File S1

Ribosomal DNA organization before and after magnification in *Drosophila melanogaster*

Additional explanatory detail

A summary of prior work on the mechanism of magnification: It has been proposed that the magnification-triggering event is production of double-strand breaks within the rDNA (Marcus *et al.* 1986; Paredes and Maggert 2009). This hypothesis is based on two observations. First, magnification is absent in flies that are mutated for genes involved in repair of double-strand breaks (Marcus *et al.* 1986). Second, reversion of the bobbed phenotype has been observed in some bobbed alleles in response to the expression of *I-Cre*I (Paredes and Maggert 2009): a trans-gene that codes for an endonuclease that produces double-strand breaks within the rDNA (Maggert and Golic 2005).

Although pre-meiotic *Ybb*$^-$-induced magnification events are reported (Hawley and Tartof 1985; Endow and Komma 1986), our experiments have never given clustered magnification events. We suspect that reported clusters of weak, and seemingly unstable, changes may be an artefact of variable expression and of the phenotypic overlap between bb and bb$^+$ individuals (Boschi 2006 ; Boschi *et al*. in preparation). Whether clusters of magnified offspring of *Ybb*$^-$ males are pre-meiotic heritable events or a hangover of somatic compensation, they do seem to result from a compensatory process induced by rDNA deficiency (Endow and Atwood 1988), since only bobbed males produce these clusters. Meiotic, non-clustered, events, in contrast, are not rDNA dose-dependent. The rest of this summary is focused on non-clustered events.

The *Ybb*$^-$ chromosome carries very few rDNA copies (Tartof 1973; Endow 1982b), but its induction of magnification seems unrelated to that lack of rDNA; the ability to induce magnification is retained even if another full rDNA array is transposed to the *Ybb*$^-$ chromosome (Endow *et al.* 1984; Hawley and Tartof 1985). Magnification of *Xbb* chromosomes in *Xbb/Ybb*$^-$ males has been assessed using two different genetic approaches, giving different results. In both schemes, the phenotype is examined in flies in which the bobbed locus being studied is the only source of rDNA, so that an increase of rDNA copy number can be detected as a bb to bb$^+$ phenotypic reversion.

Ritossa in 1968 designed a test, the '*Ybb*$^-$ assay', to assess X-chromosome rDNA magnification. In this test, *Xbb*/*Ybb*$^-$ males are crossed to *attached-X*/*Ybb*$^-$ females for several generations, and the frequency of Xbb to Xbb$^+$ revertants among *X/Ybb*$^-$ sons is scored at each generation. In early generations, almost all sons present only a slight amelioration of bobbed phenotype, but in subsequent generations the phenotype continues to improve and stabilizes as a bb$^+$ phenotype. The few fully bb$^+$ sons seen in the first generation seemed to have an unstably-inherited bb$^+$ phenotype (Ritossa 1968).

Tartof in 1971 designed another test, the 'sc^4sc^8 assay', to assess X-chromosome rDNA magnification. In this test, *Xbb*^{2}/*Ybb*$^-$ males are crossed with females carrying an rDNA-free, *In(1)*$sc^{4L}sc^{8R}$ X chromosome. At the first generation, less than 20% of all daughters revert to bb$^+$ and this phenotype remains stable in subsequent generations as shown through test-crosses

with *Xbb*$^{-}$/*Y* males (Tartof 1971; 1974a, 1974b). Moreover, in addition to the magnification events, reduction events occur at a frequency of 3% (Tartof 1974a, 1974b).

Discrepancies between Ritossa's and Tartof's results were clarified by applying both assays to magnification of the same *bb*^{2} allele (Marcus *et al.* 1986). At the first generation only 10% true and persistent magnified revertants are produced. The other non- persistent bobbed-phenotype ameliorations found at the first generation with Ritossa's assay (about 37%) are apparently not magnification events; but the synergistic and epistatic effects of autosomal modifiers segregating in the genetic background.

Two general classes of model have been proposed to explain rDNA magnification: clonal over-replication (either *in situ* or extra-chromosomal) and unequal recombination between sister chromatids. According to the model of clonal over-replication *in situ* (Terracol 1987), specific rDNA units amplify intra-chromosomally up to 3.5-fold, lengthening the rDNA array. According to the model of extra-chromosomal over-replication (Ritossa *et al.* 1971; Ritossa 1972; Ritossa 1976), the bobbed condition determines the production of extra-chromosomal rings consisting of various rDNA units. Subsequently, rings amplify and, in the germ line, reintegrate into the original chromosome. This model could explain the apparent instability of bb^{+} reversion in early generations and the gradual improvement of bobbed phenotype in subsequent generations that Ritossa observed. Although episomal rDNAs have been observed (Graziani *et al*. 1977), neither replicative model is compatible with the observed inability of ring chromosomes to undergo magnification (Coen *et al.*1982; Indik and Tartof 1980; Tartof and David 1976; Yagura *et al.* 1979). Sister-strand exchange transforms ring chromosomes into non-heritable, dicentric chromosomes (McClintock 1938); what would have been *bb*^{M} products in rod chromosomes can not be recovered. In contrast, simply integrating a stretch of DNA into a ring should not damage it so magnification by extrachromosomal replication should not be suppressed.

Unequal mitotic exchange between rDNA arrays of two sister chromatids could produce a magnified chromatid having increased rDNA content, and its reciprocal having fewer copies (Tartof 1974a, 1974b). This model is compatible with almost all of the observations concerning the bobbed locus, except for the apparent instability of magnified products obtained in the original Ritossa-style screen and Terracol's observation of a 3.5-fold increase in band intensity. It explains the nearly equal frequencies of magnification and reduction events and is consistent with the stability of the magnified chromosomes recovered in the *sc*^{4}*sc*^{8} magnification scheme. It is also consistent with the observed inability of ring chromosomes to magnify (Tartof 1974b; Endow *et al.* 1984; Komma and Endow 1986). At meiotic anaphase II, *ring-X*, *bb*/*Y*, *bb*$^{-}$ males present many aberrant circular structures: especially dicentric ring-chromosomes, but also individual and interconnected broken chromosomes (Endow *et al*. 1984). Moreover, both sex chromosomes are transmitted at the same frequency in *ring-X*/*Y* males , while *ring-X*/*Ybb*$^{-}$ males transmit more Y than ring-X chromosomes. However, the observed refractoriness of ring-X chromosomes to meiotic magnification is consistent with, but is not incontrovertible proof, that magnification is caused by unequal sister chromatid recombination; the absence of magnification could also depend either on structural peculiarities of the particular ring-X chromosome (Tartof 1974b),

or on intrinsic features of this specific bb allele. The critical test, opening and re-closing the *bb*-bearing ring to demonstrate that it is circularity *per se* that prevents magnification, has never been done.

