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Long-range chromosomal engineering is more efficient *in vitro* than *in vivo*

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Abstract

Cre/LoxP mediated chromosomal engineering in embryonic stem (ES) cells has a variety of applications, including the creation of model systems for studying aneuploidy. Targeted meiotic recombination (TAMERE) was proposed as a high efficiency *in vivo* alternative to effect Cre-mediated recombination, in which Cre recombinase under control of the Synaptonemal Complex 1 promoter is expressed during male meiosis in transgenic mice. TAMERE has been successfully used with LoxP sites up to 100 kb apart. We tested TAMERE for a chromosome engineering application in which LoxP sequences were integrated into sites 3.9 Mb apart on the same (*cis*) or opposite (*trans*) copies of mouse Chromosome 16 (MMU16). TAMERE was ineffective in generating either a deletion or a translocation *in vivo*. The TAMERE method may be of limited use for large genomic rearrangements. The desired translocation was achieved with an *in vitro* method that can be used in any ES cell line. Mice produced from the reciprocal duplication/deletion of MMU16 in a region homologous to human chromosome 21 provide models that are useful in studies of Down syndrome.

Introduction

The creation of large, precise genomic alterations in mice provides experimental models for human aneuploid conditions. The Cre/LoxP system (Tronche et al., 2002) can be used to choose specific endpoints for deletions, inversions, and duplications depending on the placement of 34 bp LoxP sites in the genome. If LoxP sites are located on the same chromosome (i.e., in *cis*) in the same orientation, Cre recombination will result in deletion of the intervening sequence. When LoxP sites are in *cis* in opposite orientations, the intervening sequence will be inverted. Cre-mediated recombination between LoxP sites located in the same orientation in *trans* results in a reciprocal translocation.

The most common method used to date to express Cre recombinase for chromosome engineering is an *in vitro* culture system (Yu & Bradley, 2001). The major advantage of this approach is the ability to select for rare recombinants by reconstitution of selectable markers. However, this system requires several consecutive transfection and selection steps. ES cell lines can become aneuploid and accumulate mutations after extensive culture and manipulation, causing them to lose the ability to colonize the germline (Liu et al., 1997; Longo et al., 1997).

TAMERE was described as an alternative method which requires fewer *in vitro* manipulations of ES cells and therefore can minimize problems associated with extended cell culture (Herault et al., 1998). This strategy utilizes a transgenic mouse which expresses Cre recombinase during spermatogenesis by virtue of the

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Synaptonemal Complex Protein 1 (*Sycp1*) promoter (Vidal et al., 1998). The extremely high frequency of *in vivo* recombination (12%) reported initially between LoxP sites separated by 5.4 kb in *trans* demonstrated its utility (Hernandez & Fisher, 1999). A subsequent study indicated that sex-specific methylation of LoxP sites occurs in males carrying the *Sycp1-Cre* transgene, which complicates breeding strategies for TAMERE (Rassoulzadegan et al., 2002). Recent reports demonstrate that TAMERE-mediated recombination can occur over regions up to 100 kb, albeit at lower efficiency of 0.2–5% (Herault et al., 2002; Genoud et al., 2004; Zakany et al., 2004). In theory, TAMERE should work for large distances on paired chromatids except at (and across?) the small segments where they are rigidly linked in the synaptonemal complex. TAMERE has not been tested for Mb size regions.

We confirmed a high frequency of TAMERE over short distances and the previously reported methylation of LoxP sequences inherited from Sycp1-Cre males. We found TAMERE to be ineffective at producing recombination in *cis* or *trans* over a 3.9 Mb region of MMU16. A vector system that is compatible with any ES cell line was used to create the desired asymmetrical reciprocal translocation *in vitro*, producing new mouse models for the study of Down syndrome.

Materials and methods

Animals

Sycp1-Cre mice (Herault et al., 1998) and Z/AP mice (Lobe et al., 1999) were obtained from the Jackson Laboratories (Bar Harbor, ME). Fixation and staining for testes from adult male Z/AP mice were performed as described (Lobe et al., 1999). All experiments for the TAMERE strategy used mice that were established on a C57BL6/J background (Jackson Laboratories, Bar Harbor, ME) by more than five generations of repeated backcrossing. Mice were maintained in a virus antibody-free colony and given breeder chow and water ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee.

