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Multiplex ligation-dependent probe amplification (MLPA) genotyping assay for mouse models of down syndrome

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1. ABSTRACT

The Ts65Dn mouse is the most widely investigated segmentally trisomic mouse model of Down syndrome. Quantitative PCR based methods are the preferred way of detecting the trisomic segment for genotyping purposes. However, identification of a 1.5 fold difference in target DNA is at the limit of detection of most quantitative PCR based methods, and in practice this can lead to difficulties in assigning genotypes. We report a 100% accurate multiplex ligation-dependent probe amplification (MLPA) assay for genotyping the Ts65Dn mouse that is also applicable to all other segmentally trisomic mouse models of Down syndrome.

2. INTRODUCTION

Down syndrome is caused by trisomy of all or part of human chromosome 21 (HSA21). Modeling Down syndrome in the mouse is complicated by the size of the trisomic region and the fact that the mouse genomic segments orthologous to HSA21 are distributed amongst mouse chromosomes (MMUs) 10, 16 and 17. Consequently, a range of approaches have been used to generate a variety of mouse models (1-5). These include transgenic models that over-express individual or a combination of genes, transgenic mice that have large pieces of foreign DNA introduced by yeast artificial chromosomes (YACs) or bacterial artificial chromosomes (BACs), models which are trisomic for all or segments of MMU16 (distal MMU16 is the largest region of synteny to HSA21), and most recently an aneuploid mouse strain engineered to carry 92% of HSA21.

Currently, the segmental trisomies are the most commonly studied models of Down syndrome (Figure 1). These include Ts65Dn, the largest segmental trisomy and most widely investigated model, which bears a small marker chromosome stretching from *Mrpl39* to *Znf295* (6, 7). A chromosomal rearrangement of the Ts65Dn marker chromosome to MMU12 subsequently created a stable Robertsonian chromosome producing the Ts[Rb(12.17^{16})]2Cje model (8). The Ts1Cje model has a smaller region of trisomy and was derived through a translocation of the distal end of MMU16 (*Sod1* to *Znf295*, *Sod1* is not functional) associated with homologous