RESULTS

Coherence of the proximal limits of variant distributions among the starting and magnified chromosomes and a consensus map of the proximal limits: Reintegration of extra-chromosomally replicated rDNA repeats would yield different marker orders in the starting bb^2 and magnified arrays. A search for changes of maker order was done in two steps.

First, the pairwise order of the proximal limits was established for each individual map. For example, consider the S3887 marker paired with each of the seven other IGS markers (S3266; S3542; S3079; S3518; S3030; S2876; S3887) for the bb^2 data (Figure 8). The proximal limit of S3887 is proximal to these of S3266, S3542 and S3079 and indistinguishable from the proximal limits of the other four markers. In Table S5 the order of each pair of markers was annotated as P for the marker whose most proximal limit is nearer the centromere, D for the marker with the more distal proximal limit, or (—) when the two limits were indistinguishable. The R2059, S3887, S3518, S3030, S2876 and S2785 markers, that have the centromere as the proximal limit in all of the alleles, were grouped together as C.

The pairwise orders of the bb^2 map (the first allele listed in Table S5) was then compared with pairwise orders in the maps of the magnified arrays (remaining columns), looking for possible PD reversals. For example, the proximal limit of R2916 in every map where the order can be established is distal to those of all but one (R1767) of the other markers; there have been no reversals of order between R2916 and any other marker. Indeed, there are no reversals of order at all in the entire data set, although there were 152 opportunities for detecting one (= total number of DP annotations minus number of marker pairs).

The absence of any reversal of proximal limit order argues against both models of extra-chromosomal amplification and allows us to establish a single order of exchanges for all of the arrays as shown in Figure 9. m24 was produced by the most proximal exchange in the bb^2 set and contains fewer markers (R2059, S3887, S3518, S3030, S2876 and S2785) than the most proximal exchange in all of the other sets of minichromosomes. The proximal limits of these six markers are therefore at the centromere. The next exchange is m28, also from the bb^2 set; it picks up S3079. The proximal limit of S3079 is therefore m24. The next more distal exchange is m1, from the bb^{M3} set; it picks up S3542. The proximal limit of S3542 is therefore m28. The next four exchanges (m27 from the bb^2 set, m7 from the bb^{M3} set, and m103 and m107 from the bb^{M18} set) are indistinguishable because they all pick up R2166, and the proximal limit of R2166 is therefore at m1. We continue in the same manner, ordering all of the remaining exchanges. The ordering of m53 from the bb^{M1} set is, however, uncertain because R0750 and R1355 were not classifiable in the bb^{M1} and bb^{M3} gels. Similarly, the orderings of m17 from the bb^{M4} set, and of m108 and m112 from the bb^{M18} set, are uncertain because R1767 was not classifiable in either the bb^{M4} or bb^{M18} gels. Note that the three markers that

cause uncertainty becuase they were not classifiable in some gels are the least abundant, and hardest to score, marker variants.

Quantitative internal controls: For the IGS gels, the fortuitous presence in one of the stocks of a unique IGS variant that segregated independently of the rDNA provided a particularly reassuring internal control. Ectopic copies of the R1 and R2 retrotransposons were not present in our stocks, however. Although it might be possible to engineer a distinct ectopic sequence with homology to the 28S-sequence R1/2 primers, we have not done so, and we therefore have no similar control for the R1/2 gels.

Nevertheless, although requiring a rather tedious explanation, both sets of gels actually provide another internal control that is nearly as convincing.

First, consider a variant, whether IGS or retrotransposon, that is present in but one copy in only one of the target chromosome's arrays. Depending on where an exchange occurs, a minichromosome can then carry either one copy or no copies of this variant. If intensity of this variant is uniform across a set of crossovers, the gel loading and PCR reactions were also uniform across this set.

Now consider a variant present in a single copy in both of the target chromosome arrays. Depending on where they are located, and where the exchanges occur, minichromosome may contain zero, one or two copies. Once again, however, if a band shows uniform intensity across a set of crossover chromosomes, either the loading and reaction conditions were uniform, or their variance was exactly compensated by fortuitous (and unlikely) positioning of the exchange sites.

Lastly, consider a variant present in multiple copies, whether it be a marker suitable for mapping because it is present in only one array, or a non-marker present in both arrays. Now, recombination will cause an even wider variation in band intensity, but, once again, if we find a band that is uniform across a set of crossovers, we either have to conclude that the PCR reactions are comparable, or that the crossovers-created variation exactly conterbalanced the experimental variation.

Inspection of the gels shows that there are many bands, both IGS and R1/2 and marker and non-marker, that have visually uniform intensity across a set of crossovers. While a single such band might be explainable by contrary effects of experimental and crossover variation, it is exceedingly unlikely that multiple bands with uniform intensity across an entire set of minichromosomes could be produced by anything except reasonably uniform gel loading and reaction conditions.

We think it important to note that this control, and that provided by the ectopic IGS variant, are controls for uniformity within each set of crossovers on a single gel. They do not provide a control for comparability of different gels and we have scrupulously avoided any consideration of cross-gel intensity differences in the mapping.

