Polymerase chain reaction method to identify Down syndrome model segmentally trisomic mice

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Abstract

The Ts65Dn segmentally trisomic mouse possesses an extra copy of a segment of chromosome 16 translocated to chromosome 17. This segment includes the mouse homolog of the Down syndrome critical region of human chromosome 21. The Ts65Dn mouse serves as a useful model to study the developmental regulation of the Down syndrome phenotype. To identify mice bearing the extra chromosome 16 segment, we developed a polymerase chain reaction (PCR) method as an alternative to karyotyping. Conditions under which segments of genes on chromosome 16 (App and Dyrk1a) could be coamplified with a control gene on chromosome 8 (Acta1) so that the yield of each PCR product was proportional to the amount of its template were determined. The amplification products were resolved and quantified by two methods. In the first method, the DNA segments were separated by agarose gel electrophoresis and stained with ethidium bromide. The fluorescence yields were quantified by photodensitometry. In the second method, the fragments were resolved and quantified by the high-performance DNA analysis system, a high-throughput, multichannel, microcapillary electrophoresis instrument. The results of both methods were within 10% of the expected ratio of 1.5. Application of these methods has allowed the maintenance of a Ts65Dn breeding colony through six generations and should permit the precise and efficient identification of trisomic and disomic animals at any developmental stage with minimally invasive procedures.

Keywords: EUR; Trisomic mice; Down syndrome; Capillary electrophoresis; Chromosome analysis

The Ts65Dn segmentally trisomic mouse is a model widely used to study Down syndrome (DS) 1 [1]. This mouse strain carries an additional chromosome, 17 c16, which is composed of a chromosome 17 centromere recombined with the distal segment of chromosome 16 spanning the locus Mrpl39 to Znf295 [2]. This locus is syntenic with the region of the long arm of human chromosome 21 believed to cause the major features of DS, the so-called “Down syndrome critical region” (DSCR). Ts65Dn mice show physiological and neurological impairments that resemble DS; consequently, the study of developmental gene expression in this model is likely to reveal clues about abnormalities in gene expression that cause DS [3].

To begin to study the differences in developmental gene expression induced by this partial trisomy, it was necessary to identify young trisomic females to establish a breeding colony. Since Ts65Dn male mice are sterile, Ts65Dn mice are bred from Ts65Dn females and
disomic mating strain males (B6EiC3Sn-a/A) [1]. The extra chromosome is randomly distributed among the offspring, and there are no gross abnormalities in the young mice; therefore, the trisomics are currently identified by chromosome analysis from peripheral blood [4]. This method is a time-consuming, labor-intensive process applicable only to dividing cells, thus requiring skill and experience. In situ hybridization (FISH) is another method used in some laboratories [5,6]. To identify trisomic mice at any developmental stage, one needs a precise, rapid, minimally invasive, and simple procedure for gene quantification. To achieve this goal, we developed a method using mouse tail tissue and the polymerase chain reaction (PCR) to proportionally amplify gene segments on chromosome 16 and on a control chromosome.

The method relies upon the coamplification in the same reaction mixture of a gene segment in the DSCR of chromosome 16 and a control gene segment not on chromosome 16 or 17. We chose to amplify the sequences in the DSCR genes encoding the β-amyloid peptide precursor (App) and the minibrain kinase (Dyrk1a) and in a control gene encoding α-actin (Acta1) on chromosome 8. The amplification products were resolved and quantified by two independent methods: agarose gel electrophoresis and capillary electrophoresis. Although the ratio of the amounts of amplification products (DSCR product/control product) was not precisely 1.0 in disomic DNA or 1.5 in trisomic DNA, the ratio of these products in trisomic DNA to that in disomic DNA was within 10% of 1.5. Using this method we have successfully maintained the trisomic line through six generations. These results led us to conclude that the coamplification and quantification of these gene fragments provides a simple, rapid, and reliable alternative to chromosome analysis for the identification of segmentally trisomic mice.

### Materials and methods

#### Preparation of template DNA

DNA was isolated and purified using a DNeasy kit (Qiagen). A tail segment about 0.5 cm long was clipped from each mouse and treated with proteinase K at 55°C overnight or until the tissue was completely lysed, according to the manufacturer’s protocol. The products were analyzed by agarose gel electrophoresis in the presence of ethidium bromide to confirm that the DNA was not degraded. The fluorescent bands were photographed, and their relative intensities were determined by photodensitometry using IP Lab Gel software from Scanalytics (Fairfax, VA). The relative fluorescence intensities were used to normalize the amounts of DNA to be added to the amplification mixtures.

