Genetic mapping of species differences via in vitro crosses in mouse embryonic stem cells

Stefano Lazzarano*, Marek Kučka*, João P. L. Castro#, Ronald Naumann*, Paloma Medina*,1, Michael N. C. Fletcher*,2, Rebecka Wombacher*,3, Joost Gribnau*, Tino Hocheptien*,4, Marc Van Montagu*,5,6, Claude Libert*,4 and Yingguang Frank Chan1,4

*Friedrich Miescher Laboratory of the Max Planck Society, 72076 Tübingen, Germany; †Max Planck Institute of Cell Biology and Genetics, 01307 Dresden, Germany; ‡Department of Reproduction and Development, Erasmus University Medical Center, 3015 CE Rotterdam, The Netherlands; §Department of Biomedical Molecular Genetics, Ghent University, 9052 Ghent, Belgium; ¶Center for Inflammation Research, Vlaams Instituut voor Biotechnologie, 9052 Ghent, Belgium; #Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium; and #Center for Plant Systems Biology, Vlaams Instituut voor Biotechnologie, 9052 Ghent, Belgium

Contributed by Marc Van Montagu, February 26, 2018 (sent for review October 11, 2017; reviewed by Frank W. Albert, Martien Kas, and Joseph Schacherer)

Discovering the genetic changes underlying species differences is a central goal in evolutionary genetics. However, hybrid crosses between species in mammals often suffer from hybrid sterility, greatly complicating genetic mapping of trait variation across species. Here, we describe a simple, robust, and transgene-free technique to generate “in vitro crosses” in hybrid mouse embryonic stem (ES) cells by inducing random mitotic cross-overs with the drug ML216, which inhibits the DNA helicase Bloom syndrome (BLM). Starting with an interspecific F1 hybrid ES cell line between the Mus musculus laboratory mouse and Mus spretus (~1.5 million years of divergence), we mapped the genetic basis of drug resistance to the antimetabolite tioguanine to a single region containing hypoxanthine–guanine phosphoribosyltransferase (Hprt) in as few as 21 d through “flow mapping” by coupling in vitro crosses with fluorescence-activated cell sorting (FACS). We also show how our platform can enable direct study of developmental variation by rederiving embryos with contribution from the recombinant ES cell lines. We demonstrate how in vitro crosses can overcome major bottlenecks in mouse complex trait genetics and address fundamental questions in evolutionary biology that are otherwise intractable through traditional breeding due to high cost, small litter sizes, and/or hybrid sterility. In doing so, we describe an experimental platform toward studying evolutionary systems biology in mouse and potentially in human and other mammals, including cross-species hybrids.

Significance

How species differ from each other is a key question in biology. However, genetic mapping between species often fails because of sterile hybrid crosses. Here, we have developed a technique called in vitro recombination to circumvent breeding. We induced genetic reshuffling through mitotic recombination with the drug ML216 and mapped trait variations in a dish. Starting with hybrid embryonic stem cells between the Mus musculus laboratory mouse and Mus spretus spanning ~1.5 million years of divergence, we show that it is possible to map the gene responsible for differential resistance to the drug tioguanine in as few as 21 days. Our technique opens up experimental avenues in genetic mapping of various traits and diseases across mouse species.

Author contributions: S.L., M.N.C.F., M.V.M., and Y.F.C. designed research; S.L., M.K., J.P.L.C., R.N., P.M., M.N.C.F., R.W., and Y.F.C. performed research; J.G., T.H., C.L., and Y.F.C. contributed new reagents/analytic tools; S.L., M.K., J.P.L.C., and Y.F.C. analyzed data; and S.L., M.K., J.P.L.C., R.N., P.M., M.N.C.F., R.W., J.G., T.H., C.L., and Y.F.C. wrote the paper.

Reviews: F.W.A., University of Minnesota; M.K., University of Groningen; and J.S., University of Strasbourg.

The authors declare no conflict of interest.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. PRJNA390071). Scripts are available at https://github.com/evolgenomics/HybridMiX.

*Present address: Department of Molecular Genetics, German Cancer Research Center, 69120 Heidelberg, Germany.

*Present address: Department of Molecular Genetics, German Cancer Research Center, 69120 Heidelberg, Germany.

