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# Molecular Genetic Linkage Mapping in Tribolium Beetles

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**Abstract:** Genetic linkage mapping is often taught using sex-linked traits in *Drosophila*. Modern linkage mapping rarely uses phenotypes. This exercise is designed to demonstrate more realistically how linkage maps are created today. The exercise uses RI lines of *Tribolium* beetles along with RAPD-PCR to create a large dataset of molecular markers that is then analyzed by a computer program to determine linkage. In the process, students learn how a DNA extraction is done, how large numbers of RAPD-PCR reactions can be set-up efficiently, how to run an agarose gel, and how to score and analyze molecular data to create a map.

**Keywords:** Molecular, linkage, mapping, *Tribolium*, RAPD-PCR, Recombinant Inbred lines

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## Introduction

This laboratory exercise was created for sophomore level genetics students as a replacement for the typical exercise about genetic linkage using *Drosophila*. It was also intended to introduce modern molecular techniques and analyses over several weeks. The exercise is based on an original research project (Yezerksi et al. 2003) and is designed to collect additional data for the project in addition to being a learning experience on modern genetic linkage mapping for the students.

The exercise begins with a typical phenol-chloroform DNA extraction from *Tribolium* beetle Recombinant Inbred lines. These lines were created as part of the aforementioned research project, and continue to be maintained in my laboratory. Although there are several hundred lines still extant, only forty-two of these lines are used for this experiment along with six representatives of the parental lines. Since completing forty-eight extractions is quite a workload for any one person in one afternoon, the beetles are divided among the laboratory groups and the resulting DNA is shared after the extraction is completed. This is possible since this method results in enough DNA from each beetle to run approximately 2000 of the RAPD-PCR reactions used in this protocol.

After the DNA is extracted and distributed, the second week involves the set-up of RAPD-PCR reactions (Williams et al. 1993). These small reactions (12  $\mu$ L total volume) require only 1 microliter of the extraction DNA that has been diluted 1 to 100. Every student group is provided with a different RAPD-PCR primer so that students are not simply reproducing the same data, but rather gathering a large amount data to be shared with the class. Since each group will be setting up a reaction for all 48 beetle samples, students also become familiar with a low-scale high-throughput method. This involves calculating a master mix as well as an opportunity to demonstrate either multiple-channel, or electronic pipettor if available. Since the set-up of these reactions is not exceptionally time consuming, the extra time available in both this and the next week of the exercise can be used for other purposes such as the worksheet in Appendix A.

The third week of the exercise simply involves loading and running the electrophoresis gels. This is best done with a large gel rig that can hold 48 or 96 reactions and a multi-channel pipettor. These pieces of equipment are very impressive to undergraduates, but smaller rigs and single channel loading are also possible.

The final week is used for “scoring” the gels and analyzing the data. Students are provided with a picture of their results. It is helpful to have previously done, successful, results pictures on file as JPEG, in the case of experimental error. This allows all students to learn how to score even if their personal data is not available. Data can be analyzed with the free software program, MapManager Manly 1998; Manly and Olson 1999.

As a result of this four week long exercise students learn DNA extraction, high-throughput PCR, gel electrophoresis, as well as how DNA data is analyzed for modern genetic linkage maps. Although, the complete process requires four sessions, the exercises in the last three weeks do not fill a typical laboratory time, thus allowing for concurrent exercises in other topics. While the exercise can be done with all of the most modern equipment, it is also possible to finish the methods on a smaller scale.

While most linkage mapping projects concentrate on the creation of scorable progeny, this one concentrates instead on molecular technology. While other organisms and genetic lines can be used, representatives of the recombinant inbred lines of these beetles can be gotten from my laboratory for \$115 for the transfer, packaging and mailing of 48 frozen beetles.

