

**Appendix D - UNIVERSITY HONORS PROGRAM
SENIOR PROJECT - APPROVAL**

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PROJECT TITLE: CLONING OF THE DNA SEQUENCES ASSOCIATED WITH A
GENE, THE MUTATION OF WHICH CAUSES FEMALE STERILITY OF DROSOPHILA
MELANOGASTER

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: Ranjan Ganguly, Faculty Mentor

Date: 5/16/97

Comments (Optional):

Cloning of the DNA sequences associated with a gene, the
mutation of which causes female sterility in *Drosophila*
melanogaster

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Acknowledgment

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Abstract

A recessive female sterile mutation in *Drosophila melanogaster*, known as *fs(100C)*, occurred when the genomic DNA at the 100C region of the third chromosome was disrupted by a P-transposon insertion. The purpose of this project was to clone the genomic DNA which was disrupted in the *fs(100C)* flies in order to use it for isolation of the complete gene apparently involved in female fertility. This was first attempted by using the inverse PCR method, but this experiment did not provide any positive results. Therefore, a second method was employed, and this required the construction and screening of a genomic library of the mutant flies. The genomic library of the *fs(100C)* flies was constructed in λ bacteriophage. About 10 genomic equivalents of phages were screened using P-element DNA as probes. Of the 16 recombinant phages which hybridized to the probes, one was chosen for DNA isolation. Southern blot hybridization was performed and a restriction map was constructed for this clone. Through these analyses, it was determined that a minimum of 2.3Kb and a maximum of 14Kb of the recombinant λ clone DNA is, in fact, the genomic DNA disrupted by the P-transposon in the *fs(100C)* flies. This DNA can now be used to clone the gene involved in female fertility in *Drosophila melanogaster*.

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INTRODUCTION

Evolutionary success is defined as being fit enough to reproduce in the environment in which a species lives. As important as being environmentally fit is being fertile enough to produce viable offspring which themselves will survive to reproductive age. This can be most important in species such as invertebrates who must produce large numbers of viable offspring in order to have a few descendants live to propagate. *Drosophila melanogaster* is such an invertebrate species, and it has been a vital tool for genetic research. This organism has been studied quite extensively in regard to its fertility and the mutations affecting it. Female sterile mutations have been thoroughly characterized and most can be classified into two main categories: female sterile and embryonic lethal mutations (Spradling 1993). Through molecular analysis, many of the genes affected by such mutations have been identified, and indeed many have homologues in vertebrate species (Spradling 1993). It is for this reason that such studies are important, as the understanding of fertility factors and processes lends itself to obliterating some blights of human infertility. It is from research such as this that an appreciable comprehension of oogenesis in *Drosophila melanogaster* has emerged, and in order to understand female sterile mutations in this organism, oogenesis must be examined, as the two are intertwined and inseparable.

I. Oogenesis and female sterility in *Drosophila melanogaster*

Located in each of the two ovaries of *D. melanogaster* are about sixteen ovarioles (Fig. 2). Each ovariole is its own entity in which there is a sequence of oogonia developing. These oogonia actually form from a cell known as a cystoblast (Fig. 2). A

single cystoblast will go through four mitotic divisions, each with an incomplete cytokinesis stage which leaves sixteen cells connected by cytoplasmic bridges (Fig. 2). One of the two cells which possesses four cytoplasmic bridges will become the actual oocyte, and the remaining 15 cells become nurse cells (Fig. 2). These nurse cells become polyploid by replicating without undergoing nuclear division or cytokinesis until approximately 1000 copies of each chromosome are present. This assists the cells in accumulating ample amounts of cytoplasm and DNA from which different RNA molecules are synthesized and passed to the oocyte through the cytoplasmic bridges.

In addition to the nurse cells, oocytes are aided by follicle cells (Fig. 1). These are somatic cells which surround the oocyte and its 15 nurse cells, and their purpose is to synthesize the vitelline envelope and the chorion, which form the eggshell, as well as to assist in procuring yolk proteins. The oocyte, its nurse cells, and the follicle cells surrounding them together make up the egg chamber. Within an ovariole, there is a sequence of egg chambers which are in differing stages of maturity, the most mature located toward the posterior portion of the ovariole (Fig. 1). Mature eggs are surrounded by an eggshell with dorsal appendages located on the anterior end whose purpose is to promote embryonic respiration.

Many female sterile mutations affect different stages of oogenesis (Spradling 1993), and from a brief overview, it is obvious that there are numerous points at which defects and alterations can cause problems. Some, such as *rudimentary* mutations, cause the females to have very small ovaries with often no germ cells present. Others, such as *tumorous* mutants, cannot form egg chambers. *Open chorion* mutants have eggs without anterior chorions and *dorsalized* mutants have chorion all around the anterior end of the

egg, preventing formation of the two dorsal appendages necessary for embryonic respiration. Similarly, *ventralized* mutants have no dorsal appendages, but they also have eggshell changes. *Thin chorion* mutants have a great deal less chorion than found in average eggs, and *collapsed egg* mutants produce eggs which fall apart when held in the ovary. As these are but a few types of female sterile mutations, it can be seen that they can manifest themselves in myriad alterations of the normal mechanisms essential to oogenesis and production of viable offspring.

In addition to female sterile mutations, there also exists a group of genes known as maternal effect genes which are needed for embryonic development (see Kalthoff 1996 for details). In most cases, mutations of these genes cause the females to appear sterile. This usually occurs when the female is homozygous for a mutation of one of many maternal effect genes and thus fails to deposit a particular gene product in the oocyte. As a result of this lacking gene product, the embryo will die. For example, if the maternal effect gene *bicoid* is mutated in females, the embryos produced will have no head or thorax and will therefore be unable to survive, rendering the females sterile. Thus, with mutations which manifest themselves as female sterile mutations, it is essential that every aspect of the alteration be examined to ascertain whether the cause is either a true female sterile mutation or perhaps a mutation of a maternal effect gene.

II. Research objectives

The focus of this project was a female sterile mutation named *fs(100C)*. *fs(100C)* originated when a P transposon carrying the *Cyp6a8* gene of *D. melanogaster* was microinjected into *ry*⁵⁰⁶ embryos for germ-line transformation. The original purpose of microinjecting this transposon was to study the regulation and function of the *Cyp6a8* gene. Through *in situ* hybridization, it was determined that the P transposon had integrated at the 100C region of the third chromosome. During subsequent genetic crosses, it was observed that only the females homozygous for this P transposon insert failed to produce offspring. It was for this reason that this female sterile mutation was named *fs(100C)*. However, the females which are heterozygous for the insert reproduce normally, and the homozygosity or heterozygosity of this insert does not affect male fertility (Narita et al, unpublished observations).

It was hypothesized that the P transposon had disrupted a gene which codes for an essential factor needed for female fertility. It is not known, however, if the insert has interrupted the transcribed region of this unidentified gene. Therefore, the objective of this project was to obtain a portion of the genomic DNA that was disrupted by the P transposon. Once isolated, this DNA can then be used as a probe in future studies to clone this gene involved in female fertility.