†To whom correspondence may be addressed. Email: marc.vamontagu@ugent.vib.be or frank.chan@tue.mpg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1717474115/-/DCSupplemental.
We set out to establish a universal method that allows genetic mapping in mammals without breeding, even across divergent species with viable but otherwise sterile hybrids. We opted to use mouse embryonic stem (ES) cells to take advantage of the full spectrum of genetic manipulations available in tissue culture. A minimal system will have the two following features: an ability to induce on-demand extensive genetic exchange; and genetic (and trait) variation such as those found in F1 hybrid ES cells, ideally between species.

Intriguingly, the technique to create genetic variation through recombination has been in broad use in the mouse genetics community, albeit never explicitly in F1 hybrid ES cells with the goal of genetic mapping. In 2004, two independent groups showed that recessive, biallelic mutants could be reliably recovered in mouse ES cells without breeding by suppressing the DNA helicase Bloom syndrome (Blm; Fig. 1A) (16, 17). Yusa and coworkers (18) showed that these recessive phenotypes were revealed through mitotic recombination between homologous chromosomes. We reasoned that the same mechanism could be leveraged to generate genome-wide random mitotic recombination. This mechanism enabled the creation of panels of arbitrary size carrying recombinant genomes, while avoiding the limitations of hybrid sterility or inbreeding depression (Fig. 1B).

**Results**

To test if Blm inhibition could lead to elevated homologous recombination rates in mitosis, we inhibited Blm in a number of mouse ES cell lines using a recently discovered small-molecule inhibitor, ML216 (Fig. 1C) (19). As a first test, we started with F1 ES cells between the laboratory mouse strains C57BL/6J (“BL6” in short) and 129 that carried a targeted transgene as a hemizygous allele at the ROSA26 locus on distal chromosome 6. We estimated homologous recombination by counting colony survival under fialuridine (FIAU) treatment, which selected against the transgene consisting of hygromycin phospho-transferase–thymidine kinase (HyTK) and a green fluorescent protein (GFP; Fig. S1). We found that Blm inhibition led to highly elevated rates of homologous recombination, as revealed by increased numbers of FIAU-resistant colonies [Fig. 1C; in vitro recombination (IVR) rate: 2.9 × 10⁻⁴ per cell per generation] and the appearance of mosaic GFP expression within a colony (Fig. 2A, Right). This is consistent with reported rates under Blm suppression or disruption (targeted tetracycline inactivation) in mammalian species with viable but otherwise sterile hybrids. (A) F1 hybrid ES cell line (BL6 × CAST) F1 (20) derived from BL6 and Mus castaneus (CAST/EJ, abbreviated to CAST; diverged ~0.5 million years ago, or 7.9 SNPs per kbp; compare 1.7 SNPs per kbp between BL6 and 129) with ML216 but otherwise grown without selection. We screened 136 randomly picked ML216-treated clones for loss of heterozygosity (LOH) recombinants and recovered recombinants in both BL6/BL6 and CAST/CAST directions on chromosome 1. Sequencing of representative clones revealed conversion from F1 heterozygous genotypes toward both homozygous genotypes at the telomeres (clones 21 and 50; note also additional recombination on chromosome 13). In contrast, control nonrecombinant clones retained heterozygosity at the telomeres (clones 54 and 56). However, even here we discovered a single clone carrying additional internal recombinants on chromosome 1 (Fig. 2C).

