Transgenic Fish and Its Application in Basic and Applied Research

Transgenic Animals in Research

- **Early embryonic development**
- **Regulation of gene expression**
- **Biological function(s) of desirable gene**
- **Bioreactors for producing valuable proteins for pharmaceutical purposes**
- **Providing animal models for biomedical research**
- **Producing and improve genetic broodstock in aquaculture**

Advantages of Using Fish for Transgenic Research

- **Small fish, such as medaka and zebrafish, can produce large numbers of eggs**
- **Very short life cycle**
- **Eggs are transparent, easy for embryological manipulations**
- **Can be easily maintained in the laboratory.**

Considerations of Transgene

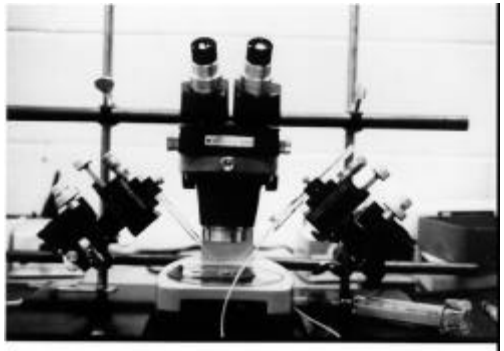
- **Suitable promoter for gene expression**
- **Genes with desirable traits, such as growth enhancement, disease resistance, low temperature tolerance, et al.**
- **Proper selection marker for screening of transgenics**

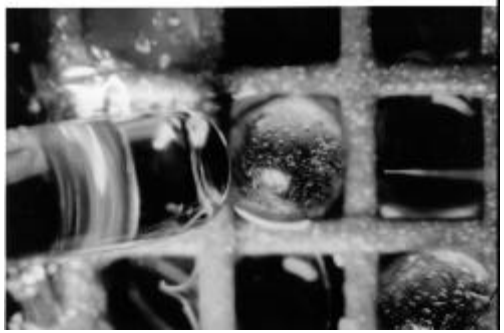
Transgenesis in Finfish and Shellfish

- **For those finfish and shellfish species that fertilized or unfertilized eggs are readily obtainable, transgenesis can be routinely accomplished by microinjection or electroporation**
  - **e.g., rainbow trout, carp, catfish, salmon, tilapia, medaka, zebrafish, scallop and abalone etc.**

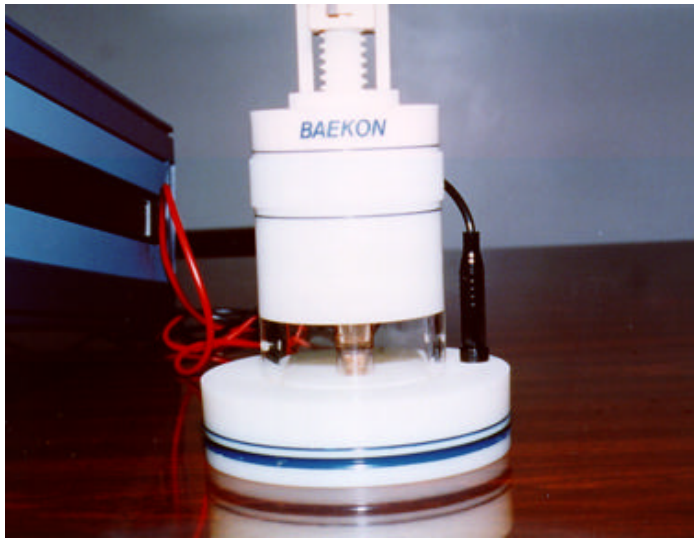
Production of Transgenic Fish

Microinjection





Electroporation



Microinjection vs. Electroporation

- **Microinjection:**
  - **one egg per injection**
  - **labor intensive**
- **Electroporation:**
  - **mass gene transfer in a short time period**
  - **cost effective**