MATERIALS AND METHODS

I. Southern blot hybridization

DNA samples were digested with appropriate enzymes and then electrophoresed on a 0.8% agarose gel containing 5µg/ml ethidium bromide. After electrophoresis, the gel was denatured for one hour in a denaturing solution containing 1.5M NaCl and 0.5M NaOH and neutralized by two 45 minute washes with neutralizing solution containing 3M NaCl and 0.5M Tris, pH 7.5 (Sambrook et al., 1989). A Hybond nylon membrane, moistened with 20X SSC, was placed over the gel and the DNA was blotted onto the membrane by capillary action. After approximately 16 hours, the DNA was crosslinked to the membrane by UV radiation and placed in a plastic envelope for storage at 4°C until prehybridization.

The blots were prehybridized for approximately 1.5 hours at 65°C in a prehybridization solution consisting of 6X SSC and 5X Denhardt's solution. Afterwards, the prehybridization solution was removed and the blots were then hybridized at 65°C for 16-24 hours in a solution comprised of 6X SSC, 5X Denhardt's solution, 0.1% SDS, 0.05 mg/ml denatured herring sperm DNA, and 40-60 x 10⁶ cpm of radiolabeled probe (Sambrook et al., 1989). After hybridization, the blots were washed three times at 60°C, 10 minutes each, in 0.1X SSC and 0.1% SDS. The blots were then air dried, wrapped in Saran Wrap, and placed in a cassette for exposure to X-ray film.

II. Polymerase chain reaction (PCR) amplification

For PCR, a commercially available kit (AmpliTaq) was used. The reaction mixtures contained 1X PCR Buffer II, 200 μ M of each dNTP, 20pmol of each primer, 4mM MgCl₂, 2 units of Taq DNA polymerase (5 units/ μ l), and 10ng of the template DNA. The reactions were run for 30 cycles with the following temperature parameters: denaturing temperature of 94°C for 2 minutes, extension temperature of 39°C for 2 minutes, and annealing temperature of 72°C for 1 minute.

III. Radiolabeling of DNA

Radiolabeling of DNA fragments was performed by random priming (Sambrook et al., 1989). For this procedure, a mixture was used which contained bovine serum albumin (10mg/ml), 2mM each of dATP, dGTP, and dTTP, 50 μ ci α^{32} P-dCTP, and Klenow enzyme (5 units/ μ l) (Sambrook et al., 1989). The reaction mixture was placed at 25°C for 3 hours. The reaction was then stopped by adding an equal volume of stop buffer, containing 50mM EDTA and 1% bromophenol blue. The radiolabeled DNA probes were then separated from unincorporated ^{32} P-dCTP by Sephadex (G50) column chromatography.

IV. Library construction

A. Partial restriction digestion of the genomic DNA:

To prepare the DNA for cloning into λ phages, genomic DNA from the *fs(100C)* flies was partially digested with Sau3AI and electrophoresed on a 0.8% agarose gel. A portion of the gel containing DNA fragments ranging from 9- to 23-kb was cut out and

placed in a dialysis bag filled with water and electrophoresed until the DNA fragments moved out into the bag. The solution was removed from the bag and mixed with a one-tenth volume of 10X DNA extraction buffer (DEB) and an equal volume each of DEB-saturated phenol and chloroform. The mixture was centrifuged at 12,000 rpm for 2 minutes, the aqueous phase was removed, and it was extracted again with one volume of chloroform. The final aqueous phase was removed and to this was added a one-tenth volume of 2M NaOAc, pH 7.5 and 2 volumes of 95% ethanol. The DNA was precipitated overnight at -20°C. It was then pelleted by centrifuging at 12,000 rpm for 20 minutes. The supernatant was discarded and the DNA pellet was washed with 70% ethanol, dried, and resuspended in 20µl of 1X shrimp alkaline phosphatase (SAP) buffer (United States Biochemicals).

B. Dephosphorylation of the DNA fragments

To dephosphorylate the ends of the restricted fragments, shrimp alkaline phosphatase (SAP) was used. The reaction mixture contained the SAP buffer, enzyme, and the eluted Sau3AI genomic DNA fragments. The reaction mixture was incubated at 37°C for 80 minutes and then at 65°C for 15 minutes. The DNA was then phenol/chloroform extracted and ethanol-precipitated overnight. The DNA pellet was recovered by centrifugation, dried and washed as before, and then resuspended in 1X TE (10mM Tris, pH 7.4, and 1mM EDTA).

C. Ligation and packaging

The long and short arms of λ EMBL3 phage DNA (0.5 μ g) and the dephosphorylated Sau3AI DNA fragments (1 μ g) were ligated by incubation at 15°C for 20 hours in a 10 μ l reaction mixture containing 1X ligation buffer and 1 unit of T4 DNA ligase. The ligation mix was then ethanol-precipitated, washed with 70% EtOH, dried, and resuspended in 5 μ l of 0.1X TE. This ligation mix was added to the freeze-thaw and sonic λ packaging extracts (Stratagene, San Diego) and incubated at 22°C for 1.5 hours. After incubation, 20 μ l chloroform and 500 μ l SM (100mM NaCl, 8mM MgSO₄ 7 H₂O, 50mM Tris-Cl, pH 7.5, and 0.1% gelatin) were added (Sambrook et al., 1989). The packaged recombinant phages were stored at 4°C.

To titer the library, two plates were made. For one plate, 1 μ l of phages was used to inoculate 100 μ l of the P2392 strain of *Escherichia coli*, and for the other plate, 10 μ l of phages was used for infection. These cells and phages were incubated at 37°C for 20 minutes. Then top agar made in LB medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.01% casamino acids, and 2% MgSO₄ 7 H₂O) was mixed with each sample and plated on separate LB bottom agar plates (Sambrook et al., 1989). The plates were incubated overnight at 37°C. After overnight growth, the phages were counted and the titer of the library was determined.

V. Library screening

For library screening, Hybond nylon membrane filters were placed on the agar plates containing approximately 10,000 recombinant phages and blotted for 1-2 minutes.

The filters were then placed for 3 minutes in denaturing solution containing 1.5M NaCl and 0.5M NaOH (Sambrook et al., 1989). After blotting on Whatman filter paper, the nylon membranes were then incubated twice, five minutes each time, in a neutralizing solution containing 2X SSCP and 0.2M Tris, pH 7.5. The DNA was then crosslinked to the membranes by UV radiation and subsequently the membranes were baked in a vacuum-oven for 1.5-2hrs. The filters were sealed in a plastic pouch and stored at 4°C until prehybridization.

The membranes were prehybridized, hybridized, and washed according to the protocol used in Southern blot hybridization. After hybridization, the filters were air dried and exposed to X-ray film.

VI. Rapid phage DNA isolation

Approximately 10^8 phages were added to a 0.1ml overnight culture of P2392 *E. coli* and incubated at 37°C for 20 minutes. These cells were then plated on agar plates using top agar. Both these agars contained 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% bactoagar, and 1mM $MgCl_2$ (Sambrook et al., 1989). The plates were incubated at 37°C for 6 hours to allow confluent lysis to take place. After incubation, 5 ml of λ diluent containing 10mM Tris, pH 8.0, and 2mM $MgCl_2$ was added to each plate and incubated overnight at 4°C to release the phage particles from the agar.

The next day, lysate from each plate was passed through a 2ml DE-52 cellulose column which was equilibrated with 10mM Tris, pH 8.0. The column was then washed first with 5ml of chase buffer (10mM Tris, pH 8.0, 10mM $MgOAc$ 4 H_2O , and 60mM $NaOAc$) and then 0.8ml of elution buffer (10mM Tris, pH 8.0 and 50mM $MgOAc$ 4

H₂O). The phage particles were then eluted by washing the column with 1.2ml of elution buffer.

