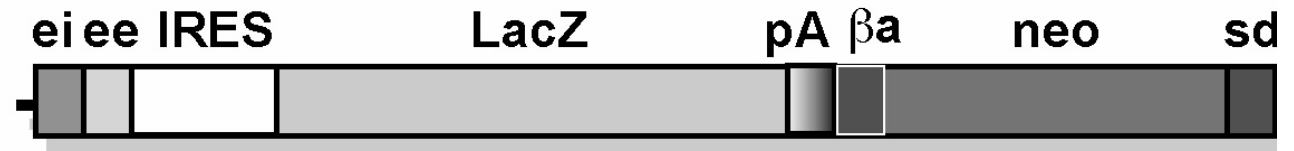


Genetrap vectors

IRES β geo



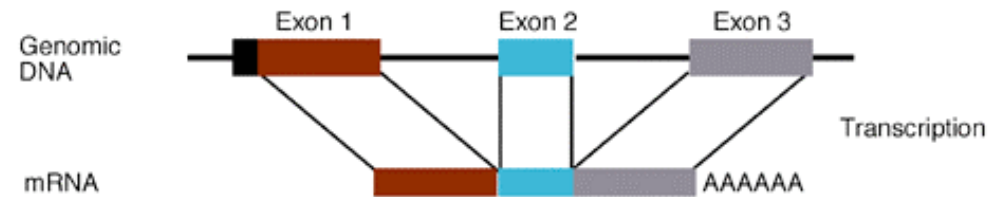
IRES β galNeo(-pA)



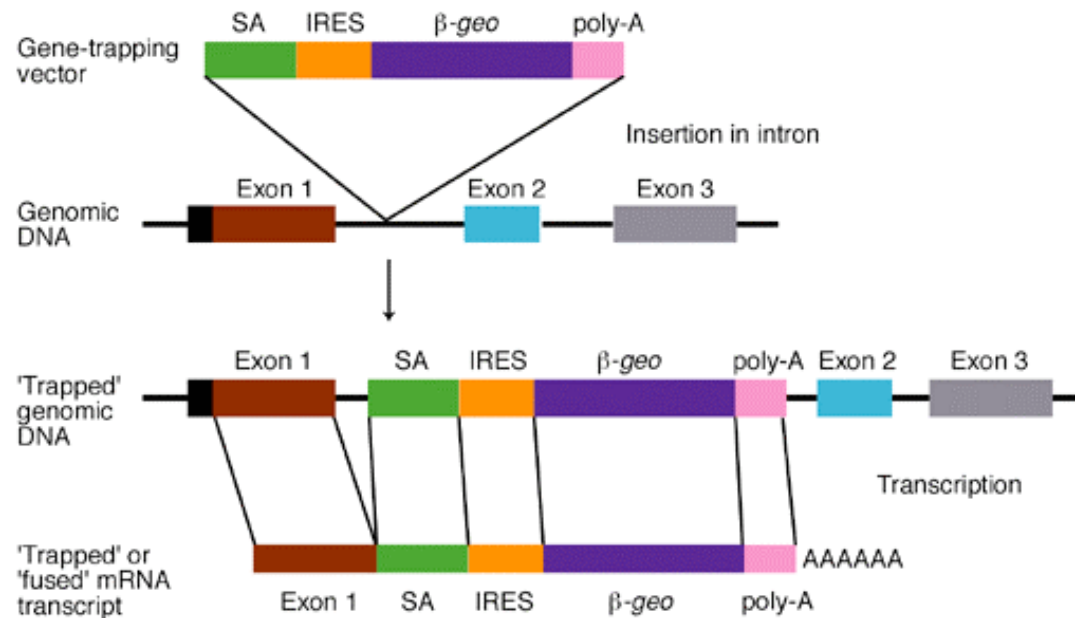
a IRES β geo gene-trapping vector



b Gene structure before insertion of gene-trapping vector



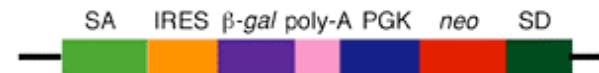
c Gene structure after insertion of gene-trapping vector



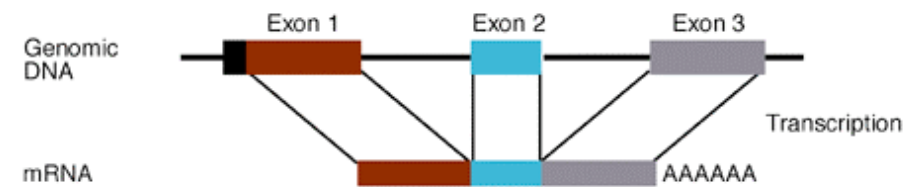
The IRES β geo gene-trapping vector and gene-trapping strategy

Expert Reviews in Molecular Medicine © 2000 Cambridge University Press

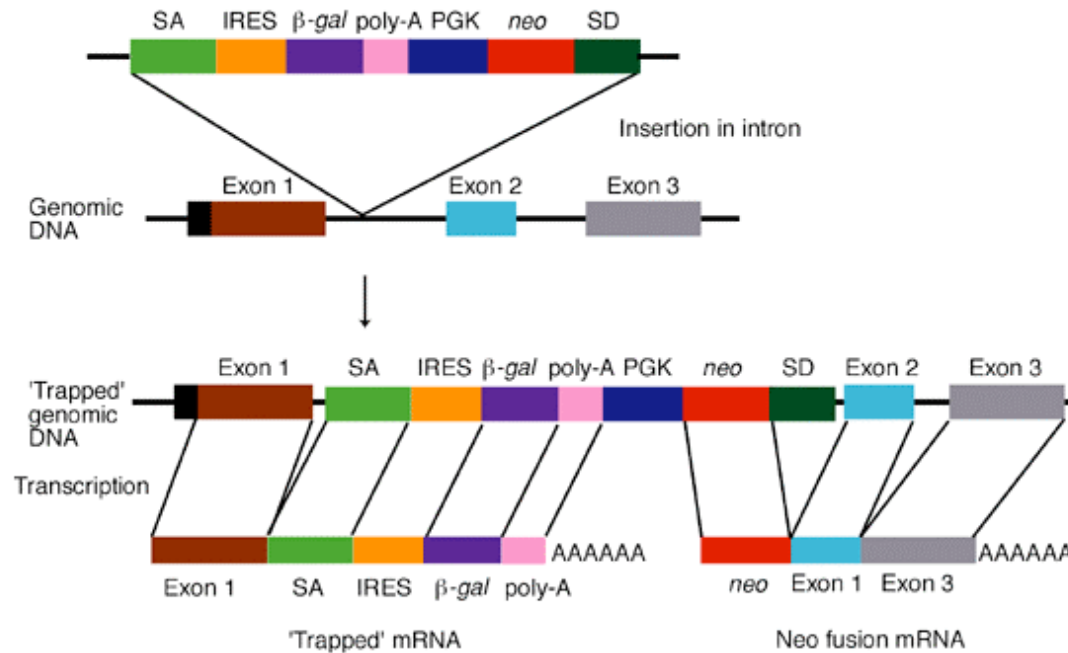
a IRES β galNeo(-pA) gene-trapping vector