Genotyping

Sycp1-Cre mice were genotyped by PCR with primers Cre1 (5′ TGATGGACATGTTCAGGGATC 3′) and Cre2 (5′ CAGCCACCAGCTTGCATGA 3′). PCR reactions contained 2 mM MgCl2 and were performed for 30 cycles of denaturation (30 s, 94°C), annealing (1 min, 59°C), and extension (45 s, 72°C). Z/AP mice were genotyped by staining ear punches as described (Lobe et al., 1999). Typing for Cbr-Lox and Mx-Lox mice has been described previously (Olson et al., 2003; Olson et al., 2004).

Bisulfite sequencing

Mouse tail DNA (10 μg) was denatured in a final concentration of 0.3 M NaOH for 15 min at 37°C, then treated with an equal volume of bisulfite solution (1 mM hydroquinone/2 M sodium metabisulfite/60 mM NaOH) overnight at 55°C. Treated DNA was cleaned with Qiaquick PCR purification kit (Qiagen, Valencia, CA). DNA was treated with a final concentration of 0.3 M NaOH for 15 min at 37°C and neutralized with a final concentration of 3 M NH_4OAc at room temperature for 5 min. Three volumes of ethanol were added and DNA was recovered by centrifugation, washed in 70% ethanol, and resuspended in 40 μl 10 mM Tris–HCl/1 mM EDTA. The PCR reactions (20 μl) contained 4 μl DNA, 1 mM dNTPs, 5 mM MgCl2, and 1 pM each primer: MXPR-M/U (5′ AAAARCRCCTCCCCTACCC 3′) and MXPF2-M/U (5′ GGAYGGTTGGAGAAGAAGGT 3′). PCR parameters were: 30 cycles of denaturation (30 s, 94°C), annealing (30 s, beginning at 65°C and dropping 0.5°C each cycle), and extension (15 s, 72°C), followed by 20 cycles at an annealing temperature of 50°C. PCR products were TA cloned (Invitrogen, Carlsbad, CA), sequenced, and scored for conversion of C to T.

Cre vector testing

Cre vectors were tested by electroporating 10 μg circular plasmid at 0.32 kV and 250 μF using a BioRad (Hercules, CA) electroporator into 1.4×10^6 floxed thymidine kinase cells (kindly provided by M. Dehoff). After three days, gancyclovir (InvivoGen, San Diego, CA) was added to a final concentration of 2 μM. After a

further three days under selection, plates were fixed in methanol and cells were stained with Giemsa (VWR, West Chester, PA).

Results

Testing TAMERE in the Z/AP reporter mouse line

In the TAMERE strategy, Cre recombinase controlled by the *Sycp1* promoter is expressed in male spermatocytes at the time of chromosomal pairing in meiosis (Herault et al., 1998). As a positive control for TAMERE efficacy, we mated Sycp1-Cre mice to Z/AP mice (Lobe et al., 1999), a double-reporter mouse line that expresses *lacZ* prior to Cre-mediated recombination and alkaline phosphatase after recombination. Testes from three adult male double heterozygotes (Z/AP/+, Sycp1-Cre/+) and two Z/AP only control mice were stained to assess Cre activity. Control Z/AP mice exhibited *lacZ* staining but no alkaline phosphatase staining, whereas Z/AP/+, Sycp1-Cre/+ double heterozygous mice exhibited multiple patches of alkaline phosphatase staining (Figure 1). This pattern is expected due to synchronized regions of spermatogenesis. Therefore, TAMERE was effective in generating a deletion between LoxP sites separated by 4.7 kb in *cis*.

Targeted recombination of LoxP sites

We generated two copies of MMU16 with LoxP sites in *trans* at asymmetrical positions using homologous recombination in ES cells (Figure 2a) (Olson et al., 2004). The proximal LoxP sequence was targeted to the *Cbr1* gene (Cbr-Lox), and the distal LoxP sequence was targeted to genomic sequence between the *Mx1* and *Mx2* genes near the distal end of MMU16 (Mx-Lox). The sites are separated by 3.9 Mb on MMU16 and by 5.4 Mb on human chromosome 21. These endpoints are the most commonly cited boundaries of the 'Down syndrome critical region' (Delabar et al., 1993; Korenberg et al., 1994). The cells, and mice derived from them, were used here to test both *in vitro* strategies and TAMERE.