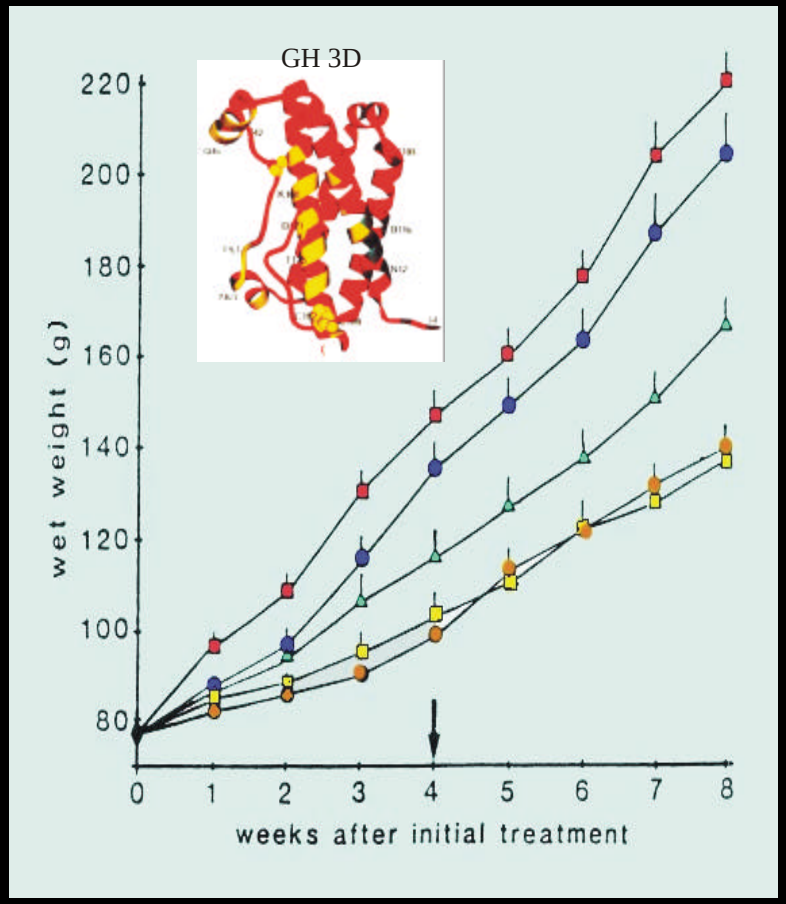
Medaka (*Oryzias latipes)*

Rainbow Trout (*Oncorhynchus mykiss)*

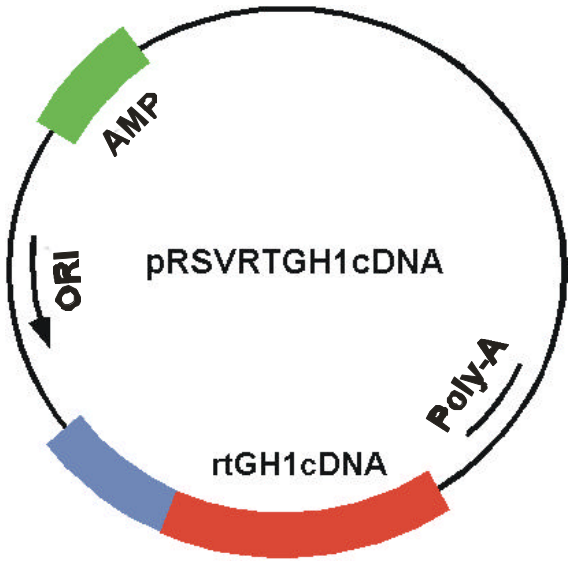
Functions of Growth Hormone

- **Promote somatic growth**
- **Increase immune functions**
- **Increase lipase activities**
- **Osmoregulation**

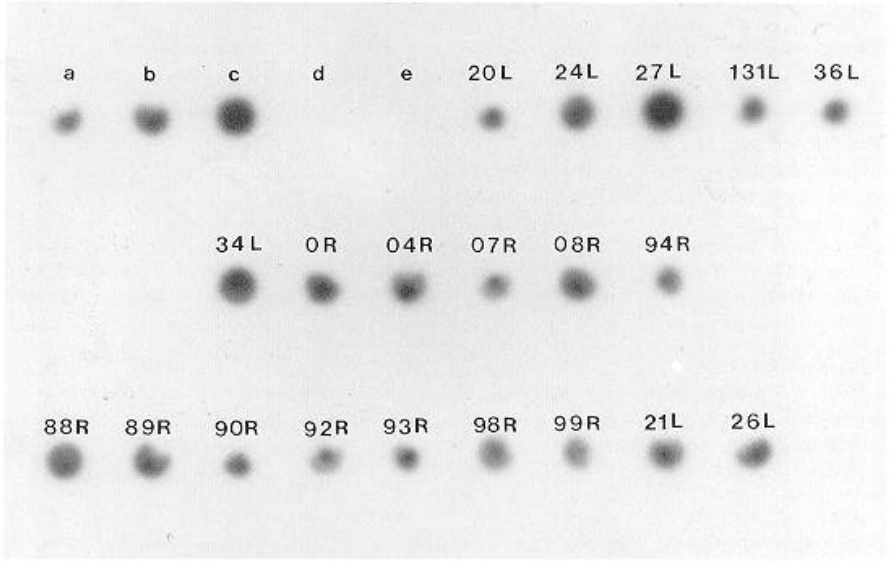
Effects of Recombinant rtGH on the Somatic Growth of Rainbow Trout



