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A new protocol to type the Ts65Dn mouse model for Down syndrome by FISH in newborn or embryo tissue imprints

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The mouse chromosome 16 (MMU16) contains a large region of conserved sinteny with human chromosome 21 (HSA21). Mice that are trisomic for MMU16 have been used as model for Down syndrome (DS). However, as they do not survive the perinatal period, a new model has been developed, Ts65Dn, which has segmental trisomy 16. This mouse carries an extra chromosome that contains the distal end of MMU16, which is the portion homologous to HSA21 (Ref. 1). A total of 18 loci have been mapped in Ts65Dn with a gene order consistent with their HSA21 counterparts (Ref. 2). The conserved region spans from *App* to *Mx1*. Here we report on a rapid and very simple method to type the progeny of Ts65Dn mice based on fluorescence *in situ* hybridization (FISH) in imprints of several fresh or frozen tissues.

The only method to distinguish the Ts65Dn progeny from the non-trisomic counterparts has been, up to now, to perform a karyotype to identify the presence or absence of the marker chromosome (Ref. 3). An alternative way of doing this typing is to perform FISH on metaphases or interphase nuclei using mouse probes from the trisomic region. The main inconveniences of this protocol are that blood has to be drawn from live animals, a culture has to be set up and mice can only be typed after 5–6 weeks of age.

We have developed a new protocol based on FISH in interphase nuclei from imprints of a vast number of tissues, even frozen tissues (Figure 1). We have succeeded in typing the Ts65Dn progeny by FISH analysis in imprints of brain, lung, liver, spleen, kidney and tail. Our protocol has also been successfully applied to the typing of mouse

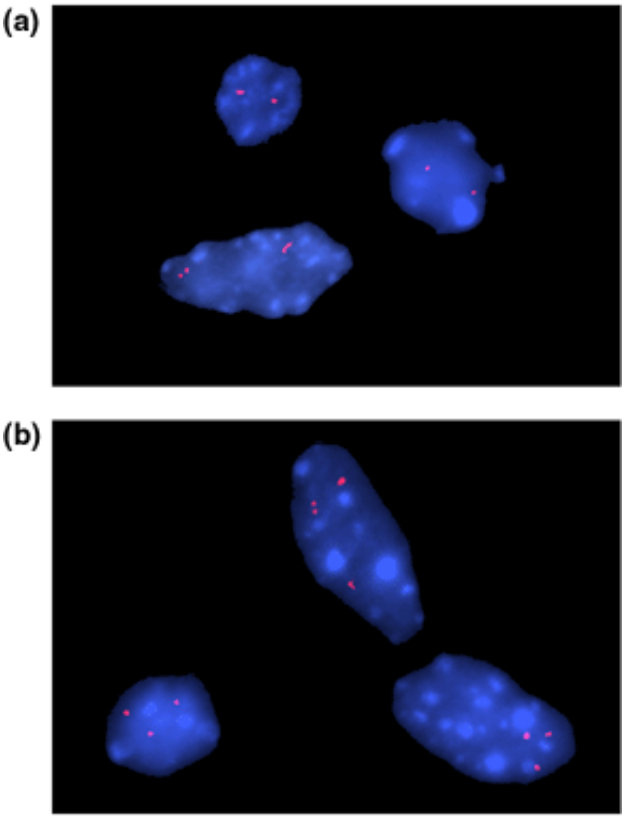

FIGURE 1. FISH analysis of the mouse conserved region of HSA21 in imprints of adult mice bladder. a) Cells from the bladder showing two FISH signals, indicating that the mouse is wild type. b) Cells from an imprint of the bladder of a Ts65Dn mouse showing three signals. All hybridizations were performed with a mouse P1 probe (pAd10-SacBII) from the conserved region of HSA21, labelled with biotin and detected with avidin-TRITC.

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embryos (stage E15) by performing an imprint from a small piece of the tail. To assess the efficiency and the reliability of this protocol we performed a blind analysis of trisomic and non-trisomic tissues that had been typed previously by karyotype analysis. The results indicated that the protocol is totally reliable; there were no discrepancies between karyotype and FISH analyses of the tissue imprints in 20 mice. The method described here is much faster than karyotyping and it allows the typing of both embryos and newborns. It can be used for typing at any age provided mice do not need to be kept alive (e.g. for breeding). To circumvent this inconvenience we are currently assessing the efficiency of the method that is described here in imprints of tail sections from live animals and our preliminary results indicate that, despite the low number of cells obtained, it will be completely reliable. This protocol can also be applied to the study of other transgenic mice.

Protocol

Imprints

A small piece (about 3 mm^2) of the tissue is cut and put on a clean slide. With another clean slide, slight pressure is made over the tissue to make the imprint. This step is repeated two or three times along the slide.

Slides are then dried at room temperature. This pressure causes sufficient cells to attach to the glass to perform FISH. At this stage, slides can be kept frozen for a long period of time or used immediately. If the tissue is frozen, it is allowed to defrost at room temperature before proceeding to make the imprints.

Fixation and permeabilization

Before hybridization, the slide area is completely covered with cold methanol:acetic acid (3:1) and dried under a bulb light.

Once the slides are dry, they are incubated at 55°C for about 30 min. The slides are then ready for hybridization.

Fish

2 μg of each PAC or cosmid mouse probe from the conserved region of HSA21 were labelled with biotin-16dUTP or digoxigenin -11dUTP (Boehringer Mannheim) in a standard nick translation reaction and purified by gel filtration. 400 ng of labelled probe were precipitated along with 1 μg of Cot1 DNA (GIBCO BRL) and 1 μg of salmon sperm DNA (Sigma), and the pellet was resuspended in hybridization mix that contained 50% formamide and 50% dextran sulphate in 12×SSC.

10 μl of the hybridization mix was applied to each slide. After heat denaturation of the probe and the preparations, slides were incubated overnight in a humidified chamber at 37°C. Post-hybridization washes were performed in 3 changes of 50% formamide/2×SSC at 42°C, followed by 3 changes of 0.1×SSC at 60°C.

For detection of the signals, slides were incubated at 37°C with avidin-TRITC (Vector Laboratories) for 20 min, and then washed in 3 changes of 4×SSC/Tween 20 at 37°C. None of our probes needed further amplification of the signal. Slides were mounted with 40 μl of antifade solution (Vector Laboratories) containing 150 ng ml^{-1} of DAPI (Ref. 4).

Slides were viewed under a fluorescence microscope VANOX (Olympus) that was equipped with the appropriate filter set for detecting the avidin-TRITC fluorescence. A total of 200 nuclei were scored for each of the probes used.

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Products Used

Anti-digoxigenin antibody: Anti-digoxigenin antibody from Boehringer Mannheim
biotin: biotin from Boehringer Mannheim
sperm DNA: sperm DNA from Sigma
avidin-TRITC: avidin-TRITC from Sigma
antifade: antifade from Oncor Inc