## Materials

(underlined items have their recipes listed in Appendix D)

### Day 1: Extraction

#### *Equipment*

- Chemical hood if using phenol
- Water bath or heat block set at 60°C
- Microcentrifuge
- Micropipettors with matching tips
  - P10
  - P100
  - P200
  - P1000
- Biohazard bags
- UV spectrophotometer

#### *Supplies* (per one group of four students)

- Gloves
- 6 frozen beetles (each in a 1.5 mL microcentrifuge tube)
- Container of autoclaved microcentrifuge tubes (at least 20)
- Container of autoclaved 0.5 mL microcentrifuge tubes (at least 30)
- 1.5 mL of lysis buffer with Proteinase K
- 8 homogenizer pestles
- 1 mL of high salt lysis buffer
- 20 mL 1:1 phenol:chloroform (stored in 50 mL Falcon tube, kept in hood – shake before using)
- 20 mL chloroform (stored in 50 mL Falcon tube, kept in hood)
- 50 mL Sodium acetate
- 10 mL TE buffer
- 50 mL 100% EtOH kept in freezer

### Day 2: RAPD-PCR

#### *Equipment*

- Thermal cycler

#### *Supplies* (per one group of four students)

- Half 96 well PCR plate with seal
- 1 mL sterile water
- One tube of Invitrogen PCR buffer without magnesium
- One tube of Invitrogen MgCl<sub>2</sub>
- 50 mL of dNTP's
- 50 mL of primer (Primers listed in Appendix E)
- Invitrogen Native Taq (keep one tube in freezer for full class's use)
- 1 mL sterile mineral oil

### Day 3: Agarose gel electrophoresis

#### *Equipment*

- Power supply
- Gel rig (recommend Bio-Rad's 96 well gel apparatus)

#### *Supplies* (per one group of four students)

- 2 or 3% agarose gels (one gel will run two group's reactions)
- 1x TBE buffer (2 liters, enough to cover gels)
- 50 mL loading dye
- 100 mL Extended base pair ladder
- Container of Ethidium bromide
- Photographing apparatus or gel imaging system

### Day 4: Analysis

#### *Equipment*

- Macintosh computer loaded with MapManager software and Microsoft Excel or other mapping software

## Notes for the Instructor

*N.B. This exercise is based on Yezerksi et al. (2003), which gives additional details about thermal cycler times and reagent specifics. A reprint of this paper can be sent if requested by email.*

### **Day 1: Extraction**

- Our class sizes are generally around 16 students and we usually have two sections. Therefore, I have students work in groups of four with each group being responsible for the extraction of 6 beetles. This would be 8 groups each with 6 beetles, resulting in the 48 extractions we do.
- Although phenol works best for this exercise, isopropyl alcohol can be substituted if hoods are not available. However, the results may not be as consistent.
- There are several wait times during this exercise. I usually have the students come in and immediately put the beetles in the homogenization buffer, crush them, and incubate. Then I used the free time periods to explain the process and further procedure.
- Make sure the caps on the microcentrifuge tubes are tightly closed before inversion. If not and they leak, the chemicals will cause all labeling to run.
- I usually have the students come in on their own time to resuspend, dilute and distribute their DNA to each group. However, I have each group label a 10 x 5 freezer box and put it in the freezer (See Appendix B for an example). I put a diagram on the door of the freezer as to which place in each box the samples will go. I also have the students make seven sets of tubes (one set for each of the other seven groups) pre-labeled with their line numbers and each containing 99  $\mu\text{L}$  of TE ready for the 1  $\mu\text{L}$  of sample that will be added to make their dilutions.

### **Day 2: RAPD-PCR**

- Each group gets a plastic 96 well PCR plate that has been cut in half.
- Each half of the group can make a master mix for half of their samples.
- Because of pipettor error, it is generally a good idea to make enough master mix for the number of reactions needed plus five extra reactions
- Half the group can quantify their DNA extractions on the spectrophotometer while the other half adds their 1  $\mu\text{L}$  samples.
- Even in a thermal cycler with a heater lid, reactions of this size can evaporate without mineral oil

### **Day 3: Agarose gel electrophoresis**

- Prepare the gels ahead of time.
- Using a 3% gel gets the best resolution but a 2% gel works reasonably well if time-limited.
- While I have historically used Ethidium bromide to stain, Cyber Green also works well.
- The best results of staining for this would be to overstain the gel for half an hour and then destain in running tap water for one hour.
- Any size standard ladder can be used, but I recommend FMC BioProduct's Extended Base Pair Ladder since the range is very appropriate.

