Male mice with large inversions or deletions of X-palindrome arms are fertile and express their associated genes post-meiosis.

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Abstract

Large (>10 kb) palindromic sequences are enriched on mammalian sex chromosomes. In mice, these palindromes harbor gene families (≥2 gene copies) expressed exclusively in post-meiotic testicular germ cells, at a time when most single-copy sex-linked genes are transcriptionally repressed. This distinct expression pattern led to the hypothesis that containment within palindrome structures or having ≥2 gene enables post-meiotic gene expression. We tested these two hypotheses by using CRISPR to precisely engineer large (10’s of kb) inversions and deletions of X chromosome palindrome arms for two regions carrying the mouse 4930567H17Rik and Mageb5 gene families. We found that 4930567H17Rik and Mageb5 gene expression is unaffected in mice carrying palindrome arm inversions, suggesting that palindromic structure is not important for mediating palindrome-associated gene expression. We also found that 4930567H17Rik and Mageb5 gene expression is reduced by half in mice carrying palindrome arm deletions, allowing us to test whether palindrome-associated genes are sensitive to reduced expression levels resulting in spermatogenic defects. Male mice carrying palindrome arm deletions of 4930567H17Rik or Mageb5, however, are fertile, have normal testis histology, and show no aberrations in spermatogenic cell population frequencies via FACS quantification. Together, these findings suggest that large palindromic structures on the sex chromosomes are not necessary for their associated genes to evade post-meiotic transcriptional repression and that these genes are not sensitive to reduced expression levels. Large sex chromosome palindromes may thus be important for other reasons, such as the long-term evolutionary stability of their associated gene families.
Introduction:

In humans and mice, the sex chromosomes are enriched for large (>10kb), nearly identical (>99% nucleotide identity) segmental duplications in palindromic orientation\textsuperscript{1-4}. In mice, genes harbored within large X chromosome palindromes are expressed predominantly or exclusively in post-meiotic testicular germ cells\textsuperscript{3}. This specific expression pattern is surprising, because most single-copy X-linked genes are transcriptionally silenced post-meiosis\textsuperscript{5-8}. The mechanism by which palindrome-associated genes escape transcriptional repression is unknown; however, two hypotheses have been suggested to explain this distinct expression pattern. First, palindromes may form secondary structures (e.g. palindrome arms pairing to form a hairpin) enabling their associated genes to evade transcriptional repression\textsuperscript{3}. Intrachromosomal synapsis of palindrome arm pairing could facilitate the evasion of post-meiotic gene repression, which itself is a consequence of asynapsis-triggered meiotic sex chromosome inactivation\textsuperscript{9-11}. Second, X-palindromic genes may be sensitive to reduced expression levels and thus require ≥2 gene copies\textsuperscript{3}. Consistent with this, the mouse X chromosome carries other non-palindromic genes that are in multiple copies and are expressed specifically in post-meiotic cells\textsuperscript{3}. To test the two hypotheses, individual palindrome arms must be genetically manipulated, in vivo.

To rigorously test whether palindrome structure or gene copy number is important for post-meiotic expression, we genetically dissected two mouse X-palindromes. We utilized CRISPR to generate large-scale (10’s of kb) inversions and deletions in mice of two X-palindrome arms harboring the 4930567H17Rik and Mageb5 (Melanoma antigen gene family member b5) gene families. We chose these two X-palindromes because of their canonical features of palindromes across mammals; they have >99% percent nucleotide identity between the two arms, the arms are >10kb, and they harbor a gene family expressed specifically in post-meiotic testicular germ cells. We also selected these two gene families because they have sequence family variants.
between the two gene copies, enabling detection of palindrome arm-specific expression. We found that for the 4930567H17Rik and Mageb5 palindromic gene families, palindrome structure is not necessary for regulating their associated post-meiotic gene expression. We observed that deletion of a single palindrome arm, for both the 4930567H17Rik and Mageb5 gene families, reduces gene expression levels by half; however, reduced expression levels did not lead to male infertility or spermatogenic defects in either case. This suggests that palindromes enrichment on the sex chromosomes is important for other reasons and that there are alternative, unknown mechanisms for palindrome-associated genes to evade post-meiotic repression.