#### Primers

Primers for the genes App (GenBank Accession No. D10603), Dyrk1a (No. US8497), and Acta1 (No. M12347) were designed to be of similar length, GC content, and melting temperature so that the Acta1 gene segment could be amplified together with either the App or the Dyrk1a gene segment in the same reaction mixture. In addition, the primers were selected to amplify fragments of sizes that could clearly be separated by agarose gel electrophoresis. The primers amplified fragments of the following sizes: App, 105 bp; Acta1, 197 bp; Dyrk1a, 238 bp. The following applications, available on the Internet, were used in primer selection: Primer 3 (www-genome.wi.mit.edu), Gene Fisher Primer Calculator 1.3 (bibierv.techfak.uni-bielefeld.de; by Chris Schleiemacher and Folker Meyer), and DNASTAR Primer Select Oligo Analyzer (calculator.idtdna.com/nicaris/dna_star.asp;Integrated DNA Technologies).

The following primer sets were used:

- **Acta1-5’** 5’CTCTTCCAGCCTTCCTTTAT, **Acta1-3’** 5’GATGCTGTTGTAGGTGGTCTTC, **App-5’** 5’TG CAGCAGAACGGGATAGAAGA, **App-3’** 5’CGCT GTCCAAGTTCAGAGG, **Dyrk1a-5’** 5’CTTGGAGT AGAAACAGGAGGA, and **Dyrk1a-3’** 5’GAAGACT GAGACTGCTCCAT.

#### Amplification assay

Each primer (100 pmol) for Acta1 and for either App or Dyrk1a were added to 100 ng of template DNA and 1 U of Taq polymerase (Promega) in 50 μl of 1× buffer containing 1.25 mM dNTPs. The following PCR program using the PE Applied Biosystems PCR 9700 was used: an initial denaturation step of 5 min at 94°C, annealing for 2 min at 56°C, and extension at 72°C for 1.5 min. After 25 cycles, the final extension step was extended to 10 min.

#### Agarose gel electrophoresis and quantification

Aliquots (5 to 10 μl) of the amplification mixture were applied on a 2% agarose gel in 40 mM Tris-acetate buffer [7] and separated at 80 V alongside 100-bp molecular weight markers from BioLabs. After electrophoresis, the gel was stained with ethidium bromide (1 μg/ml) for 5 min and destained for 15 min in water. The DNA fragments were viewed over a UV light box and photographed with a Polaroid camera using an exposure time short enough to prevent saturation of the film. The films were digitized on an Epson Model 1200U 36-bit scanner, and the bands were quantified using the application IP LabGel H. In an alternative procedure, the fluorescent bands were captured on a Diana CCD camera system (Raytest) and directly quantified using Raytest’s Aida application. The results of the two methods were similar.
Capillary gel electrophoresis and quantification

The bench-type high-performance DNA analysis (HDA) GT12 system with GCK-5000 cartridge kit provided by eGene (Irvine, CA) was utilized as the standard test instrument [8]. The system was operated according to eGene’s operation manual. Amplified DNA (5 μl) was mixed with 15 μl of 10 mM Tris buffer containing 1 mM EDTA in a 0.2-ml vial and was placed in the instrument sample tray. The DNA samples were automatically injected into the capillary channel and subjected to electrophoresis by selecting the M500.mtd method from eGene’s BioCalculator software. The quantification of DNA fragments was based on the integrated peak area determined by eGene’s BioCalculator software.

Chromosome analysis

Mouse chromosomes were prepared and analyzed from bone marrow samples according to previously published protocols [1,4,9] with some modifications. A yeast suspension (0.2 ml) prepared according to the protocol by Oncor, (Gaithersburg, MD) (3 g sucrose and 2 g Fleischmann’s yeast in 15 ml of water warmed to 37°C for 15 min), was injected subcutaneously 48 h before intraperitoneal injection of 0.1 ml of 0.5% colchicine (Sigma). After 20 min of colchicine exposure, animals were euthanized by CO2; then bone marrow samples were removed from both femurs, suspended in 0.075 M KCl for 20 min at 37°C, fixed with 3:1 methanol: acetic acid, and air-dried on microscope slides. Chromosomes were then Giemsa (G)-banded and analyzed according to Akeson and Davisson [4], Davisson et al. [1], and Sanz et al. [9] for chromosome number and for the presence of chromosome 1716, the small derivative chromosome 17 that also contains the chromosome 16 region syntenic to the DSCR on human chromosome 21. G-banded findings were confirmed using a fluorescein-isothiocyanate-labeled whole chromosome 16 kit (Cambio, Cambridge, UK: Cat. No. 1189-16NF-01) and a FISH protocol made available by the manufacturer (Cambio).