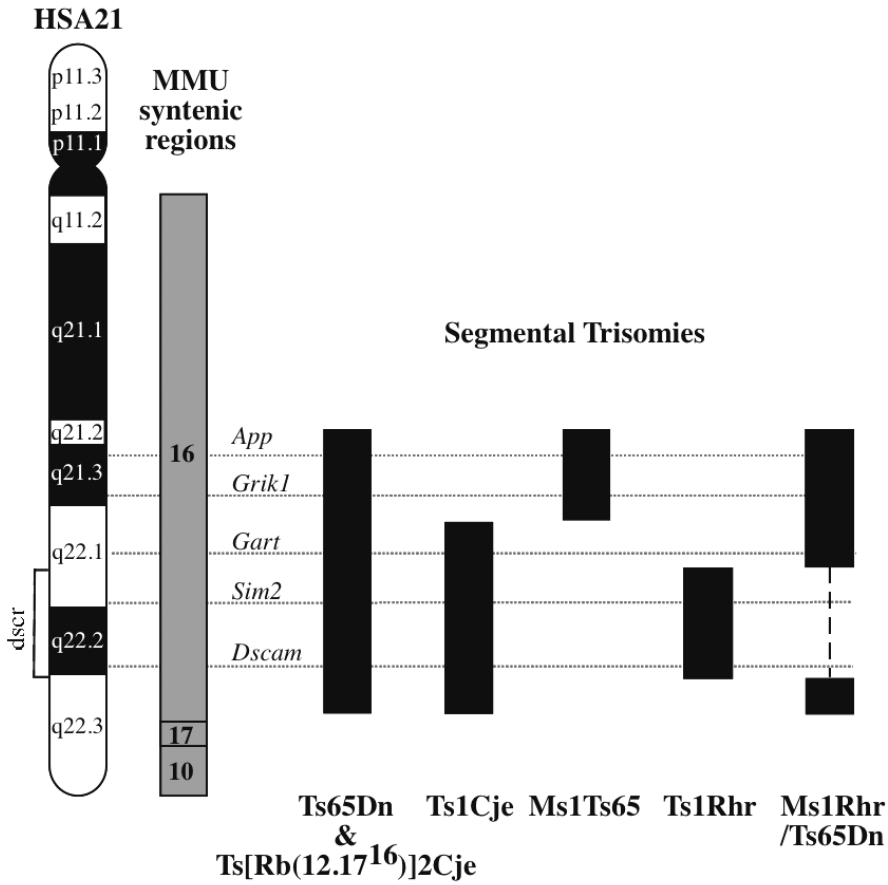

Figure 1. Segmentally trisomic mouse models of Down syndrome. Distal HSA21 is syntenic to MMU10, 16 and 17 represented in grey. A comparison of the regions of MMU16 triplicated in the different segmentally trisomic mouse models is shown using black rectangles. A dashed line indicates the region of deletion in the Ms1Rhr/Ts65Dn model. The distribution of MLPA target genes is highlighted demonstrating that the assay can be used for genotyping of all the segmental mouse models of Down syndrome. dscr = Down syndrome critical region.

recombination during a gene targeting event for *Sod1* (9). A fourth model, Ms1Ts65 which is trisomic for the region between *Mrp139* and the disrupted *Sod1*, also exists (5). Finally, using an elegant chromosomal engineering strategy, mice trisomic (Ts1Rhr) for the so called "Down syndrome critical region" (DSCR) and mice trisomic for genes found in the Ts65Dn fragment with the DSCR deleted (Ms1Rhr/Ts65Dn) have also recently been created (4).

Whilst the majority of these models can be genotyped by simple PCR due to the introduction of foreign DNA during their creation, detection of the Ts65Dn and Ts[Rb(12.17^{16})]2Cje trisomic segments is not so straightforward. An ideal genotyping protocol for the Ts65Dn and Ts[Rb(12.17^{16})]2Cje models would involve PCR amplification across the translocation breakpoint. However, the exact location of the breakpoint is currently unknown.

The trisomic segments in the Ts65Dn and Ts[Rb(12.17^{16})]2Cje models were, until recently, identified by chromosome analysis from peripheral blood or fluorescent in situ hybridization (FISH) (10-12). More recently, rapid, high throughput and less labor-intensive quantitative PCR based methods have been developed that compare the amplification products of trisomic and disomic genes (13, 14). However, detection of a 1.5 fold difference in amplification product over and above natural sample variation stretches these techniques to their limits and consequently differentiation of trisomic and disomic animals is often difficult.

We report a single tube, high throughput, 100% accurate genotyping protocol for Ts65Dn and Ts[Rb(12.17^{16})]2Cje using multiplex ligation-dependent probe amplification (MLPA). MLPA is a method used to establish the copy number of multiple target regions in the genome and is primarily used for diagnostic purposes in a wide range of human diseases, including Down syndrome (15-18). Briefly, sequence specific probes of varying lengths containing universal end sequences are hybridized to each target region. Each probe is then amplified simultaneously using one pair of fluorescently labeled primers complementary to the universal sequence. Amplification products are subsequently separated by

acrylamide gel electrophoresis. In contrast to other quantitative PCR methods, MLPA utilizes a single pair of PCR primers resulting in minimal variation in amplification efficiency between target regions. Comparison of target probe to control probe amplification products and of unknown samples to those of known genotype indicates which samples contain partial trisomy of MMU16. In addition, the distribution of the probes along MMU16 allows the differentiation of all published segmentally trisomic mouse models of Down syndrome with varying lengths of trisomy. Furthermore, this MLPA assay may be applied to the other mouse models of Down syndrome and even to other aneuploid mouse models by simply altering probe sequences.

3. MATERIALS AND METHODS

3.1. Mice

Ts65Dn, Ts[Rb(12.1716)]2Cje, Ts1Cje mice and their diploid littermates were used in the development of an MLPA genotyping assay. All mice were originally obtained from The Jackson Laboratories (Bar Harbor, ME, USA). DNA from the original Ts[Rb(12.17^{16})]2Cje and disomic control mice sent from The Jackson Laboratories (Bar Harbor, ME, USA) were used as trisomic and disomic controls throughout the development of the MLPA assay. A set of Ts65Dn and diploid tail clips were also obtained from The Jackson Laboratories (Bar Harbor, ME, USA).