Mapping the *Tp(1;1)sc^{V2}* rDNA array: To verify the mapping methodology, and to gather further information about the *Rex*-induced exchange process, the *Tp(1;1)sc^{V2}* rDNA array, the constant sub-telomeric array used as crossover partner for mapping the *bb^{2}* and magnified arrays, was also mapped. We identified eight *Tp(1;1)sc^{V2}*-specific variants (R2754, R2322, R1903, R1832,

R1179, R0883, R0666 and S2021; Figure 7) and they were classified in all forty-five minichromosomes (Figure 7, Figures S1-4 and Table S10), except for the R1179 band that was not analyzable in the bb^{M18} set (see Figure S4 panel A).

The ordering of the exchange points in the $Tp(1;1)sc^{V2}$ array is based on the number of different markers carried by each minichromosome: those minichromosomes produced by more distal exchanges carry fewer $Tp(1;1)sc^{V2}$-specific markers than those produced by more proximal exchanges. Three incongruities were found in this ordering. For the bb^{2} gels (Figure 7 and Table S10), m28, m25 and m33 each carry seven $Tp(1;1)sc^{V2}$ markers. Six markers (R2754, R2322, R1903, R1832, R0666 and S2021) are present in all of these minichromosomes, but m28 also carries R0883, while m25 and m33 carry the R1179 variant. It seems likely that the R1179 variant was deleted during the *Rex*-mediated spiral recombination that produced the m28 minichromosome. Similarly, in the bb^{M3} gels (Figure S2 and Table S10) m4 and m8 each carry five $Tp(1;1)sc^{V2}$ markers. Four markers are common to both minichromosomes, but m8 lacks R1832, which is carried by m4 and, *vice versa*, m4 lacks R1179, which is present in m8. The absence of R1179 in m4 will place it distal to m8 when the individual maps are combined (see below), but the absence of R1832 in m8 again appears to be a deficiency. Considering the bb^{2} gels (Figure 7 and Table S10), m30 is distal to m29 because their relative minichromosomes carry four and six different $Tp(1;1)sc^{V2}$ specific markers respectively. However, the R0666 marker, that is present in m30, is not in the m29 minichromosome. This incongruity, looking at only the bb^{2} gels, can be explained either as a deficiency of R0666 in m29, or as the appearance of a new variant in m30 that just happens to be 666 bp long. This ambiguity, however, is resolved when the data from all of the gels are combined.

The bb^{M3} data alone did not order m4 and m8, but the combined ordering shown in Figure 13, constructed as previously described for the basal array, places m4 distal to m8 because it lacks R1179 as do all further-distal crossovers. R1832, however, is present in numerous more-distal crossovers. Hence its absence in m8 is classed as a deficiency. Nine R0666-free minichromosomes (m4, m103, m8, m10, m46, m14, m20, m22, and m29) are produced by exchanges that are proximal to m30. To us, the appearance of a new 666 bp long variant in m30 seems more likely than the simultaneous loss of the R0666 marker in nine independent exchanges. The comparison of all of the qualitative data of all of the gels also allows us to identify the *Rex*-induced deficiency of R1832 in m30 and m31. Moreover, the R1179 variant, that was not analyzable in the bb^{M18} gel, is likely to be carried by m112, m107, m111. and m100, but be missing in m106, m110, m108, m104 and m102. The ordering of m103, however, remains uncertain because of the lack of information for R1179.

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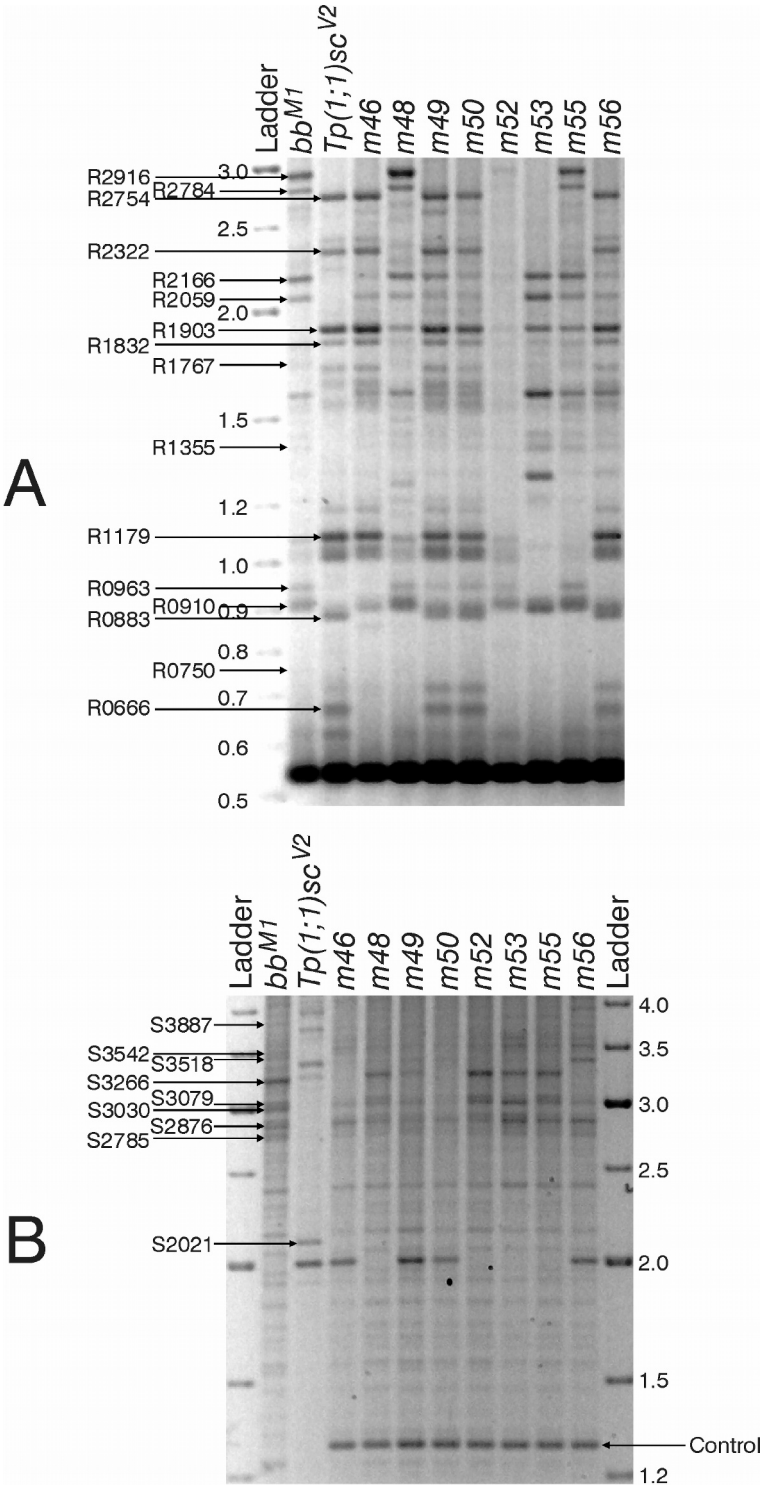