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**Day 4: Analysis**

- See Appendix A for a training worksheet on how to score gels
- It may be a good idea to try this experiment yourself first and then keep good results in a JPEG picture file in case a group does not get results.
- See Appendix C for a list of primers and their typical results
- Data can be analyzed using several free software programs. I recommend Map Manager since it has the ability to use RI lines built in (<http://iubio.bio.indiana.edu:7780/archive/00000064/>). Although it is an older program it is used extensively for publications of genetic linkage maps.
- The printable manual and a tutorial for Map Manager QT can be found at: <http://mapmgr.roswellpark.org/MMM/MMMBookTOC.html>
- However, if you use a PC or want to find a different program, see this site for a very complete list of all genetic analyses programs: <http://linkage.rockefeller.edu/soft/list.html>
- If you would like your students to try some calculations by hand, Appendix F shows how Recombinant Inbred lines are used for mapping.

## Student Outline

### Introduction:

Genetic maps have been in the news recently especially with the reports of the Human Genome Project meeting its first goals of mapping the entire genome. But what are these maps really, and what use are they? There are really two main types of genetic maps; genomic maps and linkage maps. Genomic maps are very time consuming since the goal is to find the placement of every gene on the genome of an organism and eventually to sequence the genome in its entirety and find the actual functions and interactions between all genes - a very daunting task. Linkage maps are easier to make and can be very useful in gathering preliminary information on the arrangement of genes and DNA markers for an organism and serve as a basis for studies used to identify genes that contribute to certain phenotypes. Linkage maps describe the pattern of inheritance of regions of DNA through generations. These maps are really looking for regions of the genome that tend to be inherited together like blond hair and blue eye genes or red-hair and putative "assertiveness" genes. However, just because genes are inherited together does not mean that they are physically linked on the same chromosome. They very well might be but there are also other reasons regions are inherited together that we will not go into now. So, a genetic linkage map is describing a pattern of inheritance of genes in order to show which tend to be inherited together or "linked."

To make a genetic linkage map two main components are necessary: a breeding design where you know which offspring came from which parents, and "markers." "Markers" are really polymorphisms, that is some trait, whether it be morphological or molecular, that differs between two strains, or races, of organisms of interest. Molecular markers look for differences in the proteins, RNA, or DNA of the organism of interest. Our markers will be found using Random Amplified Polymorphic DNA's or RAPD's. Refer to Figure 1 to see how this works. RAPD primers are short, ~10-mer, primers that find several sites on the genome and amplify them. Some of the regions that are amplified can be informative polymorphisms if a band is consistently present in one strain and consistently absent in the other. If we know which strain an allele (here presence or absence) came from than we can trace that

