Analysis of Transgenic Animal Pedigrees Using the Junction-Polymerase Chain Reaction

Michael Ward,
Wanda Abramow-Newerly,
Susan Clapoff, and
John C. Roder

Division of Molecular Immunology
and Neurobiology, Samuel Lunenfeld
Research Institute, Mount Sinai
Hospital, Toronto, Ontario, Canada
M5G 1X5

Here we describe a modification of the polymerase chain reaction—junction-PCR (J-PCR)—that has been designed to facilitate the identification and subsequent screening of transgenic animals and their offspring. This procedure targets a unique recombination junction, which is present in the concatamerized transgene insert, for amplification. Although several non-radioactive PCR-based strategies have recently been described for analyzing transgenic mice,^(1,2) each suffers from the possibility of identifying false-positive mice due to contaminating plasmid DNA. Since J-PCR involves amplifying a recombination junction generated by the concatamerization of transgene inserts, the interpretation of the amplification reaction is not affected by overt levels of contaminating plasmid DNA.

As shown in Figure 1A, a defined junction is generated upon transgene concatamerization and insertion. In animals that carry more than one copy

of the transgene, this junction represents a unique DNA fragment that can be the target of J-PCR analysis using appropriately positioned primers. The location of these primers allows amplification of both circular plasmid DNA (pMBP/p72MAG) and of linear transgenic DNA such that two discrete and diagnostic bands are produced. J-PCR analysis produces a 3.2-kb band from plasmid DNA or a 0.9-kb band from transgene-positive DNA (see Fig. 1c).

We have used this procedure successfully to verify the multicopy nature of several transgenic lines established using the vector outlined in Figure 1 (unpublished). Specifically, the founder mice represented in lanes b, c, d, and f of Figure 1C were positive by the J-PCR analysis (note the 0.9-kb band), whereas mouse e tested negative by both Southern analysis of tail biopsy DNA and by J-PCR (Fig. 1C,D). Amplification of circular plasmid DNA failed to generate the diagnostic 0.9-kb fragment (lanes h and i), demonstrating

FIGURE 1 (A) The linear concatamerized derivative of pMP302AS⁽³⁾ containing the cDNA sequence of the myelin-associated glycoprotein⁽⁴⁾ is shown together with the binding sites for primers MBPAS (5'-GTTCCTCCATCTACCCACTG)⁽⁵⁾ and RBpA (5'-CATTGCAATAGTGTGT-TGGA)⁽⁶⁾ and the expected 0.9-kb J-PCR product. (B) The circular pMBP/p72MAG plasmid showing primer binding sites and the expected 3.2-kb PCR product. (C) Ethidium bromide-stained gel of J-PCR analysis: (lane a) 1-kb ladder; (lanes b-f) DNA from founder animals; (lane g) no DNA control; (lane h) 1 ng of pMBP/p72MAG; (lane i) 10 ng of pMBP/p72MAG; (lane j) 1 ng of pBS plasmid. PCR was performed as described⁽⁷⁾ using 200 ng of each primer and cycle times of 2 min at 94°C, 2 min at 55°C, and 4 min at 72°C. (D) Southern analysis of genomic DNA obtained from tail biopsy. The 2.6-kb transgene positive signal is highlighted with a closed triangle. Lanes b-f correspond to founder mice b-f above.

that plasmid DNA does not generate a positive signal in the J-PCR analysis. As a further control we have demonstrated that a common laboratory plasmid contaminant, pBS, fails to generate the 0.9-kb fragment (Fig. 1C, lane j).

However, we were concerned that high levels of contaminating plasmid DNA may squelch the signal generated by the transgene insert. Therefore, we performed reconstruction experiments in which varying amounts of plasmid vector were added to 1 μ g of transgene-positive genomic DNA obtained from offspring of founder animal c (see Fig. 1D, lane c; densitometry scan suggests that this animal has a transgene copy number of two to three). This experiment allowed us to estimate the point at which amplification from contaminating plasmid DNA was able to squelch the signal generated from the integrated transgene. As shown in Figure 2, the transgene-positive signal can be squelched by contaminating plasmid in excess of 10 ng (lanes D and E). However, at levels below this threshold, the J-PCR product is reproduced faithfully (lanes E–J).

Because J-PCR uses a recombination junction as a unique template, the transgene insert being analyzed must necessarily exist as a tandem insert of two or more copies. In our experience, the majority of transgenic animals produced (18/18 of our founder animals) do, in fact, contain several copies of the transgene that can be integrated at one or more sites within the genome. We believe that J-PCR represents a reliable means of avoiding discrepancies that may arise in pedigree analysis due to low levels of plasmid contamination.

Although we have demonstrated the use of J-PCR for analyzing "head-to-tail" transgene inserts, less frequent "head-to-head" or "tail-to-tail" arrangements can be accommodated simply by altering the position of the J-PCR primers.

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FIGURE 2 Reconstruction experiment to examine the effect of plasmid contamination on J-PCR. Serial dilutions of the circular plasmid pMBP/p72MAG were added to reactions containing 1 μ g of transgene-positive genomic DNA and then PCR was performed, as described (see Fig. 1). (Lane A) Transgene-negative DNA with no added vector; (lane B) transgene-positive DNA with no added vector; (lane C) transgene-negative DNA with 100 ng of vector; (lanes D–J) transgene-positive DNA with 100 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 0.1 pg, respectively; (lane K) no DNA. The positions of the 0.9- and 3.2-kb bands are shown.

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