Ts65Dn mice were genotyped previously using the real-time quantitative PCR assay with the probe set for *App* described by Lui *et al*. (14). Ts1Cje mice were genotyped previously using a duplex PCR amplification of the neomycin resistance sequence and *App* as an internal control, primer sequences have been previously described in Olson *et al*. (4) and Lui *et al*. (14) respectively.

3.2. Sample Preparation

High quality genomic DNA, was prepared by conventional methods and stored in Tris EDTA. DNA concentration was established using a UV spectrophotometer (Varian, Palo Alto, CA, USA) and MLPA reactions were performed on 50-200nanograms of genomic DNA.

3.3. Multiplex Ligation-Probe Dependent Amplification (MLPA)

MLPA was completed as described by Shouten *et al*. (15). However, 2 synthetic oligonucleotides were used rather than one being M13-derived, an adaptation previously described by White *et al*. (16).

3.3.1. Probe Design

Pairs of probes were designed for 16 regions of the mouse genome, including 9 specific for chromosome 16, and 7 control pairs for chromosomes other than 16 (Table 1, Figure 2). To control for potential effects of amplification product size on electrophoresis and PCR efficiency, the assay was designed so that the control probe amplification products had no size bias and were distributed throughout the size-range of all products.

To allow simultaneous amplification by a single primer pair all probes have identical sequences at their 5' and 3' ends as described in Schouten *et al*. (15). To permit separation of PCR amplification product, each probe set was designed to produce products that differ by between 3 and 7bp; including universal sequences the products range from 100 to 174bp (Table 1). Probes were designed according to instructions on the MRC-Holland website (http://www.mrc-holland.com). Briefly, the hybridization sequences of the probes were complementary to the genomic sequence from the University of Santa Cruz website (UCSC; http://genome.ucsc.edu), with a GC content between 45-60%, and a Tm >70^{o}C (defined using the RAW program MRC-Holland, Amsterdam, The Netherlands). The BLAT program from the UCSC website was used to ensure specific hybridization of probes only to target sequence (19).

Probe oligonucleotides were ordered from Sigma Genosys (Australia, http://www.sigma-genosys.com/) at a synthesis scale of 0.05micromoles and were cartridge purified. The 5' end of each downstream oligonucleotide was phosphorylated to allow ligation of the probe. The probe mix was prepared by combining each oligonucleotide to a final concentration of 0.9femtomoles/microlitre.

3.3.2. Product Separation and Data Analysis

Product separation was performed using capillary electrophoresis on the ABI3730 DNA Analyzer with the GeneScan™ 500 LIZ™ size standard (Applied Biosystems, Foster City, CA, USA).

Genemapper Software version 3.7 (Applied Biosystems, Foster City, CA, USA) was used to analyze sample files collected by the Data Collection Software version3.0 (Applied Biosystems, Foster City, CA, USA). Quantitative analysis was completed in Excel (Microsoft; www.microsoft.com) essentially as described by White *et al*. (16). The 3 disomic probes Gad2-1, Edar-1 and Pls-2 were used as reference peaks. Rather than using the median ratio across all samples for normalizing each probe to 1.0, we took the median of the 2 disomic control samples for all probes for chromosomes other than X and Y. For Y chromosome specific probes we took the median of all samples giving a reading (i.e. not female) and doubled it. For X chromosome specific probes the median of all female samples was used.