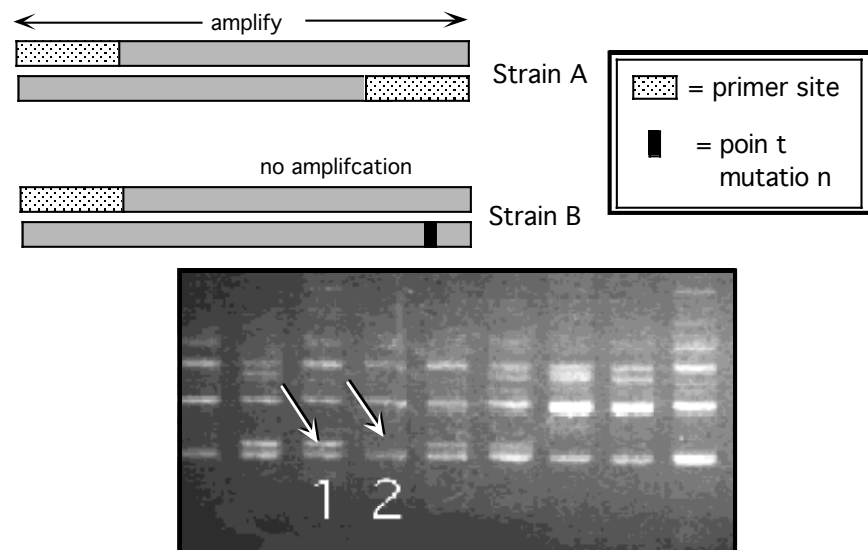


Figure 1. How a RAPD marker works. If the primer in the PCR reaction finds two sites in strain A you get a band on a gel at a certain size given between the two primer sites. However, in strain B a point mutation has eliminated the primer site and thus a band is not produced. The result on a gel would be like Lane 1 for strain A and Lane 2 for Strain B.

RAPD primers are short, ~10-mer, primers that find several sites on the genome and amplify them. Some of the regions that are amplified can be informative polymorphisms if a band is consistently present in one strain and consistently absent in the other. If we know which strain an allele (here presence or absence) came from than we can trace that

allele in the progeny just like any other marker (see Figure 2). Now we need to trace them within known crosses.

### Recombinant Inbred lines



**Figure 2.** A picture of a real mapping gel showing three polymorphisms between b-+ and b-Pakistan (b-Pak) (markers 1, 2, and 3). The RI lines have various combinations of these alleles. The first and last lanes are size standard ladders (L). The next four lanes are from b-Pakistan parents followed by four lanes from b-+ parents. The numbered lanes are 32 different representatives from RI lines. For an explanation of how this leads to a map, see the text.

The crosses we are using are called Recombinant Inbred Lines (RI lines). They are similar to intercrosses but with a twist. These lines are created by first crossing essentially homozygous parental lines, here b-+ with b-Pak, and creating essentially all heterozygous progeny. If we cross the heterozygous progeny, we will get a mix of the parental genes in the  $F_2$  generation. This would be an intercross. The additional step for RI lines is that after the second cross the resulting progeny are inbred for several generations by full sibling mating until they are essentially homozygous again. The results are lines that have various mixes of the original parental genes, but all the individuals in one line are virtually genetically identical. This is helpful in that you can treat every individual within each line as a genetic twin.

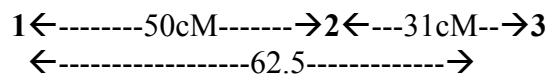
To create the genetic linkage map we will trace the alleles defined by the RAPD polymorphisms within these lines. If we imagine these lines are intercrosses then we just need to find out which parental allele for each marker is found within each line and then find out how often alleles from the same parent have segregated by recombination. If we designate all those alleles from b-Pak as "A" and those from the b-+ as "B" we can look for instances where there is a mix between "A's" and "B's" in a particular line. For example, look at Figure 2. You can see that for marker 1, presence of a band is a b-Pak allele, for marker 2 presence is a b-+ allele and for marker 3, presence is a b-Pak allele. So the scoring system for the RI lines shown would be as shown in the following table:

Marker/Line#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A	B	A	A	B	B	A	A	A	B	A	B	B	A	B	B
2	A	A	B	B	A	A	B	A	B	B	A	A	B	B	A	B
3	A	B	B	B	A	A	B	A	B	A	A	A	B	A	A	B

Marker/Line#	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	B	B	A	A	A	B	A	A	B	B	B	B	B	A	A	A
2	A	B	A	B	B	B	A	A	A	B	B	B	A	A	A	B
3	A	B	A	B	A	A	B	B	A	B	A	B	A	B	B	B



Now take just marker 1 and marker 2 and counting up the number of times you see an "A" and a "B" together in the same line rather than either two "A's" or two "B's" you can get the number of recombinants. From this you can get the Recombination frequency which is simply, the number of recombinants/ total offspring (here the # of lines) x 100 = %. This value tells you how often the alleles got separated during crossing-over events in meiosis. A 1% recombination frequency ( $R_f$ ) is called a centiMorgan (cM). So marker 1 and marker 2 are  $(16/32) \times 100 = 50\% = 50\text{cM}$  apart on our map. Doing the same for the other two combinations, 1 and 3, and 2 and 3, you get the cM distance between each of the three pairs (62.5cM and 31cM respectively). From this you can guess their order on the map: 2 and 3 are about 31 cM apart and, 1 and 3 are 62.5 cM apart so the order seems to go as follows:



The extra distance is partly because of double crossovers especially prevalent in RI lines (why would this be?). Technically an  $R_f$  value of 50% or greater indicates the markers are essentially on two different linkage groups, but with enough markers you can find linkages between distinct markers which put them together by a sort of transitive property. Now it's time to collect your own map data.

### **Procedure:**

#### *DAY 1*

##### *A. DNA Extraction:* WEAR GLOVES!!!

1. Obtain the beetles to be extracted from your lab instructor and place in 1.5 mL microcentrifuge tube. Note in your lab notebook the labels for each beetle. These labels must be carried through the procedure.
2. Add 100  $\mu\text{L}$  of lysis buffer with proteinase K to each tube on top of the beetle.
3. Using the blue "homogenizers", thoroughly crush the beetles in the solution until no large parts are left.
4. Incubate in the 60°C water bath for 15 minutes.
5. Add 35  $\mu\text{L}$  high salt lysis buffer to your extraction and mix.
6. Incubate an additional 15 minutes.
7. Remove tubes from incubator and let cool.
8. IN THE HOOD: Add 135  $\mu\text{L}$  of the 1:1 phenol:chloroform mixture and cap tightly.
9. Invert the tubes 20 times.
10. Spin in microcentrifuge for 2 minutes.
11. While centrifuging, label the new 1.5 mL tubes appropriately.
12. Add 135  $\mu\text{L}$  of pure chloroform to this tube.

13. Take original tubes out of microcentrifuge and carefully pipette off the top layer (using the 200  $\mu\text{L}$  pipette set at 135  $\mu\text{L}$ ).
14. Transfer this **top** layer into your new chloroform-containing tube.
15. Cap the original tube, AND THROW IN BIOHAZARD BAG.
16. Invert your new tube 20 times
17. Centrifuge for 2 minutes.
18. While centrifuging, label the new 1.5 mL tubes appropriately and add 13.5  $\mu\text{L}$  of sodium acetate (NaAc) to these tubes.
19. Take original tubes out of microcentrifuge and carefully pipette off the top layer (using the 200  $\mu\text{L}$  pipette set at 135  $\mu\text{L}$ ).
20. Transfer this **top** layer to your new tube and invert. Cap the original tube, AND THROW IN BIOHAZARD BAG.
21. Add 200  $\mu\text{L}$  of ice-cold ethanol and invert.
22. Put tube in freezer for 15 minutes.
23. Centrifuge for 15 minutes.
24. Gently pour off the supernatant.
25. Leave tube, labeled with cap open, in hood for air-drying.

*B. Spectrophotometry (can be done the next day or the next week)*

1. Add 20  $\mu\text{L}$  TE buffer to air-dried sample.
2. Heat in 60°C water bath for 10 minutes to resuspend DNA.
3. In a new tube, make a 1:100 dilution of your sample with enough volume for detection in your UV spectrophotometer.
4. Follow your instructor's instructions for using the UV spectrophotometer.
5. Obtain readings for 260 nm, 280 nm, 260/280, 280/260, and concentration.
6. Calculate the concentration for your original sample and record.
7. Label eight new tubes for each sample that you extracted.
8. In each of the new tubes make a 1:100 dilution of your extracted DNA.
9. Keep one tube and distribute the other 7 copies to the other lab groups so that every group will eventually have a copy of all extracted beetle DNA. This must be done and in the freezer before the next week.