**Materials and Methods:**

*Generation of 4930567H17Rik and Mageb5 palindrome arm inversions and deletions*

To generate mice carrying palindrome arm inversions and deletions we used CRISPR with dual sgRNAs (single guide RNAs). sgRNAs were designed to unique sequences flanking the targeted palindrome arm, as close to the edge of the arm as possible. Since sgRNA cutting efficiency varies between sgRNAs, we tested their activity via pronuclear injection of an sgRNA/Cas9 expression plasmid, pSpCas9(BB)-2A-GFP (pX458)12 in mouse zygotes. The surviving mouse zygotes were allowed to develop into 64-128 cell blastocysts and the cutting efficiency of each sgRNA was determined by PCR of the cut sites and Sanger sequencing of purified PCR products to identify local edits at the target sites.

Once active sgRNAs were identified for both sides of the targeted palindrome arm, two pX458M plasmids encoding the sgRNAs and Cas9 together with a single-stranded oligonucleotide were pronuclear injected into hybrid C57BL/6JxSJL hybrid mouse zygotes. We added single-stranded oligonucleotides with sequence homology flanking the junction boundaries, to promote either inversions or deletions. The zygotes were generated from mating C57BL/6J females to
C57BL/6J x SJL males so that all targeted X chromosomes were C57BL/6J. Blastocysts from the pronuclear injection were implanted into pseudopregnant females. Genomic DNA from resulting pups was screened via PCR and Sanger sequencing for the inversion and deletion junctions. At least two independent mouse lines were obtained for inversions and deletions of the 4930567H17Rik and Mageb5 palindrome arms. Male and female mice were able to germline transmit both the 4930567H17Rik and Mageb5 deletions and inversions, thus their overall health was unaffected by our CRISPR-mediated chromosome engineering.

**Mice and testis sample collection**

All mice carrying an inversion or deletion of a single palindrome arm were backcrossed to C57BL/6J (N2-N7). Backcrossing was performed to minimize any possible CRISPR-mediated off-target effects. For assessing fecundity (average litter size per male), we mated mutant males to CD1 females. Testes were collected from 2-6 month old males for all experiments. We used wild-type littermate controls whenever possible and if not available, we used age-matched controls from the same breeding line. The alleles for the first mouse lines of each type were named 4930567H17RikInvArm1, 4930567H17RikDelArm1, Mageb5InvArm1, Mageb5DelArm1 with respective registered symbols Del(X4930567H17Rik)1Jbmu, In(X4930567H17Rik)1Jbmu, Del(XMageb5)1Jbmu, In(XMageb5)1Jbmu. All experiments performed on mice were approved by the University of Michigan Committee on Use and Care of Animals, and all experiments followed the National Institutes of Health Guidelines of the Care and Use of Experimental Animals.

**Preparation of adult testis cDNA for qPCR**

Intron-spanning primers, when possible, were used to perform qPCR on adult testis cDNA preparations. One testis per mouse was used to isolate RNA using Trizol (Invitrogen) following the manufacturers recommendations. 5 μg of total RNA was DNase treated with TurboDNase.
(Ambion) and reversed transcribed using Superscript II (Invitrogen) and oligonucleotide (dT) primers following the manufacturers protocol. qPCR was performed in triplicate using Power SYBR Green master mix (Thermo Fisher Scientific) on a 7500 Real-time PCR thermalcycler (Applied Biosystems). Trim42 (*Tripartite motif-containing 42*) was used as a normalization control, because it is expressed specifically in the same post-meiotic testicular cells and at similar levels as 4930567H17Rik and Mageb5. The Delta-delta Ct method, with Trim42 as the normalization control was used determine gene expression differences.

**Testis Histology**

Testes were fixed overnight in Bouin’s solution, paraffin embedded, sectioned to 5 µm, and stained with Periodic acid Schiff (PAS) and hematoxylin. Sections were visualized under a light microscope and specific germ cell populations were identified by their location within a tubule, nuclear size, and chromatin pattern\(^{13}\).

**FACs-based estimates of round spermatid frequencies**

We largely followed a previously published protocol\(^ {14}\) to isolate round spermatids (1n) and spermatocytes (4n). Briefly, we disassociated cells from a single testis by enzymatic treatment with Collagenase type I, DNase I (Worthington Biochemical Corporation), and Trypsin (Life Technologies). The cell suspension was passed through cell strainers (100 µm and 40 µm) and incubated with Hoechst 33342 (Life Technologies) for DNA content and propidium iodide (Acros Organics) for cell viability. Cell sorting was performed on a FACSaria II cell sorter (BD Biosciences). The purity of each sort was determined via fluorescence microscopy visual inspection of 100 cells morphology and nuclear staining with DAPI.