The Cbr-Lox targeting created a null allele of *Cbr1* (Olson et al., 2003). Mice homozygous for the Mx-Lox allele are normal with no observed effect on viability or fertility. Two lines of transgenic mice were created from ES cells carrying either the proximal or distal LoxP site and

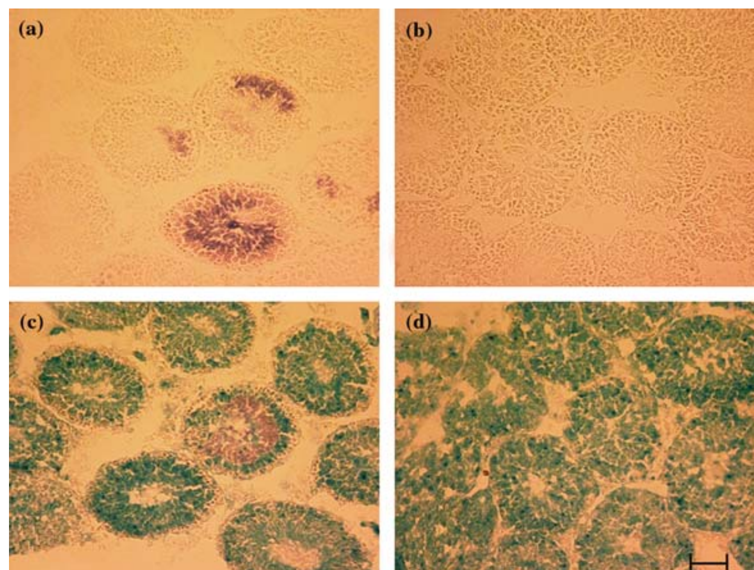

Figure 1. TAMERE is effective in the Z/AP mouse reporter line. Testes from adult male Z/AP and Sycp1-Cre double heterozygous (a and c) mice and Z/AP only (b and d) mice were stained for alkaline phosphatase (a and b) or LacZ plus alkaline phosphatase (c and d). LacZ is expressed prior to Cre recombination and alkaline phosphatase is expressed after Cre recombination. Regional expression in a subset of seminiferous tubules is expected due to the synchronization of spermatogenesis. Bar equals 100 μm.

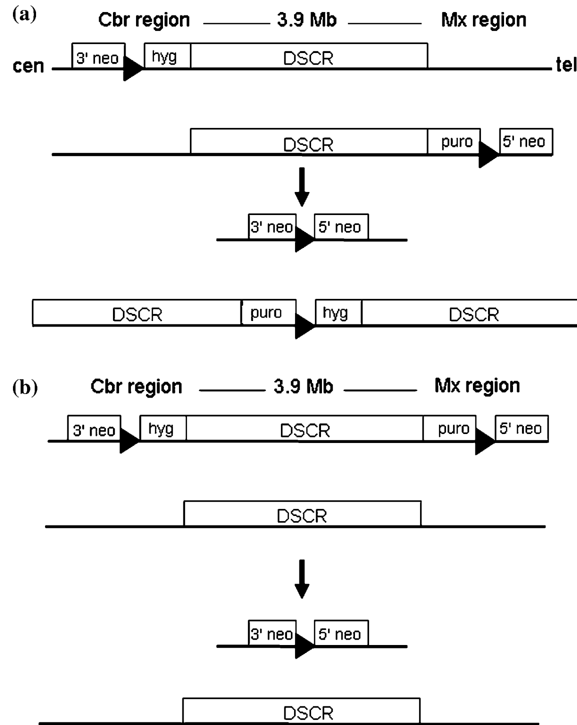

Figure 2. Chromosome engineering strategy to create a deletion and duplication of a region of MMU16. (a) When LoxP sites are in *trans*, Cre-mediated recombination will produce a duplication and a deletion. Each targeting vector contained a LoxP site (triangle), an antibiotic resistance gene (hyg, hygromycin resistance; puro, puromycin resistance), and half of the neomycin resistance gene (5′ or 3′ neo). DSCR, region orthologous to the hypothesized 'Down syndrome critical region' on MMU16; cen, centromere; tel, telomere. (b) A deletion occurs when LoxP sites are in *cis*.

backcrossed to C57BL6/J for five or more generations. These lines were intercrossed to produce mice with LoxP sites in *trans*.