b Gene structure before insertion of gene-trapping vector



c Gene structure after insertion of gene-trapping vector



The IRES β galNeo(-pA) gene-trapping vector and gene-trapping strategy

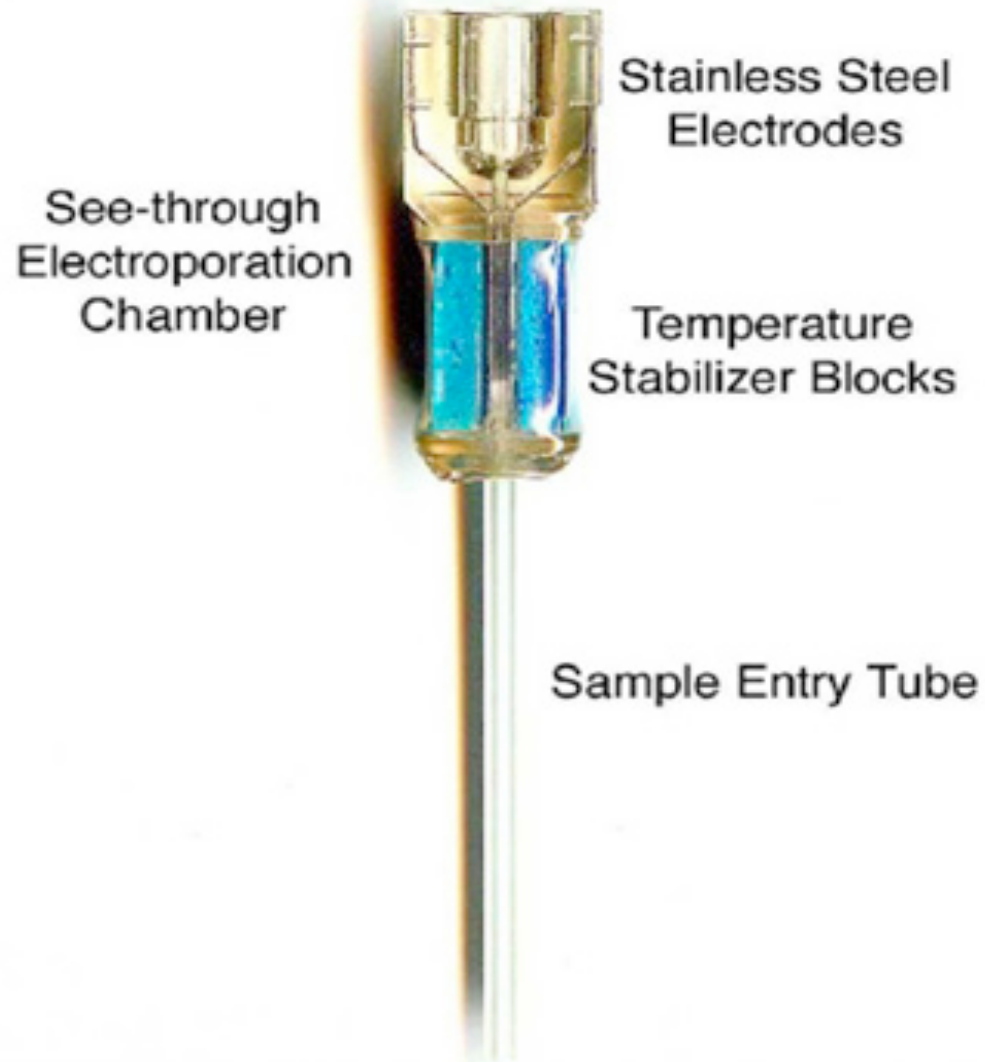
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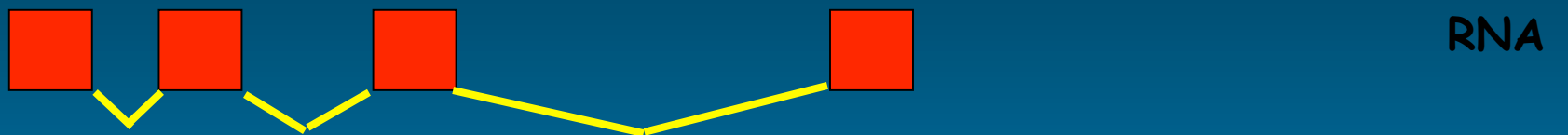
La strategia gene trap

- 1. Può essere applicata su larga scala**
- 2. Consente l'isolamento di nuovi geni**
- 3. Consente la determinazione del profilo d'espressione del gene intrappolato**
- 4. È di per sé mutagenica: dà luogo ad un knockout**

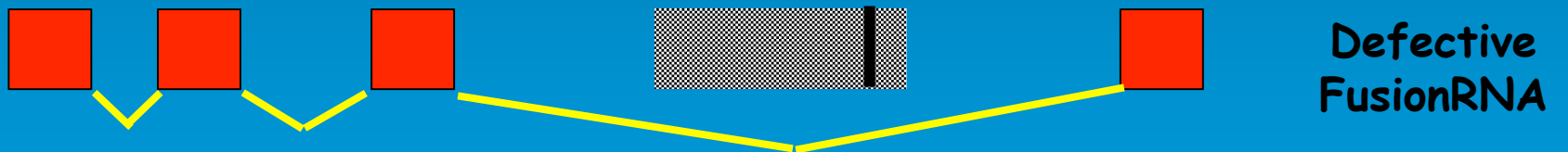
Transfezione di cellule in coltura I. Lipofectamina

II. Elettroporazione





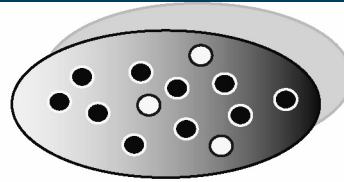
MA.....



(SPICE-AROUND)

La sequenza della strategia gene trap

1. Electroporation of the gene trap cassette into ES cells



2. Selection of recombinant ES cell clones



3. Aggregation of the ES clone with preimplantation mouse embryos

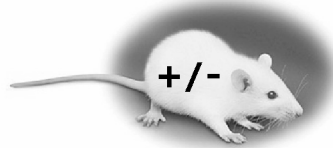
4. Identification of the trapped gene by 5' RACE Technology



5. Analysis of the trapped gene expression pattern in mouse embryo



6. Generation of transgenic offspring and phenotypic analysis



1. Il sequenziamento del gene intrappolato

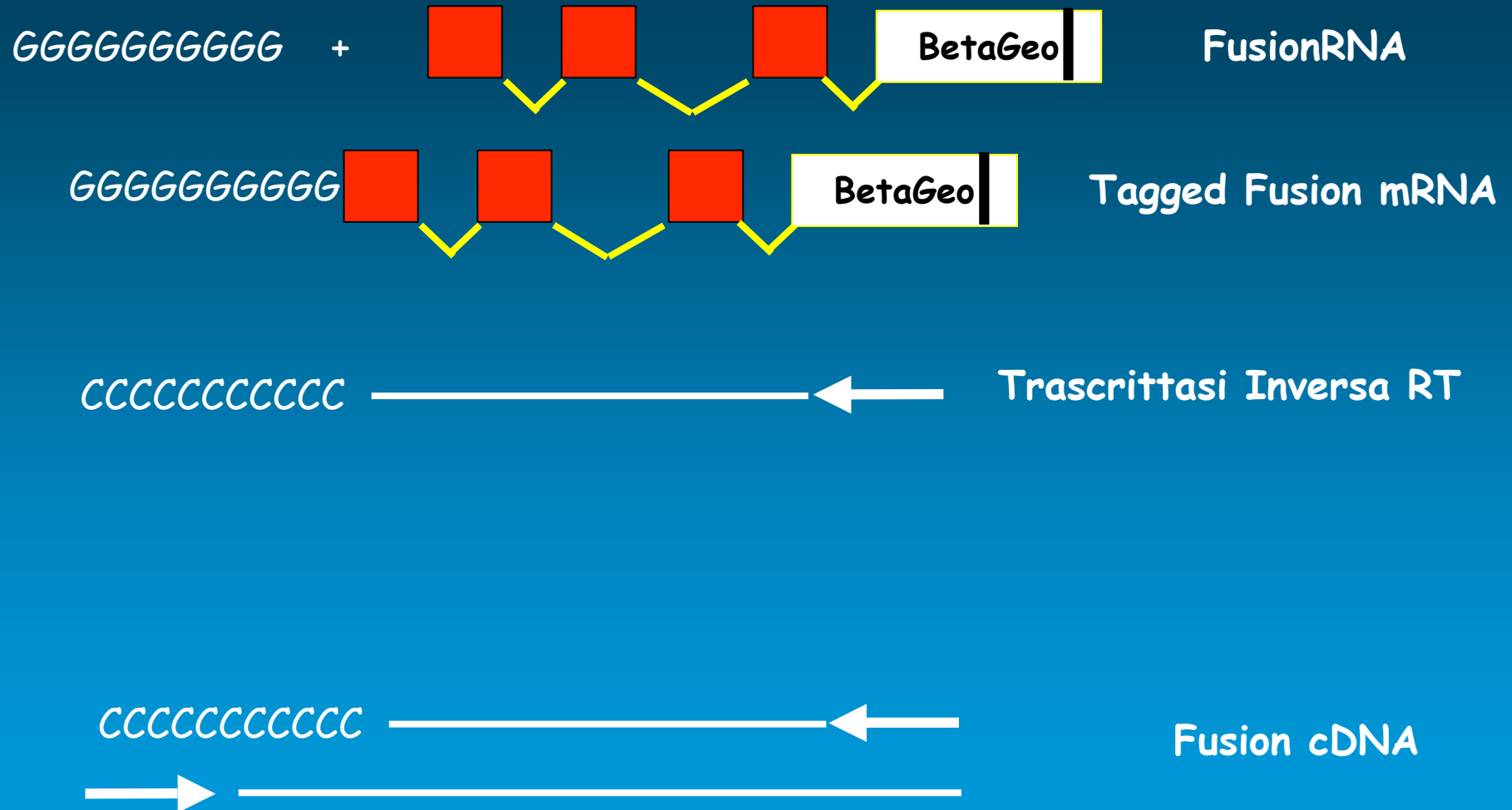


TABLE 1

Subcellular β -gal Localization Patterns in Cell Lines
Obtained with pGT1.8geo

Diffuse ^a	49 (42%)
Cytoplasmic ^b	12 (10%)
Dot ^c	10 (8%)
Cytoplasmic + dot ^d	29 (25%)
Cell surface ^e	6 (5%)
Nuclear ^f	11 (9%)

Note. Frequencies were calculated on the total number of β -gal-positive cell lines.

