CRISPR-mediated Isolation of Specific Megabase Segments of Genomic DNA

Supplementary Information

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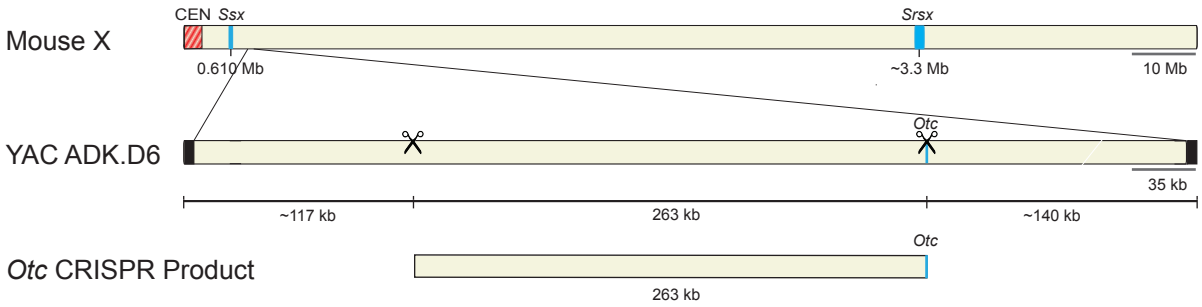

Figure S1. A large genomic locus on the mouse X chromosome, carried on the ~520 kb YAC, ADK.D6, is targeted for *in vitro* CRISPR. A pair of sgRNAs (✂) are designed to flank a 263 kb segment of the mouse X chromosome containing most of the *Otc* gene. *In vitro Otc* CRISPR on ADK.D6, producing ~117 kb, ~140 kb, and 263 kb DNA segments, serves as a positive control for *in vitro* CRISPR digestion of mouse genomic DNA. Mouse genomic DNA is shown in yellow (▯). YAC vector sequences are displayed in black (▮). The genomic location and estimated size of each of the two large mouse X chromosome DNA segments for *Ssx* and *Srsx* are shown in blue (▮).