4. RESULTS AND DISCUSSION

Twenty known Ts65Dn and 20 disomic littermate control DNA samples of mixed sex, and 20 Ts1Cje and 20 disomic littermate control DNA samples of mixed sex were randomized blindly and then genotyped using the MLPA assay. Results were compared to genotyping results obtained previously using a Taqman quantitative PCR assay for Ts65Dn mice and PCR for the neomycin resistance sequence for Ts1Cje mice (4, 14). An example of typical MLPA results is shown in Figure 3. The MLPA results were 100% concordant for both genotype and sex of all 80 mice, demonstrating that MLPA can be used to differentially genotype segmentally trisomic mouse models

Table 1 Probe information

Probe	Chr.	Hybridizing sequence 5'-3'	Product Size (bp)	Purpose
Gad2-1 U	2	CCTGGTGAGTGCCACAGCTGGAACCACC	100	1
Gad2-1 D		GTGTATGGGGCTTTTGATCCTCTCTTGGC		
Gart-1 U	16	CCTGACCTCTGCAGGAGTACGATGC	104	3
Gart-1 D		TTTGGCCCCACAGCACAAGCAGCTCAATTAGAGTCC		
Sim2-1 U	16	GGTCATTCACTGCAGCGGCTACCTAAAGATC	110	5
Sim2-1 D		AGACAGTACATGCTGGACATGTCCCTGTACGACTCC		
Sry-1 U	Y	CAGCAGCAGTTCCACGACCAGCAGCTTACCTACTT	115	2
Sry-1 D		ACTAACAGCTGACATCACTGGTGAGCATACACCATAC		
Dscam-1 U	16	CAGTCCGTGTGGAGGACCAGAAAACCATGAGAGGCAATGTC	120	5
Dscam-1 D		GCGGTGTTCAAGTGCATTATCCCCTCCTCGGTGGAG		
App-1 U	16	CGACAAGTTCCGAGGGGTAGAGTTTGTATGCTGCCCGTTGGCCGAGGA	125	4
App-1 D		AAGCGACAGCGTGGATTCTGCGGATGCAGAGGAG		
Dcx-1 U	X	GTGTACGCTGTTTCTTCTGACCGTTTTCGTAGTTTTGATGCGTTGCTGG	130	2
Dcx-1 D		CTGACCTGACCCGATCCTTGTCTGACAACATTAACCTG		
Gart-2 U	16	CAATGATCACAGTGCCCTCGCTCAGTTCTGCAAGGATGAA	135	3
Gart-2 D		AAGATTGAACTCGTAGTTGTCGGACCAGAGGCCCCTCTGGCTGCAGGTAACC		
Edar-1 U	10	GATGCCTCCTCTGAGAACGAGCAGTTGCTAAGTCGCAGTGTGGACAGTGATG	140	1
Edar-1 D		AAGAGCCAGCCCCGGACAAGCAGGGGTCCCCAGAGCTATGTCTGC		
Grik1-1 U	16	GGTCCTTCCCACAGCTCCTCCGTCAGTGCTGTACAGTCTATTTGCAATGCTCTGGAA	145	4
Grik1-1 D		GTTCCACACATTCAGACTCGCTGGAAACACCCTTCTGTGGACAAC		
Gpc3-1 U	X	CGTGGATGATATGGTCAATGAATTGTTCGACAGCCTCTTTCCAGTCATCTACACCCAG	150	2
Gpc3-1 D		ATGATGAACCCAGGCCTGCCTGAGTCAGTCTTAGACATCAACGAGTGCC		
Sim2-2 U	16	CGAGTTTTACGAGCTGGCCAAGCTGCTCCCGCTGCCTTCGGCCATCACCT	155	5
Sim2-2 D		CGCAGCTGGACAAAGCGTCCATCATCCGACTCACCACCAGCTACCTGAAGATGCGCGCGGTC		
App-2 U	16	GGTCATGAGAGAATGGGAAGAGGCAGAGCGTCAAGCCAAGAACTTGCCCAAAGCTGACAAGAAGGCCGTTATCC	162	4
App-2 D		AGGTAACACCCCGGTCCCACCTACACCAGACAGAACATGCCAC		
Sry-2 U	Y	CTGGGATGCAGGTGGAAAAGCCTTACAGAAGCCGAAAAAAGGCCCTTTTT	165	2
Sry-2 D		CCAGGAGGCACAGAGATTGAAGATCCTACACAGAGAGAAATACCCAAACTATAAATATCAGCCTCATCGGAG		
Dscam-2 U	16	CATCCACGGGAGCCTTGTATATTAAAGATGTACAGAACGAAGATGGGCTGTACAACTACCGCTGC	170	5
Dscam-2 D		ATCACGCGGCACAGATACACAGGGGAGACGAGACAAAGCAACAGCGCGAGACTGTTCGTGTC		
Pls-2 U	19	CCGAATGATGTTGCTGATGAACATCAAGACCTATCCAGTAACGAGTCCAGAAACCCAGC	174	1
Pls-2 D		AGACGAAGCCTTCCGACGAAGGCTGATTGCAAACCTGGCTGAGCACATTCTCTTCAGTAAGTAAGCTCACAC		