*DAY 2**C. RAPD-PCR Reactions*

1. In a 1.5 mL microcentrifuge tube, make a master PCR reaction mix enough for all the samples you are responsible for plus one additional reaction. Calculate the amounts needed of each component below:

Component	For 1 reaction	For _____ reactions
sterile water	6.98 ul	
PCR buffer	1.2 ul	
MgCl <sub>2</sub>	0.5 ul	
dNTP's	1.2 ul	
Your primer (Name _____)	1.0 ul	
Taq polymerase	0.12 ul	
<b>TOTAL</b>	11 ul	

2. Add the Taq last (keep in freezer until use) and vortex VERY well by using the vortexer and inverting the tube.
3. In your portion of a 96 well plate, add 11  $\mu$ L of the Master Mix to each well in which you will have a reaction.
4. Add 1  $\mu$ L of each DNA template (diluted 1:100) to the appropriate well, changing tips each time.
5. Layer 10  $\mu$ L of mineral oil on top of each reaction and seal the plate.
6. Label clearly and give to the instructor to run in the thermal cycler.

*DAY 3**D. Agarose gel and scoring the markers*

1. Add 2  $\mu$ L loading dye to each reaction.
2. Load 10  $\mu$ L of each reaction into a pre-prepared 2% agarose gel keeping the samples in numerical order. Load 10  $\mu$ L of 100bp extended range DNA ladder to appropriate wells.
3. Run the gel at 125 volts for 3 hours (your instructor will stain with Ethidium bromide)
4. Take a photograph of the gel for scoring purposes.
5. To find polymorphisms examine and compare the b-+ and b-Pak lanes for bands consistently present in one strain while consistently absent in the other.

6. Name these markers by the primer used and the approximate size of the band when present (i.e. a band at 600bp using primer A04 would be called "A04-600.")
7. Put these names on the data chart under "marker names."
8. Put the line names across the top of the following chart. To score the RI lines for genotype, for each marker note the presence or absence of a band at the appropriate size in each line.
9. b-Pak will be "A" and b-+ will be "B." If a polymorphic marker had the presence of a band in b-+, any line having that band should be given a "B" as a score. If a band was present in b-Pak, any line having that band will be given an "A" as the score for that marker.
10. Score each marker separately to avoid confusion since presence will not always be "A" or vice versa.
11. Combine the classes' data and enter into an Excel Spreadsheet.
12. Your instructor will show you how to use the mapping program to create a map from your data.

*Questions:*

1. On average, how many markers per primer were found? You will have to find out how many markers were found for each of the other groups' primers to answer this.
2. From the class data, did more markers have a presence in b-+ or b-Pak? Why might this be?
3. How big is your map and how many linkage groups does it have? Add up all the centiMorgan distances for each linkage group to get the total.
4. What is the average spacing of markers (in centiMorgans)? Divide the total map size by number of markers on the map.
5. Using the example in the introduction as a guide, hand-calculate the recombination fractions between any three markers that were discovered and explain if your findings match the computer results.
6. How many markers should be on a genetic linkage map before it would be considered useful and informative? Why do you think so?
7. What other types of markers are used on maps? How do they compare? Check the recent literature of genetic linkage maps.

### Literature Cited

- Manly, K. F. 1998. User's Manual for Map Manager Classic and Map Manager QT.
- Manly, K. F. and J. M. Olson 1999. Overview of QTL mapping software and introduction to map manager QT. *Mamm Genome* 10(4): 327-34.
- Williams, J. G. K., M. K. Hanafey, K. J. Livak, J. A. Rafalski and S. V. Tingey 1993. Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymology* 218: 704-740.
- Yezerksi, A., L. Stevens and J. Ametrano (2003). A genetic linkage map for *Tribolium confusum* based on Random Amplified Polymorphic DNA's (RAPD's) and Recombinant Inbred (RI) lines. *Insect Molecular Biology* 12(5): 517-526.