FIGURE S1 The *bb*^{M1} set of minichromosome crossovers: The eight minichromosomes derived from *bb*^{M1} were analyzed in the same gels along with *bb*^{M1} and *Tp*(*1;1*)*sc*^{V2}. (A) PCR amplification using the R2/1 primer pair. (B) PCR amplification using the IGS primer pair. Markers specific to one or the other parental chromosome are indicated. All of the minichromosome samples, but neither *bb*^{M1} nor *Tp*(*1;1*)*sc*^{V2}, contain the 1.25 kb long IGS variant that was used as a quantitative internal control (see text and Figure 10).

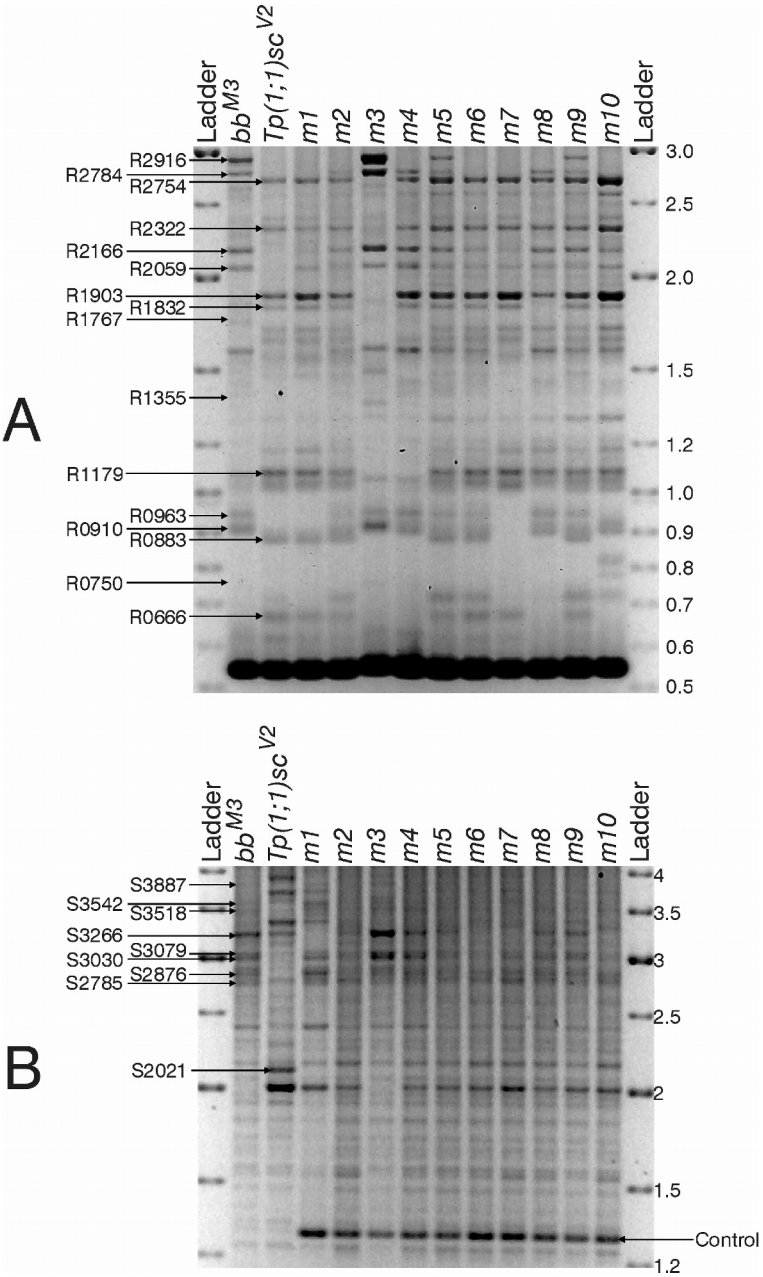

FIGURE S2 The *bb*^{M3} set of minichromosome crossovers: The ten minichromosomes derived from *bb*^{M3} were analyzed in the same gels along with *bb*^{M3} and *Tp*(*1;1*)*sc*^{V2}. (A) PCR amplification using the R2/1 primer pair. (B) PCR amplification using the IGS primer pair. Markers specific to one or the other parental chromosome are indicated. All of the minichromosome samples, but neither *bb*^{M3} nor *Tp*(*1;1*)*sc*^{V2}, contain the 1.25 kb long IGS variant that was used as a quantitative internal control (see text and Figure 10).