^a β -gal staining was found throughout the cell.

^b β -gal staining was evenly distributed in the cytoplasm.

^c β -gal staining was restricted to single cytoplasmic dots.

^d β -gal staining was present both in cytoplasm and dots.

^e β -gal staining was found at the periphery of the cell.

^f β -gal staining was present only in the nucleus.

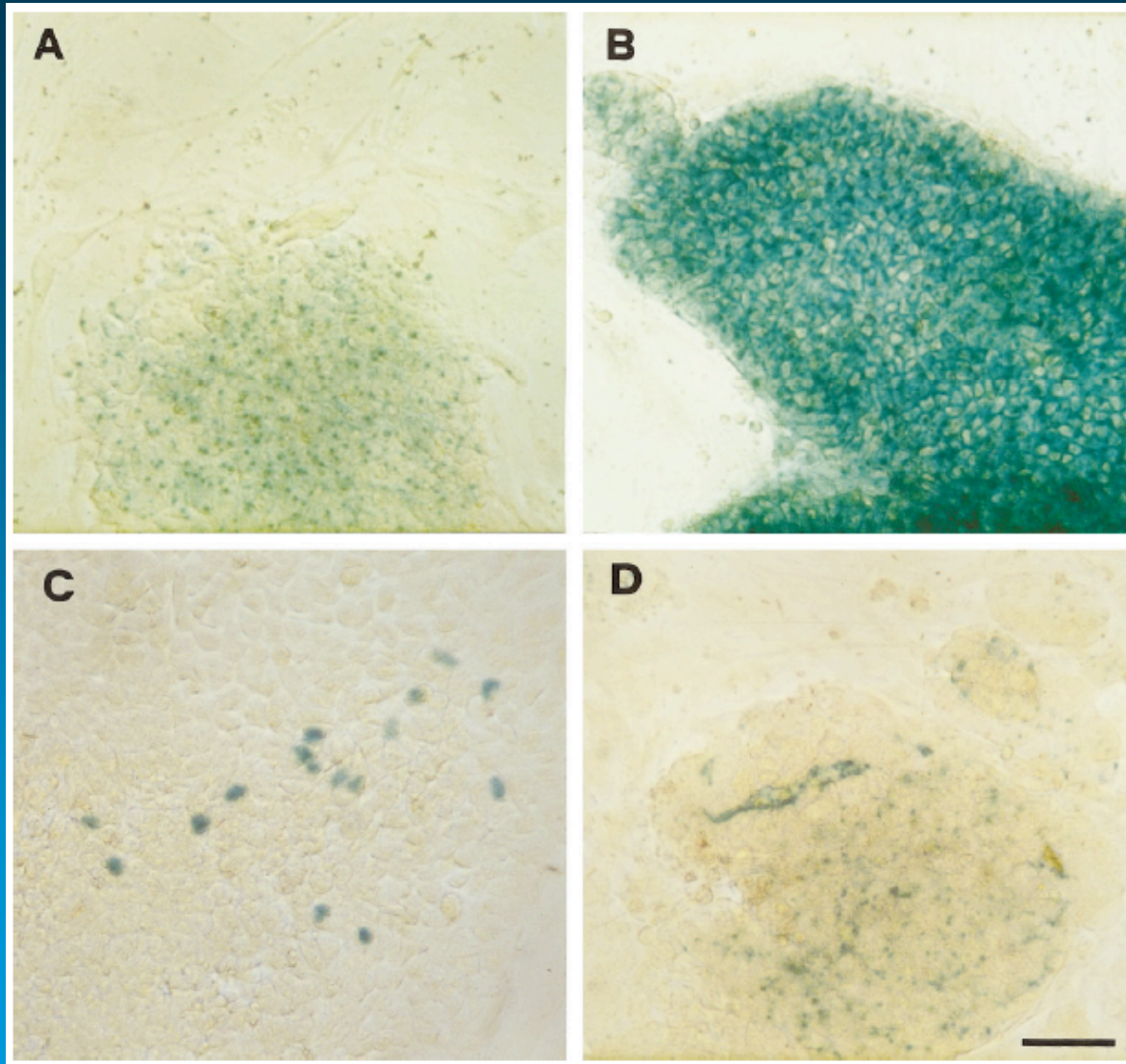


TABLE 2

Gene Trap Efficiencies of pGT1.8geo and IRES β geo Vectors

Experiment	Vector	No. of cells ^a	G418 ^{Rb}	β -gal + ^c
1	pGT1.8geo	$8 \cdot 10^6$	15	5 (33%)
2	pGT1.8geo	10^8	131	36 (30%)
3	pGT1.8geo	$9 \cdot 10^7$	84	20 (24%)
4	pGT1.8geo	$8 \cdot 10^7$	89	26 (30%)
5	pGT1.8geo	$6 \cdot 10^7$	90	33 (37%)
6	pGT1.8geo	10^7	21	6 (29%)
7	IRES β geo	10^7	39 ^d	28 (72%)
8	IRES β geo	$8 \cdot 10^7$	172 ^d	157 (91%)
9	IRES β geo	10^7	77	55 (71%)
10	IRES β geo	10^7	83	64 (77%)

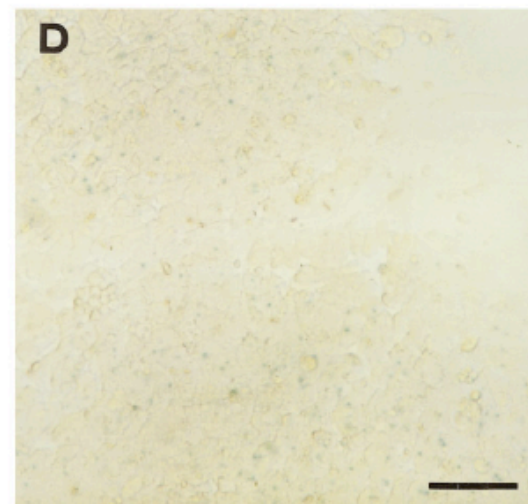
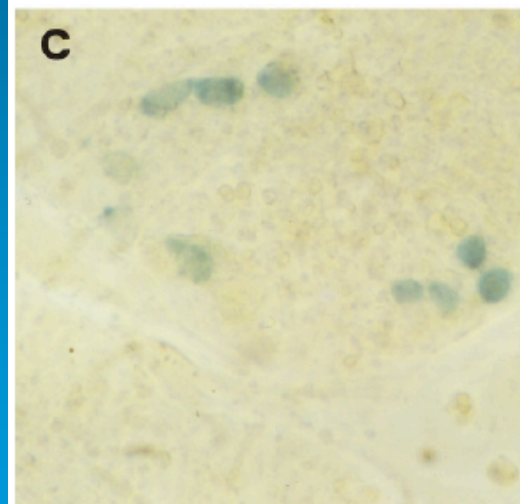
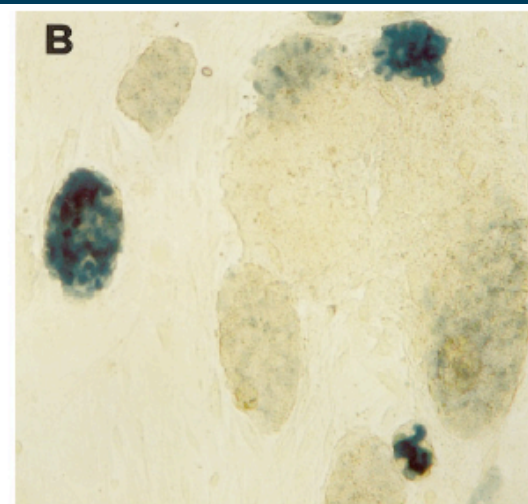
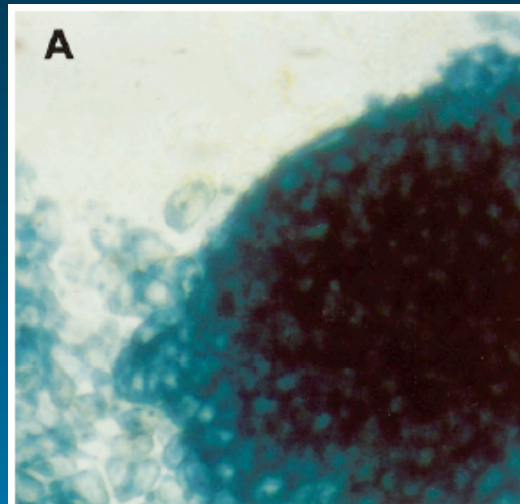
^a Number of ES cells used for each electroporation experiment.^b Number of G418-resistant colonies obtained on each experiment. The average number of G418^R colonies/ 10^7 electroporated cells was 12 (pGT1.8geo) and 80 (IRES β geo).^c Number of β -gal-positive colonies detected on each experiment. The proportion of β -gal-positive colonies among the total G418-resistant colonies is shown in parenthesis. The mean frequencies of β -gal-positive colonies were 29% (pGT1.8geo) and 82% (IRES β geo).^d Only some G418-resistant colonies were picked in these two experiments (remaining colonies were not included in the counting).