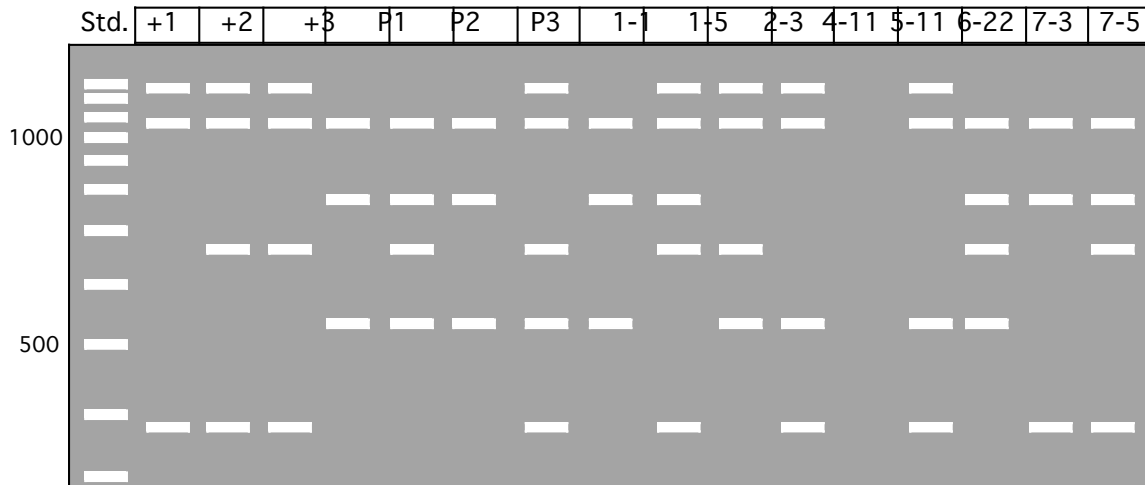
### About the Author

**Ann Yezerksi** received her B.A. in Biopsychology from Juniata College and M.S. as well as Ph.D. from the University of Vermont. Since 1999 she has been a member of the Biology Department at King's College teaching lectures and labs for Introductory Biology, Genetics, Physiology, and Evolutionary Analysis and Bioinformatics. Ann also conducts an active research program involving undergraduates on the genetics, physiology, ecology and behavior of the flour beetles of the *Tribolium* genus. Lately, Ann has expanded her research to include collaborations in the topics of microbiology, ecotoxicology, neuroscience and behavior with members of three other departments within King's College.

## Appendix A: Mapping Worksheet

Genetic mapping worksheet

Name \_\_\_\_\_



1. How many useful polymorphic markers can you identify? What criteria do you use?
2. Give an appropriate name to each of your new markers.
3. On a separate sheet of paper, score your markers in each of the RI lines. Calculate the recombination frequency between each pair of markers.
4. Looking specifically at the results for line # 6-22, what could explain what you see? Recalculate your recombination frequencies after eliminating the results of 6-22 on a separate sheet of paper.
5. Using the most appropriate data (either from #3 or #4) give a reasonable map of the markers you identified. Explain your results and your reasoning for using the data that you chose.
6. How many markers should be on a genetic linkage map before it would be considered useful and informative? Why do you think so?
7. What other types of markers, besides RAPD's are used on maps? How do they compare? Check the recent literature of genetic linkage maps.
8. What do you do with the information gained from creating a genetic linkage map of an organism? in other words, what is the point of mapping? What further research is conducted once a reasonable linkage map has been created?

## Appendix B: Example of freezer box chart

b-P1	b-P2	b-P3	b-+1	b-+2	b-+3	1-1	1-16	1-25	1-26
1-28	1-29	1-32	1-33	1-34	2-3	2-5	2-6	2-9	2-13
2-18	2-20	2-22	2-23	2-24	3-9	3-10	3-12	3-14	3-17
3-18	3-19	3-20	3-21	3-22	3-23	3-24	4-1	4-2	4-6
4-9	4-10	4-11	4-13	4-17	4-18	4-23	4-25		

### Appendix C: Marker list with Operon Primers

Primer	Primer sequence*	Known markers (size in bp)	Presence	Absence
AY10	CAAGGCC CT	680	B	A
		780	A	B
		1230	B	A
AY12	CTCGTCGG CGT	470	A	B
		490	A	B
G03	GAGCCCTC CA	315	A	B
		990	A	B
G04	AGCGTGTC TG	580	A	B
		600	B	A
		680	B	A
		1105	A	B
G14	GGATGAG ACC	390	B	A
		775	A	B
		1050	A	B
		1700	B	A
G17	ACGACCG ACA	300	A	B
G19	GTCAGGGC AA	600	A	B
		620	A	B
		660	B	A
		795	A	B
K04	CCGCCCAA AC	730	A	B
		800	B	A
		1050	A	B