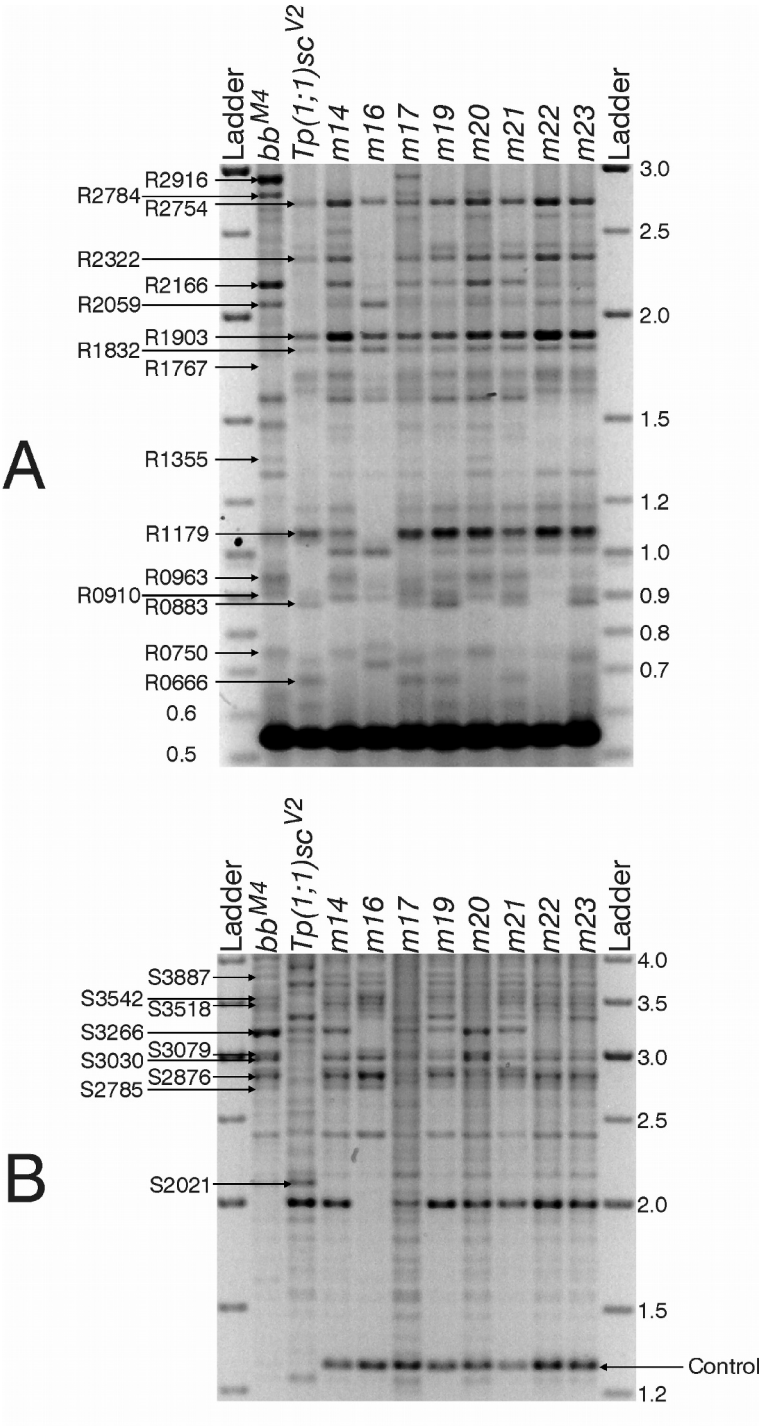

FIGURE S3 The *bb*^{M4} set of minichromosome crossovers: The eight minichromosomes derived from *bb*^{M4} were analyzed in the same gels along with *bb*^{M4} and *Tp*(*1;1*)*sc*^{V2}. (A) PCR amplification using the R2/1 primer pair. (B) PCR amplification using the IGS primer pair. Markers specific to one or the other parental chromosome are indicated. All of the minichromosome samples, but neither *bb*^{M4} nor *Tp*(*1;1*)*sc*^{V2}, contain the 1.25 kb long IGS variant that was used as a quantitative internal control (see text and Figure 10).