Production of Transgenic Carp

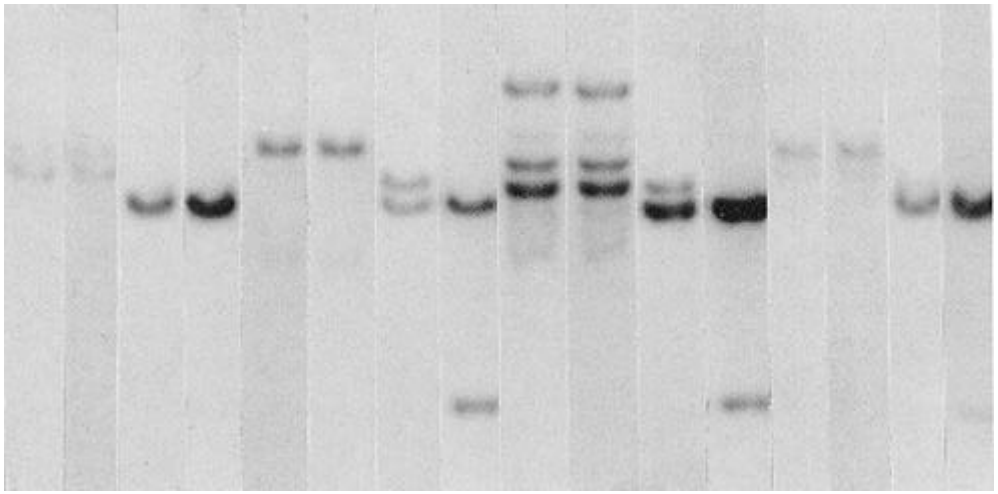


(A) Gene construct

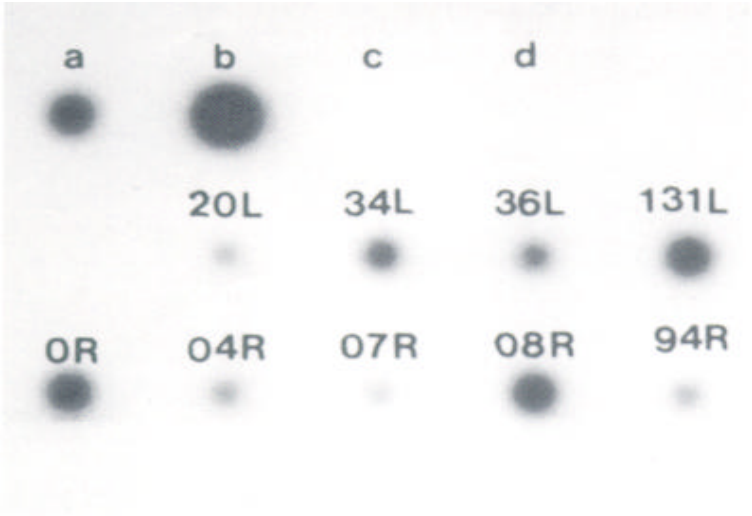


(B) Screening by dot hybridization

Production of Transgenic Carp

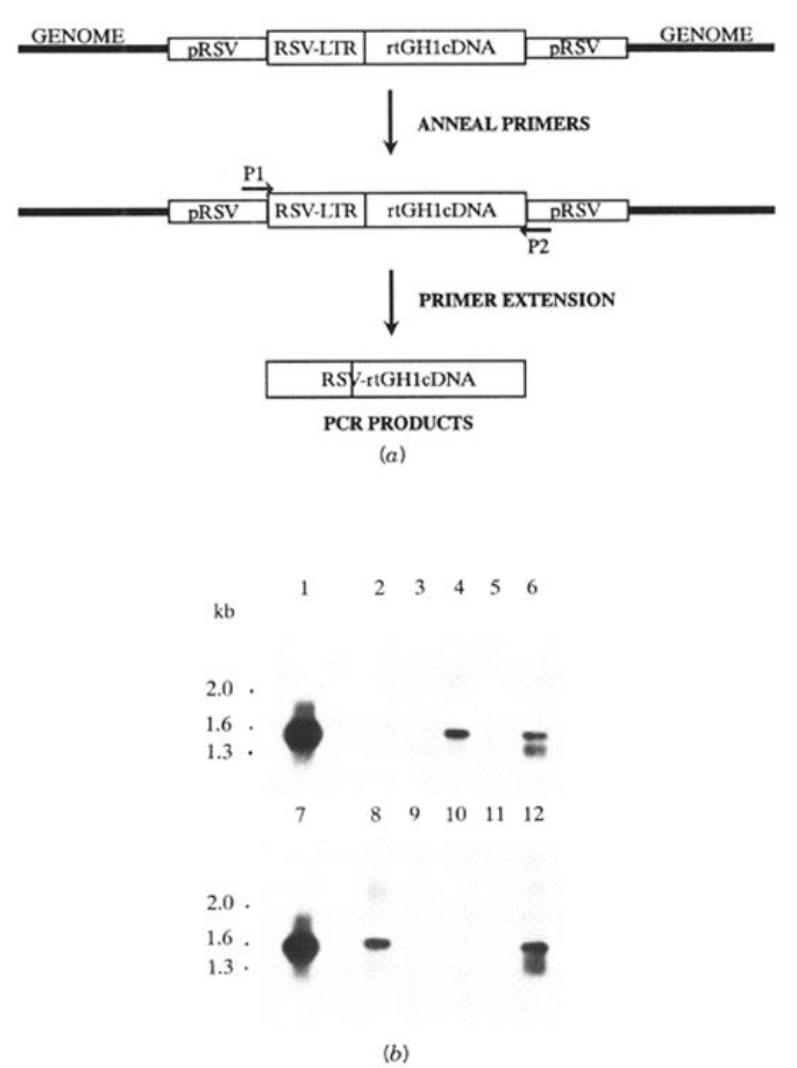


(C) Genomic Southern hybridization



(D) Immunodetection of GH

PCR Screening of Transgenic Fish



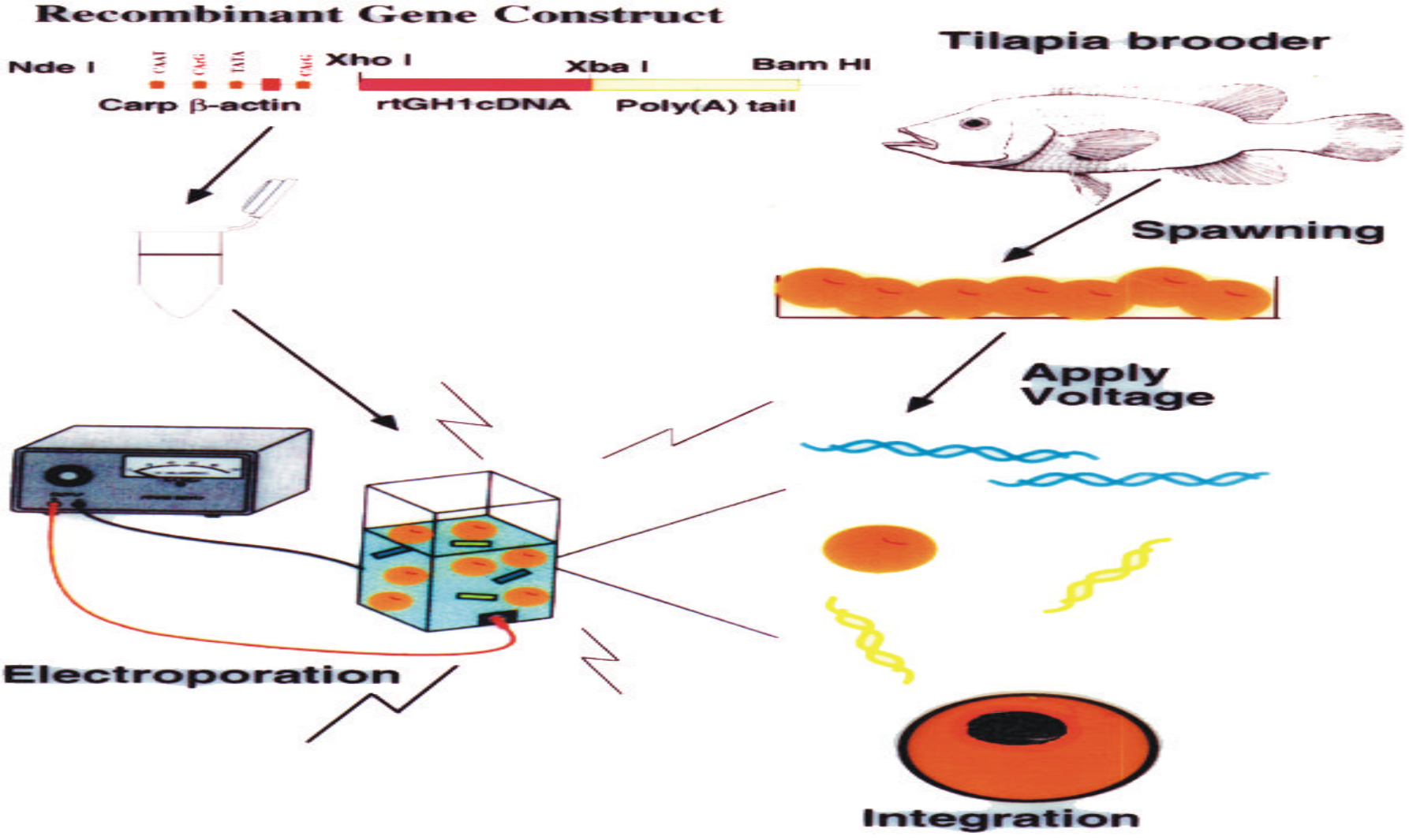
Percent of F_1 Progeny Inheriting pRSVLTR-rtGH1 Transgene

Family	Crosses	N	Observed inheritance (%)	Expected inheritance (%)
1	P_1 x control	17	0	50
2	P_1 x control	96	32	50
3	P_1 x control	26	42	50
4	P_1 x control	4	100	50
5	P_1 x P_1	28	21	75
6	P_1 x P_1	99	21	75
7	P_1 x P_1	312	31	75
8	P_1 x P_1	93	30	75