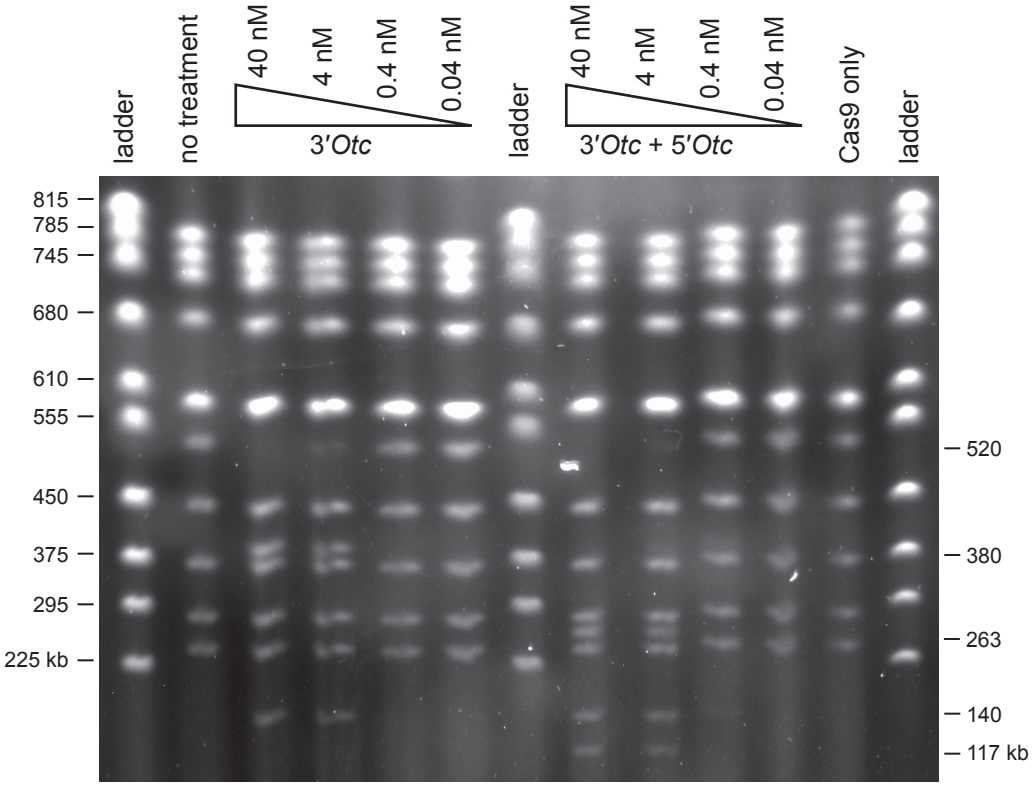

Figure S2. Assessing the optimal sgRNA/Cas9 concentration for complete *in vitro* CRISPR digestion of YAC ADK.D6. *In vitro Otc* CRISPR of approximately 4 µg of YAC ADK.D6 DNA in 40 µl agarose blocks is tested for completion with a range of sgRNA/Cas9 concentrations: 40 nM, 4 nM, 0.4 nM, and 0.04 nM. Each sgRNA is mixed 1:1 with Cas9 and allowed to digest the target DNA for 16 hours; a single sgRNA at 40 nM is mixed with 40 nM Cas9 and a pair of sgRNAs at 40 nM each is mixed with 80 nM Cas9. The expected *in vitro Otc* CRISPR digest products of the YAC (Supplementary Figure S1), labled on the right, appear with increasing concentration of sgRNA/Cas9. Following *in vitro Otc* CRISPR digestion with 40 nM of each sgRNA/Cas9 combination, the ~520 kb intact YAC is not detectable on the PFG with Diamond stain (Promega). The Yeast Chromosome PFG size marker (Bio-Rad) serves as size ladders, labled on the left.