The unique sequence of the two halves of each hybridization probe are given, these are combined with the universal sequences described in Schouten *et al*. (15). 1 = Disomic control, 2 = Copy number control, 3 = Trisomic in Ts65Dn, Ts[Rb(12.17^{16})]2Cje, Ms1Rhr/Ts65Dn and Ts1Cje, 4 = Trisomic in Ts65Dn, Ts[Rb(12.17^{16})]2Cje and Ms1Rhr/Ts65Dn, 5 = Trisomic in Ts65Dn, Ts[Rb(12.17^{16})]2Cje, Ts1Rhr and Ts1Cje, U, upstream hybridizing sequence, D, downstream hybridizing sequence, Chr., chromosome, bp base pair. For further clarification of the purpose of the trisomic probes in differentiating between the different segmentally trisomic mouse models refer to Figure 1.

of Down syndrome and determine their sex with a sensitivity and specificity of 100%. In addition, the MLPA assay has been used to genotype 195 Ts[Rb(12.17^{16})]2Cje mice and successfully maintain the colony for 11 months. From the MLPA genotyping results we have designated 6 new female breeders that have all produced trisomic progeny.

Karyotypic methods available for genotyping of Ts65Dn and Ts[Rb(12.17^{16})]2Cje mice include chromosomal analysis and interphase FISH (10-12). Chromosomal analysis takes 2-3 days, involves cell culture and is difficult to use on mice younger than 6-7 weeks of age as a mature immune system is required (11). Interphase FISH is labor intensive and cumbersome and takes about 18 hours (10, 12). More recently published quantitative PCR based methods that compare the copy number of trisomic genes to disomic genes are more accessible, less labor intensive and less time consuming (13, 14). Liu *et al*. (14) reported a real-time quantitative PCR method based on

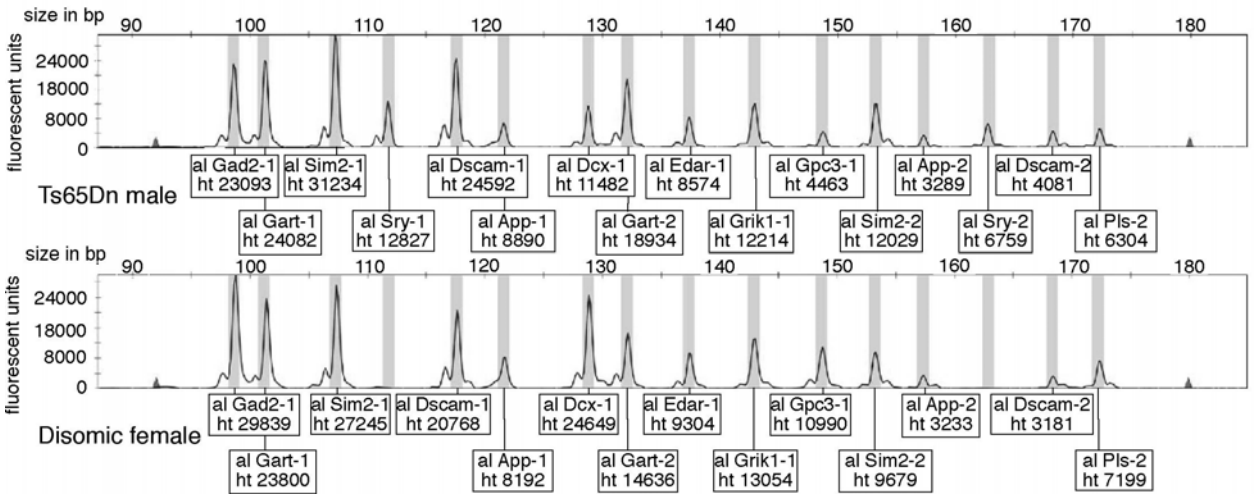

Figure 2. Electropherogram traces. The traces are a visual representation of the raw data demonstrating the size distribution in base pairs (*x* axis) and level of fluorescence in fluorescent units (*y* axis) of the amplification products in a male Ts65Dn compared to a female disomic mouse. al = allele, ht = peak height.