TABLE 3

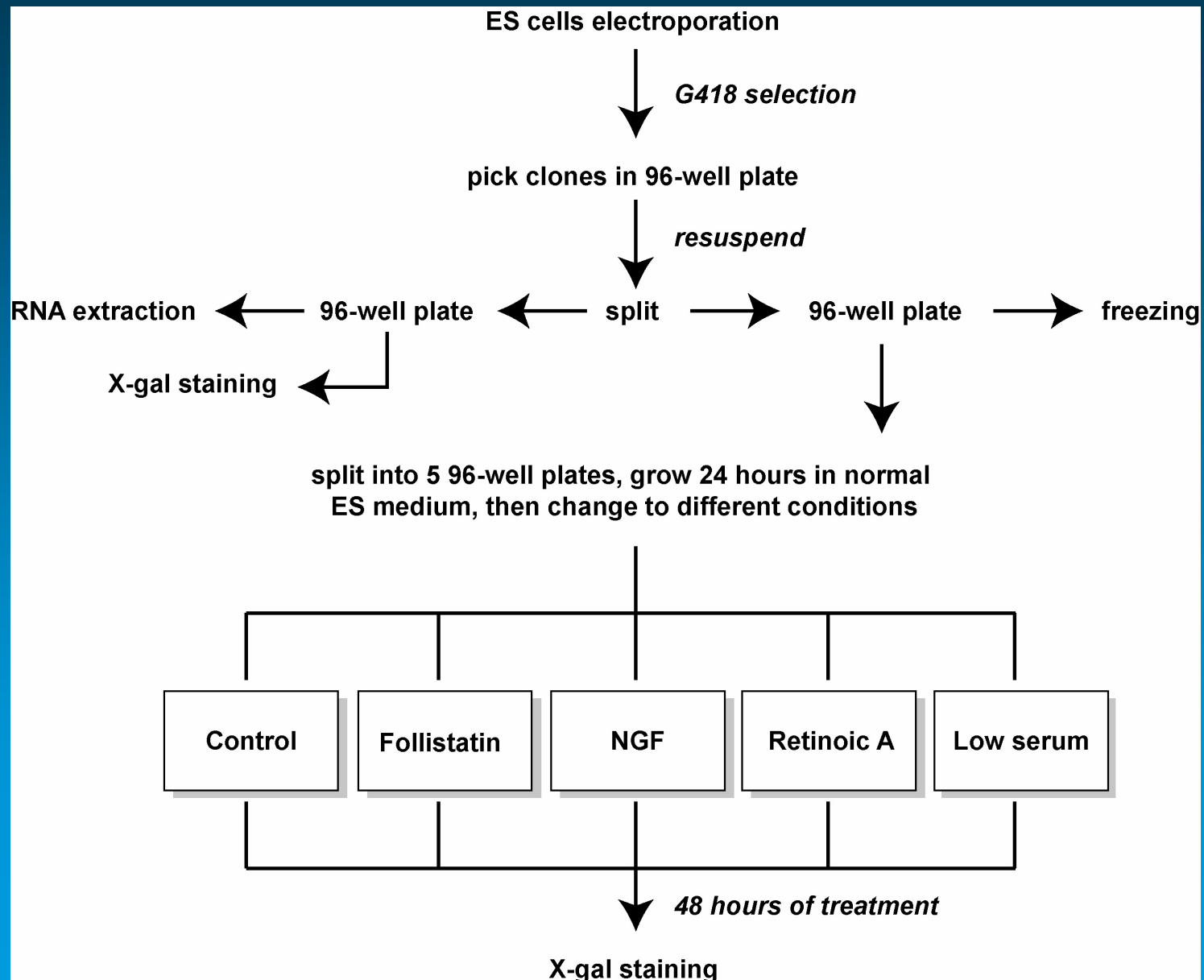
Distribution of *lacZ* Expression in Gene-Trapped Cell Lines

<i>In vitro lacZ</i> expression ^a	β geo ^b	IRES β geo ^b
Ubiquitous	18 (14%)	43 (15%)
Widespread	25 (20%)	22 (8%)
Ubiquitous/widespread, regulated	n.d. ^c	96 (34%)
Restricted	34 (27%)	51 (18%)
Very restricted	50 (39%)	69 (24%)
Faint (dot) expression ^d	n.d. ^c	68 (24%)

^a β -gal staining patterns of trapped cell lines were classified into five different groups as follows: ubiquitous (all cells in the dishes were positive, Fig. 3A), widespread (60–95% of the cells were positive), regulated (low levels of β -gal staining in most cells and much stronger staining in few cells, Fig. 3B), restricted (20–50% of the cells were positive), very restricted (1–15% of the cells were positive, Fig. 3C).^b Frequencies of lines for each group were calculated on the total of β -gal-positive lines (127 lines for pGT1.8geo and 281 lines for IRES β geo).^d *lacZ* expression was only detected after 48 h staining as a faint dot present in a variable proportion of cells (Fig. 3D).^c n.d., not determined.