For DNA isolation, 20µl of 0.1mg/ml Proteinase K and 48µl 10% SDS were added to each 1.2ml of eluted phages. The solutions were mixed and left at room temperature for 5 minutes. Then 200µl of 3M KOAc, pH 8.7-9.0, was added and this mixture was heated at 88°C for 20 minutes. The mixture was cooled in ice for 15 minutes and centrifuged at 12,000 rpm for 15 minutes. The supernatant was removed and mixed with a one-tenth volume of 2M NaOAc, pH 7.5, and an equal volume of 100% isopropanol. After precipitation at -20°C for 1 hour, the samples were centrifuged at 12,000 rpm for 30 minutes to pellet the DNA. The supernatant was discarded and the DNA pellet was washed with 100% ethanol, dried, and resuspended in 1X TE.

RESULTS AND DISCUSSION

I. Inverse polymerase chain reaction (PCR)

In order to obtain a portion of the genomic DNA flanking the transposon at the 100C region of the third chromosome, inverse PCR was employed. In this method, a restriction fragment is obtained which carries a portion of the transposon as well as the flanking genomic DNA (Ochman et al., 1989). This fragment is then circularized *in vitro* via compatible or identical restriction sites at its ends. Once circularized, PCR amplification is done by using oppositely oriented primers located within the transposon DNA (Ochman et al., 1989). Many cycles of PCR is expected to yield a large amount of amplified DNA which will contain both the flanking genomic DNA and a portion of the transposon.

To be able to use inverse PCR, a restriction fragment of 4-kb or less is preferable (Ochman et al., 1989). In order to obtain this fragment, it was necessary to construct a restriction map of the flanking genomic DNA by Southern blot hybridization. For the first Southern blot hybridization, genomic DNA isolated from the homozygous flies was restricted separately with SstI, BamHI, and PstI. SstI and BamHI are known to have sites close to the left-hand side of the P transposon (Fig. 3), and PstI was used to ascertain whether it would generate a suitable fragment. The resulting Southern blot was hybridized with a 0.9-kb EcoRI/BamHI fragment (probe A) located at the left-most end of the *Cyp6a8* transgene (Fig. 3). Several bands hybridized with the probe (Fig. 4). In the BamHI lane, there was an intensely hybridizing 7-kb band which originated from the *Cyp6a8* gene located within the P transposon (Fig. 3) on the third chromosome as well as

the endogenous gene located on the second chromosome. Mild hybridization was also observed with a 4.3-kb BamHI fragment (Fig. 4). According to the published restriction map of the *Cyp6a8* gene (Maitra et al., 1996), no BamHI fragment other than the 7-kb band is expected to hybridize with this probe. It is possible that this 4.3-kb BamHI band is part of another Cyp gene which may have sequence similarity with probe A. Cytochrome P450 is a multigene family and therefore cross-hybridization is not unusual.

In lane 2 (Fig. 4), the 7-kb and 6-kb SstI bands were thought to be partially digested DNA fragments. The 2.2-kb fragment originated from the natural *Cyp6a8* gene located on the second chromosome. The strongly hybridizing 0.6-kb band was actually a doublet; one copy originated from the transgene and the other from the *Cyp6a8* gene located on the second chromosome. The 4.3-kb SstI band was thought to be the only one which originated from the transgene and contained a portion of the transposon as well as the genomic DNA flanking the transposon. This DNA fragment was thus chosen as the template for inverse PCR.

This 4.3-kb SstI fragment was gel-purified and circularized via self-ligation (Fig. 5). Using primers located in the left P-element (Fig. 5), the inverse PCR was performed. However, when the PCR product was electrophoresed on the agarose gel, a smear was observed rather than the single band which would have been expected had the experiment been successful. This experiment was probably unsuccessful due to the fact that the 4.3-kb fragment chosen for this inverse PCR was simply too large, making it difficult for the DNA to self-ligate.

II. Genomic library screening

Due to the lack of positive results from the inverse PCR method, a genomic library was created. When the genomic library was titered, it was determined that the size of the library was 3.65×10^5 plaque-forming units (pfus). Approximately 70,000 pfus were plated on agar plates and blotted onto nylon membrane filters. The filters were hybridized with probe B, representing a portion of the 5' end of the *ry*⁺ gene (Fig. 3). Twenty-two plaques hybridized intensely with probe B, and of these, 14 were chosen for the second round of screening. All 14 clones hybridized again with probe B, illustrating that the previous signals were not false. Six of these clones were then arbitrarily chosen for a third round of screening. Duplicate plaque lifts were prepared from each clone and one set of filters was hybridized with probe B while the second set of filters was probed with DNA from the right P-element (probe C, Fig. 3). This was done to distinguish those clones which may have had a portion of the endogenous *ry*⁺ gene from those which had the *ry*⁺ transgene. The clone carrying the endogenous *ry*⁺ DNA was not expected to hybridize with probe C because the right P-element is not present in this naturally-occurring *ry*⁺ gene. Clones $\lambda 2$, $\lambda 8$, and $\lambda 15$ hybridized to both probes and therefore were thought to contain the *ry*⁺ gene as well as the right P element. The $\lambda 2$ clone was grown for further analysis.

DNA from the $\lambda 2$ clone was used for Southern blot analysis to determine if it contained both the transposon and a portion of the flanking genomic DNA. In order to do this, the $\lambda 2$ DNA was first restricted with both EcoRI and SalI enzymes. These enzymes were chosen because they have sites within the transposon, thus facilitating deductions concerning the location of these enzymes' sites in the flanking DNA. The restricted DNA

was run in two lanes which were then separated, blotted, and probed separately with probes B and C (Fig. 3). Both lanes of the restricted DNA hybridized to both probes at 5.8-kb (Fig. 6). However, since the DNA had been digested simultaneously with SalI and EcoRI, it was impossible to determine whether the fragments were generated by either one or both of the EcoRI and SalI enzymes. Therefore, a second Southern blot was prepared.

For this Southern blot analysis, EcoRI and SalI enzymes were used separately to restrict the λ 2 DNA. Upon examination of the restricted DNA on an agarose gel (Fig. 7), it became clear that the 5.8-kb band which had hybridized to both probes in the first Southern blot (Fig. 6) was most likely generated by EcoRI. Therefore, the EcoRI digestion was focused upon, and four of the six fragments produced by this restriction enzyme were chosen for further analysis (Fig. 7). The DNA fragment which migrated as a 23-kb band was not chosen because it was the longer arm of the λ phage. The 4.1-kb EcoRI band was not chosen because it was the downstream half of the ry^+ gene present in the transposon (Fig. 3). Therefore, the 9-kb, 5.8-kb, 1.2-kb, and a doublet of 0.9-kb and 0.8-kb bands were eluted for further analysis.

The 5.8-kb EcoRI DNA fragment was digested with HindIII for Southern blot analysis. This enzyme was chosen because the restriction map showed that the ry^+ gene and the right P-element are separated by a HindIII site (Fig. 3). It was therefore thought that digestion of this fragment with HindIII would generate a more complete restriction map of the flanking genomic DNA. The other three EcoRI fragments (9.0-kb, 1.2-kb, and a 0.9/0.8-kb doublet; Fig. 7) were also electrophoresed without further digestion in parallel with the HindIII-digested 5.8-kb EcoRI DNA for blotting. The three undigested

fragments were hybridized with Probe D (Fig. 3) to ascertain whether they were portions of the *Cyp6a8* gene. However, the HindIII-digested 5.8-kb DNA was probed with DNA fragment C (Fig. 3). The results showed that the 9-kb, 1.2-kb, and 0.9-kb/0.8-kb doublet were not part of the *Cyp6a8* gene because they did not hybridize with probe D (Fig. 8, lanes 2-4). Therefore, all or most of these DNA fragments are parts of the genomic DNA flanking the right P element of this transposon. It also became clear, however, that genomic DNA flanking the transposon had been obtained.