\* Originally from Operon -- listed 5' to 3'

**A=came from b-Pak**

**B=came from b-+**

## Appendix D: Reagent formulations

### Lysis Buffer

For 500 mL:

3.0285 g Tris

5g CTAB

21.915 g NaCl

10 mL 0.5 M EDTA (pH8)

\*Add Tris first and pH with HCl before adding other ingredients

### High Salt Lysis Buffer

For 500 mL:

3.0285 g Tris

5g CTAB

43.83 g NaCl

10 mL 0.5 M EDTA (pH8)

\*Add Tris first and pH with HCl before adding other ingredients

### TE Buffer

For 500 mL:

0.1 mL 0.5M EDTA (pH8)

5 mL 1 M Tris pH 7.6

(60.55 g Tris in 500 mL dH<sub>2</sub>O)

Bring up to 500 mL with dH<sub>2</sub>O

### 10x TBE

For one liter:

108 g Tris

55 g Boric acid

20 mL 0.5 M EDTA (pH 8)

Bring pH to 8 with HCl

Dilute 1:10 for a working solution

### Phenol:Chloroform

10 mL Phenol

10 mL Chloroform

\*Shake well before use!

### Primers:

Supplier should provide enough information to use: <http://www.changbioscience.com/primo/oligo.html> in order to

Make a 50000 nM stock

Dilute 1:10 for a working solution

### Loading dye

For 10 mL:

Dissolve 3 g sucrose in 4 mL of dH<sub>2</sub>O and 2 mL 0.5M EDTA

Add:

50 mg Bromphenol Blue

50 mg Xylene cyanol

0.5 g SDS

Bring to 10 mL with dH<sub>2</sub>O

### Extended Base Pair Ladder

To 150 µL in tube add:

250 µL 6x dye

1110 µL dH<sub>2</sub>O

### dNTP's

For 1 mL:

Add 10 µL of each dNTP to 960 µL dH<sub>2</sub>O



### Appendix E: Materials Suppliers

<b>Item</b>	<b>Company</b>	<b>Catalogue #</b>
48 Frozen beetles	Ann Yezerksi	Ask by email
10 ml Falcon tubes	USA Scientific	1475-1611
50 mL Falcon tubes	USA Scientific	1500-1811
1.5 mL microcentrifuge tubes	USA Scientific	1615-5500
0.5 mL microcentrifuge tubes	USA Scientific	1605-0099
SDS	Sigma	L 4390
NaCl	Sigma	S 3014
Tris	Sigma	T6066
EDTA	Sigma	E7889
Proteinase K	Invitrogen	25530-015
Homogenizers	VWR	KT749521-1590
Phenol	Sigma	P4557
Chloroform	Sigma	C2432
Sodium acetate	Sigma	S5889
100% ethanol	Sigma	E7023
Boric Acid	Sigma	B 6768
Sucrose	Sigma	S 0389
Xylene cyanol	Sigma	X 4126
Bromphenol blue	Sigma	B5525
Ethidium bromide	Sigma	E 8751
Sterile water	Sigma	W3500
Taq w/buffer and Mg separate	Invitrogen	18038-042
dNTP's	Invitrogen	10297-018
Primers	See Appendix C for sequences	
96 Well Plates	USA Scientific	2796-3330
Plate Seals	USA Scientific	2920-0000
Mineral Oil	Sigma	M5904
Agarose	Invitrogen	15510-019
100 bp xtended range ladder	FMC Bioproducts	50322
8 Channel Multi- pipettor	Rainin	L8-20
Sub Cell 96 system	Bio-Rad	170-4500

### Appendix F: Recombinant Inbred Line Handout