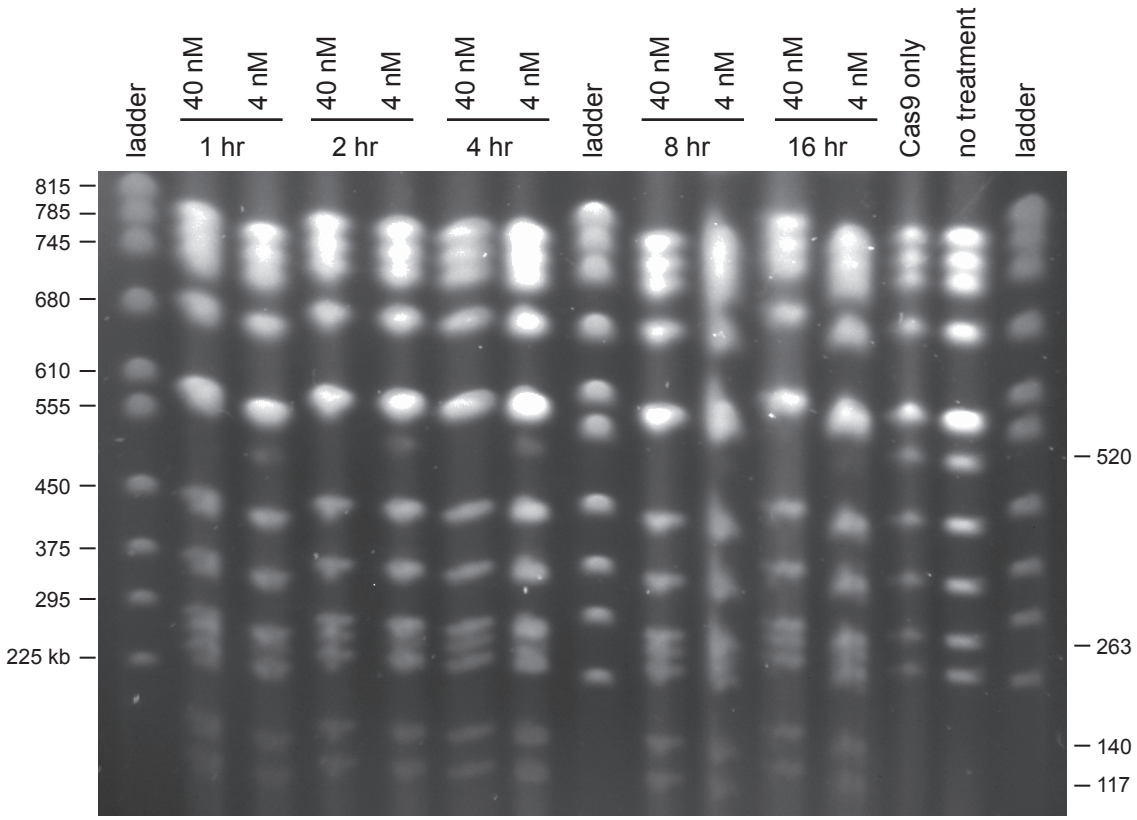

Figure S3. Assessing the optimal incubation time for complete *in vitro* CRISPR digestion of YAC ADK.D6. *In vitro Otc* CRISPR digestion of approximately 4 µg YAC ADK.D6 DNA in 40 µl agarose blocks is tested for completion with a range of incubation times: 1 hour through 16 hours. Reactions contain either 40 nM or 4 nM of each sgRNA, 3′*Otc* and 5′*Otc*, mixed 1:1 with Cas9. The expected *in vitro Otc* CRISPR digest products of the YAC (Supplementary Figure S1), labled on the right, are detected in all samples on the PFG with Diamond stain (Promega). After one hour of *in vitro Otc* CRISPR digestion with 40 nM of the sgRNAs, the ~520 kb intact YAC is not detectable. Increasing duration of the incubation with 4 nM of the sgRNAs does not improve the *in vitro Otc* CRISPR digestion of the YAC DNA. The Yeast Chromosome PFG size marker (Bio-Rad) serves as size ladders, labled on the left.