Percent Difference in Body Weight of Transgenic Carp and Their Nontrasngenic Siblings

Crosses	Genotype	N	Mean body weight (g)	% Difference
P_1 x control	T	31	120.6	20.8
	NT	65	99.3	
P_1 x control	T	11	206.0	40.1
	NT	15	147.0	
P_1 x P_1	T	7	5.8	-26.6
	NT	21	7.9	
P_1 x P_1	T	28	66.1	58.5
	NT	65	41.7	
P_1 x P_1	T	17	14.7	21.5
	NT	82	12.1	
P_1 x P_1	T	97	114.2	-14.5
	NT	215	133.6	
P_1 x P_1	T	15	72.2	-1.5
	NT	48	73.3	

Produce Transgenic Tilapia

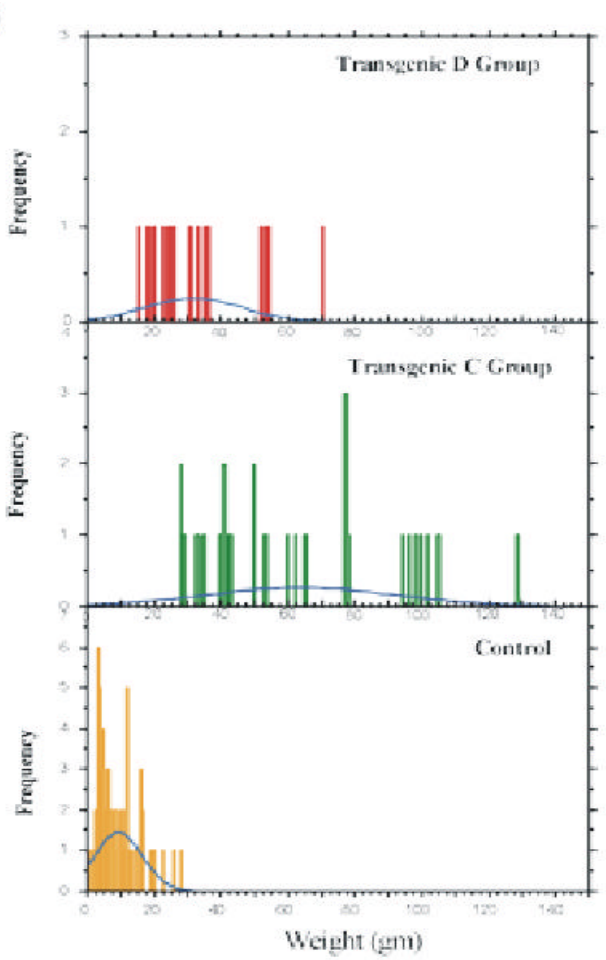


Growth Enhancement of Transgenic Tilapia

(A)

(B)

(C)



GH Transgenic Fish

Carp (*Cyprinus carpio*)

Catfish (*Ictalurus punctatus*)

Tilapia (*Oreochromis niloticus*)

Production of Transgenic Mollusks and Crustaceans

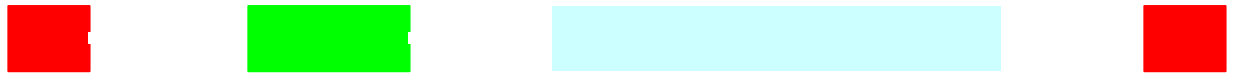
Major Bottlenecks in Crustacean Aquaculture

- **Incomplete control of the reproductive cycles**
  - **Many crustacean species fail to spawn when kept under captivity**
- **Infection by pathogens and disease outbreak**
  - **pathogens: water quality and nutrients**
- **Slow growth in some cold water species**

Alternative Gene Transfer Methods

- **Alternative gene transfer methods need to be developed for:**
  - ***Finfish or shellfish species that newly fertilized or unfertilized eggs are unavailable***
  - ***Marine fish species that striping sperms and eggs can lead to severe mortality***