**Fig. 1.** IVR via Blm helicase suppression. (A) Blm encodes a helicase normally active during mitosis. Loss of Blm activity leads to increased improper sister chromatid exchange as well as recombination between homologous chromosomes. Mitotic recombination can give rise to recombinant diploid daughter cells with LOH between the breakpoint and the telomeres. (B) IVR allowed the circumvention of hybrid sterility in crosses between the laboratory mouse, e.g., BL6, and a murine sister species SPRET. (BL6 × SPRET) F1 hybrid mice were viable and allowed derivation of F1 ES cells despite male sterility (25). Applying IVR to F1 ES cells allowed rapid and efficient generation of recombinant ES cell panels for genetic mapping. (Scale bar: 50 μm.) chr, chromosome. (C) Efficiency of IVR was estimated by colony survival assay. We estimated the recombination rate between homologous chromosomes with cells hemizygous for a dominant selectable marker (HyTK; green). We induced IVR by adding a small-molecule Blm inhibitor, ML216 (19), to the culturing medium for 1 or 5 d. Under FIAU negative selection, cells having undergone mitotic recombination to become homozygous for the wild-type BL6 alleles (blue) survived, while nonrecombined cells or recombinant cells retaining the HyTK transgene metabolized FIAU, resulting in cell death due to misincorporation of toxic nucleotide analogs (top and middle cells with red chromosomes). Under ML216 treatment (25 μM), IVR rate was estimated to be 2.9 × 10⁻⁴ per cell per generation, yielding 800–1,500 FIAU-resistant colonies per million following treatment.
Widespread IVR across a range of evolutionary divergence. (A) Selection cassette transgene (HyTK-GFP-Neo). ES cell colonies displayed mosaic GFP expression within a colony when cultured with ML216, but not under control conditions, consistent with homologous recombination and loss of GFP through IVR. Recombination between homologous chromosomes could result in daughter cells with two wild-type (BL6 allele; dark) or transgenic copies (129 allele; bright). Early recombination events followed by random cell loss during clonal expansion could produce completely dark colonies. (Scale bars: 100 µm) (B) Double-selected clones. After expansion under negative selection against the transgene (both ganciclovir and FIAU kill cells expressing HyTK), 11 ganciclovir-resistant and GFP-negative colonies were recovered and sequenced each by fluorescence-activated cell sorting (FACS; “live”) proportions under ML216 treatment vs. live proportions under 6-TG treatment, n = 5 paired treatments; Kruskal–Wallis test, χ² = 13.17, df = 1, P < 0.0003; Fig. 3A and Fig. 6b). We separately recovered and sequenced each “resistant” (6-TG⁻) and “susceptible” (6-TG⁺) pool (Fig. 3A). Under both 5- and 21-d ML216 treatment, a large skew toward 6-TG susceptibility observed on chromosome X in the 6-TG⁻ relative to the 6-TG⁺ pool (Fig. 3A and B; differential SPROt bias as a fraction of all reads in adjacent megabase windows between the two pools, with raw SPROt bias ranging from 1 for SPROt-only to ~1 for BL6-only). This was in stark contrast to the genomic background, which showed little bias for either SPROt or BL6 contributions (Fig. 3A and B, also Fig. 5c). The region with the greatest SPROt bias was found on chromosome X near Hprt (Fig. 3C; common region found in both 5- and 21-d ML216 treatments between 49 and 80 Mb; also see chromosome X in Fig. 6f and discussion in SI Text). Here, our forward genetic mapping for 6-TG susceptibility clearly identified a single locus, suggesting that 6-TG susceptibility depended only on Hprt genotypes. To confirm the role of Hprt in mediating differential 6-TG susceptibility beyond bulk sequencing, we also sequenced 46 individual 6-TG⁻ IVR clones after 10-d ML216 treatment to determine their recombination breakpoints (Fig. 3C). Echoing the skewed cross-over patterns centromeric to the HyTK selection cassette (Fig. 2B), we observed more SPROt-to-BL6 than BL6-to-SPROT centromeric recombinants (n = 35 vs. 8, P ≤ 2 × 10⁻⁵, exact binomial test, h₁ ≥ h₀) and also ruled out aneuploidy or deletion of Hprt as major contributors to 6-TG resistance. We note, however, that despite the strongly skewed ratio of 27 BL6/B6 homologous clones at the Hprt locus, of 46 total recovered clones, we still observed 9 heterozygotes and 10 SPROt/SPROT homozygous clones (BL6/BL6 at the Hprt locus: 58.6%; χ² test
Discussion

A central goal of evolutionary genetics is to identify how mutations arose during evolution and influenced phenotypes. For many organisms, a major barrier has been the inability to reliably generate diverse and large mapping panels of sufficient evolutionary diversity. Here, we describe a simple and robust method to make "in vitro crosses," resulting in panels with inter-cross-like homozygous genotypes from otherwise sterile interspecific hybrid crosses. Being able to bring forth genetic diversity in a Petri dish creates the unique opportunity to conduct mouse genetic mapping at unprecedented speeds with "flow mapping" similar to "X-QTL" in yeast (28) or arbitrarily large panels unmatched by most other model organisms, except possibly yeast (21, 28). As renewable stem cells, IVR panels can be expanded, archived, and shared, offering a cellular resource with many of the advantages sought from traditional community resources such as recombinant inbred (RI) line panels. Furthermore, we have shown that our IVR method works in a broad range of ES cells. With millions of potentially recombinant (thus genetically distinct) ES cells in a Petri dish, we demonstrated how IVR enabled mapping of QTLs for drug resistance in as few as 6 d (with an estimated total of five doublings over 5 d). Putting this in context, such an experiment using traditional mouse crosses would have taken 450 d, based on the typical mouse generation
time of 90 d, assuming that hybrid sterility could be overcome and allowing for selfing.