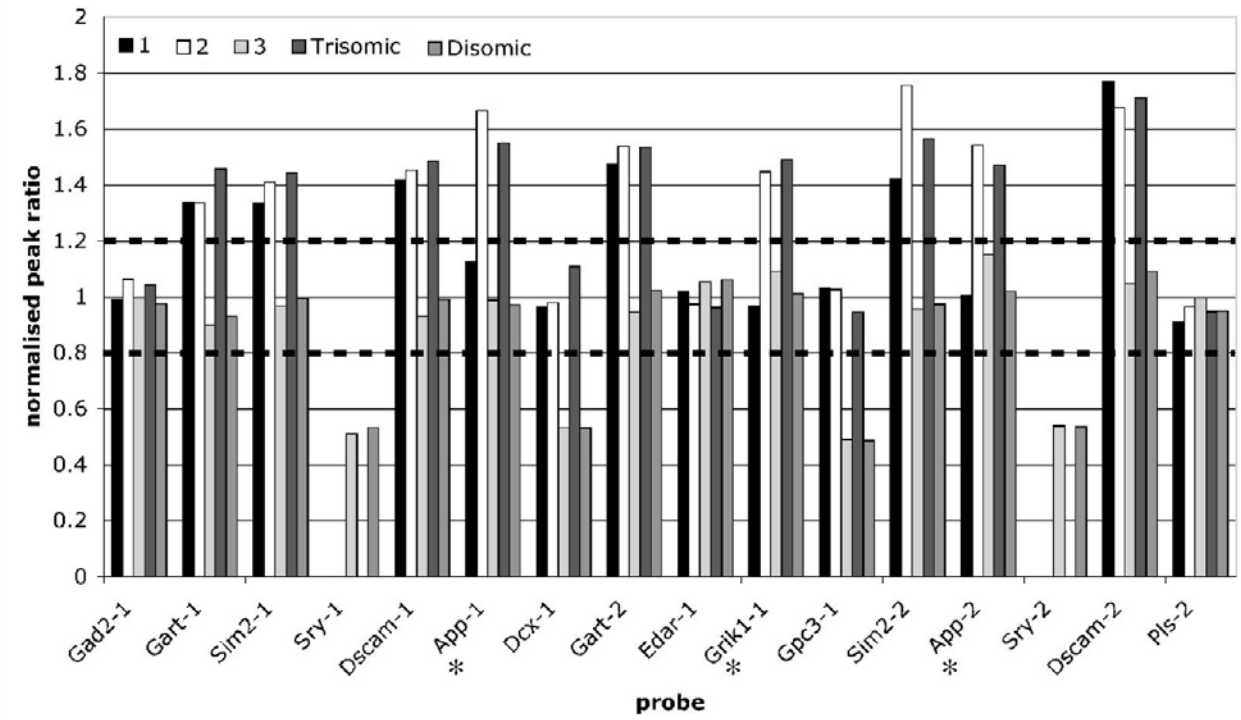

Figure 3. Typical MLPA results. Graph showing the copy number of each target probe relative to the disomic control probes. Probes that fall within the dotted lines are considered to be disomic with a copy number of 2. The trisomic animals can be easily separated from disomic animals as samples that have normalized peak ratios of >1.2 for probes that lie within the trisomic region. Probes highlighted with an asterisk (*) allow differentiation of Ts65Dn from Ts1Cje mice due to their position on MMU16. For further information on probe distribution refer to Table 1 and Figure 1. 1-3 = mice with unknown genotype, Trisomic = trisomic control, Disomic = disomic control. Sample 1 is a Ts1Cje female, sample 2 is a Ts65Dn female and sample 3 is a disomic male mouse.

