

Brown, Robert E. III

Identification of Insertion Points of the Macrophage Fas-Induced Apoptosis (Mafia) Transgene

Faculty Mentor: Dr. Sandra Burnett, Ph.D., MMBio

Problem: Without site direction, transgenes integrate randomly into the genome of target cells. The transgene used in macrophage fas-induced apoptosis (Mafia) mice was randomly incorporated and indications of multiple insertion sites were observed. Our goal in performing this study was to detect the exact insertion point of the transgene so as to ultimately develop a genetic test to verify expression of the transgene in Mafia mice. In Figure 1 the premise of our experimental design is explained. Polymerase Chain Reaction (PCR) is a scientific technique that is used to amplify a specific segment of DNA. To determine the insertion points of interest, inverse nested-set PCR was used to identify the sequence adjoining the 5' end of the Mafia transgene.

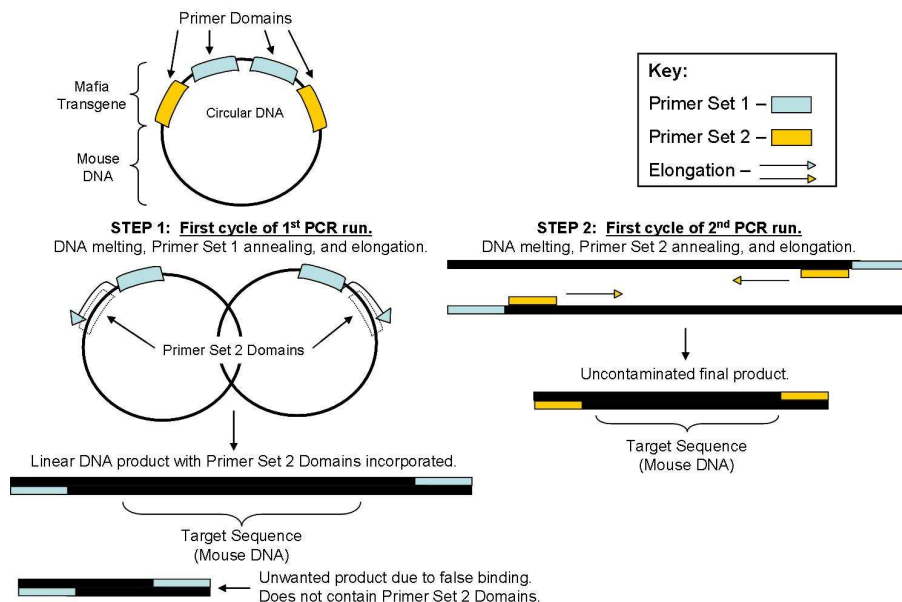


Figure 1: Inverse Nested-Set PCR – to determine insertion points, inverse nested-set PCR was used to identify the sequence adjoining the 5' end of the Mafia transgene.

Approach: After isolating and sequencing DNA fragments which were made up of a Mafia transgene segment contiguous with native mouse DNA, we were confident that one of the insertion points of the transgene was on mouse chromosome 18. To confirm the location on chromosome 18, five new primers were designed upstream of the transgene for PCR products that would overlap genomic and transgene DNA. The resulting 20 sequences matched the same location on chromosome 18 as the initial nested-set experiment. Four additional primers were designed further downstream on the transgene to confirm its integration with chromosome 18. The 8 resulting sequences overlapped chromosome 18 and a larger portion of the Mafia transgene. All 8 sequences again matched the 5' end of the transgene and chromosome 18 at the

hypothesized location. We have defined a consensus sequence that places the Mafia transgene in chromosome 18 at position 58267742 which falls within the mouse *cfms* sequence.

Results: Figure 2 is an image of the agarose gel which we obtained by electrophoresis of various PCR products. Notice the banding pattern of each group. The three groups each had different forward primers that were constructed on the Mafia transgene. The four lanes in each group utilize the same reverse primers (1-4) constructed from genomic DNA found on mouse chromosome 18. This banding pattern is consistent with what we predicted because the reverse primers we constructed were made at increasing distances from our hypothesized transgene insertion point thus producing PCR products that increased in size relative to primer distance.

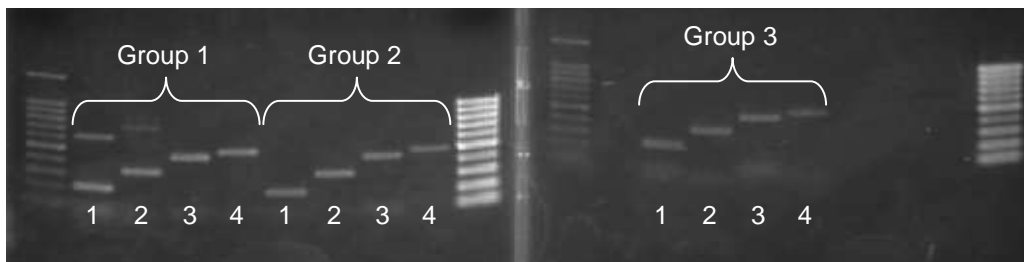


Figure 2: PCR products run on a gel by electrophoresis.

The more convincing results came when we actually sequenced the above bands and were able to construct a consensus sequence that showed a single PCR product matching a portion of the Mafia transgene and the location where we predicted the transgene's insertion on chromosome 18 as well.

Pending Work: Currently, we are in the beginning stages of publishing a paper in *Molecular and Cellular Biology*. We have outlined the information that we want to include in the publication and we have obtained the formatting criteria which the journal editors look for when deciding to accept an article or not. We hope to publish our results by summer 2009.

Contributions to research: I had the privilege of attending the Autumn Immunology Conference which took place in Chicago, IL on November 21-24, 2008. It was my responsibility to present a poster outlining our research to a relevant audience and answer any questions I was posed with. It was intriguing to have a few individuals that had similar issues with trying to find a transgene insertion point ask detailed questions about our approach and congratulate us on the successes we had. Ultimately, this research will benefit the distributors of Mafia transgenic mice, The Jackson Laboratory. They will be able to use the information generated by our research to make a genetic test to verify the genotype and functionality of the Mafia mice and to guarantee expression of the transgene for researchers who order the mice around the world.

My Experience: I am so grateful that I was able to receive an ORCA grant which gave me added motivation to work towards finishing this genetics project. I have learned that really thinking through an experiment and working it out on paper will greatly enhance one's ability to achieve valid results. I have also learned that perseverance and a good attitude are keys to being an accomplished scientist.