E' possibile *pre-screenare* in vitro le cellule ES



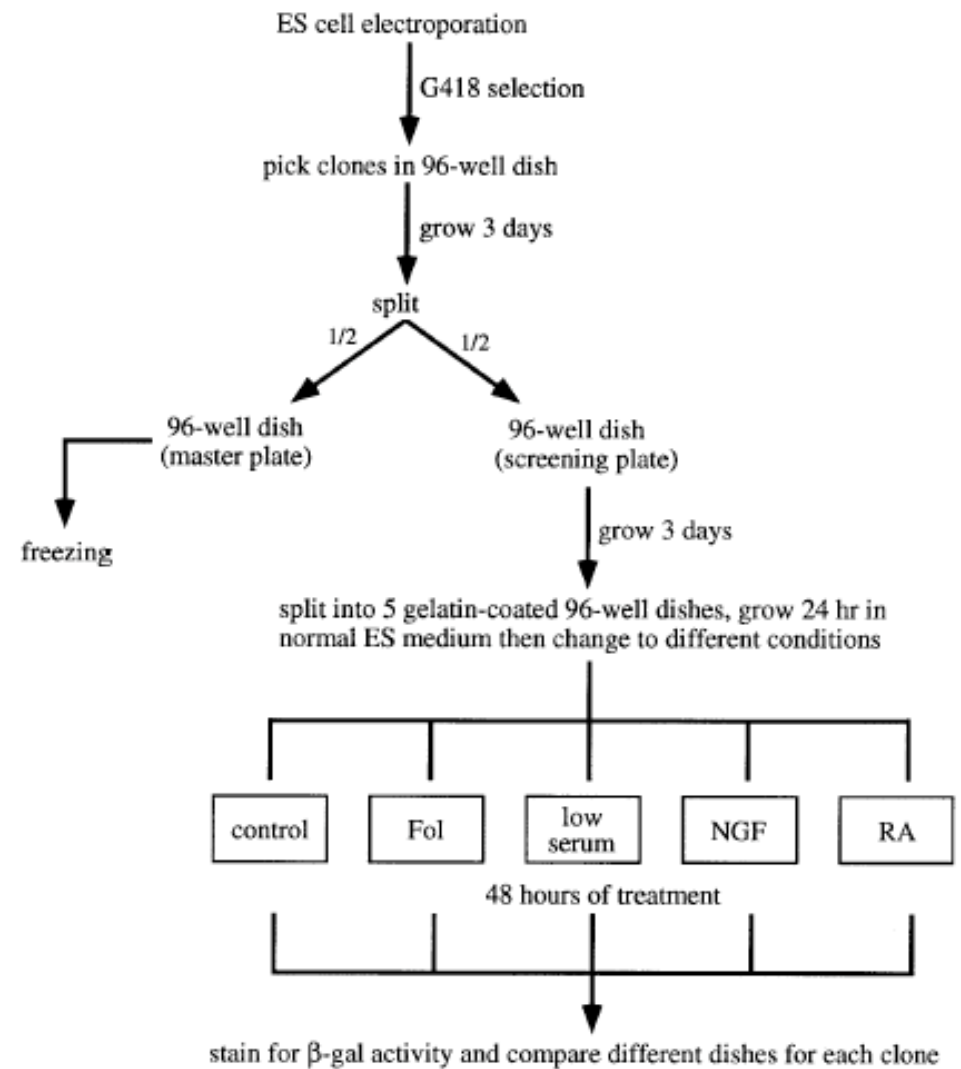
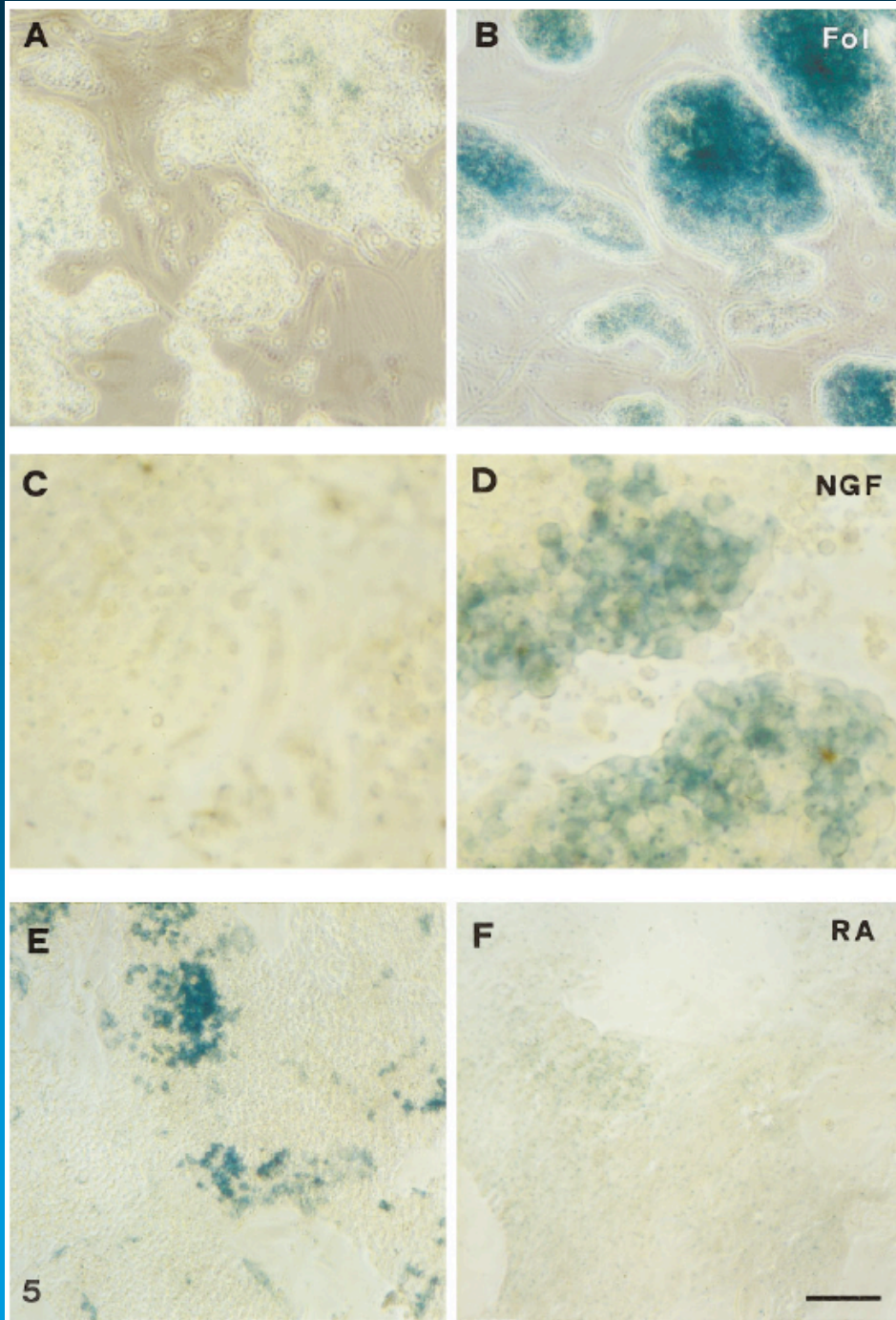


TABLE 4
Comparison of *lacZ* Expression Patterns
in Trapped Cell Lines and in Mice

Expression <i>in vitro</i> ^a		Expression <i>in vivo</i> (9.5–14.5 dpc) ^b		
		Ubiquitous	Restricted	Negative
Ubiquitous/widespread	20	14	1	4
Ubiquitous/widespread, regulated	14	6	7	1
Restricted	13	2	8	3
Very restricted	7	0	3	4
Faint (dot) expression	7	1	5	1
Negative	12	0	1	11

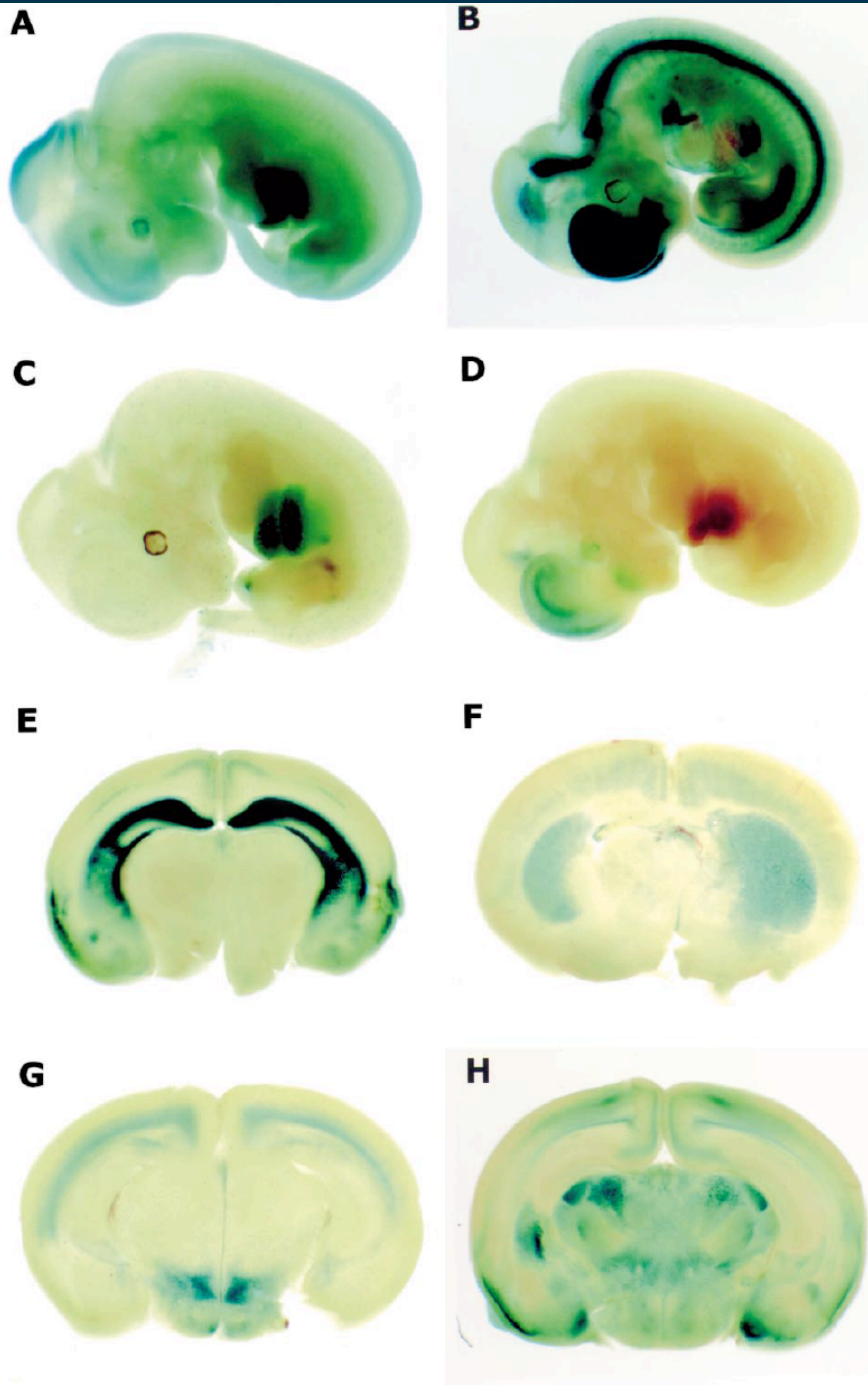
^a For the classification of β -gal staining patterns *in vitro*, see Table 3.