TAMERE did not generate trans *recombination*

We initially generated *'trans-loxing'* fathers carrying the Sycp1-Cre transgene and the Cbr-Lox and Mx-Lox sites in *trans* by mating as in (Figure 3a). Subsequently, it was demonstrated that a LoxP site can become methylated when it is transmitted through the male germ line with Sycp1-Cre, and this methylation inhibits Cre-mediated recombination (Rassoulzadegan et al., 2002). Methylation of LoxP in these *trans*-loxing fathers was confirmed using bisulfite sequencing around the Mx-Lox site in a mouse which inherited the LoxP allele from a Sycp1-Cre expressing father (Figure 3a). The different CpG dinucleotides near LoxP were methylated in 7–36% of sequence reads (n = 14 reads). Clones from the same mouse showed different methylation patterns, reflecting varying degrees of methylation in different cells. This may be due to the young age at which tail biopsies were obtained in this study (ten days), since methylation was reported previously to increase with age from three weeks to three months (Rassoulzadegan et al., 2002). We screened 142 pups for translocation products by PCR from four of these *trans*-loxing fathers. All were negative for the deleted and duplicated chromosomes.

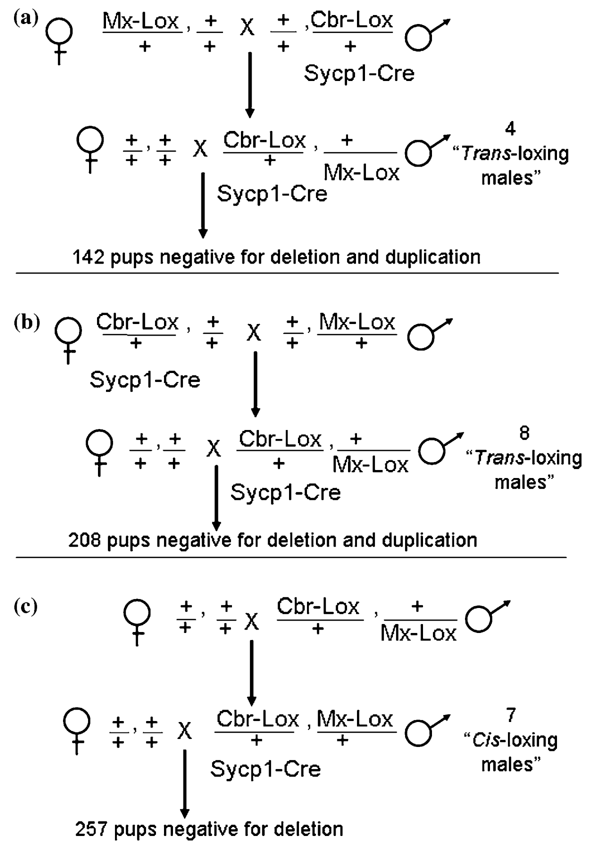

Figure 3. TAMERE mating strategy. (a) *trans*-loxing fathers which inherit a LoxP allele with the Sycp1-Cre transgene through the paternal germline undergo methylation of LoxP sites which inhibits recombination. No deleted or duplicated chromosomes were found in the offspring of these matings. (b) Methylation is avoided when the Sycp1-Cre transgene is inherited through the maternal germline. No deleted or duplicated chromosomes were found in the offspring of these matings. (c) Mice with LoxP sites in *trans* were mated to produce animals with LoxP sites in *cis* following meiotic recombination. These *cis*-loxing mice were used in TAMERE but no deletions were detected.