**RNA-seq analyses**

RNA-seq analyses were conducted by analyzing previously published datasets. Specifically,
mouse tissue panel data was analyzed from GSE41637\textsuperscript{15}, ovary data from GSE43520\textsuperscript{16} and sorted testicular germ cell populations from GSE49624\textsuperscript{17}. Alignments were performed using Tophat\textsuperscript{18} with the mm10 mouse reference genome, a refFlat file with RefSeq gene annotations and --max-multihits set to 240; otherwise standard default parameters were used. We used Cufflinks\textsuperscript{18} using the refFlat gene annotation file to estimate expression levels as fragments per kilobase per millions of mapped fragments (FPKM).

\textit{Dot plots}

Self-symmetry triangular dot plots that show repeats within a sequenced region were generated from a custom Perl script that can be found at http://pagelab.wi.mit.edu/material-request.html.

\textbf{Results:}

\textit{The mouse X chromosome harbors eight singleton palindromes.}

Large palindromes on mammalian sex chromosomes are typically found as isolated pairs of palindrome arms (singleton palindromes) or in complex arrays of palindromes. We investigated singleton palindromes, because they are more commonly found across mammalian sex chromosomes and can be genetically manipulated in vivo more precisely. Of the eight singleton palindromes on the mouse X chromosome (Table 1 and Figure 1A), we selected two harboring the 4930567H17Rik and Mageb5, because they share canonical features of sex chromosome palindromes: >10kb, >99\% nucleotide identity between palindrome arms, harbor genes expressed specifically in testicular germ cells, and have a spacer sequence between the palindrome arms (Table 1 and Figure 1B). Additionally, the palindrome carrying the 4930567H17Rik gene family has the longest palindrome arm (65kb), for a singleton palindrome, which if we can delete and invert in mice, will serve as a proof of principle for the manipulation of shorter palindrome arms.
We wanted to ensure that the \textit{4930567H17Rik} and \textit{Mageb5} gene families are expressed exclusively in post-meiotic round spermatids and that both copies are expressed. By reanalyzing previously published RNA-seq datasets, we find that both gene families are expressed exclusively in round spermatids (Figure 1C). We consider the low levels of expression in spermatogonia to be due to contamination of round spermatids in the spermatogonial populations during sorting. To determine if both \textit{4930567H17Rik} and \textit{Mageb5} gene copies are expressed, we utilized individual nucleotide differences between gene copies. Sequencing of RT-PCR products for both \textit{4930567H17Rik} and \textit{Mageb5} show that both gene copies are expressed (Figure 1D). Having confirmed that both gene copies are expressed exclusively in post-meiotic round spermatids, we proceeded to delete and invert individual palindrome arms to assess the importance of palindrome structure and gene copy number.

\textit{Generation of mice carrying precise inversions and deletions of individual X-palindrome arms via CRISPR.}

We utilized CRISPR/Cas9 technology to precisely invert and delete large X-palindromes arms in mice. Pronuclear injection of mouse zygotes with single guide RNAs (sgRNAs), targeting unique flanking regions of each palindrome arm, and a single stranded oligonucleotide donor enabled us to generate large (29 kb and 65 kb) inversions and deletions of individual arms for the \textit{4930567H17Rik} and \textit{Mageb5} X-palindromes (Figure 2A). The single stranded oligonucleotide donors were used to promote inversions and deletions. We screened founder mouse lines with all combinations of primers flanking the sgRNA cut sites in order to detect and distinguish inversions and deletions (Figure 2B). We validated inversion and deletion junctions by Sanger sequencing (Figure 2C). After \textasciitilde 300 pronucelar injections we obtained 2 and 3 independent mouse lines carrying deletions of a single palindrome arm for the \textit{4930567H17Rik} and \textit{Mageb5} gene families, respectively. Similarly, after \textasciitilde 300 pronuclear injections we obtained 4 and 2
independent mouse lines carrying inversions of a single palindrome arm for the 4930567H17Rik and Mageb5 gene families, respectively. The independently obtained inversion and deletion junctions differed from each other by only a few nucleotides. We also confirmed the deletions via Sanger sequencing of RT-PCR products to show that only a single sequence family variant was expressed (Figure 1D) in each mouse line. We selected two deletion and two inversion independent mouse lines for each X-palindromic region. Throughout this study, the genotypes for the eight deletion and inversion carrying male lines are 4930567H17Rik^{InvArm/Y}, 4930567H17Rik^{DelArm/Y}, Mageb5^{InvArm/Y}, and Mageb5^{DelArm/Y}, followed by a 1 or 2 depending on the mouse line. Our dual sgRNAs combined with single stranded oligonucleotides approach was successful in generating multiple independent mouse lines carrying 29 kb and 65kb inversions and deletions of single palindrome arms from a single round of ~300 pronuclear injections.