^b β -gal staining was checked using mouse embryos heterozygous for the gene trap insertion. At least three different developmental stages were stained for each line: 9.5/10.5, 11.5/12.5, 13.5/14.5 dpc (days post coitum). Ubiquitous: most or all tissues were positive. Restricted: staining was restricted to specific tissues or organs in all the stages. Negative: no β -gal activity was detected in any of the stages.

Profili d'espressione di cloni gene trap

E10.5

pn0



Ma a tanti interessanti profili d'espressione Corrispondono sempre altrettanti fenotipi?

Table 1
Statistical analysis of LacZ expression patterns shown from mouse gene trap lines generated with different vectors

Vector	Negative (%)	Extended (%)	Restricted (%)	CNS (%)
IRES β geo ^a (45)	20	44	36	31
IRES β galNeo(-pA) ^b (56)	29	7	64	43

ES cell clones selected on the basis of a restricted LacZ activity pattern (blue staining in only 10% of the colonies in a 2 cm dish and in 50% of the cells within a colony) under standard/undifferentiated culture conditions have been used to generate the mouse lines. The expression patterns have been analyzed during embryonic development at stage e9.5, e12.5 and e15.5 and on brain samples from postnatal day 9 (pn9). The individual expression patterns of the mouse lines have been classified into negative, extended (activity in different cell types, organs and structures) and restricted (activity restricted to one cell type, organ or structure). Within the restricted patterns, the subgroup of lines showing expression in the CNS has been determined. The number of analyzed mouse lines is indicated in brackets.

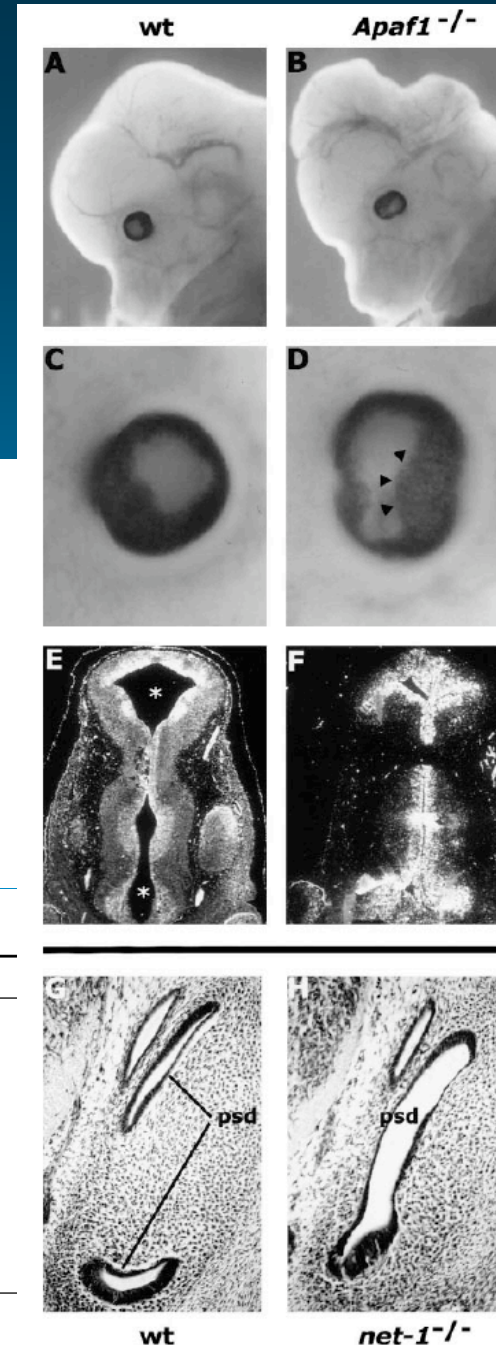
^a[14,15].

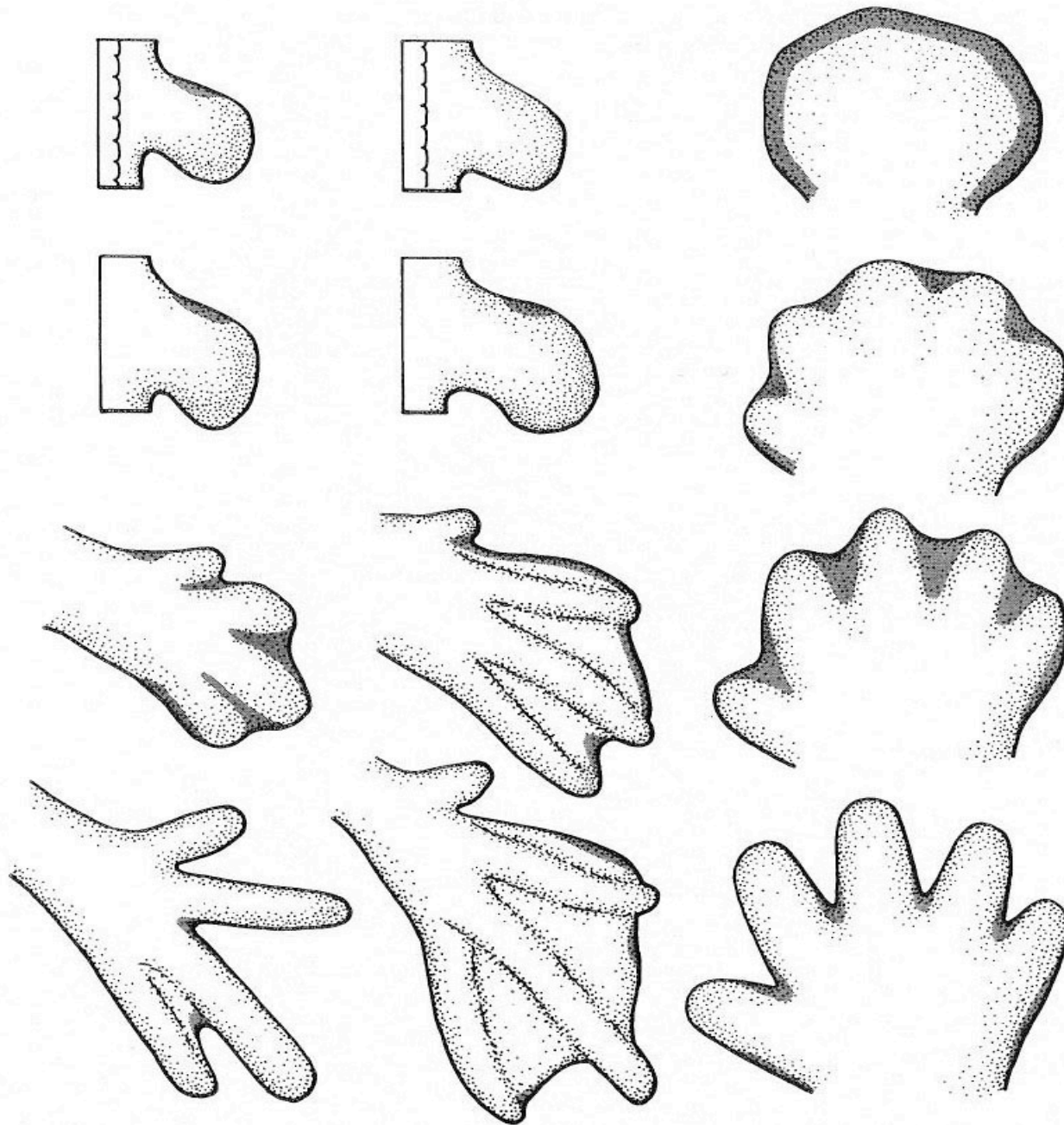
^b[16].