the average change in cycle threshold of target gene *App* or *Mx1* from that of an internal control *Apob*. Ramakrishna *et al*. (13) reported a PCR method, which relies on the co-amplification of target genes *App* and *Dyrk1a* with a control gene *Acta1* and resolution and quantification by two independent methods: agarose gel electrophoresis and capillary electrophoresis. The difference in copy number of trisomic genes between a trisomic and disomic animal is 1.5 fold. Detection of such a small difference over and above natural variation in amplification product stretches

the detection limit of most relative quantification methods leading to unreliable results. One of the main sources of variation in amplification between samples is the variation in amplification efficiency due to the use of different primer pairs specific to each of the target genes in the PCR. A major benefit of MLPA is that each probe is amplified using a universal primer pair and therefore with more analogous efficiency.

A further advantage of the MLPA assay is the large number of target sequences that can be analyzed simultaneously. This allows for the investigation of multiple trisomic targets, disomic controls and copy number controls in one reaction resulting in a more conclusive result and less requirement for repetition. Out of 16 probes, 9 are trisomic in Ts65Dn and Ts[Rb(12.17^{16})]2Cje mice, 3 are disomic controls and 4 are copy number controls. The copy number controls are probes specific to the X and Y-chromosomes and the MLPA assay can therefore also act as a sex determination test.

The real-time quantitative PCR assay is currently the most frequently used genotyping method for Ts65Dn and Ts[Rb(12.17^{16})]2Cje mice. Similar to the real-time assay, initial set up of the MLPA method is relatively expensive due to the cost of long probe oligonucleotides and 5' phosphorylation. However, 50nanomoles of each oligonucleotide is enough for 37.5 million reactions and therefore they are unlikely to require reordering. As a result, in the long-term the MLPA assay is cheaper than the real-time quantitative method. The MLPA assay is easy to perform and takes less than 22 hours to complete, with actual hands on experimental and analysis time of approximately 3 hours. Despite similar hands-on time, the real-time assay is comparatively more rapid, taking approximately 4 hours from start to finish. However, in our opinion, the simplicity and increased accuracy of genotyping using the MLPA assay more than compensates for the increase in assay duration.

In summary we present a simple, high throughput, 100% reliable, genotyping MLPA method for the identification of segmentally trisomic mouse models of Down syndrome.

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6. REFERENCES

1. I. Kola & P. J. Hertzog: Down syndrome and mouse models. *Curr Opin Genet Dev*, 8, 316-21 (1998)
2. M. Dierssen, C. Fillat, L. Crnic, M. Arbones, J. Florez & X. Estivill: Murine models for Down syndrome. *Physiol Behav*, 73, 859-71 (2001)

3. A. O'Doherty, S. Ruf, C. Mulligan, V. Hildreth, M. L. Errington, S. Cooke, A. Sesay, S. Modino, L. Vanes, D. Hernandez, J. M. Linehan, P. T. Sharpe, S. Brandner, T. V. Bliss, D. J. Henderson, D. Nizetic, V. L. Tybulewicz & E. M. Fisher: An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. *Science*, 309, 2033-7 (2005)

4. L. E. Olson, R. J. Roper, L. L. Baxter, E. J. Carlson, C. J. Epstein & R. H. Reeves: Down syndrome mouse models Ts65Dn, Ts1Cje, and Ms1Cje/Ts65Dn exhibit variable severity of cerebellar phenotypes. *Dev Dyn*, 230, 581-9 (2004)

5. H. Sago, E. J. Carlson, D. J. Smith, E. M. Rubin, L. S. Crnic, T. T. Huang & C. J. Epstein: Genetic dissection of region associated with behavioral abnormalities in mouse models for Down syndrome. *Pediatr Res*, 48, 606-13 (2000)

6. P. Kahlem, M. Sultan, R. Herwig, M. Steinfath, D. Balzereit, B. Eppens, N. G. Saran, M. T. Pletcher, S. T. South, G. Stetten, H. Lehrach, R. H. Reeves & M. L. Yaspo: Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of down syndrome. *Genome Res*, 14, 1258-67 (2004)

