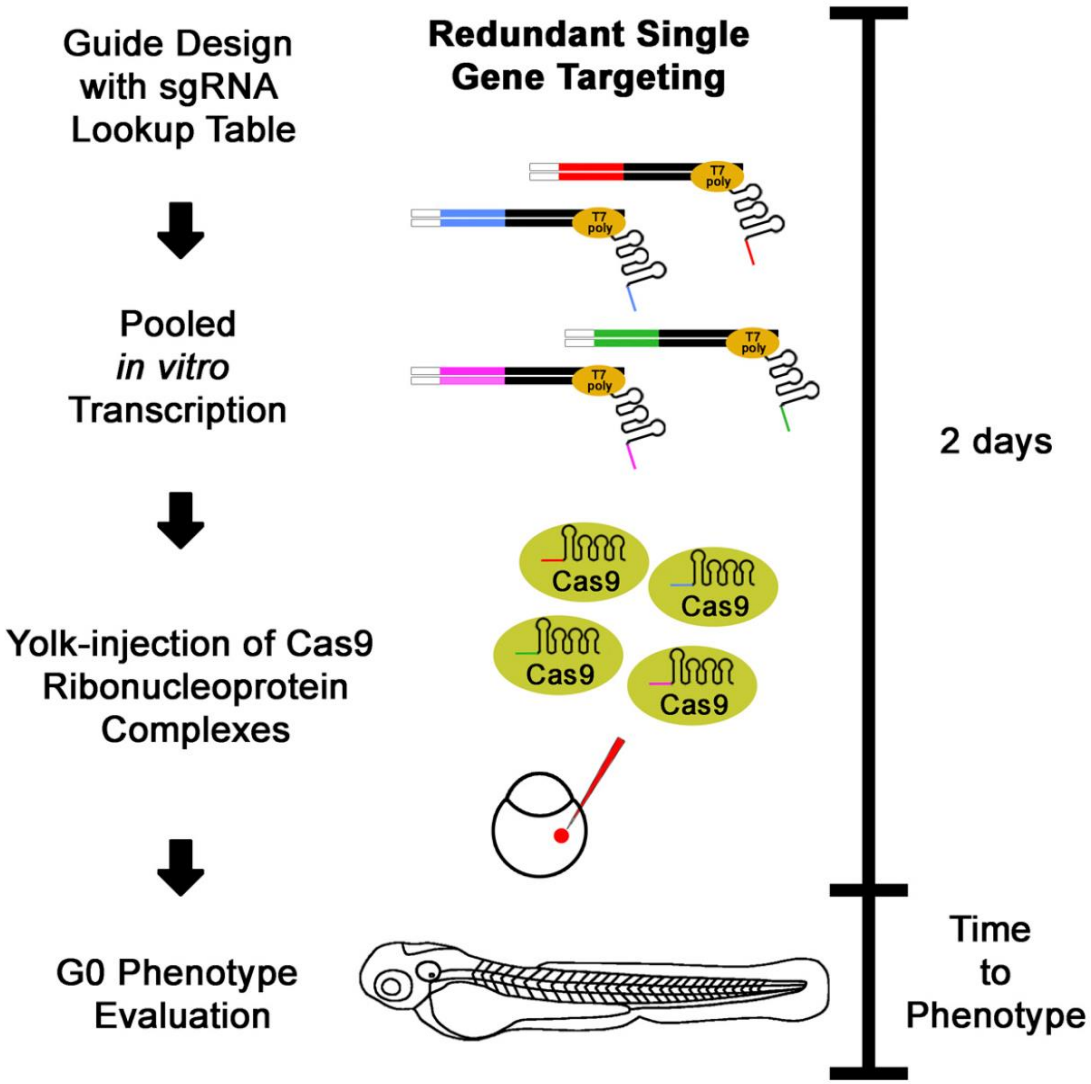


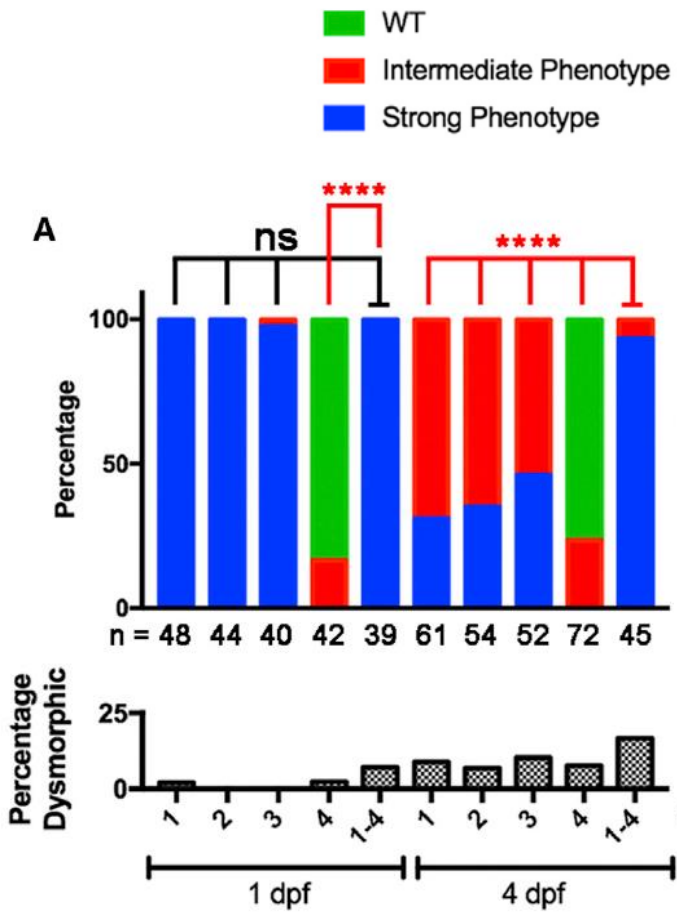


Developmental Cell

A Rapid Method for Directed Gene Knockout for Screening in G0 Zebrafish

Roland S. Wu, Ian I. Lam, Hilary Clay, Daniel N. Duong, Rahul C. Deo, Shaun R. Coughlin





https://youtu.be/aV3asP07KJw