We see IVR as a complementary extension to classical organismal genetic mapping panels. In the mouse, the largest organismal RI panel BXD contains ~160 lines (with most published work based on the ~35 original BXD strains) (29), and attempts in generating panels incorporating greater diversities encountered enormous challenges (30). Nevertheless, mouse RI resources represented some of the most powerful tools available to dissect system genetics in the mouse, the prime biomedical model organism (31). Seen in this light, IVR represents an alternative approach that could greatly extend the available renewable resources, not least because the genotype combinations between divergent species are hitherto impossible to obtain in the first place.

Recently, Sadhu et al. (21) also achieved a major advance in genetic mapping using CRISPR/Cas9-mediated mitotic recombination in yeast. In contrast to CRISPR targeting, our transgene-free approach offers the simplicity of inducing genome-wide recombinants by the simple addition of a single inexpensive small molecule to the tissue culture medium. Going forward, we envision a combined, complementary approach to IVR: using BLM inhibition for mapping panel generations and efficient QTL identification, then switching to targeted transgene-based screening or CRISPR/Cas9-based IVR for fine-scale mapping.

In addition to the traits we have investigated, M. spretus and the Mus musculus laboratory mouse differ in a number of distinct traits, such as longevity and telomere lengths (32), cancer and inflammation resistance (33, 34), and metabolism (35). Many of these traits have tissue or cellular models suitable for IVR mapping panels or flow mapping through fluorescent detection of specific proteins or metabolites. Future experiments may also probe even greater evolutionary divergence: Early work has shown that F1 hybrids spanning as much as 6 million years between M. musculus and Mus caroli was viable (36). Given active development in single-cell genomics and disease modeling from patient-specific induced pluripotent stem cells, including organoids or organ-on-a-chip microfluidics systems, we anticipate that the in vitro recombinant platform can be broadly applied to accelerate the identification of the genetic basis of many traits and diseases.

Materials and Methods

**Animal Care and Use.** All animal experiments have been approved by the applicable animal welfare ethics committees: Faculty of Sciences, Ghent University (reference no. 06022); and Landesdirektion Sachsen, Germany, permit 24-9168.11-9/2012-5.

**Cell Culture.** Unless otherwise stated, ES cell lines were cultured on SNL76/7-4 feeder cells in attachment factor (AF)-coated plates under 2i medium, supplemented with leukemia inhibitory factor (LIF) (see SI Methods for details).

**BLM inhibition Using ML216.** BLM inhibition was performed by using 25 μM ML216 (Sigma-Aldrich) in 2i/LIF medium on inactivated feeders.

**Generation of HyTK-EGFP-Neo Cell Line.** Starting with G4 ROSALUC B12 ES cells (37), we replaced the cassette at the ROSA26 locus with a cassette carrying two selectable markers, HyTK and enhanced GFP (EGFP; selectable in fluorescence-assisted cell sorting: Fig. S1; see SI Methods for details). Successful replacement of the cassette was confirmed through selection by using geneticin (G148; Thermofisher Scientific) and genotyping.

**Colonial Survival Assay.** HyTK-EGFP-Neo cells were seeded at a density of 5 × 10^4 per 10 cm AF per feeder plate, followed by 25 μM ML216 treatment for 1 or 5 d. Before the start of negative selection, cells were replated at 2 × 10^5 per 10 cm AF per feeder plate, and FIAU (0.2 μM; Sigma-Aldrich) or ganciclovir (10 μM; Sigma-Aldrich) selection was applied for 5 d. Colonies were stained with the alkaline phosphatase kit (EMD Millipore), photographed, and counted to determine survival rates. The entire procedure was repeated multiple times, and the survival colony counts were averaged to determine IVR rate.

**Screening for Spontaneous Recombinant ES Cell Colonies.** Cells were treated with ML216 for 2 d at a concentration of 5 μM and then for 3 d at a concentration of 25 μM. Cells were then replated and cultured for 5 d in 2i/LIF without ML216. A total of 189 colonies were randomly picked (without selection), of which 136 were screened with multiplexed genotyping.