We then altered breeding strategies to avoid this methylation by introducing Sycp1-Cre and one LoxP site through the maternal germline (Figure 3b). No methylation was observed in a mouse which inherited a LoxP allele and Sycp1-Cre from a female ($n = 9$ sequence reads). However, none of 208 progeny from eight of these *trans*-loxing males contained the deletion or the duplication. To determine whether rare recombinations occurred but were not detected as progeny, DNA isolated from testes of *trans*-loxing fathers was screened for recombination by PCR; no recombination was detected.

TAMERE with LoxP sites in cis *did not generate deletions*

Several *in vitro* studies have shown a higher frequency of Cre-mediated recombination when LoxP sites were in *cis* (producing a deletion, Figure 2b) than when they were in *trans* (producing a translocation, Figure 2a) (Ramirez-Solis et al., 1995; Lindsay et al., 1999; Puech et al., 2000). To test the efficacy of TAMERE for long range recombination of LoxP sites in *cis*, we first generated doubly heterozygous mice with the Cbr-Lox and Mx-Lox sites in *trans* by breeding the singly-targeted mouse lines together. These mice were mated with wild type mice and pups were screened for the presence of both LoxP sites, demonstrating recombination between *Cbr1* and *Mx1*. These genes are separated by 4.2 cM on MMU16 (www.informatics.jax.org). Two recombinant offspring were recovered from 70 pups (2.9%) and used to generate '*cis*-loxing' fathers expressing Sycp1-Cre (Figure 3c). We screened 257 progeny from seven *cis*-loxing fathers by PCR and all were negative for the deletion. PCR of testis DNA from *cis*-loxing fathers did not detect deletions.

Efficacy of Cre expressing plasmids in ES cells

The efficacy of TAMERE may be target-dependent. We used an established *in vitro* method involving the transfection of a Cre expressing plasmid in ES cells to confirm that Cre-mediated recombination between Mx-Lox and Cbr-Lox constructs can occur. To optimize the *in vitro* strategy, Cre expression controlled by several different promoters was tested in the 'floxTK' ES cell line (M. Dehoff, unpublished). FloxTK cells contain a thymidine kinase gene flanked by LoxP sites (floxed). The tested promoters included the major immediate early promoter of human cytomegalovirus (Sauer, 1993), the chicken β-actin/rabbit β-globin hybrid promoter, CAGGS-Cre (Niwa et al., 1991; Puech et al., 2000), and the elongation factor 1a promoter (Gagneten et al., 1997).

FloxTK cells die in the presence of gancyclovir unless the thymidine kinase gene is deleted by Cre-mediated recombination. We transfected the different plasmids into an equal number of cells, selected with gancyclovir, and visualized surviving colonies by Giemsa staining. The CAGGS-Cre vector yielded >10-fold more colonies than the other vectors (Figure 4).

Generation of a T(16C4;16C4)1Rhr translocation in vitro

To demonstrate that a reciprocal translocation could be induced by Cre, we created ES cell lines containing both LoxP sites by transfecting the vectors successively (Olson et al., 2004). Five karyotypically normal ES cell lines were

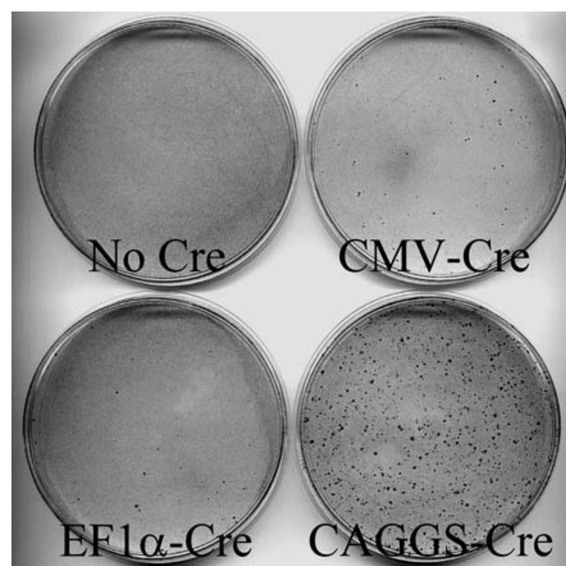

Figure 4. CAGGS-Cre is much more effective than other vectors at deleting a floxed thymidine kinase gene. Cre-mediated recombination confers resistance to gancyclovir by deleting the thymidine kinase gene. Colonies were stained with Giemsa six days after transfection (three days of gancyclovir selection). CMV, cytomegalovirus promoter; EF1α, elongation factor 1α promoter; CAGGS, chicken β-actin/rabbit β-globin hybrid promoter.

produced that contained both Cbr-Lox and Mx-Lox sites. It was unknown whether these sites were located in *cis* or *trans* in a given line.