Prototype of Defective Pantropic Retroviral Vector



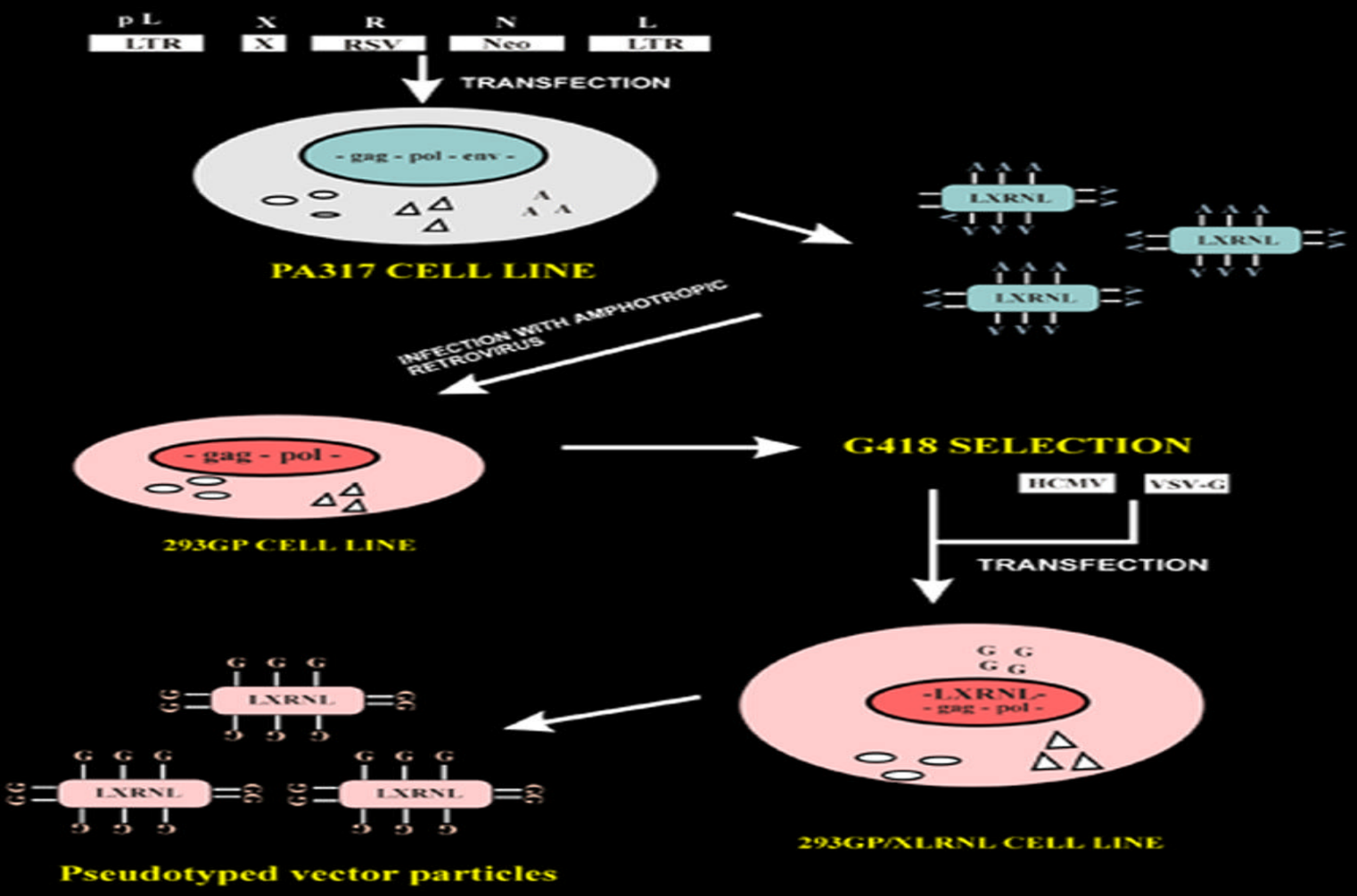
MMLV **NEO** **TRANSGENE** **LTR**

(1) LTR sequence

(2) Selection Marker

(3) Transgene sequence

Production of VSV-G Pseudotyped Retroviral Vectors

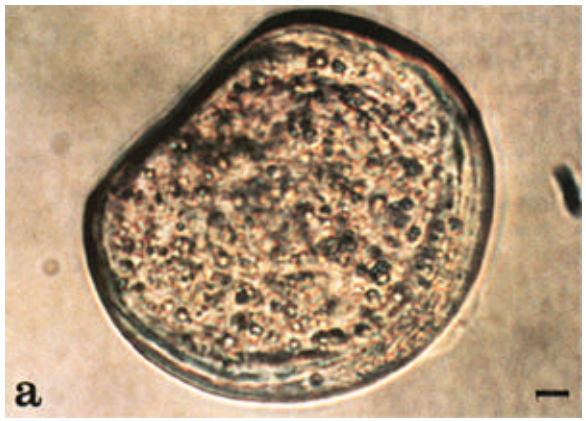


Surfclam
(Mulinia lateralis)

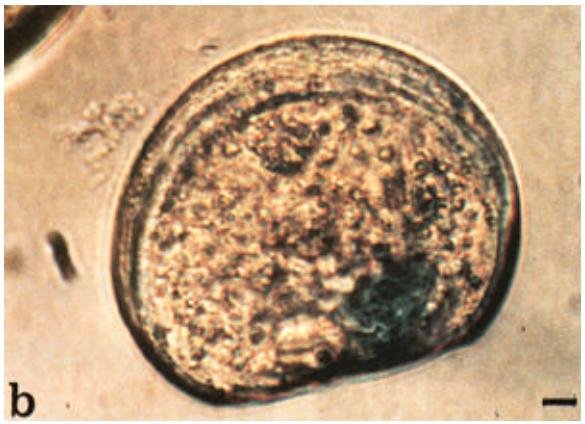
Production of Transgenic Surfclam (Mulinia lateralis)

- **Post-fertilized eggs (one hour)**
  - **100,000 to 150,000 eggs**
- **Electroporation**
  - **Baekon 2000**
  - **Vectors: pLSRNL, pLRGNL**
  - **Vector concentration: $1x10^4$ cfu/ml**

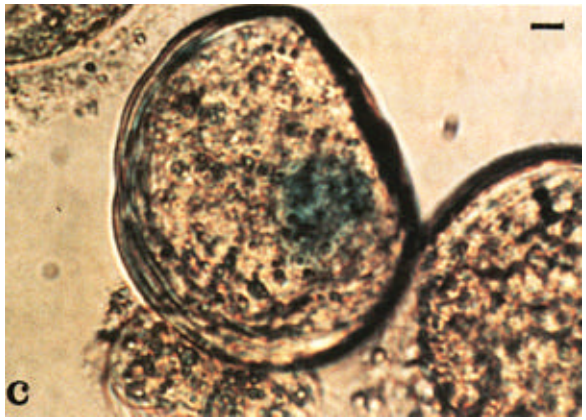
Expression of β-gal *Gene in the Transgenic Surfclams*



