

MBLG2972 - GENE MAPPING WITH MOLECULAR MARKERS

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Introduction

A molecular marker is a DNA polymorphism within an individual's nucleotide sequence that may differ from other individuals within the same population. These markers do not cause an observable change in phenotype as a visible marker does, but they are much more common. This means they can be used to create a linkage map with much more resolution than a map using phenotypic markers alone (Griffiths et al, 2008).

A useful way of finding DNA polymorphisms is to cut the target DNA with a restriction enzyme. Each restriction enzyme has a specific recognition site, and site differences between individuals will lead to varying restriction fragment lengths after digestion. Sequencing of the DNA is not necessary. The molecular markers detected with this technique are known as Restriction Fragment Length Polymorphisms (RFLPs) (Griffiths et al, 2008).

This experiment focused on *Bactrocera tryoni*, known as the Queensland Fruit Fly. This tiny insect is a serious agricultural pest of fruit in Eastern Australia (MBLG Lab Manual, 2010). Additional knowledge about its genome is valuable because it could potentially lead to improved biological control of the species. Several papers have been published on the genome of *B. tryoni* by the Fruit Fly Research Centre at the University of Sydney (including Frommer & Bennett, 1997; Zhao et al, 1998; Zhao et al, 2003).

The aim of this experiment was to use an RFLP along with known microsatellites and a phenotypic marker (*white marks* or *wm* for short) to locate the *white* gene of *B. tryoni* on its chromosome map. Male *B. tryoni* are not thought to exhibit crossing over during meiosis, which means that genes on the same chromosome will be transmitted together and linkage analysis can be done with a smaller number of progeny (Zhao et al, 2003).

Materials & Methods

In brief, the following methods were used. A male backcross of *B. tryoni* was performed in order to assign the *white* gene to linkage groups. A fragment of the *white* gene of the 16 G2 progeny was amplified using the Polymerase Chain Reaction (PCR). The PCR product was digested using the restriction enzyme *RsaI*, and gel electrophoresis was performed with the products of digestion. Fragment size was calculated using a lane on the gel containing pUC19 standard. For exact details of methods, please refer to the Molecular Biology and Genetics B Practical Manual (2010). There was one change from the Practical Manual - gel electrophoresis of the undigested PCR product was also performed as a control.

Results

All the fly DNA, except for that of one progeny fly, was successfully visualised on both the cut and uncut gels. The missing sample (from G2-16) was not present on either gel, and was most likely not successfully amplified in the PCR. All fragments on the gel of restriction product were consistent with the parental types,

apart from two. The first was an extra band apparent in the P2 lane (lane 3). This band is the same size as the uncut product on the other gel, and appears to be remnants of undigested DNA. Nearly all the lanes also contained a faint band approximately 70bp in size. This is consistent with left over primer dimers from the PCR process. All of the PCR product run on the control gel was uncut, which is what we would expect.

Table 1 lists the estimated sizes of the fragment classes found at the *white* locus, which correspond to the different alleles of the *white* RFLP. The phenotypes for all individual flies are listed in Table 2.

Table 1: Fragment sizes corresponding to different *white* alleles after digestion with *RsaI*. Size was estimated using pUC19 standard and rounded to the nearest 10.

ALLELE	NO OF FRAGMENTS	FRAGMENT SIZE (bp)
R^a	3	400, 290, 190
R^b	2	>500, 190

Table 2: Phenotypes of the Parental, F1 and G2 1-16 flies for *w^m*, the microsatellite loci *Bt1*, 2, 5, 7, 11, 15 and 17, and the *white* RFLP. Flies marked with * indicates data taken from Lena's Thursday group results.

FLY	SEX	<i>w^m</i>	Bt1 Chr 2	Bt2 Chr 5	Bt5 Chr 3	Bt7 Chr 2	Bt11 Chr 6	Bt15 Chr 6	Bt17 Chr 4	<i>white</i> RFLP
P1	♂	WT	LL	LL	SS	LL	SS	LL	LL	$R^a R^a$
P2	♀	<i>w^m</i>	SS	SS	LL	SS	LL	SS	SS	$R^b R^b$
F1	♂	WT	SL	SL	SL	SL	SL	SL	SL	$R^a R^b$
G2-1	♀	<i>w^m</i>	SS	SL	SL	SS	LL	SS	SL	$R^a R^b$
G2-2	♀	<i>w^m</i>	SS	SL	LL	SS	SL	SL	SS	$R^a R^b$
G2-3	♀	<i>w^m</i>	SS	SS	SL	SS	SL	SL	SL	$R^b R^b$
G2-4	♀	<i>w^m</i>	SS	SS	LL	SS	SL	SL	SS	$R^b R^b$
G2-5	♂	<i>w^m</i>	SS	SL	SL	SS	SL	SL	SL	$R^a R^b$
G2-6	♂	<i>w^m</i>	SS	SL	SL	SS	LL	SS	SS	$R^a R^b$
G2-7	♂	<i>w^m</i>	SS	SS	SL	SS	LL	SS	SL	$R^b R^b$
G2-8	♂	<i>w^m</i>	SS	SL	SL	SS	LL	SS	SS	$R^a R^b$
G2-9	♀	WT	SL	SL	SL	SL	SL	SL	SS	$R^a R^b$
G2-10	♀	WT	SL	SS	LL	SL	LL	SS	SL	$R^b R^b$