Results and discussion

DNA was extracted from tail samples of six female trisomic mice and six disomic male mice previously karyotyped by The Jackson Laboratories. A segment of the DSCR gene App or Dyrk1a was coamplified with the control Acta1 gene segment. Annealing temperature, template concentration, primer concentrations, and number of cycles were optimized for each chromosome 16 gene (App or Dyrk1a) and the control Acta1 gene. Analysis of the amplification mixtures by agarose gel electrophoresis (Fig. 1) revealed that, based upon its

![Fig. 1. Separation of PCR products by agarose gel electrophoresis. Five microliters of the amplification product was subjected to electrophoresis on a 2% agarose gel at 80 V for 4 h and analyzed as described under Materials and methods. Lanes 1–9 and 10–12 shown are from separate amplifications. Lanes 1–6 are disomic and 7–12 are trisomic samples.](image-url)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Fluorescence intensity ratios of ethidium–DNA complexes</th>
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<td>Genotype</td>
<td>Disomic</td>
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<td></td>
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<td>A. Calculated from photodensitometric scans</td>
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<td>PCR prod. ratio</td>
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<td>Dyrk1A/Acta1</td>
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<td>B. App/Acta1 fluorescence intensity ratios calculated from capillary electrophoresis data</td>
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The genotypes were determined by Jackson Laboratories; μ, mean fluorescence ratio; SD, standard deviation; N, number of animals; p is the probability that trisomics cannot be distinguished from disomics. ANOVA analyses was performed using the SPSS software v.11.0.
mobility compared to the DNA standards, each of the PCR products had the expected size: *App* 105 bp, *Dyrk1a* 238 bp, and *Acta1* 197 bp. The larger fragments (350–450 bp, Fig. 1A) and the ca. 380-bp band accompanying *Dyrk1a* amplification (Fig. 1B) were nonspecific products, which disappeared at a higher annealing temperature. The amplification parameters were adjusted so that disomic DNA yielded equal amounts of the *Acta1* and *App* or *Dyrk1a* PCR products, as measured by photodensitometry of the ethidium-stained bands.

The ratio of fluorescence intensity of the *App* gene segment to that of the *Acta1* gene segment amplified from disomic DNA (quantified by densitometry of photographic images) was 0.61. If ethidium fluorescence were directly proportional to DNA length, the ratio would be 0.53. The average of the ratios found for the fragments amplified from trisomic DNA was 0.96, whereas, a ratio of 0.80 would have been expected. Whether the enhanced fluorescence of the *App* bands resulted from the faster amplification of the *App* segment or from the nonproportional binding of ethidium was not determined; however, these factors can be corrected by calculating the ratio of the trisomic to disomic fluorescence ratios, 0.96/0.61 = 1.57, which is within 10% of the expected value of 1.5.

Fig. 2. Separation of *App* and *Acta1* amplification products by capillary electrophoresis on the HDA system. Five-microliter aliquots of the PCR mixture were applied to the capillary, and samples were analyzed as described under Materials and methods.
When the Dyrk1A products amplified from disomic DNA were analyzed, the ratio of the fluorescence intensity of the Dyrk1A fragment to that of the Acta1 fragment was found to be 1.36. A ratio of 1.21 would have been expected if the fragments were equally amplified and if ethidium fluorescence were directly proportional to fragment size. The ratio found for the fragments amplified from trisomic DNA was 2.17, whereas a ratio of 1.81 would have been expected. The ratio of the relative fluorescence intensities was calculated to be 1.60, which again is within 10% of the expected value of 1.5.

Multiple samples of DNA from disomic and trisomic animals were amplified with each primer set and the products were analyzed by the photodensitometric method (Table 1A). A statistical treatment of the data (ANOVA) revealed that, with either primer set, trisomics could be distinguished from disomics with an uncertainty of p < 0.001.

As an alternative to Polaroid photography, a limited number of fluorescent gel images were directly captured by a CCD camera, and the bands were digitized and quantified. The relative intensity ratios obtained were nearly identical to those calculated from the photdensitometric data (not shown).