In contrast to previous reports of Cre-mediated chromosome rearrangement which required the use of *Hprt*-null ES cell lines (Ramirez-Solis et al., 1995; Liu et al., 1998; Lindsay et al., 1999; Mills & Bradley, 2001; Yu & Bradley, 2001), our approach can utilize any ES cell line, since successful Cre recombination reconstitutes a neomycin^R cassette (Figure 2). Double LoxP-targeted cell lines were transfected with CAGGS-Cre plasmid and grown in G418 to select for those cells that had undergone Cre recombination. One cell line gave rise to two G418 resistant colonies; as these arose on the same plate, it is unclear whether they were independent clones. Both clones retained both hygromycin and puromycin resistance, indicating that the parental line contained LoxP sites in *trans* and that Cre recombination had therefore produced an asymmetrical reciprocal translocation (Figure 2a). If LoxP sites had been in *cis*, only a deletion would have occurred and cells would have lost resistance to both hygromycin and puromycin (Figure 2b).

Discussion

The TAMERE strategy, although effective for recombination with target regions up to 100 kb, may not be useful for creating deletions or duplications over distances that are substantially larger. We confirm the TAMERE strategy here in a mouse reporter line but show that it was ineffective in generating recombination either in *cis* or in *trans* on MMU16 homologues. However, we were successful in generating this translocation by optimizing an *in vitro* method for use in any ES cells. Previous approaches required the use of *Hprt*-null cell lines for chromosomal engineering (Adams et al., 2004).

Chromosomally engineered mice have many potential uses. Large inversions can be used as balancer chromosomes to maintain stocks of mutant mice (Yu & Bradley, 2001). Deleted chromosomes are advantageous for targeted mutagenesis strategies that elucidate the function of unknown genes or create novel alleles of known genes. In a typical mutagenesis scheme, recessive mutations are not detected until the third generation (G3), requiring large breeding colonies. By using mice carrying large deletions, a recessive mutation corresponding to the deleted segment will be uncovered in the G2 generation.

Chromosome-engineered mice can also be used to model human aneuploid disorders. Engineering aneuploid mice to model human development has advantages over radiation-induced (Davisson et al., 1990), fortuitously recovered (Sago et al., 1998), or transgenic (Kola & Pritchard, 1999) mouse models of aneuploidy. Endpoints for the aneuploid segment can be chosen to test specific hypotheses about dosage imbalance, eliminating complicating effects of irrelevant segments of aneuploidy or gene rearrangements that occur during spontaneous translocations. Large genomic alterations more closely resemble human aneuploid conditions; transgenic mice do not always express genes at levels analogous to those of human aneuploidy, and even large insert (BAC, PAC) transgenics may not carry all coding or regulatory sequences for a given gene. YAC transgenics often are rearranged or missing segments (Smith et al., 1995; Kola & Pritchard, 1999).

The endpoints of the translocated segment described here are the most commonly cited boundaries of the 'Down syndrome critical region', the smallest region of overlap among individuals with segmental trisomy for human chromosome 21 who share specific Down syndrome features (Delabar et al., 1993; Korenberg et al., 1994). Down syndrome features associated with this region include abnormalities of the face and craniofacial skeleton, mental retardation, joint hyperlaxity, muscle hypotonia, short stature and a variety of dermatoglyphic abnormalities. Barlow et al., have defined a congenital heart disease region on chromosome 21 that overlaps substantially with this region (Barlow et al., 2001). The mice generated with this deletion and duplication enable hypothesis testing about gene action in Down syndrome (Figure 5). Segmentally trisomic mice can be tested for quantifiable phenotypes with direct parallels to Down syndrome, and monosomic mice can be bred to currently existing mouse models such as the Ts65Dn mouse to subtract this segment from the larger Ts65Dn trisomic region (Reeves et al., 2001; Olson et al., 2004).