In the lane containing HindIII-digested 5.8-kb EcoRI DNA (Fig. 8, lane 1), four DNA fragments hybridized with probe C with variable intensities. The sizes of these fragments were 5.8-, 4.7-, 2.7-, and 1.6-kb. In addition to these bands, a 3.1- and a 1.1-kb band were also found in lane 1 (data not shown) which did not hybridize with the probe. Generation of these six DNA fragments from the 5.8-kb band can only be explained if it is assumed that the 5.8-kb EcoRI fragment contains two HindIII sites, one of which or both remained uncut in some molecules (Fig. 9). The frequency of the failure of digestion at these two HindIII sites apparently was not similar because DNA quantity and hybridization signal intensity associated with different-sized bands were not proportional to the size of the band. For example, signal intensity on the 4.7-kb band was lower than that found on the 1.6-kb band (Fig. 8). This is because in most cases the enzyme could cut at both HindIII sites, but in a small number of molecules it failed to cut at the proximal HindIII, thus producing the 4.7- and 1.1-kb bands (Fig. 9). How six different fragments can be generated from the 5.8-kb EcoRI band after HindIII digestion is explained in figure 9. Based on these analyses, it was determined that there must be a HindIII site in the flanking genomic DNA 1.6-kb from the end of the *ry*⁺ gene present in

the transposon. Similarly, there must also be an EcoRI site in the flanking DNA approximately 2.7-kb away from the end of the *ry*⁺ gene (Fig. 9).

In summary, the restriction analysis described above showed that λ 2 contains a complete copy of the 7.0-kb *ry*⁺ gene and no portion of the *Cyp6a8* gene. The λ EMBL3 phage used for construction of the genomic library of the *fs(100C)* flies can take DNA fragments ranging between 9.3-kb and 23-kb in length. Because the *ry*⁺ gene and the right P element (0.2-kb) together are 7.2-kb, the λ 2 clone must have 2.1- to 15.8-kb of flanking genomic DNA. This DNA can be obtained and used in future molecular studies to isolate and characterize this unidentified gene involved in female fertility.

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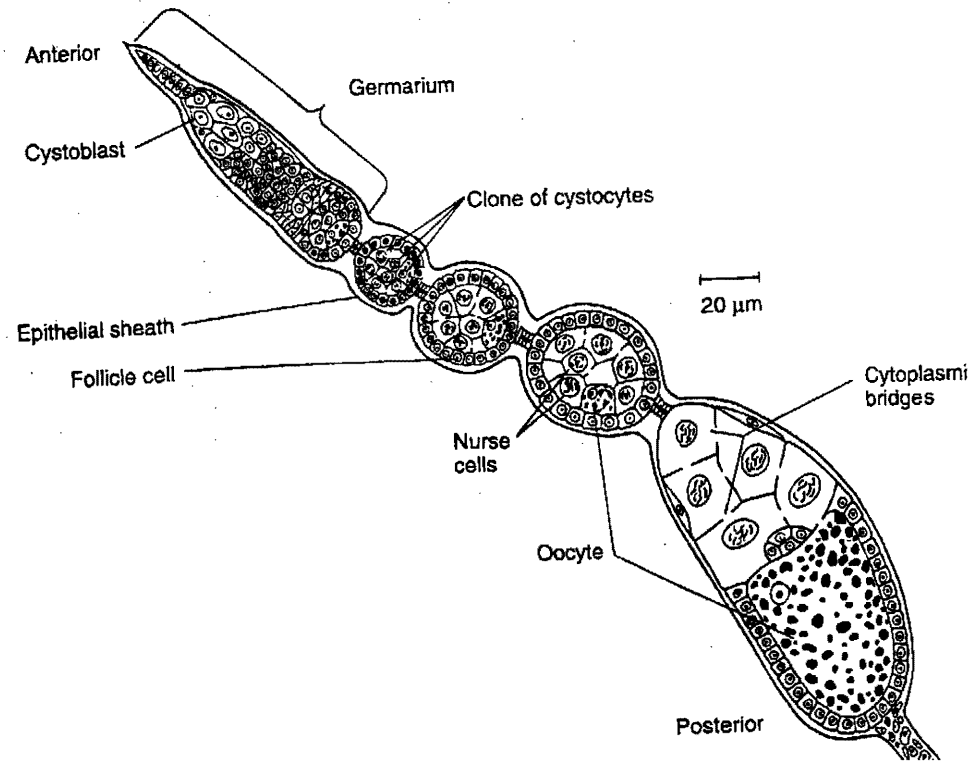


Figure 1--One ovariole with several egg chambers in differing stages of development. The most developed is located at its posterior end. Also illustrated are the nurse cells and follicle cells, which aid the oocytes, and the cystoblasts from which both the nurse cells and oocyte form.

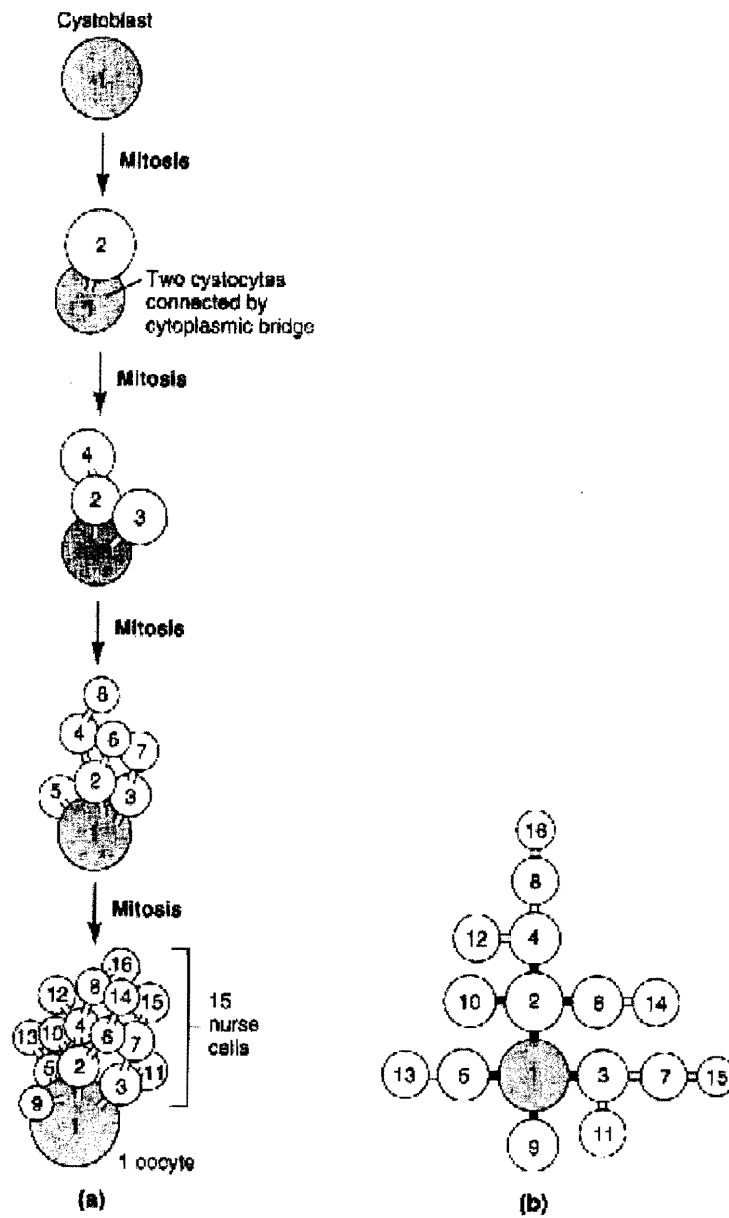


Figure 2--(a) Depiction of a cystoblast in the mitotic divisions by which it moves from a single cystoblast to an oocyte with fifteen nurse cells. **(b)** A flattened view of the cystoblast with its nurse cells.