FLY	SEX	wm	Bt1 Chr 2	Bt2 Chr 5	Bt5 Chr 3	Bt7 Chr 2	Bt11 Chr 6	Bt15 Chr 6	Bt17 Chr 4	<i>white</i> RFLP
G2-11	♀	WT	SL	SL	SL	SL	LL	SS	SS	$R^a R^b$
G2-12	♀	WT	SL	SS	SL	SL	SL	SL	SL	$R^b R^b$
G2-13	♂	WT	SL	SS	SL	SL	SL	SL	SS	$R^b R^b$
G2-14	♂	WT	SL	SL	SL	SL	LL	SS	SL	$R^a R^b$
G2-15	♂	WT	SL	SL	LL	SL	SL	SL	SS	$R^a R^b$
G2-16*	♂	WT	SL	SS	LL	SL	LL	SS	SL	$R^b R^b$

A linkage analysis was performed using the data in Table 1. The *white* RFLP phenotype was firstly compared to the wm phenotype (Table 3).

Table 3: Linkage analysis of *white* RFLP and wm phenotype

PHENOTYPES: <i>white</i> wm	G2 FLY NO.	NUMBER OBSERVED
$R^a R^b$ WT (parental F1 ♂)	G2-9, 11, 14, 15	4
$R^a R^b$ wm	G2-1, 2, 5, 6, 8	5
$R^b R^b$ WT	G2-10, 12, 13, 16	4
$R^b R^b$ wm (parental)	G2-3, 4, 7	3

There are phenotypes within the G2 progeny that are not the parental ones. Thus the *white* RFLP and the wm phenotype assort independently and are not linked. Both the *white* RFLP phenotype and the wm phenotype were then compared to the Bt1 microsatellite (Table 4).

Table 4: Linkage analysis of *white* RFLP, wm phenotype and Bt1 microsatellite (chromosome 2)

PHENOTYPES: <i>white</i> wm Bt1	G2 FLY NO.	NUMBER OBSERVED
$R^a R^b$ WT SL (parental F1 ♂)	G2-9, 11, 14, 15	4
$R^a R^b$ WT SS	-	0
$R^a R^b$ wm SL	-	0
$R^a R^b$ wm SS	G2-1, 2, 5, 6, 8	5
$R^b R^b$ WT SL	G2-10, 12, 13, 16	4
$R^b R^b$ WT SS	-	0

PHENOTYPES: <i>white</i> <i>wm</i> Bt1	G2 FLY NO.	NUMBER OBSERVED
$R^b R^b$ <i>wm</i> SL	-	0
$R^b R^b$ <i>wm</i> SS (parental P2 ♀)	G2-3, 4, 7	3

The *white* RFLP and the Bt1 microsatellite assort independently so they are not linked. However the *wm* phenotype and Bt1 do not assort independently, and the only phenotypes observed correspond to the parental phenotypes. *wm* and Bt1 are therefore linked and on chromosome 2, and the *white* RFLP is not on chromosome 2.

Since the location of the *wm* allele was now known, linkage analysis with subsequent microsatellites on other chromosomes was continued comparing only the *white* RFLP (Table 5).

Table 5: Linkage analysis of *white* RFLP and Bt2 microsatellite (chromosome 5)

PHENOTYPES: <i>white</i> Bt2	G2 FLY NO.	NUMBER OBSERVED
$R^a R^b$ SL (parental F1 ♂)	G2-1, 2, 5, 6, 8, 9, 11, 14, 15	9
$R^a R^b$ SS	-	0
$R^b R^b$ SL	-	0
$R^b R^b$ SS (parental P2 ♀)	G2-3, 4, 7, 10, 12, 13, 16	7

The only phenotypes within the G2 progeny are the parental ones. Thus the *white* RFLP and the Bt2 microsatellite are linked, and the *white* RFLP is on chromosome 5. Since the location was now known, no further linkage analyses were performed.

Discussion

The *white* gene of *B. tryoni* was successfully located on chromosome 5 by linkage analysis with the Bt2 microsatellite. This is in line with previous cytogenetic work that mapped the polytene chromosomes of *B. tryoni* (Zhao *et al*, 1998). A RFLP at RsaI sites in the *white* gene has been described and is found within intron 5 of the gene (Frommer & Bennett, 1997). The marker has been named *Rwhite* and is the same marker we have examined in this experiment.