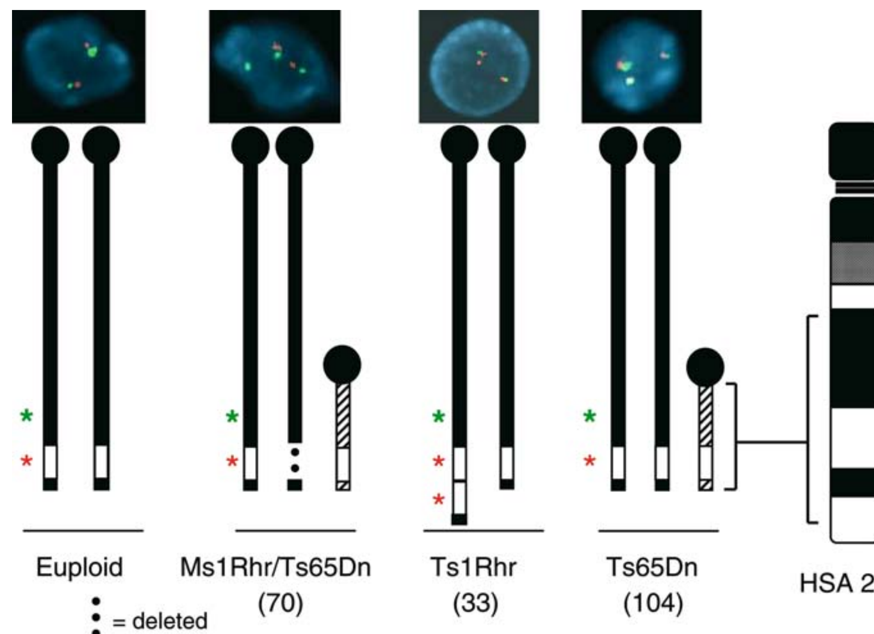

Figure 5. Chromosome engineering provides new models to study dosage sensitive genes in mouse models of trisomy. Interphase FISH analysis of chromosomes from mice containing a normal chromosome complement (euploid) and from Down syndrome models, Ms1Rhr/Ts65Dn, Ts1Rhr and Ts65Dn. The cartoons indicate the positions of FISH probes relative to triplicated or deleted segments and the segmental trisomy in each model. Superimposition of green and red signals is shown as a yellow signal in Ts65Dn. Numbers in parentheses indicate the number of conserved genes orthologous to HSA21 (Gardiner et al., 2003) that are triplicated in each model.

Chromosome engineering is a time- and resource-consuming endeavor. Consequently, few comparisons have been made of the different methods available. Our results suggest that TAMERE is most effective across short distances, while expression of Cre under optimal promoters in ES cells provides a versatile system for recombination across long distances.

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References

Adams DJ et al., (2004) Mutagenic insertion and chromosome engineering resource (MICER). *Nat Genet* **36**: 867–871.

Barlow GM et al., (2001) Down syndrome congenital heart disease: a narrowed region and a candidate gene. *Genet Med* **3**: 91–101.

Davisson MT, Schmidt C, Akeson E (1990) Segmental trisomy of murine chromosome 16: A new model system for studying Down Syndrome. *Prog Clin Biol Res* **360**: 263–280.

Delabar JM et al. (1993) Molecular mapping of twenty-four features of Down syndrome on chromosome 21. *Eur J Hum Genet* **1**: 114–124.

Gagneten S, Le Y, Miller J, Sauer B (1997) Brief expression of a GFP cre fusion gene in embryonic stem cells allows rapid retrieval of site-specific genomic deletions. *Nucleic Acids Res* **25**: 3326–3331.

Gardiner K, Fortna A, Bechtel L, Davisson MT (2003) Mouse models of Down syndrome: how useful can they be? Comparison of the gene content of human chromosome 21 with orthologous mouse genomic regions. *Gene* **318**: 137–147.

Genoud N et al. (2004) Disruption of Doppel prevents neurodegeneration in mice with extensive Prnp deletions. *Proc Natl Acad Sci USA* **101**: 4198–4203.