**Multiplexed Genotyping for Detection of LOH.** Diagnostic markers between Bl6, CAST, and Spret strains at tips of each chromosome were designed to track the presence of each allele. The markers were amplified with fluorescence-tagged primers as proposed in ref. 38 (see SI Methods and Table S1 for details). The PCRs were pooled at equimolar proportions and analyzed with a 3730xl DNA Analyzer capillary sequencer (ThermoFisher Scientific) and scored for conversion from heterozygous into homozygous genotypes (LOH) at the tips of each chromosome.

**The 6-TG Treatment and DAPI Exclusion Assay.** Before the main experiments, killing curves for 6-TG (Sigma-Aldrich) was performed by using a WST-1 assay (Roche) according to the manufacturer’s instructions (Figs. 3A and S1, Left). For the main experiments, the S18 ES cell line was cultured for 5, 10, or 21 d with 25 μM ML216. Following the designated ML216 treatment, the cells were replated and treated with 25 μM 6-TG in 2i/LIF. We determined “live/dead” cell viability by using DAPI staining (1 μg/mL; Sigma-Aldrich) after 1 d of 25 μM 6-TG treatment.

**FACS.** Flow cytometry was performed by using an Aria II Cell Sorter (Becton Dickinson GmbH). We defined the 6-TG^- and -Tg^+ populations by using the DAPI exclusion assay in reference ES cell populations. In sorting experiments, ML216-treated or control 6-TG^- and -Tg^+ populations were recovered for sequencing. For quantification, we performed post hoc analysis using the R Bioconductor package flowCore (39), principally by clustering using the forward scatter area and DAPI/Pacific Blue-A channels into live and dead clusters using mclust (Version 5.2) (40, 41) in 6-TG^-treated experiments, considering ML216-treated and controls separately (Fig. S5).

**Sequencing and Analysis Pipeline.** Sequencing libraries for high-throughput sequencing were generated by using the Nextera DNA Library Prep Kit (Illumina, Inc.) according to manufacturer’s recommendations or equivalent purified Tn5 transposase as described in ref. 42. Each sample (FACS-sorted clones, single colonies or pooled cells) was barcoded through PCR extension by using an i7-index primer (N701–N763) and the N501 i5-index primer. Pooled libraries were sequenced by a HiSeq 3000 (Illumina) and analyzed by using a custom pipeline (see SI Methods for details). We performed genotyping based on allelic coverage per megabase using known informative
variants between the BL6, CAST, and SPRET strains (Mouse Genomes Project Version 3 dbSNP Version 137 release) (24). Scripts are available at: https://github.com/evolgenomics/HybridMiX.

**Laser-Assisted Morula Injection.** ES cell-derived embryos were obtained through injection into eight-cell-stage embryos (morulae) as described in ref. 27. The introduction of excess ES cells was expected to lead to embryos with fully ES cell contributions. At 14 d after the injection and subsequent embryo transfer into surrogates (approximating developmental stage E14.5), the gestation was terminated, and embryos were dissected and fixed with 4% paraformaldehyde for microCT scanning. Due to sample preparation, genotyping of scanned embryos was not performed. Instead, control (unscanned) embryos were genotyped at diagnostic loci, confirming ES cell contributions from the respective cell lines.

**microCT.** Soft-tissue X-ray contrast staining was done via 4-d perfusion in 25% Lugol’s or iodine potassium iodide solution. Then the embryos were rehydrated, mounted in 1% low-melting agarose, and scanned with a Skyscan 1173 instrument (Bruker Corporation) at 9.96-μm resolution (0.5-mm aluminum filter, energy at 70 kV and 110 μA). Image analysis, segmentation, and visualizations were performed by using Amira (Version 6.2.0; FEI) with the XImagePAQ extension 6.2.

**ACKNOWLEDGMENTS.** We thank Felicity Jones for experimental design, helpful discussion and input, and improving the manuscript; Caroline Schmid for animal husbandry; Sebastian Kick for microCT scanning; the Y.F.C. and Jones laboratory members for support, scientific input, and improving the manuscript; Christa Lanz and Ija Bezrukov for assistance with high-throughput sequencing and associated data processing; Andre Noll for high-performance computing support; and Cornelia Grimmel and Stella Autenrieth for technical assistance with FACS. Cell sorting, flow cytometry sample acquisition, and data analysis was done on shared instruments of the Flow Cytometry Core Facility Tübingen. RV-L3-HytTK-2L and pCAG-Fipo were gifts from Geoff Wahl and Massimo Scanlandi (Addgene plasmids 11684 and 60662). The G4 ROSALUC ES cell line was a gift from Jody J. Haigh. We thank Hua Tang, David M. Kingsley, Karsten Borogvard, and Detlef Weigel for input and discussion on experimental design. We thank our reviewers for their thoughtful input, which has greatly improved the manuscript. J.P.L.C. is supported by the International Max Planck Research School “From Molecules to Organisms.” P.M. was supported by the Fullbright US Student Program. T.H. and C.L. are supported by Genth University. Y.F.C. is supported by the Max Planck Society and European Research Council Starting Grant 639096 “HybridMiX.”