Control



Transgenic P_1



Transgenic F_1

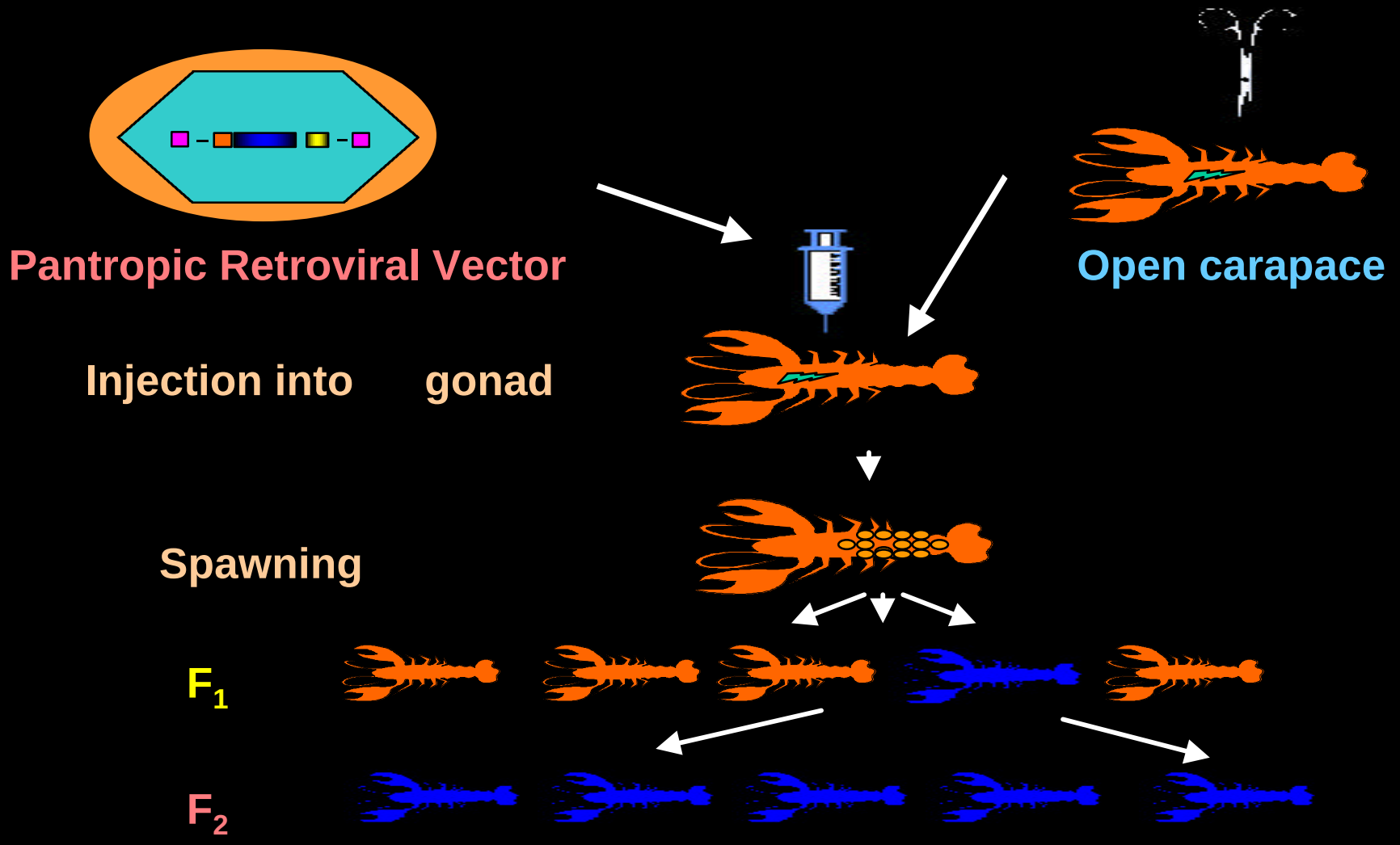
Defective Pantropic Retroviral Vectors

- **Possess broad host range specificity due to viral envelop containing VSV-G protein -- can deliver DNA into a wide range of hosts**
- **Can accommodate up to 10 Kb foreign DNA**
- **May be used to transfer genes into many different species**

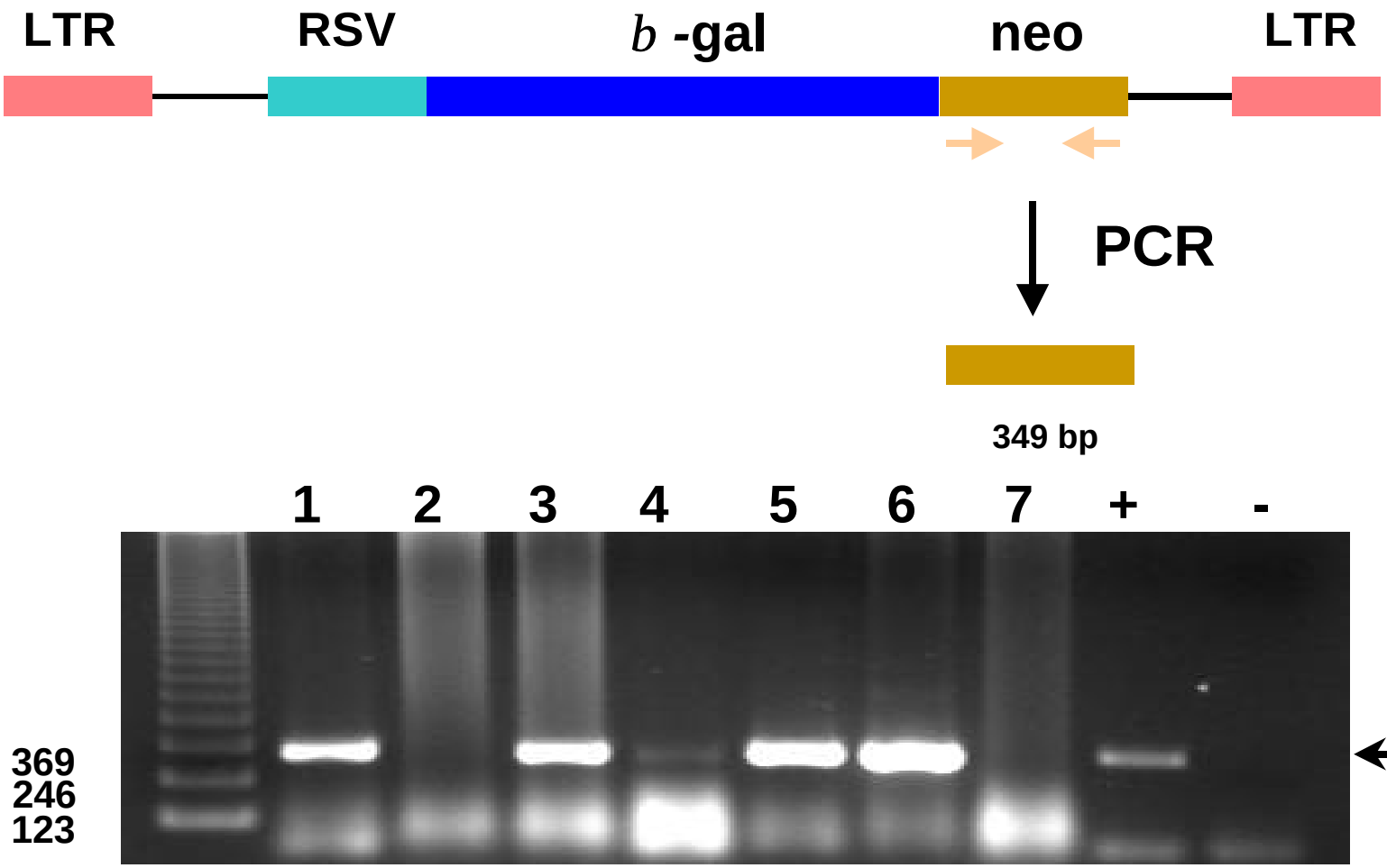
Direct Gonadal Gene Transfer

- **Defective Pantropic Retroviral Vectors may be used to directly transform the gonads of finfish, shellfish or crustaceans**
- **This gene transfer method will eliminate the problem of germ line mosaism**
- **Will provide an effective and non-invasive gene transfer alternative**