We employed a second method of analysis, which is suitable for large numbers of samples. The eGene HDA system can simultaneously analyze 12 PCR aliquots in less than 10 min. An example of the analyses of two samples of amplified App and Acta1 DNA segments is shown in Fig. 2. The App and Acta1 DNA segments, respectively, emerged as cleanly separated peaks at 2.31 ± 0.01 and 2.60 ± 0.01 min. The ratio of the area of the App peak to the Acta1 peak was 0.48 in the disomic DNA amplification mixture (Fig. 2A) and 0.73 in the trisomic DNA mixture (Fig. 2B). The quotient of these ratios, 1.52, is virtually identical to the expected value. Four sets of PCR mixtures containing amplified Acta1 and App DNA segments were also analyzed by the HDA system (Table 1B). The ratio of the peak areas, App/Acta1, of the segments amplified from trisomic DNA tended to be somewhat lower than 1.5 times the area ratio from the disomic DNA segments; nevertheless, the standard deviations were small enough to allow the identification of the genotype at the level of p < 0.003. Rerunning the samples might increase the confidence level; however, this was not done on these samples. The results in Table 1B were obtained from a single run on each sample. The reliability of each method is graphically displayed in Fig. 3.

We used the photodensitometric quantification method to identify the Ts65Dn offspring from the mating of a Ts65Dn female and a disomic male. The results in Table 2A indicate that the litter contained two groups of offspring, with App/Acta1 mean ratios of 0.56 and 0.89. These determinations were presumed to indicate that the higher ratios derived from trisomic animals. The presumed trisomic female (F1-1) was then bred with a disomic mating-strain male, and the offspring were subjected to PCR gene quantification. The second-generation litter (F2s) contained two groups (Table 2B), whose App/Acta1 fragment ratios were 0.59 and 0.93 (by the photodensitometric method), which indicated that the litter contained two trisomics and five disomics. This litter could have contained Ts65Dn mice only if the PCR

![Fig. 3](image-url)
method initially had correctly identified the maternal mouse (F1-1) as Ts65Dn.

The results of blinded G-banded analyses of bone marrow metaphase chromosomes from eight of the offspring over six generations showed that mice F1-1, F2-1, F5-10, and F6-5 exhibited a modal chromosome number of 41 due to the presence of the small translocation chromosome 17/16. The data in Table 3 show that...
among eight offspring analyzed, F1-6, F2-2, F5-3, and F6-1 exhibited a normal chromosome number of 40 with no translocation 1716. FISH analysis (Fig. 4) showed that the small marker 1716 contained chromosome 16 DNA. The results of these blind cytogenetic/FISH analyses confirmed the DNA analyses, which indicated two copies of App gene in mice F1-6, F2-2, F5-3, and F6-1 and the presence of three copies in mice F1-1, F2-1, F5-10, and F6-5 in comparison with the control gene Acta1. Additional copy of the App gene is present in the Ts65Dn translocation chromosome. The exact agreement of identification of Ts65Dn mice between the PCR-photodensitometric method and the blinded G-band and FISH analyses over six generations provides a high level of confidence in the PCR method. Traditional PCR methods are not thought to be suitable for obtaining quantitative measurements of template concentrations; however, in this case, where the gene ratios are either 1:1 or 1.5:1, the amplification parameters yielding the appropriate PCR product ratios were found. Our results demonstrate that if DNA samples from known disomic and trisomic animals are used as standards, these methods can discriminate between disomic and trisomic DNA samples accurately.

Correct identification of the translocation chromosome over six generations demonstrates the 100% accuracy of this method in identifying the genotype at each generation. The 25% frequency of Ts65Dn neonates identified by the PCR method in our litters agrees with the 19–25% reported by the qPCR method of Liu et al. [10]. We have used the method described in the present publication to identify trisomic neonates and adults for experimental and breeding purposes. The method should also be suitable for identifying trisomic fetuses at any developmental stage with equal reliability.

Some methods alternative to karyotyping, previously used to identify chromosomal aneuploidies such as trisomy 21, have relied upon blotting techniques that require multiple hybridizations with radioactive probes and cumbersome autoradiographic analyses [4,9]. Recently, Liu et al. [10] reported a real-time quantitative PCR method, which may yield results comparable to those reported here but requires specialized equipment that is not widely available.

The method presented in this paper has some advantages compared to previous methods. It is not labor intensive and does not require an additional criterion to provide Ts65Dn identification at a high confidence level. It provides precise results within a day, is relatively inexpensive, and does not require sophisticated instrumentation. This method can accommodate a high sample throughput using the HDA technology, and it can be adapted to the diagnosis of human trisomy 21. In addition, this method is applicable to both living and stored tissues from animals at any developmental stage. It requires only 100–200 μg of tissue containing 250 ng extractable DNA, an amount sufficient for multiple assays. In summary, we have developed a rapid and extremely reliable identification method, which should facilitate the study of DS and of DS model animals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.ab.2005.02.002.

References