The *wm* gene is on chromosome 2, as has also been found in previous studies of morphological markers (Zhao *et al*, 2003). There is only data for one other marker (Bt1) on chromosome 2 so no linkage maps can be derived with the results of this experiment. Similarly, Bt2 and the *white* RFLP are the only markers that we have results for on chromosome 5.

To map the *white* RFLP and *wm* gene more precisely, a different set of molecular markers should be selected that are specifically on chromosomes 2 and 5. Zhao *et al* (2003) have characterised microsatellites Bt4, Bt14

and Bt32 on chromosome 2, among others. By looking at these markers and those for chromosome 5, a linkage map could be constructed.

A larger number of progeny would also be preferable. However because crossing over is not thought to occur in *B. tryoni*, a smaller sample size can be used to establish linkage (Zhao *et al*, 2003).

References

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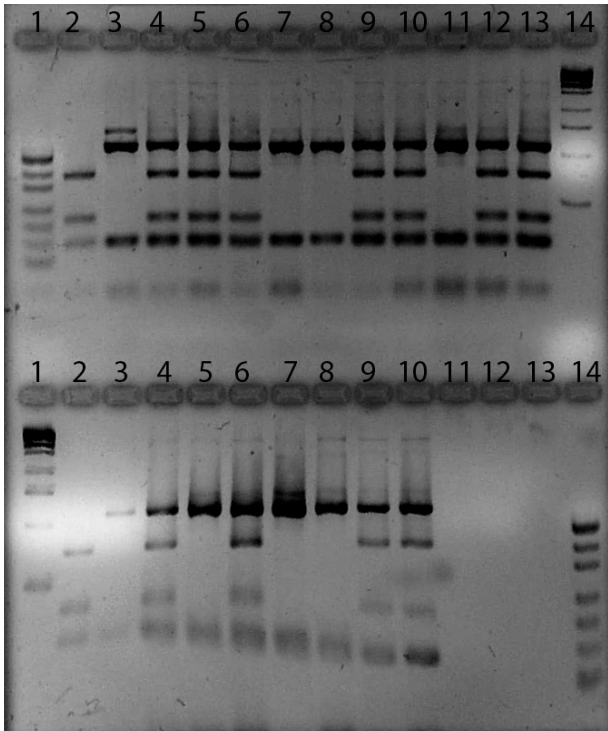
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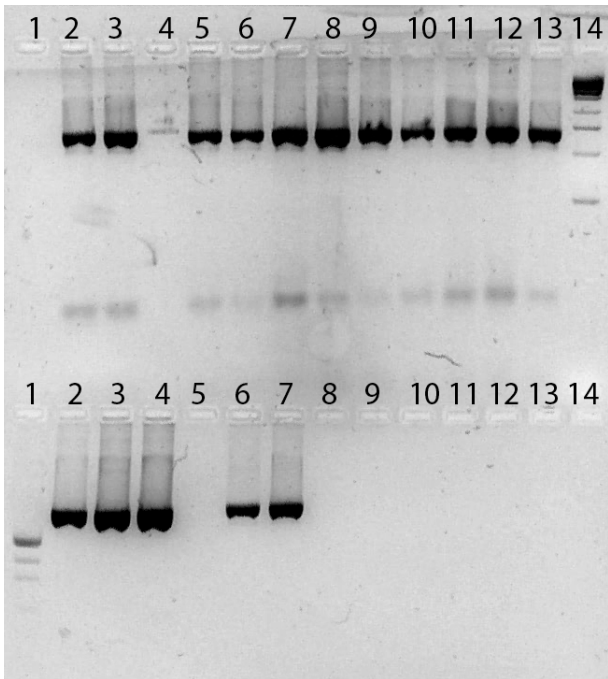
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Appendix



DNA Electrophoresis Gel 1: PCR product cut with *RsaI*

ROW 1		ROW 2	
LANE	SAMPLE	LANE	SAMPLE
1	pUC19	1	1 kb
2	P1 ♂	2	P1 ♂
3	P2 ♀	3	P2 ♀
4	F1	4	F1
5	G2-1	5	G2-10
6	G2-2	6	G2-11
7	G2-3	7	G2-12
8	G2-4	8	G2-13
9	G2-5	9	G2-14
10	G2-6	10	G2-15
11	G2-7	11	G2-16
12	G2-8	12	No DNA
13	G2-9	13	
14	1 kb	14	pUC19



DNA Electrophoresis Gel 1: Uncut PCR product

ROW 1		ROW 2	
LANE	SAMPLE	LANE	SAMPLE
1		1	1 kb
2	P1 ♂	2	G2-10
3	P2 ♀	3	G2-11
4	F1	4	G2-12
5	G2-1	5	G2-13
6	G2-2	6	G2-14
7	G2-3	7	G2-15
8	G2-4	8	G2-16
9	G2-5	9	No DNA
10	G2-6	10	
11	G2-7	11	
12	G2-8	12	
13	G2-9	13	
14	1 kb	14	