Table 2
Examples of genes isolated and mutated by gene trap

Gene	Product	LacZ ^a	Vector	Reference
<i>TEF-1</i>	transcriptional factor	extended	Rosa β -geo	[52]
<i>Fug1</i>	<i>RNAI</i> -like	extended	U3neo	[25]
<i>Netrin 1</i>	secreted factor	restricted	PGT1.8TM	[28]
			IRES β geo	[37]
α - <i>E-Catenin</i>	adhesion molecule	restricted	PGT1.8geo	[38]
<i>Jmj</i>	novel	restricted	TV2	[23]
<i>Bcl-w</i>	anti-apoptotic protein	restricted	Rosa β -gal	[53]
<i>Apaf1</i>	pro-apoptotic protein	extended	IRES β geo	[36]
<i>HSP90β</i>	Heat-shock protein	extended	PGT1.8geo	[68]
<i>Neuropilin 2</i>	TM receptor	extended	PGT1.8TM	[29]

^aLacZ expression pattern analyzed at several stages of development.

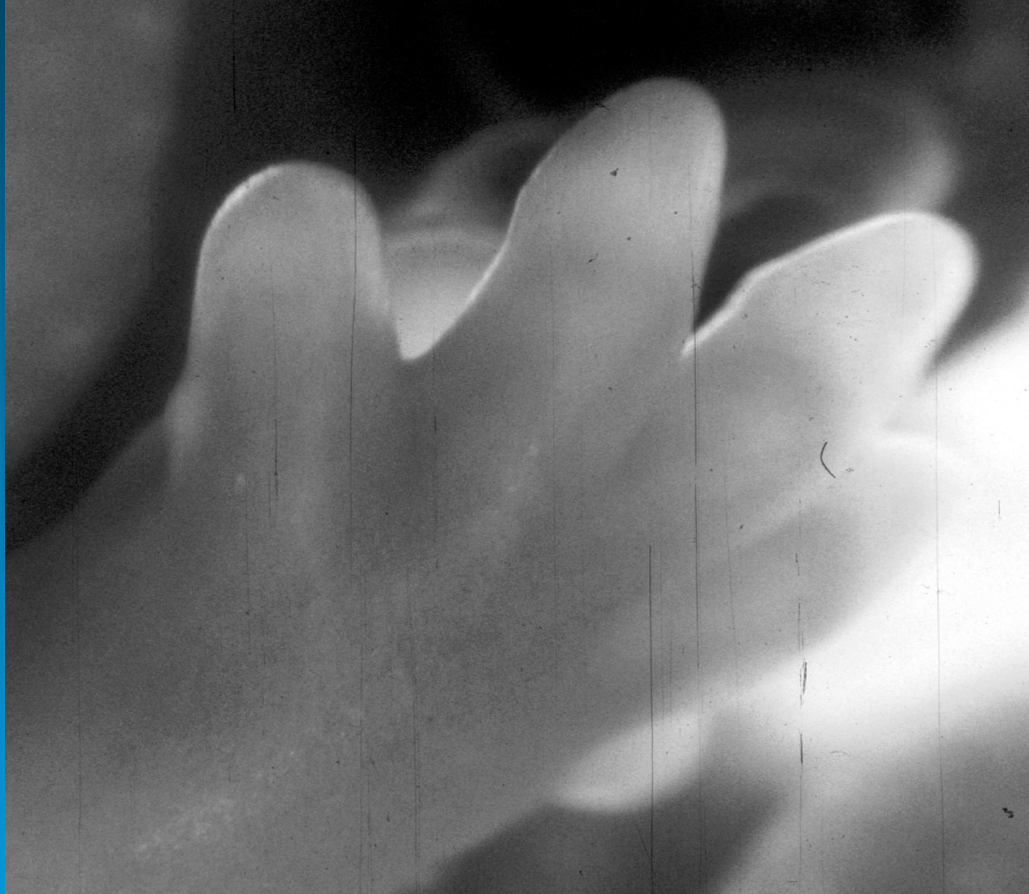




Chick

Duck

Human



IL vettore PLAP-secretorio

PLAP: placental-like alkaline phosphatase

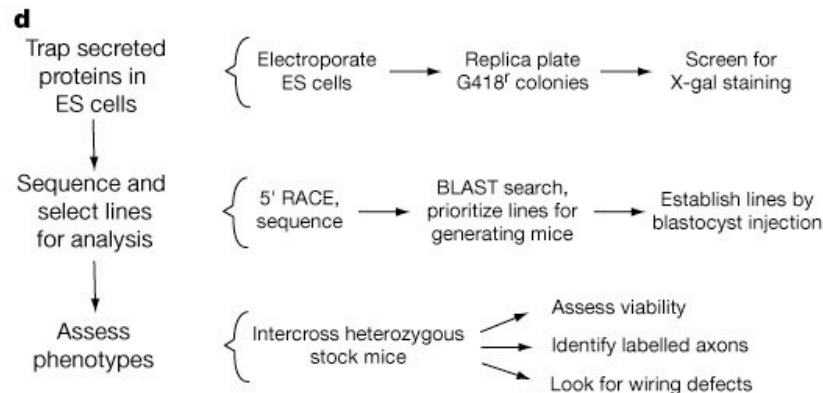
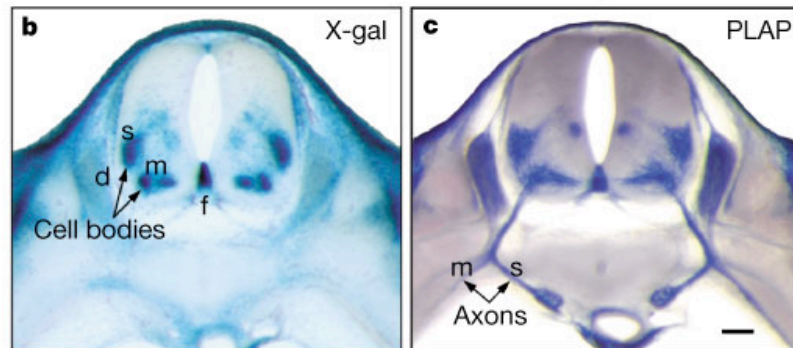
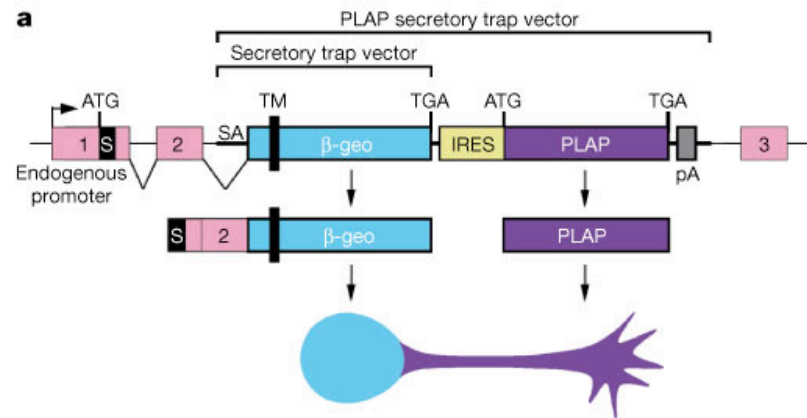


Table 1 Selected genes trapped with the PLAP secretory trap vector

Transmembrane (31)

ADAM23
 Attractin
 CD98
 CRIM-1
 Desmoglein-2
 E-cadherin
 EphA2*
 EphA4*
 E selectin ligand 1
 ICAM1
 Integrin $\alpha 5^*$
 Integrin $\alpha 6^*$
 Jagged-1
 Jam
 LDL receptor
 Lisch7
 LRP6
 LRP2 (megalin)
 mFAT1
 MFG E8
 Mph poliovirus receptor
 Neogenin*
 Notch-1
 Notch-2-like
 Notch-3
 Plexin-A1*
 pM5
 PTP κ^*
 PTP σ^*
 SDR-1
 Sema6A*

Multi-transmembrane (6)

GABA-BR1a/b
 N33
 LGR4
 p76
 RW1
 TASK-2

Secreted (15)

Agrin
 Fibulin-1
 Bikunin-related protein
 Cyr61
 Fibronectin
 Glypican-3
 Glypican-4
 GS3786
 Laminin $\alpha 1^*$
 Laminin $\alpha 5$
 Laminin $\beta 1^*$
 Laminin $\gamma 1^*$
 Netrin-1*
 Perlecan
 Protein S