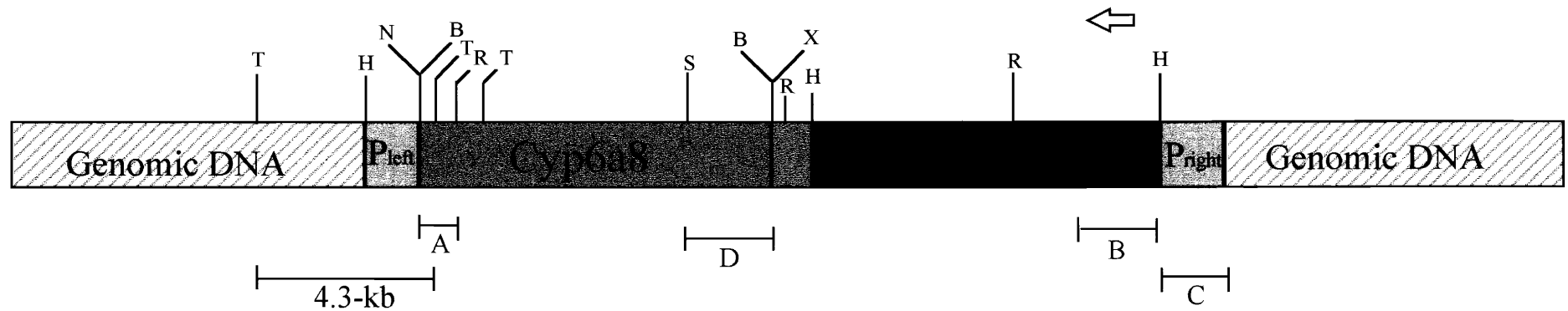


Figure 3--Restriction map of the transposon and the flanking genomic DNA. S, SalI; T, SstI; H, HindIII; N, NotI; B, BamHI; R, EcoRI; X, XhoI. A-D are the DNA fragments used in various experiments. The 4.3-kb SstI fragment was used for inverse PCR. P_{right} and P_{left} are the P elements. The arrow indicates the direction of transcription of the *ry*⁺ gene.

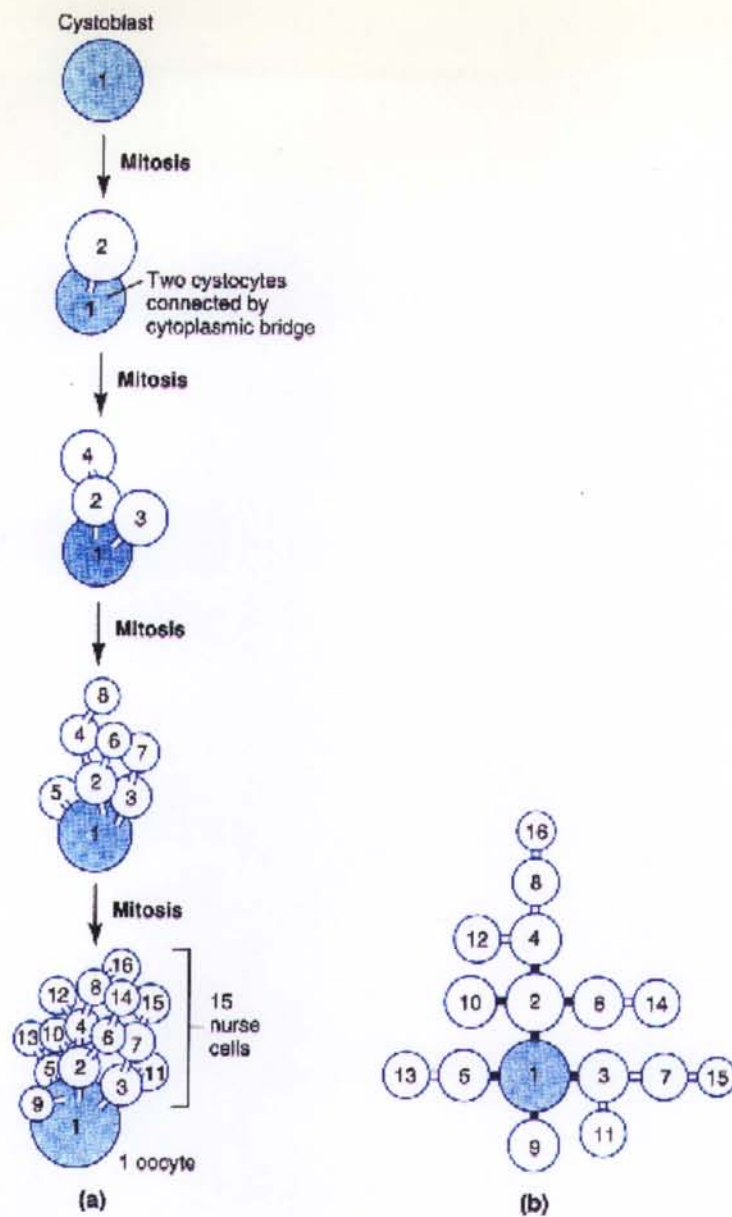


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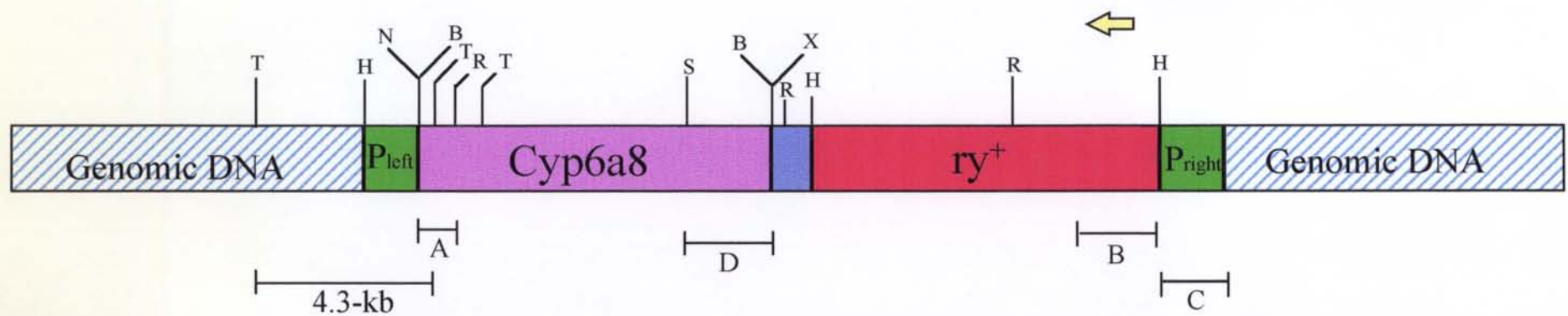