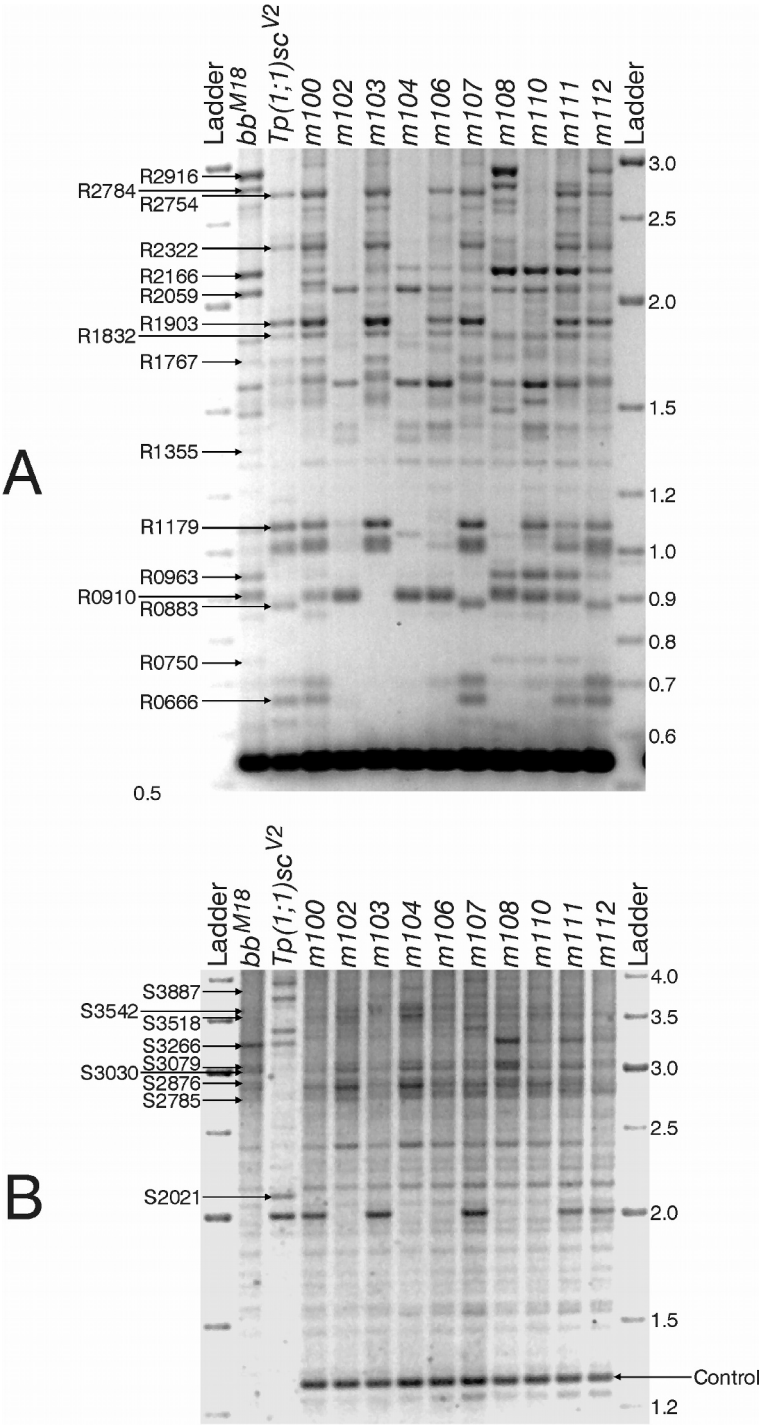

FIGURE S4 The *bb*^{M18} set of minichromosome crossovers: The ten minichromosomes derived from *bb*^{M18} were analyzed in the same gels along with *bb*^{M18} and *Tp*(*1;1*)*sc*^{V2}. (A) PCR amplification using the R2/1 primer pair. (B) PCR amplification using the IGS primer pair. Markers specific to one or the other parental chromosome are indicated. All of the minichromosome samples, but neither *bb*^{M18} nor *Tp*(*1;1*)*sc*^{V2}, contain the 1.25 kb long IGS variant that was used as a quantitative internal control (see text and Figure 10).

TABLE S1 Qualitative crossover data for the *bb*^{M1} set of crossover minichromosomes

	crossover minichromosome:							
marker	m48	m52	m55	m49	m53	m50	m56	m46
R2916	+	+	+					
R2784	+	+	+	+				
R2166	+	+	+	+	+	+	+	+
R2059	+	+	+	+	+	+	+	+
R1767	+	+	+					
R1355	nc	nc	nc	nc	nc	nc	nc	nc
R0963	+	+	+	+	Def	+		
R0910	+	+	+	+	+	+	+	+
R0750	nc	nc	nc	nc	nc	nc	nc	nc
S3887	+	+	+	+	+	+	+	+
S3542	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+
S3266	+	+	+	+	+			
S3079	+	+	+	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+

The presence of nine R2/1 and eight IGS markers was scored for the *Rex*-magnified *bb*^{M1} crossover minichromosome set (Figure S1). The minichromosomes are listed from left to right based on the number of markers carried; minichromosomes produced by more distal exchanges carry more markers than those produced by more proximal exchanges. Two markers were not scorable in this gel and there was one crossover in which an expected marker was absent (see text).

+ = marker present

nc = marker not classifiable

Def = absence of an expected marker in a more-distal exchange

TABLE S2 Qualitative crossover data for the *bb*^{M3} set of crossover minichromosomes

	crossover minichromosome:									
marker	m3	m5	m9	m4	m8	m2	m6	m10	m7	m1
R2916	+	+	+							
R2784	+	+	+	+	+	+				
R2166	+	+	+	+	+	+	+	+	+	
R2059	+	+	+	+	+	+	+	+	+	+
R1767	+									
R1355	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
R0963	+	+	+	+	+	+	+	+		
R0910	+	+	+	+	+	+	+	+		
R0750	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
S3887	+	+	+	+	+	+	+	+	+	+
S3542	+	+	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+	+	+
S3266	+	+	+	+	+	+				
S3079	+	+	+	+	+	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+	+	+

The presence of nine R2/1 and eight IGS markers was scored for the *Rex*-magnified *bb*^{M3} crossover set (Figure S2). The minichromosomes are listed from left to right based on the number of markers carried; minichromosomes produced by more distal exchanges carry more markers than those produced by more proximal exchanges. The gel image did not permit scoring two markers.

+ = marker present

nc = marker not classifiable

TABLE S3 Qualitative crossover data for the *bb*^{M4} set of crossover minichromosomes

	crossover minichromosome:							
marker	m17	m20	m14	m19	m21	m22	m23	m16
R2916	+							
R2784	+	+						
R2166	+	+	+	+	+	+	+	+
R2059	+	+	+	+	+	+	+	+
R1767	nc	nc	nc	nc	nc	nc	nc	nc
R1355	+	+	+					
R0963	+	+	+	+	+	+	+	
R0910	+	+	+	+	+	+	+	+
R0750	+	+	+	+	+			
S3887	+	+	+	+	+	+	+	+
S3542	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+
S3266	+	+	+	+	+			
S3079	+	+	+	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+

The presence of nine R2/1 and eight IGS markers was scored for the *Rex*-magnified *bb*^{M4} crossover minichromosome set (Figure S3). The minichromosomes are listed from left to right based on the number of markers carried; minichromosomes produced by more distal exchanges carry more markers than those produced by more proximal exchanges. This gel image did not permit scoring one marker.

+ = marker present

nc = marker not classifiable

TABLE S4 Qualitative crossover data for the *bb*^{M18} set of crossover minichromosomes

	crossover minichromosome:									
marker	m108	m112	m111	m110	m100	m104	m106	m102	m103	m107
R2916	+	+								
R2784	+	+	+							
R2166	+	+	+	+	+	+	+	+	+	+
R2059	+	+	+	+	+	+	+	+	+	+
R1767	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
R1355	+	+	+							
R0963	+	+	+	+	+	+	+			
R0910	+	Def	+	+	+	+	+	+		
R0750	+	+	+	+						
S3887	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
S3542	+	+	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+	+	+
S3266	+	+	+	+						
S3079	+	+	+	+	+	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+	+	+

The presence of nine R2/1 and eight IGS markers was scored for the *Ybb*$^{-}$-magnified *bb*^{M18} crossover minichromosome set (Figure S4). The minichromosomes are listed from left to right based on the number of markers carried; minichromosomes produced by more distal exchanges carry more markers than those produced by more proximal exchanges. Two markers were unscorable in this gel, and there was one instance of an expected marker being absent (see text).