1. Darwin C (1859) On the Origin of Species by Means of Natural Selection (John Murray, London, 1859).
2. Dejneger L, Libert C, Montagnuoli X (2009) Thirty years of Mus spretus: A promising future. Trends Genet 25:234-241.
3. Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.
4. Mackay TFC, Stone EA, Ayroles JF (2009) The genetics of quantitative traits: Challenges and prospects. Nat Rev Genet 10:565-577.
5. Allen Orr H (2001) The genetics of species differences. Trends Ecol Evol 16:343-350.
6. Churchill GA, et al.: Complex Trait Consortium (2004) The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat Genet 36: 1133-1137.
7. Nicod J, et al. (2016) Genome-wide association of multiple complex traits in outbred mice by ultra-low-covariance screening. Nat Genet 48:912-918.
8. Parker CC, et al. (2016) Genome-wide association study of behavioral, physiological and gene expression traits in outbred C57J mice. Nat Genet 48:919-926.
9. Poltorak A, et al. (1998) Defective LPS signalling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. Science 282:2085-2088.
10. Mihola O, Tschuchtem Z, Bicke C, Schimenti JC, Forejt J (2009) A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science 323:373-375.
11. Turner LM, White MA, Tautz D, Payne BA (2014) Genomic networks of hybrid stability. PLoS Genet 10:e1004162.
12. White MA, Ikeda A, Payne BA (2012) A pronounced evolutionary shift of the pseudautosomal region boundary in house mice. Mamm Genome 23:454-466.
13. White MA, Stubbings M, Dumont BL, Payne BA (2012) Genetics and evolution of hybrid male stability in house mice. Genetics 191:917-934.
14. Forejt J (1996) Hybrid stability in the mouse. Trends Genet 12:412-417.
15. Burgio G, et al. (2007) Interspecific recombinant congenic strains between C57Bl/6 and mice of the Mus spretus species: A powerful tool to dissect genetic control of complex traits. Genetics 177:2231-2233.
16. Guo G, Wang W, Bradley A (2004) Mismatch repair genes identified using genetic screens in Ilm-deficient embryonic stem cells. Nature 429:891-895.
17. Yusa K, et al. (2004) Genome-wide phenotype analysis in ES cells by regulated disruption of Bloom’s syndrome gene. Nature 429:896-899.
18. Yamanishi A, et al. (2013) Enhancement of microRNA-mediated genomic re-arrangements by transient loss of mouse Bloom syndrome helicase. Genome Res 23: 1462-1473.
19. Nguyen GH, et al. (2013) A small molecule inhibitor of the BLM helicase modulates chromosome stability in human cells. Chem Biol 20:55-62.
20. Barakat TS, Rentmeester E, Sleutels F, Grootegoed JA, Gribnau J (2011) Precise BAC targeting of genetically polymorphic mouse ES cells. Nucleic Acids Res 39:e121.
21. Sadhu MJ, Bloom JS, Day L, Kruglyak L (2016) CRISPR-directed mitotic recombination enables genetic mapping without crosses. Science 352:1113-1116.
22. Johnson GJ, Chapman VM (1987) Altered turnover of hypoxanthine phosphoribosyltransferase in erythroid cells of mice expressing Hprt a and Hprt b alleles. Genetics 116:313-320.
23. Mouse Genomes Project, Wellcome Trust Sanger Institute (2017) Data from Genome Evolution Browser, SPRET_EU. Available at mice-geval.sanger.ac.uk/SPRET_EU_R0105090/ InfoIndex. Accessed January 28, 2017.
24. Keane TM, et al. (2011) Mouse genomic variation and its effect on phenotypes and gene regulation. Nature 477:289-294.
25. Bochehip T, et al. (2004) Breaking the species barrier: Derivation of germline-competent embryonic stem cells from Mus spretus x C57BL/6 hybrids. Stem Cells 22: 441-447.
26. Barakat TS, Gribnau J (2010) X chromosome inactivation and embryonic stem cells. Trends Genet 26:239-242.