Disruption of palindrome structure, via inverting a single palindrome arm, does not affect the gene expression levels of the palindrome associated gene family.

With our generation of mice carrying precise inversions of two different X-palindrome arms that disrupt palindrome structure, we tested whether palindrome structure is necessary for facilitating post-meiotic gene expression. If palindrome structure is necessary for post-meiotic gene expression, then we expected to abolish gene expression of 4930567H17Rik and Mageb5 in 4930567H17Rik^{InvArm/Y} and Mageb5^{InvArm/Y} mice. Using gene-specific primers for 4930567H17Rik and Mageb5, we compared their testis gene expression levels via quantitative RT-PCR (qRT-PCR) in 4930567H17Rik^{InvArm/Y} and Mageb5^{InvArm/Y} mice carrying to wild-type controls and normalized to Trim42. After normalization, we find that 4930567H17Rik^{InvArm/Y} and Mageb5^{InvArm/Y} mice, across two independent mouse lines, express their associated genes at similar levels to wild-type mice (Figure 3A). Consistent with their similar gene expression levels, 4930567H17Rik^{InvArm/Y} and Mageb5^{InvArm/Y} are fertile and do no exhibit overt spermatogenic
defects. This suggests that palindrome structure is not necessary for the expression of the 4930567H17Rik and Mageb5 X-palindrome genes.

Deleting a single palindrome arm reduces the gene expression level of the palindrome-associated gene family by half.

Using mice carrying single palindrome arm deletions, we reduced the gene copy number of the palindrome associated 4930567H17Rik and Mageb5 X-linked genes by half (2 gene copies to 1). Concordantly, we expected 4930567H17Rik and Mageb5 gene expression levels would drop by half in 4930567H17Rik_{DelArm/Y} and Mageb5_{DelArm/Y} mice. Using gene-specific primers for 4930567H17Rik and Mageb5, we compared the testis gene expression levels of 4930567H17Rik_{DelArm/Y} and Mageb5_{DelArm/Y} mice to wild-type controls and normalized to Trim42. After normalization, we indeed find that 4930567H17Rik_{DelArm/Y} and Mageb5_{DelArm/Y} mice, across two independent mouse lines, express their associated genes at approximately half the levels of wild-type males (Figure 3B). The reduction of gene expression by half allows us to test whether the 4930567H17Rik and Mageb5 gene families are sensitive to reduced gene expression levels, resulting in males that are infertile with defects in post-meiotic spermatogenic development.

Male mice carrying deletions of individual palindrome arms do not exhibit defects in fecundity, testis histology, or numbers of round spermatids.

We performed a systematic characterization of fecundity and post-meiotic spermatogenic development in 4930567H17Rik_{DelArm/Y} and Mageb5_{DelArm/Y} mice. We found that 4930567H17Rik_{DelArm/Y} and Mageb5_{DelArm/Y} mice are fertile and produce litter sizes that are similar to wild-type controls (Figure 4A). To detect potential defects in post-meiotic spermatid development, we examined testis histological sections of 4930567H17Rik_{DelArm/Y} and Mageb5_{DelArm/Y} mice. We did not observe defects in spermatid morphology, formation of the acrosome, initiation of spermatid elongation or their formation or the presence of abnormal...
elongated spermatids in the epithelium. To assess whether the number of round spermatids were affected in 4930567H17Rik<sup>DelArm/Y</sup> and Mageb5<sup>DelArm/Y</sup> mice, we quantified the number of round spermatids per testis as the ratio of round spermatids/spermatocytes (control) via FACs. We find that the ratio of round spermatids/spermatocytes of 4930567H17Rik<sup>DelArm/Y</sup> and Mageb5<sup>DelArm/Y</sup> mice is similar to wild-type males (Figure 4B), which is consistent with their testis weights also being similar. Altogether, 4930567H17Rik<sup>DelArm/Y</sup> and Mageb5<sup>DelArm/Y</sup> mice do not exhibit detectable defects in fecundity and post-meiotic spermatid development.