7. E. C. Akeson, J. P. Lambert, S. Narayanswami, K. Gardiner, L. J. Bechtel & M. T. Davisson: Ts65Dn -- localization of the translocation breakpoint and trisomic gene content in a mouse model for Down syndrome. *Cytogenet Cell Genet*, 93, 270-6 (2001)

8. A. J. Villar, P. V. Belichenko, A. M. Gillespie, H. M. Kozy, W. C. Mobley & C. J. Epstein: Identification and characterization of a new Down syndrome model, Ts[Rb (12.1716)]2Cje, resulting from a spontaneous Robertsonian fusion between T (171)65Dn and mouse chromosome 12. *Mamm Genome*, 16, 79-90 (2005)

9. H. Sago, E. J. Carlson, D. J. Smith, J. Kilbridge, E. M. Rubin, W. C. Mobley, C. J. Epstein & T. T. Huang: Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. *Proc Natl Acad Sci U S A*, 95, 6256-61 (1998)

10. J. R. Korenberg, X. N. Chen, K. L. Devon, D. Noya, M. L. Oster-Granite & B. W. Birren: Mouse molecular cytogenetic resource: 157 BACs link the chromosomal and genetic maps. *Genome Res*, 9, 514-23 (1999)

11. M. T. Davisson & E. C. Akeson: An improved method for preparing G-banded chromosomes from mouse peripheral blood. *Cytogenet Cell Genet*, 45, 70-4 (1987)

12. J. Strovel, J. Stamberg & P. J. Yarowsky: Interphase FISH for rapid identification of a down syndrome animal model. *Cytogenet Cell Genet*, 86, 285-7 (1999)
13. N. Ramakrishna, C. Meeker, S. Li, E. C. Jenkins, J. R. Currie, M. Flory, B. Lee, M. S. Liu & D. L. Miller: Polymerase chain reaction method to identify Down syndrome model segmentally trisomic mice. *Anal Biochem*, 340, 213-9 (2005)

14. D. P. Liu, C. Schmidt, T. Billings & M. T. Davisson: Quantitative PCR genotyping assay for the Ts65Dn mouse model of Down syndrome. *Biotechniques*, 35, 1170-4, 1176, 1178 passim (2003)

15. J. P. Schouten, C. J. McElgunn, R. Waaijer, D. Zwijnenburg, F. Diepvens & G. Pals: Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*, 30, e57 (2002)

16. S. J. White, G. R. Vink, M. Kriek, W. Wuyts, J. Schouten, B. Bakker, M. H. Breuning & J. T. den Dunnen: Two-color multiplex ligation-dependent probe amplification: detecting genomic rearrangements in hereditary multiple exostoses. *Hum Mutat*, 24, 86-92 (2004)

17. H. R. Slater, D. L. Bruno, H. Ren, M. Pertile, J. P. Schouten & K. H. Choo: Rapid, high throughput prenatal detection of aneuploidy using a novel quantitative method (MLPA). *J Med Genet*, 40, 907-12 (2003)

18. T. Gerdes, M. Kirchhoff, A. M. Lind, G. V. Larsen, M. Schwartz & C. Lundsteen: Computer-assisted prenatal aneuploidy screening for chromosome 13, 18, 21, X and Y based on multiplex ligation-dependent probe amplification (MLPA). *Eur J Hum Genet*, 13, 171-5 (2005)

19. W. J. Kent: BLAT--the BLAST-like alignment tool. *Genome Res*, 12, 656-64 (2002)

Abbreviations: MLPA: multiplex ligation-dependent probe amplification, HSA21: human chromosome 21, MMU: mouse chromosome, YAC: yeast artificial chromosome, BAC: bacterial artificial chromosome, DSCR; Down syndrome critical region, FISH: fluorescent *in situ* hybridization, U: upstream, D: downstream, Chr: chromosome, bp: base pair.

Key Words: Mouse, Models,Down syndrome, Ts65Dn, Genotyping assay, Multiplex, Ligation-dependent probe, Amplification, MLPA

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