Gene Transfer in Crayfish



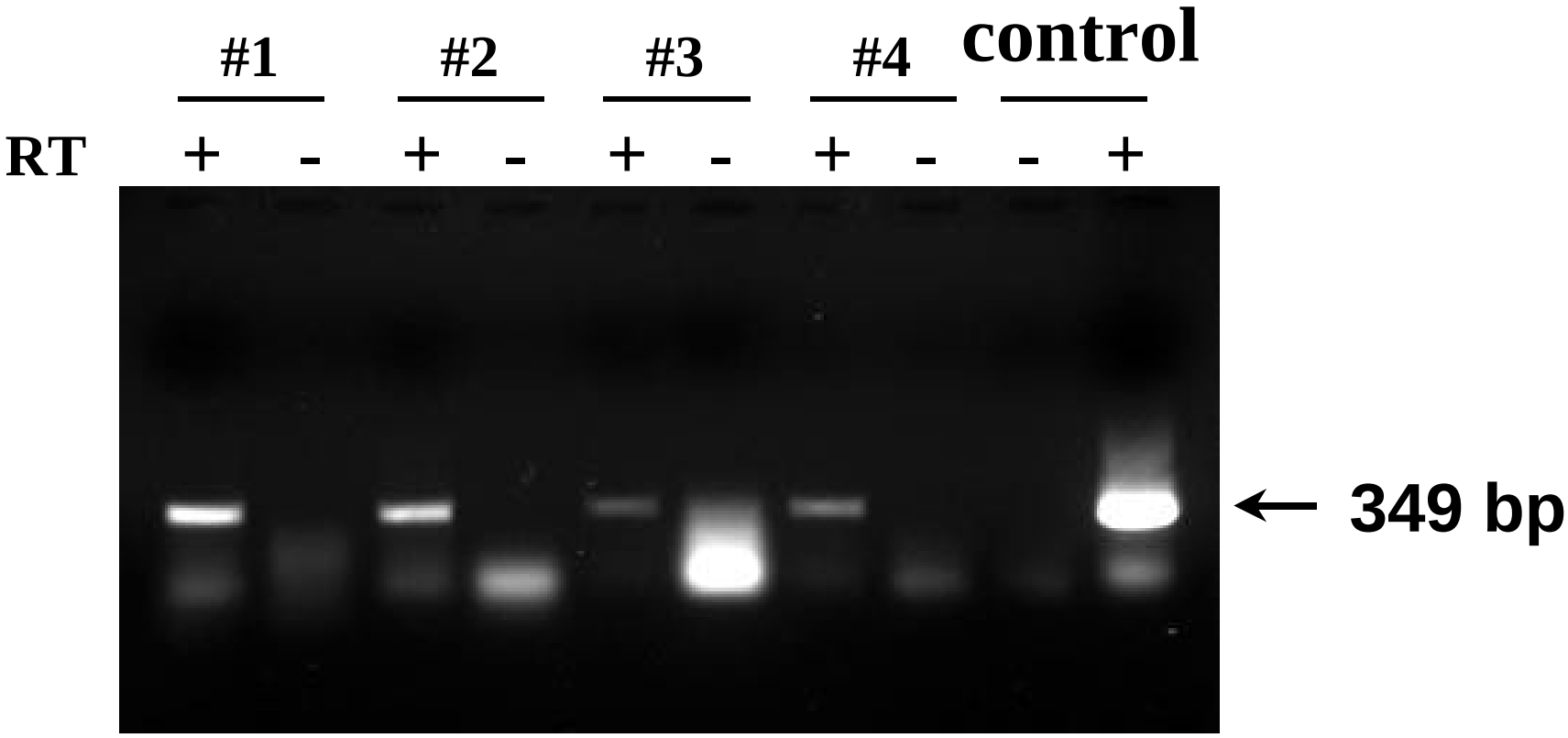
Detection of Transgenic Crayfish by PCR Amplification



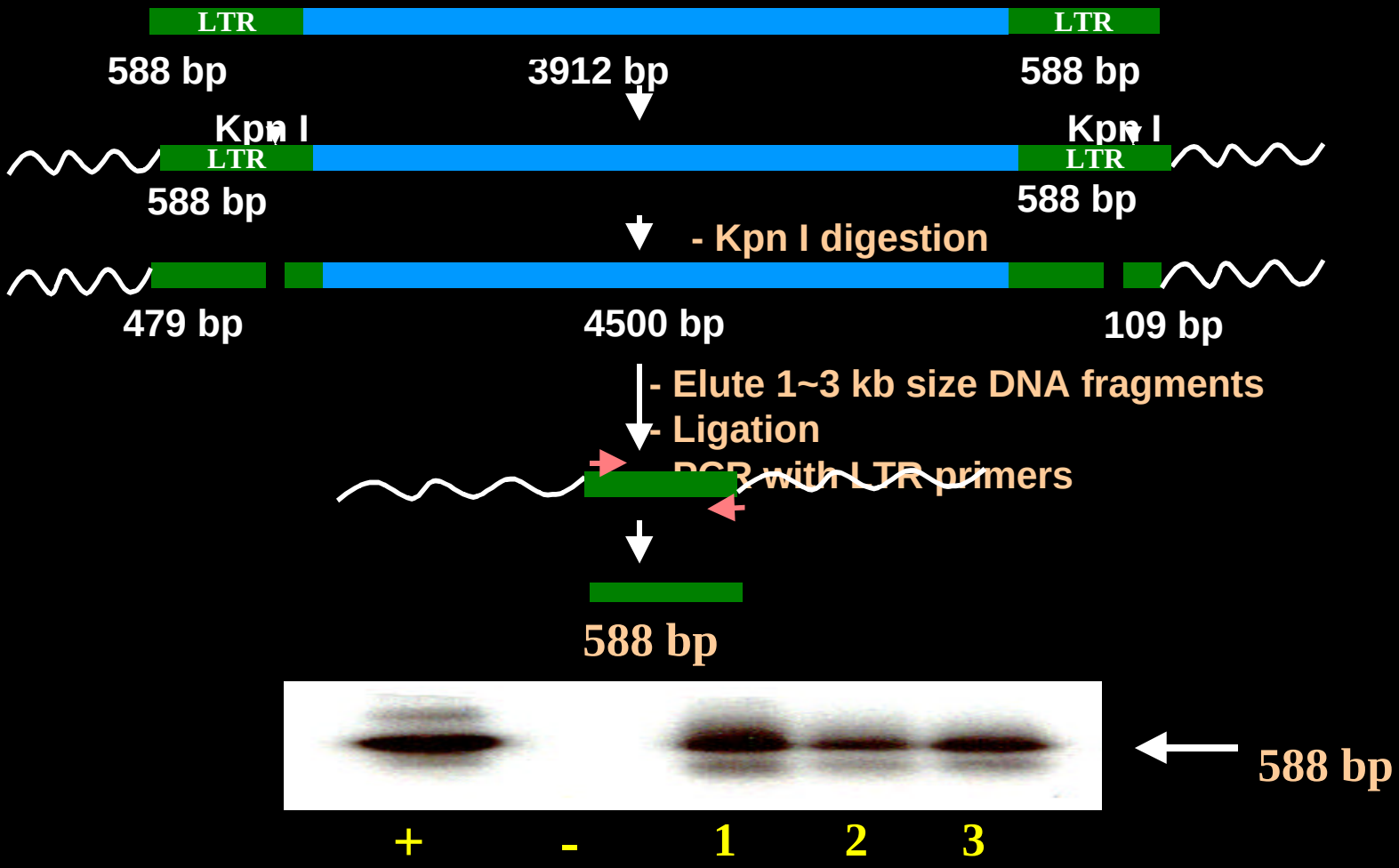
Gene Transfer in Crayfish with Pantropic Retroviral Vectors