New genes with axon guidance motifs (4/43)

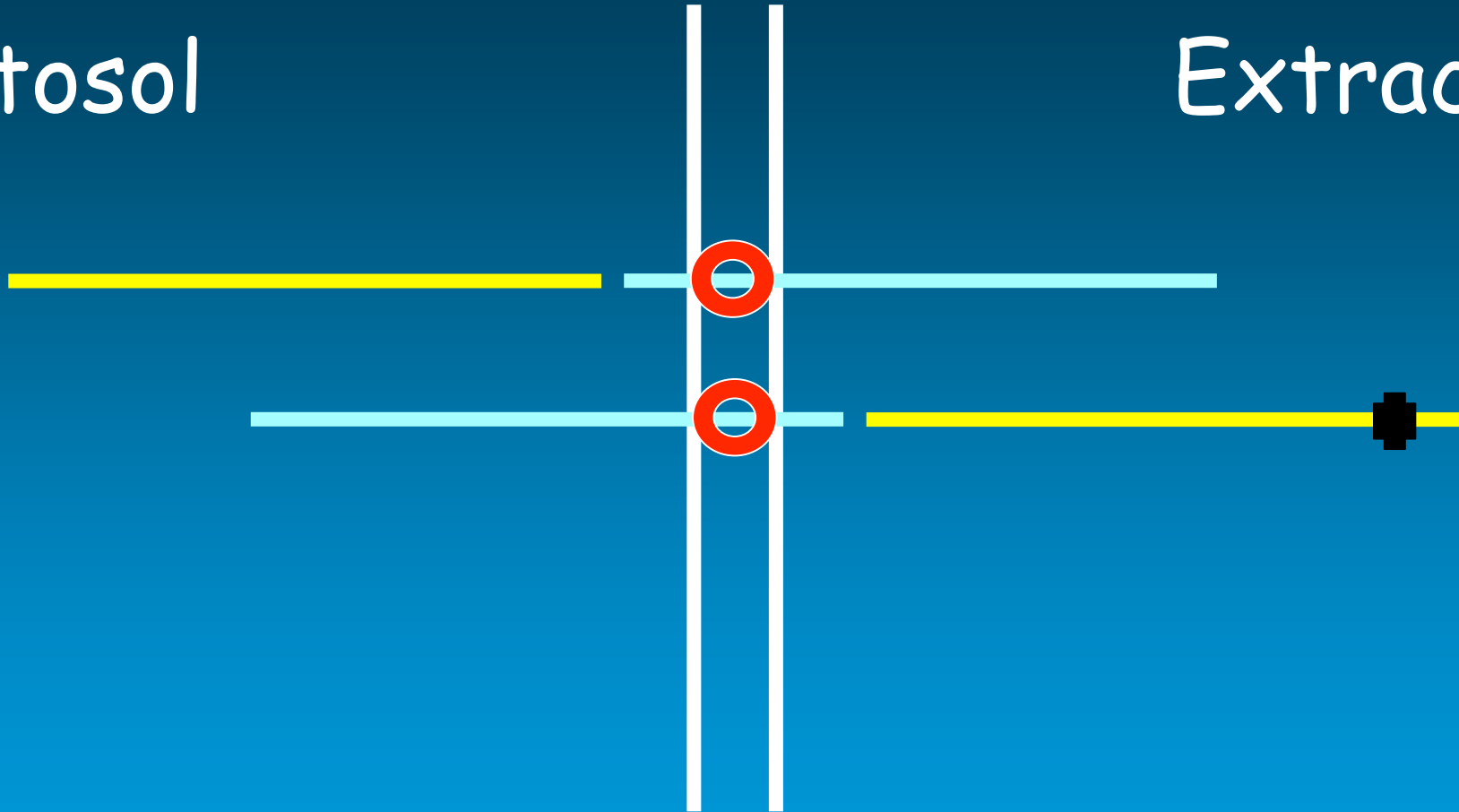
KST37 (plexin repeat)
 KST72 (Ig, FNIII repeats)
 KST225 (cadherin domains)
 LST16 (LRRs)

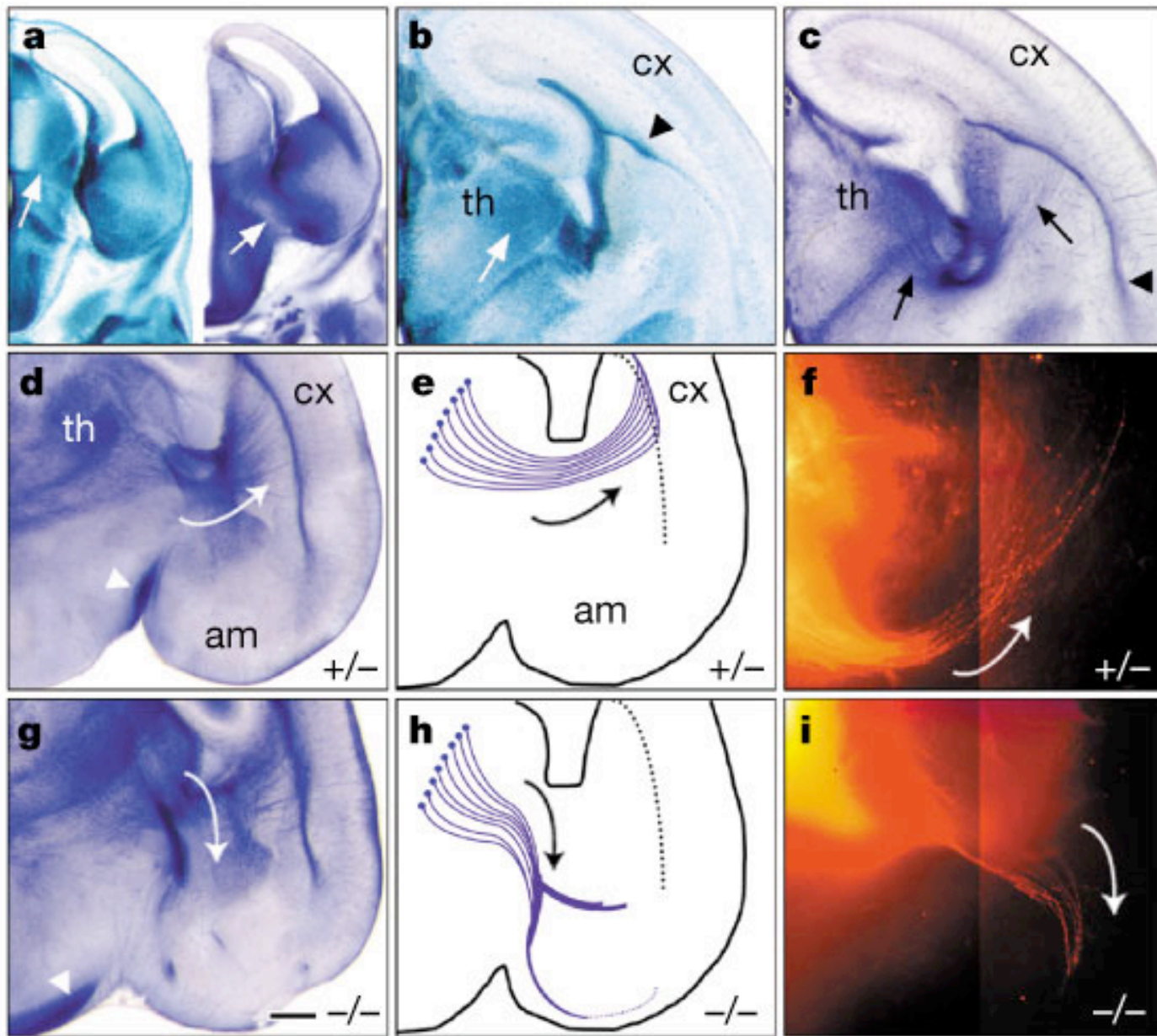
All transmembrane and secreted proteins are listed, as are several new genes with intriguing predicted structures. Ig, immunoglobulin, LRR, leucine-rich repeat. FNIII, fibronectin type III.

* Known or likely axon guidance molecules.

Cytosol

Extracell





REFERENCES

GENE-TRAP MUTAGENESIS: PAST, PRESENT AND BEYOND

William L. Stanford^{}, Jason B. Cohn^{*‡} and Sabine P. Cordes^{*‡}*

Efficient Gene Trap Screening for Novel Developmental Genes Using IRESbgeo Vector and *in Vitro* Preselection

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Defining brain wiring patterns and mechanisms through gene trapping in mice

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