+ = marker present

nc = marker not classifiable

Def = absence of an expected marker in a more-distal exchange

TABLE S5 Coherence of the order of proximal limits

Distal	Proximal	*bb*^{2}	*bb*^{M1}	*bb*^{M3}	*bb*^{M4}	*bb*^{M18}
R1767	R2916	—	—	DP	—	—
	R2784	—	DP	DP	—	—
	R1355	—	—	—	—	—
	R0750	—	—	—	—	—
	S3266	—	DP	DP	—	—
	R0963	DP	DP	DP	—	—
	R0910	DP	DP	DP	—	—
	R2166	DP	DP	DP	—	—
	S3542	DP	DP	DP	—	—
	S3079	DP	DP	DP	—	—
	C	DP	DP	DP	—	—
R2916	R2784	—	DP	DP	DP	DP
	R1355	—	—	—	DP	DP
	R0750	—	—	—	DP	DP
	S3266	—	DP	DP	DP	DP
	R0963	DP	DP	DP	DP	DP
	R0910	DP	DP	DP	DP	DP
	R2166	DP	DP	DP	DP	—
	S3542	DP	DP	DP	DP	DP
	S3079	DP	DP	DP	DP	DP
	C	DP	DP	DP	DP	DP
R2784	R1355	—	—	—	DP	—
	R0750	—	—	—	DP	DP
	S3266	—	DP	—	DP	DP

	R0963	DP	DP	DP	DP	DP
	R0910	DP	DP	DP	DP	DP
	R2166	DP	DP	DP	DP	DP
	S3542	DP	DP	DP	DP	DP
	S3079	DP	DP	DP	DP	DP
	C	DP	DP	DP	DP	DP
R1355	R0750	—	—	—	DP	DP
	S3266	—	—	—	DP	DP
	R0963	DP	—	—	DP	DP
	R0910	DP	—	—	DP	DP
	R2166	DP	—	—	DP	DP
	S3542	DP	—	—	DP	DP
	S3079	DP	—	—	DP	DP
	C	DP	—	—	DP	DP
R0750	S3266	—	—	—	—	—
	R0963	DP	—	—	DP	DP
	R0910	DP	—	—	DP	DP
	R2166	DP	—	—	DP	DP
	S3542	DP	—	—	DP	DP
	S3079	DP	—	—	DP	DP
	C	DP	—	—	DP	DP
S3266	R0963	DP	DP	DP	DP	DP
	R0910	DP	DP	DP	DP	DP
	R2166	DP	DP	DP	DP	DP
	S3542	DP	DP	DP	DP	DP
	S3079	DP	DP	DP	DP	DP

	C	DP	DP	DP	DP	DP
R0963	R0910	DP	DP	—	DP	DP
	R2166	DP	DP	DP	DP	DP
	S3542	DP	DP	DP	DP	DP
	S3079	DP	DP	DP	DP	DP
	C	DP	DP	DP	DP	DP
R0910	R2166	DP	—	DP	—	DP
	S3542	DP	—	DP	—	DP
	S3079	DP	—	DP	—	DP
	C	DP	—	DP	—	DP
R2166	S3542	—	—	DP	—	—
	S3079	DP	—	DP	—	—
	C	DP	—	DP	—	—
S3542	S3079	DP	—	—	—	—
	C	DP	—	—	—	—
S3079	C	DP	—	—	—	—

The pairwise orders of the proximal limits of all of the markers, listed in the first and the second columns, are compared for the maps of bb^2 (third column) and the magnified alleles (fourth through seventh columns).

D = distal, P = proximal

— = order can not be determined

C = R2059, S3887, S3518, S3030, S2876 and S2785; grouped together because they all have the centromere as their proximal limit in all of the arrays.

Table S6 Semi-quantitative crossover data for the *bb*^{M1} set of crossover minichromosomes

	crossover minichromosome:							
marker	m48	m52	m55	m49	m53	m50	m56	m46
R2916	+	+	+					
R2784	++	++	++	+				
R2166	+++	+++	+++	++	++	+	+	+
R2059	+	+	+	+	+	+	+	+
R1767	+	+	+					
R1355	nc	nc	nc	nc	nc	nc	nc	nc
R0963	++	++	++	+	Def	+		
R0910	++	++	++	++	++	++	++	+
R0750	nc	nc	nc	nc	nc	nc	nc	nc
S3887	+	+	+	+	+	+	+	+
S3542	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+
S3266	+	+	+	+	+			
S3079	++	++	++	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+

The band intensities of the nine R2/1 and eight IGS markers were estimated for the *Rex*-magnified *bb*^{M1} crossover minichromosome set (Figure S1). Columns are arranged from left to right, based on decreasing number or intensities of the markers. Two markers were not scorable in this gel and there was one crossover in which an expected marker was absent (see text).

+ = marker present

++ = marker distinctly more abundant than +

+++ = marker distinctly more abundant than ++

nc = marker not classifiable in gel

Def = absence of expected marker in a more-distal exchange

Table S7 Semi-quantitative crossover data for the *bb*^{M3} set of crossover minichromosomes

	crossover minichromosome:								
marker	m3	m5	m9	m4	m8	m2	m6	m10	m7
R2916	++	+	+						
R2784	+++	++	++	++	++	+			
R2166	+++	++	++	++	++	++	+	+	+
R2059	+	+	+	+	+	+	+	+	+
R1767	+								
R1355	nc	nc	nc	nc	nc	nc	nc	nc	nc
R0963	+++	++	++	++	++	+	+	+	
R0910	++	+	+	+	+	+	+	+	
R0750	nc	nc	nc	nc	nc	nc	nc	nc	nc
S3887	+	+	+	+	+	+	+	+	+
S3542	+	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+	+
S3266	++	+	+	+	+	+			
S3079	++	+	+	+	+	+	+	+	+
S3030	++	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+	+

The band intensities of the nine R2/1 and eight IGS markers were estimated for the *Rex*-magnified *bb*^{M3} crossover minichromosome set (Figure S2). Columns are arranged from left to right, based on decreasing number or intensities of the markers. The gel image did not permit scoring two markers.