**Discussion**

Our finding that palindrome structure is not necessary for facilitating post-meiotic gene expression for 4930567H17Rik and Mageb5 gene families leaves open the question as to why palindromes are heavily enriched on both the mammalian X and Y chromosomes. It is possible that palindromes, both on the X and Y chromosomes, are necessary for long-term evolutionary stability of the genes they harbor in order to rapidly purge deleterious mutations via gene conversion.<sup>19</sup>

Our findings also show that the 4930567H17Rik and Mageb5 gene families are not sensitive to reduced gene expression levels, when a single palindrome arm deletion reduces the gene copy number from 2 to 1. This, together with our findings on palindrome structures, suggests there are alternative mechanisms for X-palindromic genes to be expressed on the otherwise transcriptionally repressed X chromosome. There are a small number of X-linked single-copy genes expressed in round spermatids<sup>6,20</sup>, indicating that multiple gene copies are not a strict requirement for post-meiotic gene expression from the sex chromosomes. Thus, specific enhancers and transcription factors may have evolved to overcome the transcriptional repression associated with the rest of the sex chromosomes. Two potential transcription factors that facilitate post-meiotic sex-linked gene expression are Heat Shock Transcription Factor 1
HSF1), which localizes to sex chromatin\cite{21} and HSF2, which preferentially binds chromatin of Y-palindromic genes\cite{22}. Consistent with this, the testis is known to have specialized transcription regulation strategies in post-meiotic testicular germ cells\cite{23} and appears to have evolved a unique mechanism for palindromic and multicopy X- and Y-linked genes.

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**Figure Legends**

**Figure 1. Singleton palindromes on the mouse X chromosome.** A) The location of the eight singleton palindromic regions on the mouse X chromosome are labeled alphabetically, the sequence features of which can be found in Table 1. B) Self-symmetry triangular dot plots of the...
two singleton X-palindromes to be studied, carrying the 4930567H17Rik and Mageb5 gene families, respectively. Each dot plot represents the palindromic X chromosome sequence (4930567H17Rik = chrX:70385921-70553920 and Mageb5= chrX:91624421-91790420) plotted against itself with a sliding window of 100 nucleotides (step size=1 nucleotide). When the window of 100 nucleotides is identical to the sequence it is compared to, a dot is plotted. Segmental duplications in an inverted orientation are visualized as vertical lines. A visual representation of the palindrome arms (arrows) and the >99% identical genes harbored within them (squares) is plotted at the base of the triangular plots across the 168 kb 4930567H17Rik and 166kb Mageb5 palindromes. C) Expression levels of 4930567H17Rik and Mageb5 genes in adult tissues and sorted spermatogenic populations, shown as FPKMs (number of fragments per kilobase per million mapped fragments). D) Sanger sequencing of RT-PCR products displaying the sequence family variants that distinguish the two palindromic gene copies. In 4930567H17RikDelArm/Y and Mageb5DelArm/Y mice, expression is detected only from the remaining copy.

Figure 2. CRISPR strategy to generate large inversions and deletions of individual palindrome arms. A) Schematic of the mouse X chromosome with a diagram of a singleton palindrome shown below. Palindrome arms are shown as blue arrows. Single gRNA sites are shown as vertical red lines and primers to detect inversions and deletions are shown as black arrows. B) PCR genotyping of DNA from the two independent mouse lines for each of the deletions and inversions of 4930567H17Rik and Mageb5 palindrome arms. Numbered primers from panel A were used to amplify deletion (1+4) and inversion (1+3, 2+4) junctions. WT = wild-type C) Representative example of Sanger sequencing the deletion and inversion junctions for 4930567H17Rik palindrome arm rearrangements. Junction sites are shown with a vertical red line. The mm10 coordinates for the sequence removed in the 4930567H17RikDelArm1/Y line are
ChrX:70389542-70457358 and coordinates for the sequence inverted in the 
4930567H17RikInvArm1/Y are ChrX:70389544-70457357.