Date of treatment	# Infected	Spawning dates	Duration (days)	% Positive
8/1/97	15 females 4 males	8/28/97	28	0 (0/15)
		9/8/97	39	16.6% (2/12)
		9/12/97	43	33.3% (4/12)
		9/28/97	59	66.6% (8/12)

Detection of Transgene Expression By Reverse Transcription (RT)-PCR



Integration of Transgene in the Host Genome



Inheritance of Transgene in the F_2 Transgenic Crayfish

Family	F_2 individuals analyzed	# Transgenics	% Transgenics
F_{1a} female x non-tg	15	9	60
F_{1b} female x non-tg	14	8	57
F_{1b} female x non-tg	15	7	47

Gene Transfer in the Scallop

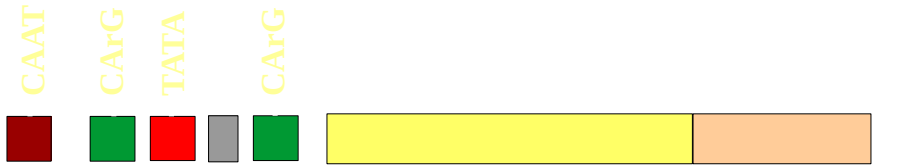
- **Improve genetic traits**

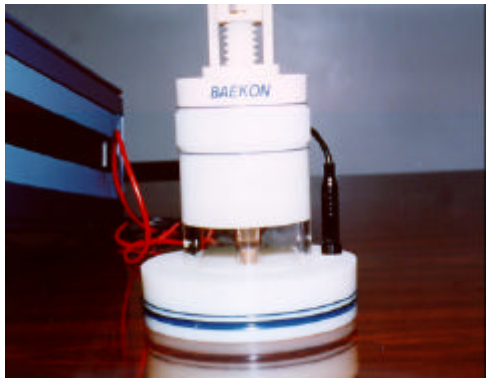
  e.g., enhanced growth rate and survival at low temperature; disease resistance etc.

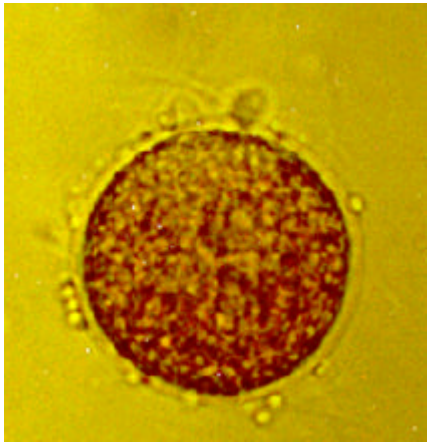
- **Markers for easy identification**

  e.g., GFP, histone gene, specific repetitive sequence etc.

Production of Transgenic Bay Scallop



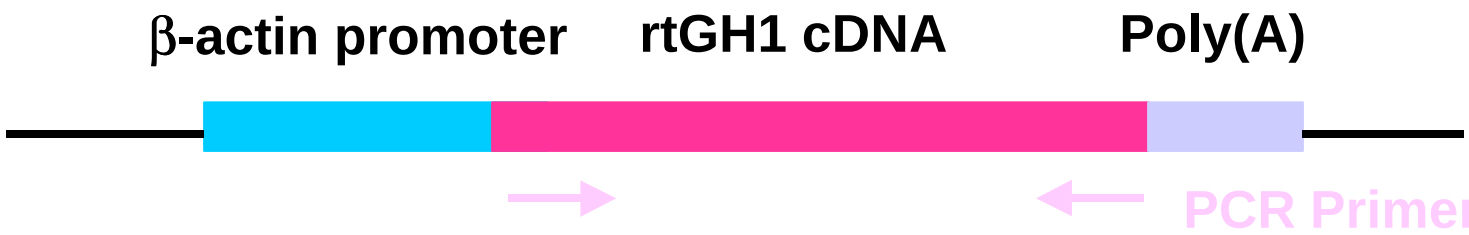




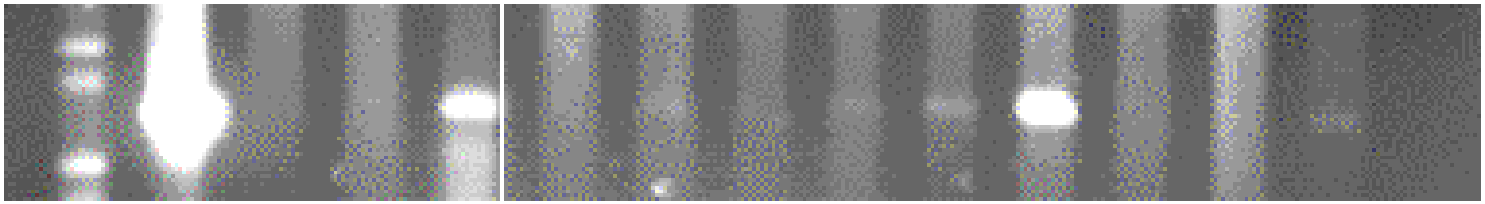
Eletroporation

Transgenic Scallops

Identification of Transgenic Bay Scallops by PCR



PCR Amplification



Summary Of Transgenic Scallops by PCR Analysis

Date Sampled	# Animals Analyzed	# PCR Positive	% PCR Positive
8/97	158	8	5%
1/98	139	20	14%
5/98	376	131	36%
12/98	340	181	53%

SUMMARY

- **Transgenic surfclam and scallop can be produced by electroporation and infection with pseudotyped retroviral vectors**

- **Gene transfer can be accomplished by direct gonadal infection with pseudotyped retroviral vectors**