Herault Y, Kmita M, Sawaya CC, Duboule D (2002) A nested deletion approach to generate Cre deleter mice with progressive Hox profiles. *Int J Dev Biol* **46**: 185–191.

Herault Y, Rassoulzadegan M, Cuzin F, Duboule D (1998) Engineering chromosomes in mice through targeted meiotic recombination (TAMERE). *Nat Genet* **20**: 381–384.

Hernandez D, Fisher EM (1999) Mouse autosomal trisomy: two's company, three's a crowd. *Trends Genet* **15**: 241–247.

Kola I, Pritchard M (1999) Animal models of Down syndrome. *Mol Med Today* **5**: 276–277.

Korenberg JR et al. (1994) Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc Natl Acad Sci USA* **91**: 4997–5001.

Lindsay EA et al., (1999) Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature* **401**: 379–383.

Liu P, Zhang H, McLellan A, Vogel H, Bradley A (1998) Embryonic lethality and tumorigenesis caused by segmental aneuploidy on mouse chromosome 11. *Genetics* **150**: 1155–1168.

Liu X et al. (1997) Trisomy eight in ES cells is a common potential problem in gene targeting and interferes with germ line transmission. *Dev Dynam* **209**: 85–91.

Lobe CG, Koop KE, Kreppner W, Lomeli H, Gertsenstein M, Nagy A (1999) Z/AP, a double reporter for cre-mediated recombination. *Dev Biol* **208**: 281–292.

Longo L, Bygrave A, Grosveld FG, Pandolfi PP (1997) The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism. *Transgenic Res* **6**: 321–328.

Mills AA, Bradley A (2001) From mouse to man: generating megabase chromosome rearrangements. *Trends Genet* **17**: 331–339.

Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* **108**: 193–199.

Olson LE, Bedja D, Alvey SJ, Cardounel AJ, Gabrielson KL, Reeves RH (2003) Protection from doxorubicin-induced cardiac toxicity in mice with a null allele of carbonyl reductase 1. *Cancer Res* **63**: 6602–6606.

Olson LE, Richtsmeier JT, Leszl J, Reeves RH (2004) A chromosome 21 critical region does not cause specific down syndrome phenotypes. *Science* **306**: 687–690.

Puech A et al. (2000) Normal cardiovascular development in mice deficient for 16 genes in 550 kb of the velocardiofacial/DiGeorge syndrome region. *Proc Natl Acad Sci USA* **97**: 10090–10095.

Ramirez-Solis R, Liu P, Bradley A (1995) Chromosome engineering in mice. *Nature* **378**: 720–724.

Rassoulzadegan M, Magliano M, Cuzin F (2002) Transvection effects involving DNA methylation during meiosis in the mouse. *Embo J* **21**: 440–450.

Reeves RH, Baxter LL, Richtsmeier JT (2001) Too much of a good thing: mechanisms of gene action in Down syndrome. *Trends Genet* **17**: 83–88.

Sago H et al. (1998) Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. *Proc Natl Acad Sci USA* **95**: 6256–6261.

Sauer B (1993) Manipulation of transgenes by site-specific recombination: use of Cre recombinase. *Method Enzymol* **225**: 890–900.

Smith DJ, Zhu Y, Zhang J, Cheng JF, Rubin EM (1995) Construction of a panel of transgenic mice containing a contiguous 2-Mb set of YAC/P1 clones from human chromosome 21q22.2. *Genomics* **27**: 425–434.

Tronche F, Casanova E, Turiault M, Sahly I, Kellendonk C (2002) When reverse genetics meets physiology: the use of site-specific recombinases in mice. *FEBS Lett* **529**: 116–121.

Vidal F, Sage J, Cuzin F, Rassoulzadegan M (1998) Cre expression in primary spermatocytes: a tool for genetic engineering of the germ line. *Mol Reprod Dev* **51**: 274–280.

Yu Y, Bradley A (2001) Engineering chromosomal rearrangements in mice. *Nat Rev Genet* **2**: 780–790.

Zakany J, Kmita M, Duboule D (2004) A dual role for Hox genes in limb anterior–posterior asymmetry. *Science* **304**: 1669–1672.