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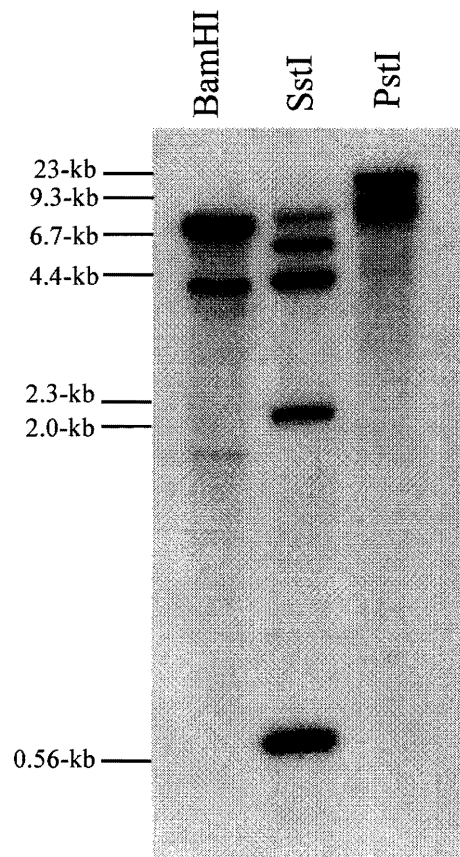


Figure 4--Southern blot hybridization of the genomic DNA from *fs(100C)* homozygous flies digested with BamHI, SstI, and PstI. DNA fragment A (Fig. 3) was used as a probe.

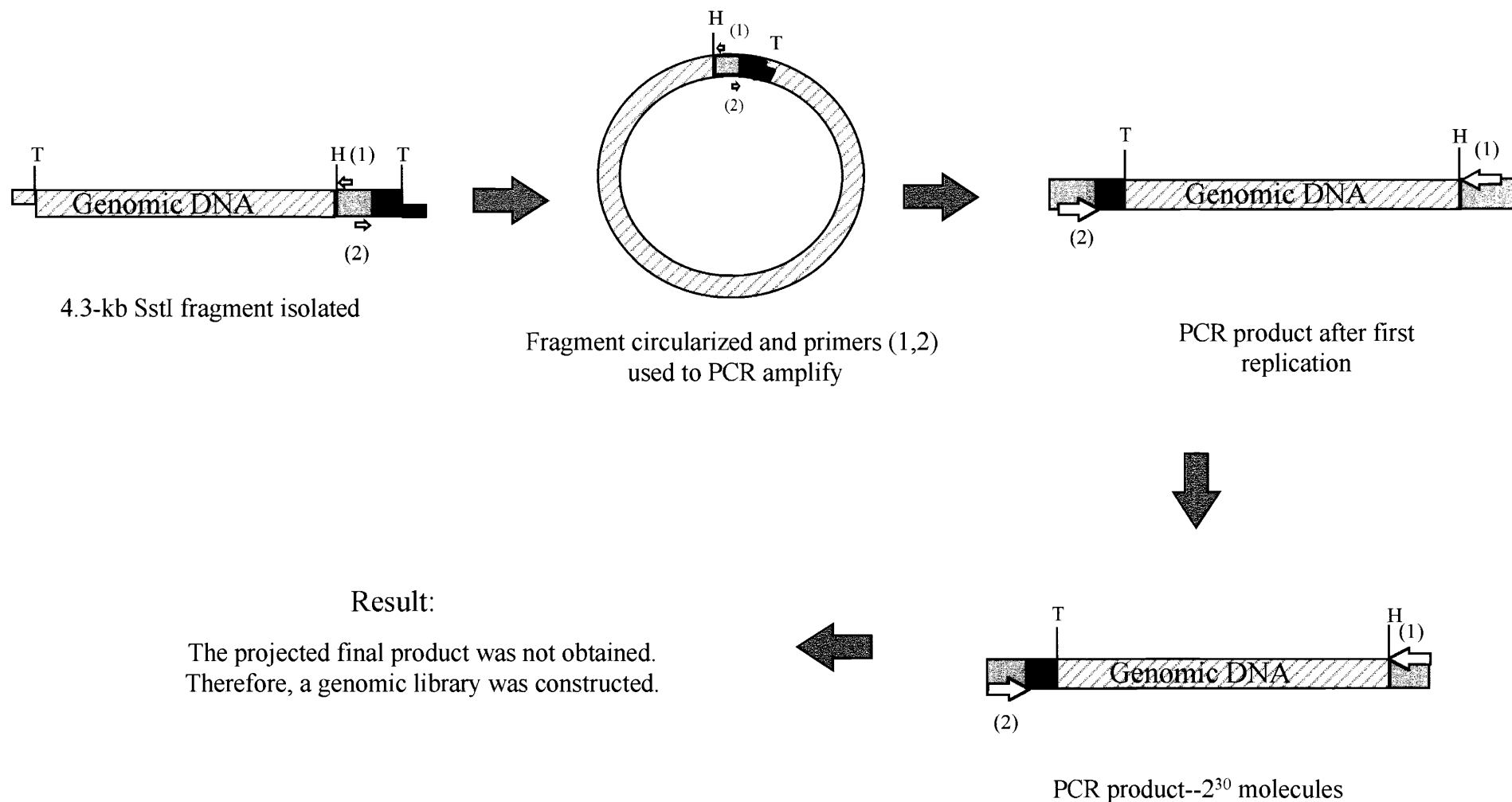


Figure 5--Inverse PCR with the 4.3-kb SstI fragment (see Fig. 3).
The arrows (1, 2) represent the primers used.