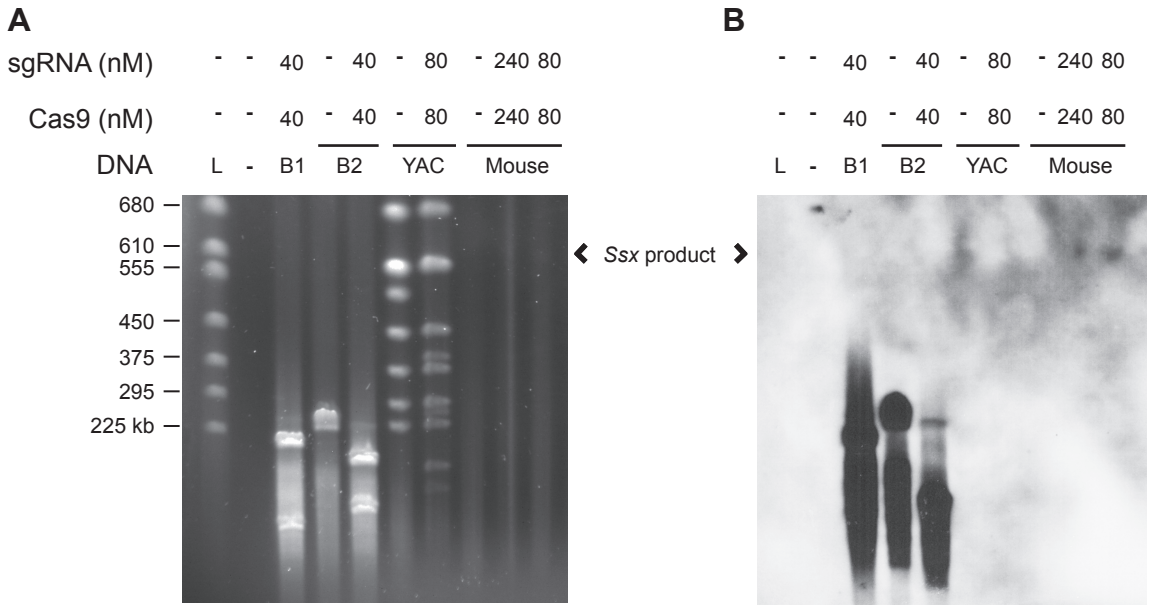

Figure S4. *In vitro Ssx* CRISPR digestion of mouse genomic DNA produces a specific, 610 kb DNA segment. (**A**) Using optimized reaction conditions, *in vitro Ssx* CRISPR digests of mouse genomic DNA and positive, bacterial artificial chromosome (BAC) clone controls (Supplementary Table S2) produce DNA segments detectable on the PFG with Diamond stain (Promega). Increasing sgRNA/Cas9 concentrations to a total of 240 nM does not improve the *in vitro Ssx* CRISPR digestion of mouse genomic DNA. The YAC, ADK.D6, is digested by *in vitro Otc* CRISPR as a postive control for CRISPR reaction components. The Yeast Chromosome PFG size marker (Bio-Rad) serves as size ladders (L), labled on the left. (**B**) The *Ssx* identity of the faint, 610 kb mouse genomic DNA segments is confirmed by the hybridization of the PFG (**A**) Southern blot with a DIG-labelled probe specific to the *Ssx* sequence.

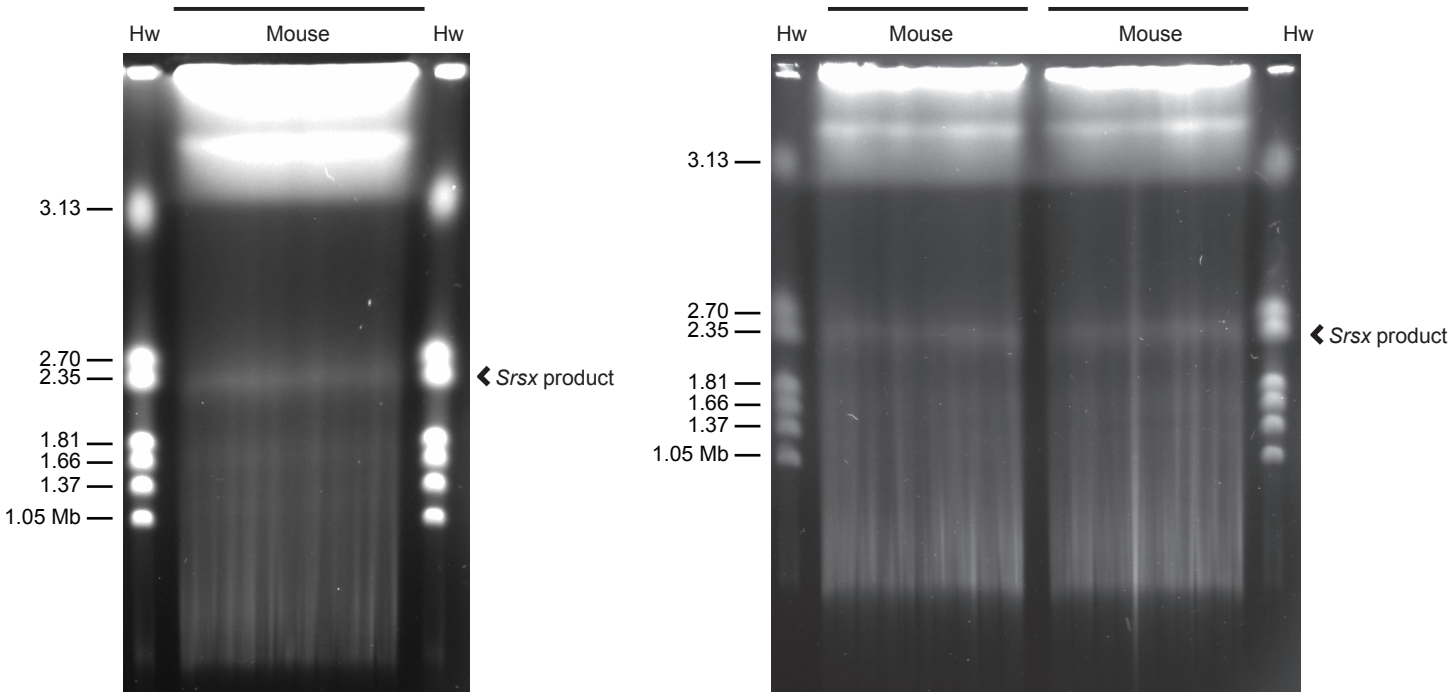

Figure S5. Resolution of the ~2.3 Mb *Srsx in vitro* CRISPR product from mouse genomic DNA by preparative PFGE, performed in triplicate. *H. wingei* (Hw) chromsomes (Bio-Rad) serve as the size ladders labeled on the left.