Figure 3. Male mice carrying 4930567H17Rik and Mageb5 palindrome arm inversions or deletions express their associated genes at wild-type and half of wild-type levels, respectively. A) 4930567H17Rik and Mageb5 gene expression levels from testes of 4930567H17RikInvArm1/Y and Mageb5InvArm1/Y, respectively. B) 4930567H17Rik and Mageb5 gene expression levels from testes of 4930567H17RikDelArm1/Y and Mageb5DelArm1/Y mice, respectively. For A and B, the gene expression values for each individual mouse (each dot) are normalized to wild-type expression (WT= 1). All expression values are normalized to Trim42, which is expressed specifically in round spermatids. Error bars represent the standard deviation of technical replicates. Gene expression levels from independent mouse lines. Welch’s two-tailed t-tests were used to determine significance.

Figure 4. Male mice carrying 4930567H17Rik and Mageb5 palindrome arm deletions are fertile and do not display defects in spermatogenic cell population frequencies. A) Multiple males from each 4930567H17RikDelArm1/Y and Mageb5DelArm1/Y line and control males were mated to multiple CD1 females to assess fertility and fecundity. B) Total testis weight (g) from 4930567H17RikDelArm1/Y and Mageb5DelArm1/Y lines were normalized to total body weight (g). Deletion males were compared to wild-type littermates. C) Representative FACS plot of spermatogenic cell populations with round spermatids (1n) and spermatocytes (4n) populations indicated by black arrows. D) The frequency of post-meiotic round spermatids was assessed by normalizing the percentage of cells in the post-meiotic round spermatid (1n) gate to the percentage of cells in the spermatocytes (4n).
| Palindrome | Genes* | Arm Size (kb)$^*$ | Spacer size (kb) | Percent Identity$^+$ |
|------------|--------|-------------------|------------------|----------------------|
| A          | 4930567H17Rik | 64.7              | 29.1             | 99.26                |
|   | Gene   | Palindrome Arm Size | Percent Identity | Maternal Percent Identity |
|---|--------|---------------------|------------------|--------------------------|
| B | Gm5640 | 47.1                | 11.7             | 99.61                    |
| C | Gm5071 | 34.6                | 44.9             | 99.88                    |
| D | Mageb5 | 30.0                | 98.8             | 99.26                    |
| E | 3010001F23Rik | 28.5            | 47.8             | 99.49                    |
| F | Xlr5a  | 27.0                | 3.7              | 99.59                    |
| G | Zxda   | 21.4                | 67.8             | 99.56                    |
| H | Gm773  | 13.3                | 25.7             | 99.36                    |

* In cases where two gene family members have different names either, only one was selected (e.g. Mageb5 was selected for gene family that has Mageb5 and Gm14781).

+ Palindrome arm size and percent identities between arms were identified from the “Segmental Dups” track of the UCSC genome browser mm10 mouse genome assembly.

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Figure A: Schematic diagram showing the genetic locations of genes 4930567H17Rik and Gm14725. The diagram indicates the deletion lines 1 and 2 of the genes.

Figure B: A tree diagram showing the expression of genes 4930567H17Rik and Mageb5 in different tissues. The expression levels are quantified using FPKM (Fragments Per Kilobase of transcript per Million mapped reads).

Figure C: A bar graph indicating the expression levels of 4930567H17Rik and Mageb5 in various tissues. The y-axis represents FPKM values, and the x-axis represents different tissues.

Figure D: A comparison of the expression patterns of 4930567H17Rik and Mageb5 in wildtype and deletion lines 1 and 2. The graphs show the changes in expression levels across different tissue types.
Mouse X Chromosome

Deletions

Inversions

Cas9, dual gRNAs

Junctions of PCR products

WT

Mageb5DelArm1/Y

Mageb5DelArm2/Y

Mageb5InvArm1/Y

Mageb5InvArm2/Y

H2O

Primer Pair

1&2

3&4

1&4

1&3

2&4

Junctions of PCR products
A

Relative gene expression level

WT  4930567H17Rikk\textsuperscript{Inv}\textsuperscript{Arm2}/Y

p = 0.73

WT Mageb5\textsuperscript{Inv}\textsuperscript{Arm2}/Y

p = 0.07

B

WT  4930567H17Rikk\textsuperscript{Del}\textsuperscript{Arm2}/Y

p < 0.0001

WT Mageb5\textsuperscript{Del}\textsuperscript{Arm1}/Y

p < 0.0001
**A**

Average Litter Size

- 4930567H17Rik
- Mageb5

Wildtype

Deletion

**B**

Total testis weight (g)/Body weight (g)

**C**

Spermatocytes (4n)

Round spermatids (n)

**D**

n/4n Cell Populations

4930567H17Rik
Mageb5

Mageb5

Mageb5

Mageb5