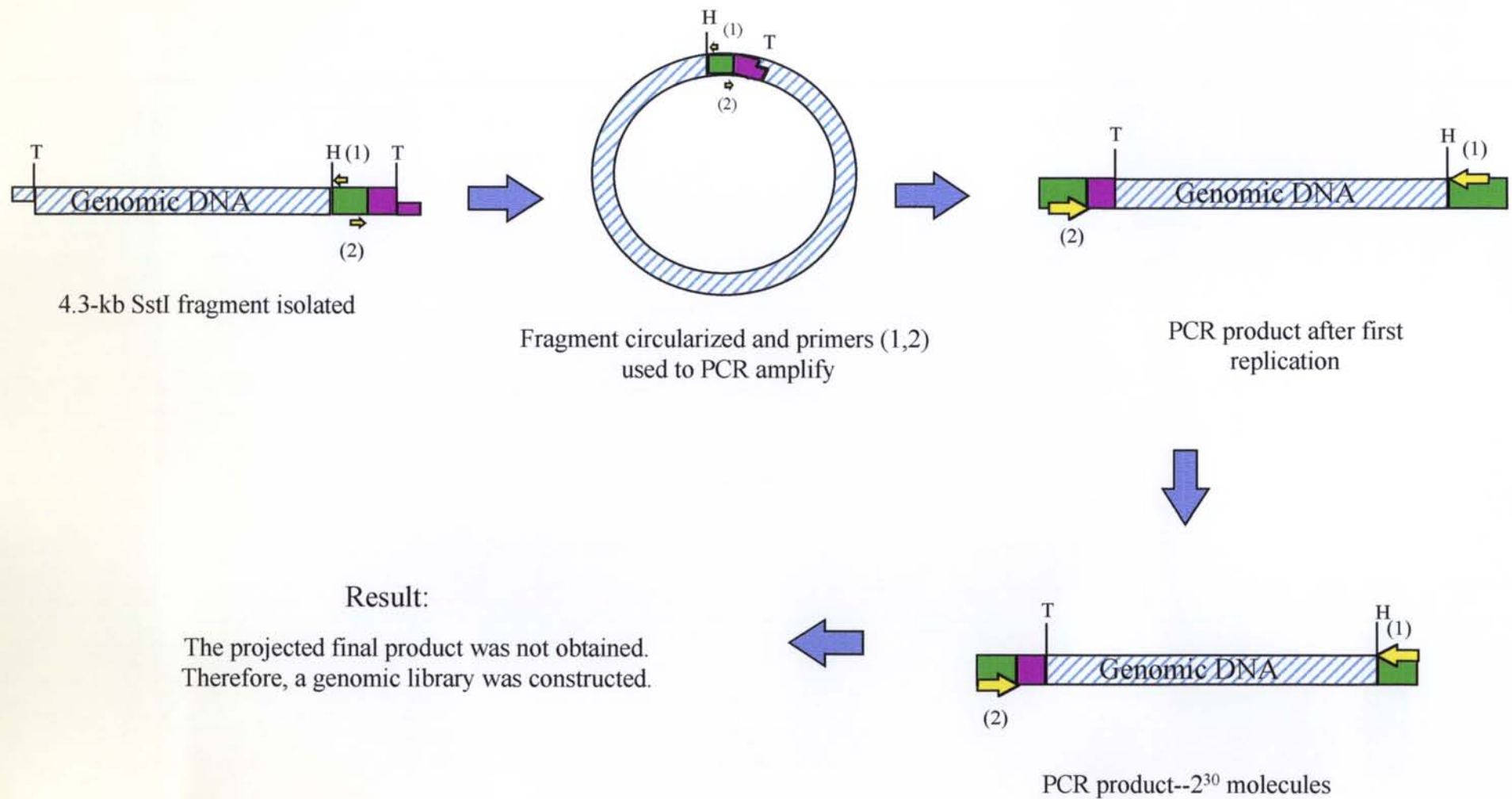


Figure 5--Inverse PCR with the 4.3-kb SstI fragment (see Fig. 3).
The arrows (1, 2) represent the primers used.

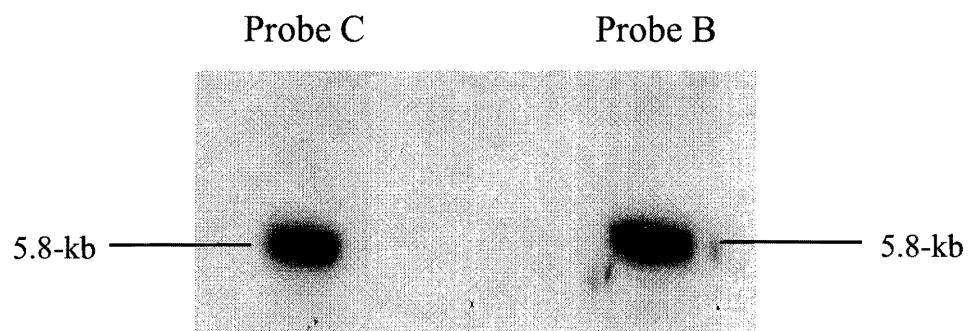


Figure 6--Southern blot hybridization of the Sall/EcoRI-digested DNA of *fs(100C)* homozygous flies. DNA fragments C and B (Fig. 3), representing different regions of the transposon, were used as probes.

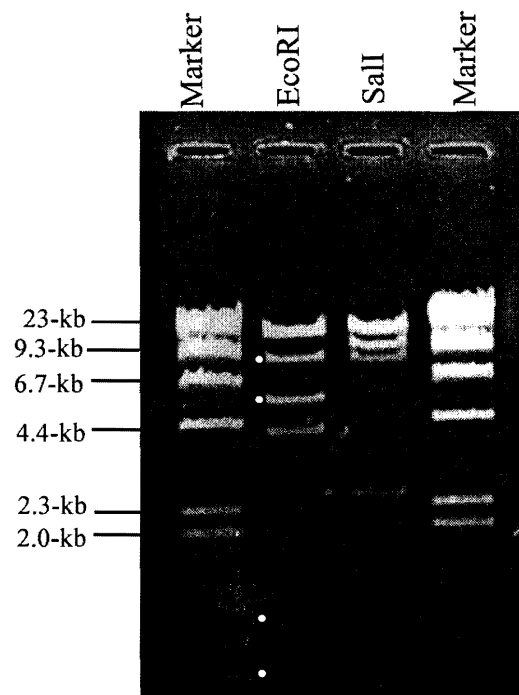


Figure 7--Agarose gel with restricted genomic DNA from *fs(100C)* homozygous flies. Lanes 1 and 4 contain HindIII-cut Lambda DNA marker. The white circles indicate the 9-, 5.8-, 1.2-, and 0.9-/0.8-kb bands which were eluted for Southern blot analysis (Fig. 8).

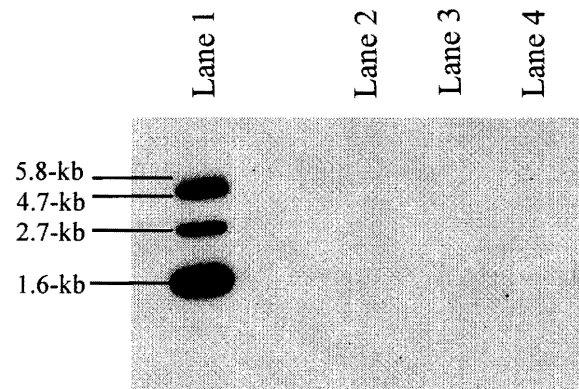


Figure 8--Southern blot hybridization of the EcoRI fragments isolated from the genomic DNA of the *fs(100C)* flies (see Fig. 7). Lane 1 is the 5.8-kb EcoRI fragment digested with HindIII; lanes 2-4 contain the 9.0-kb, 1.2-kb, and the 0.9-/0.8-kb EcoRI fragments, respectively. Lane 1 was hybridized with probe C (Fig. 3) and lanes 2-4 were probed with DNA fragment D (Fig. 3). The hybridization signal intensities are dependent upon the amount of DNA present in each band.