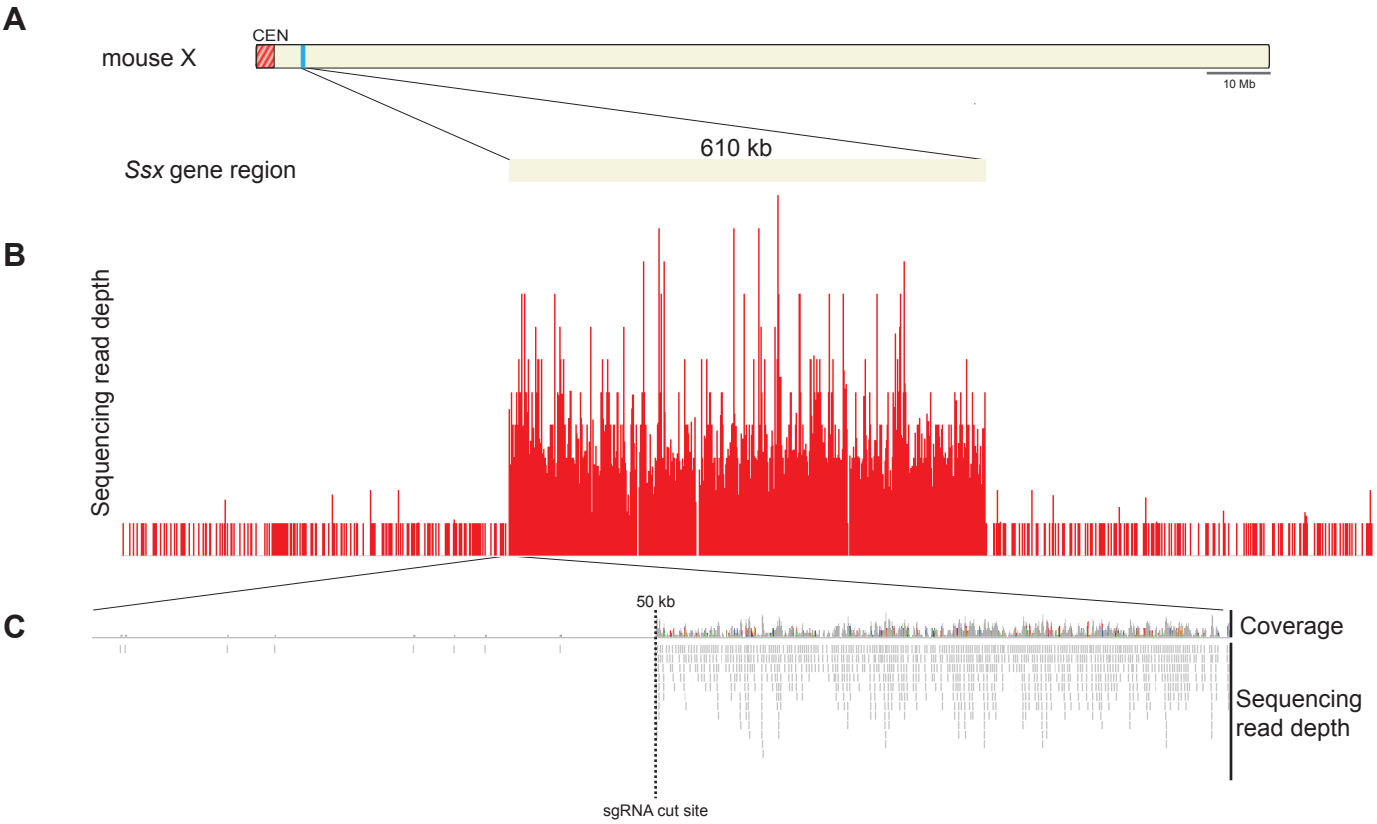

Figure S6. Enrichment of Illumina sequencing reads of the 610 kb genomic segment of the *Ssx* gene region. (**A**) Schematic of the mouse X chromosome shows the location of the *Ssx* gene region targeted by *in vitro Ssx* CRISPR for DNA sequencing. (**B**) Sequencing read depth of the 610 kb *Ssx* gene region and flanking 500 kb sequences. (**C**) Read depth of the 25 kb regions flanking the 5'*Ssx* sgRNA cut site (vertical dotted line).