+ = marker present

++ = marker distinctly more abundant than +

+++ = marker distinctly more abundant than ++

nc = marker not classifiable in gel

Table S8 Semi-quantitative crossover data for the *bb*^{M4} set of crossover minichromosomes

	crossover minichromosome:							
marker	m17	m20	m14	m19	m21	m22	m23	m15
R2916	+							
R2784	+	+						
R2166	++	++	++	++	++	+	+	+
R2059	+	+	+	+	+	+	+	+
R1767	nc	nc	nc	nc	nc	nc	nc	nc
R1355	+	+	+					
R0963	++	++	+	+	+	+	+	
R0910	++	++	++	++	++	++	++	+
R0750	+	+	+	+	+			
S3887	+	+	+	+	+	+	+	+
S3542	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+
S3266	+	+	+	+	+			
S3079	+	+	+	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	++
S2785	+	+	+	+	+	+	+	+

The band intensities of the nine R2/1 and eight IGS markers were estimated for the *Rex*-magnified *bb*^{M4} crossover minichromosome set (Figure S3). Columns are ordered from left to right based on decreasing number or intensities of the markers. This gel image did not permit scoring one marker.

+ = marker present

++ = marker distinctly more abundant than +

nc = marker not classifiable in gel

Table S9 Semi-quantitative crossover data for the *bb*^{M18} set of crossover minichromosomes

	crossover minichromosome:								
marker	m108	m112	m111	m110	m100	m104	m106	m102	m103
R2916	++	+							
R2784	++	+	+						
R2166	+++	++	++	++	++	+	+	+	+
R2059	+	+	+	+	+	+	+	+	+
R1767	nc	nc	nc	nc	nc	nc	nc	nc	nc
R1355	+	+	+						
R0963	+++	+++	++	+	+	+	+		
R0910	++	Def	++	++	++	++	++	+	
R0750	+	+	+	+					
S3887	nc	nc	nc	nc	nc	nc	nc	nc	nc
S3542	+	+	+	+	+	+	+	+	+
S3518	+	+	+	+	+	+	+	+	+
S3266	++	+	+	+					
S3079	++	+	+	+	+	+	+	+	+
S3030	+	+	+	+	+	+	+	+	+
S2876	+	+	+	+	+	+	+	+	+
S2785	+	+	+	+	+	+	+	+	+

The band intensities of the nine R2/1 and eight IGS markers were estimated for the *Ybb*$^{-}$-magnified *bb*^{M18} crossover minichromosome set (Figure S4). Columns are ordered from left to right based on decreasing number or intensities of the markers. Two markers were unscorable in this gel, and there was one instance of an expected marker being absent (see text).

+ = marker present

++ = marker distinctly more abundant than +

+++ = marker distinctly more abundant than ++

nc = marker not classifiable in gel

Def = absence of an expected marker in a more-distal exchange

TABLE S10 Qualitative data for the *Tp(1;1)sc*^{V2L} rDNA array

gel					crossover minichromosome:					
bb^{2}	m32	m31	m30	m29	m25	m33	m28	m24	m27	
R2754		+	+	+	+	+	+	+	+	
R2322		+	+	+	+	+	+	+	+	
R1903		+	+	+	+	+	+	+	+	
R1832				+	+	+	+	+	+	
R1179				+	+	+		+	+	
R0883							+	+	+	
R0666			+		+	+	+	+	+	
S2021				+	+	+	+	+	+	
bb^{M1}	m48	m52	m53	m55	m46	m49	m50	m56		
R2754					+	+	+	+		
R2322					+	+	+	+		
R1903	+	+	+	+	+	+	+	+		
R1832	+	+	+	+	+	+	+	+		
R1179					+	+	+	+		
R0883						+	+	+		
R0666						+	+	+		
S2021					+	+	+	+		
bb^{M3}	m3	m4	m8	m10	m7	m1	m2	m5	m6	m9
R2754		+	+	+	+	+	+	+	+	+
R2322		+	+	+	+	+	+	+	+	+
R1903		+	+	+	+	+	+	+	+	+
R1832		+		+	+	+	+	+	+	+
R1179			+	+	+	+	+	+	+	+

R0883						+	+	+	+	+
R0666					+	+	+	+	+	+
S2021		+	+	+	+	+	+	+	+	+
bb^{M4}	m16	m14	m20	m22	m17	m19	m21	m23		
R2754	+	+	+	+	+	+	+	+		
R2322		+	+	+	+	+	+	+		
R1903	+	+	+	+	+	+	+	+		
R1832	+	+	+	+	+	+	+	+		
R1179		+	+	+	+	+	+	+		
R0883					+	+	+	+		
R0666					+	+	+	+		
S2021		+	+	+	+	+	+	+		
bb^{M18}	m102	m104	m108	m110	m106	m103	m100	m111	m107	m112
R2754					+	+	+	+	+	+
R2322						+	+	+	+	+
R1903				+	+	+	+	+	+	+
R1832		+	+	+	+	+	+	+	+	+
R1179	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
R0883									+	+
R0666							+	+	+	+
S2021						+	+	+	+	+

The presence of seven *Tp(1;1)sc*^{V2}-specific R2/1 variants (R2754, R2322, R1903, R1832, R1179, R0883, R0666) and of the one *Tp(1;1)sc*^{V2}-specific IGS variant (S2021) was scored in the minichromosomes produced by recombination between the *Tp(1;1)sc*^{V2} rDNA array and bb^{2}, three *Rex*-magnified alleles (bb^{M1} ,bb^{M3} and bb^{M4}) and the *Ybb*$^{-}$-magnified bb^{M18} allele. Minichromosome columns are ordered distal to proximal based on the number of *Tp(1;1)sc*^{V2}-specific markers; those produced by more proximal exchanges carry more *Tp(1;1)sc*^{V2}-specific markers than those produced by more distal exchanges.

+ = marker present

nc = marker not classifiable