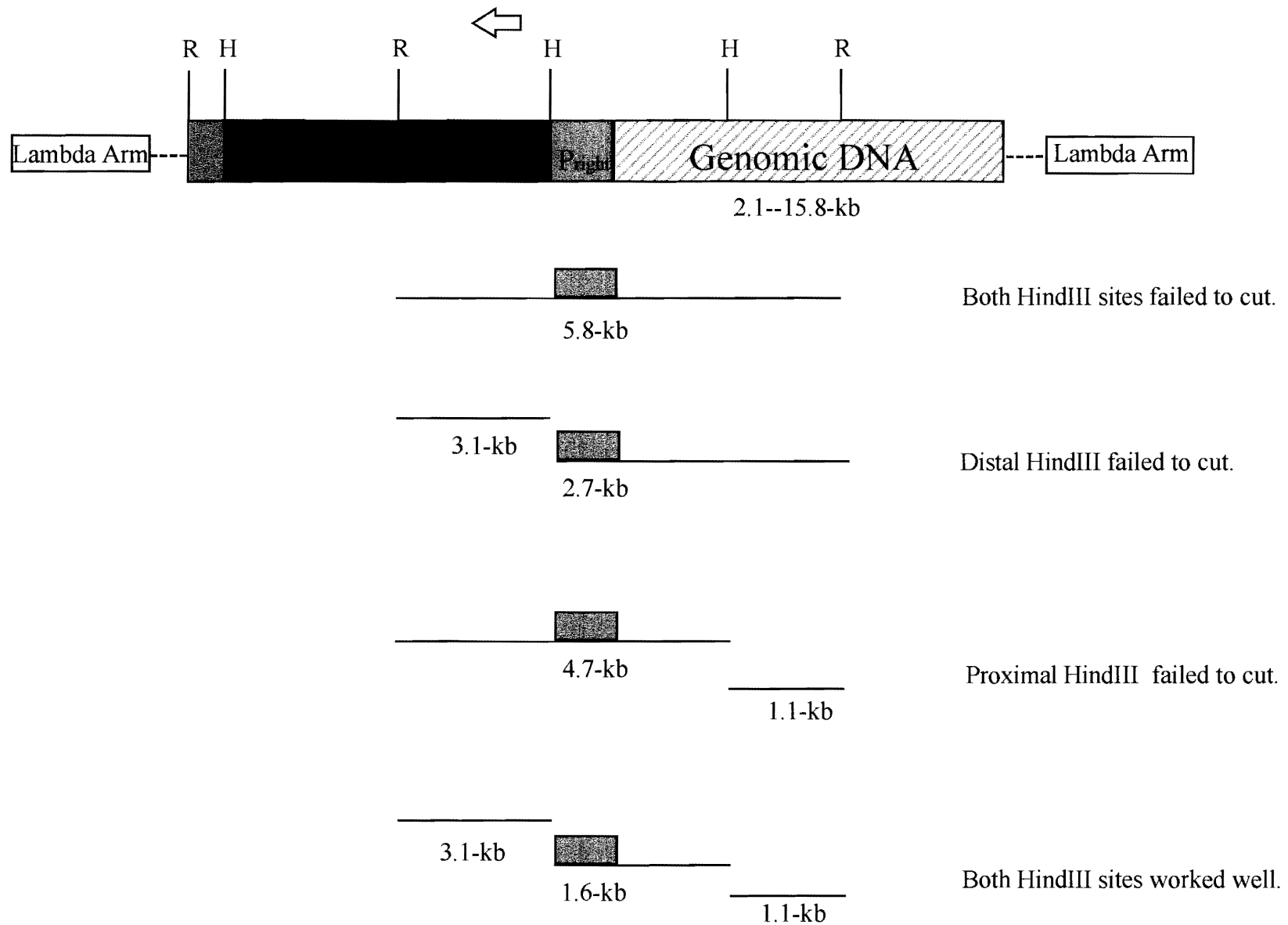


Figure 9--Analysis of the Southern blot hybridization of the 5.8-kb EcoRI fragment digested with HindIII. The probe used was DNA fragment C (Fig. 3). The arrow indicates the direction of transcription of the ry^+ gene.

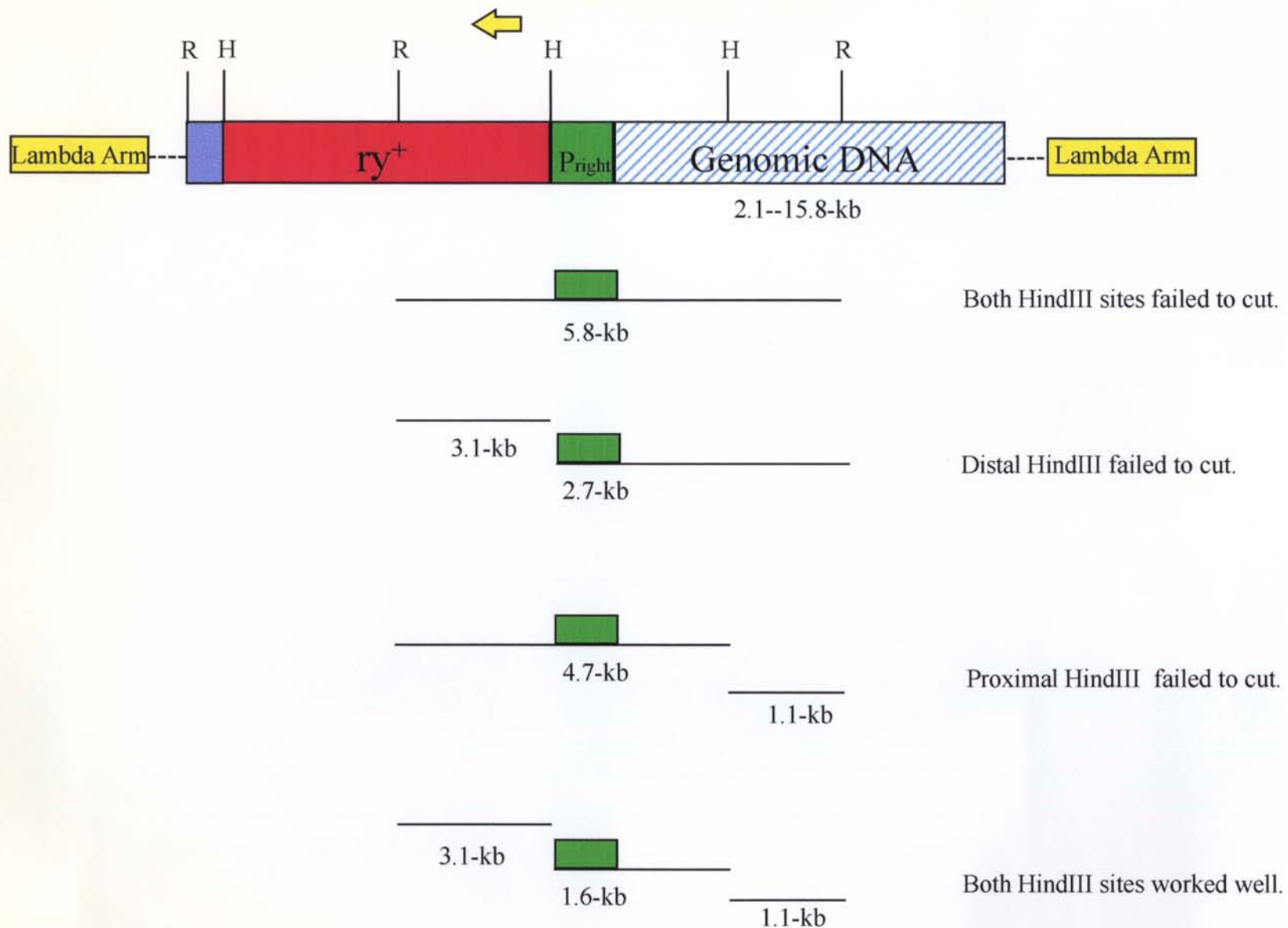


Figure 9--Analysis of the Southern blot hybridization of the 5.8-kb EcoRI fragment digested with HindIII. The probe used was DNA fragment C (Fig. 3). The arrow indicates the direction of transcription of the *ry*⁺ gene.