Table S1. Reagents

name	composition
Zymolase Solution, 1 mg/ml	1 mg Zymolyase 20-T per ml of a solution of 10 mM Sodium Phosphate and 50% glycerol, final pH 7.5
NDSK	0.5 M EDTA, 1% w/v N-laurylsarcosine, 1 mg/ml Proteinase K, final ph 9.5
PMSF stock solution, 200 mM	1 g PMSF dissolved in 28.7 ml 100% ethanol
Cas9 Buffer (Rnase Free)	200 mM HEPES, 1 M NaCl, 50 mM MgCl2, 1 mM EDTA, final pH 6.5
High SDS Prehyb/Hyb Solution	50% Formamide, 7% SDS, 5X SSC, 2% w/v Blocking Reagent, 0.1% w/v N-lauroylsarcosine, 50 mM Sodium Phosphate Buffer (pH 7.0)

Table S2. *In vitro* CRISPR reaction components

CRISPR reaction	sgRNA name	target + PAM site*	Genome location (mm10)	Segment size (bp)	CRISPR BAC controls	DIG-labled Probe PCR Primer Pair***	Probe size - Hybrid. Temp	Probe BAC controls
Otc	5'*Otc*	GCCCATGGGATAATAGC AGG	10037275-10037294		na	AAGGCCGTGACCTCCTCA	482bp - 35°C	na
	3'*Otc*	TCCATCCCAATTGTCAA TGG	10300394-10300413	263132	na	TCAGAACAGAGTTGCATTGCT		
Ssx	5'*Ssx*	AAGATATCGCGGATGGT TGG	8278972-8278991		BAC 1 (B1) = RP23-59I20	TCACTCACCATCACTGTCATGA	476-486bp - 39°C	BAC 1 = RP23-59I20
	3'*Ssx*	CCTTGAGGAGCATGCGT GGG	8889193-8889212	610237	BAC 2 (B2) = RP23-260O19	AGAGTGGGGAAAGCTGACTC		BAC 2 = RP23-260O19
Srsx	5'*Srsx*	ATAGTCAGTCGTAAGCT AGG	122911126-122911145		RP24-137N6	TGGATGGTCTGTGAATGCCT	449bp - 38°C	RP24-327G2
	3'*Srsx*	CTACCACAATGCGTCTG TGG	126299997-126300016	na**	RP24-159H9	TTCTGCCGAAAGTAGCCTCA		

**Srsx* sgRNAs were amplified from pX458 plasmids with
TTAATACGACTCACTATAgg(17mer gRNA target sequence)gtttaagagctatgctgGA forward primers and
AAAAAAAGCACCGACTCGGTGC reverse primer

**The reference sequence for the *Srsx* ampliconic region contains five gaps, prohibiting the accurate estimation of its size.

*** Annealing temprature for all primers is 60°C

Table S3. Illumina Sequencing Summary Information

Genomic Region	Target Region Size (bp)	el Purification Yield (ng)	Total Reads	Mean Coverage of Target	% on-target bases	Fold Enrichment
Otc	263,132	7.2	1,742,930	1.23	0.7	75.34
Ssx	610,205	3.2	1,090,176	2.09	3.9	173.72
Srsx_1	3,388,890	3.1	10,040,103	9.07	4.9	39.23
Srsx_2	3,388,890	7.0	2,419,266	4.12	9.5	76.73
Srus_3	3,388,890	4.1	2,236,130	4.21	10.7	85.91

Based upon single end (forward) read mapping of the Illumina libraries