



MALDI Mass Spectrometry Identification of Proteins in a Murine Transgenic Model of Apert Syndrome

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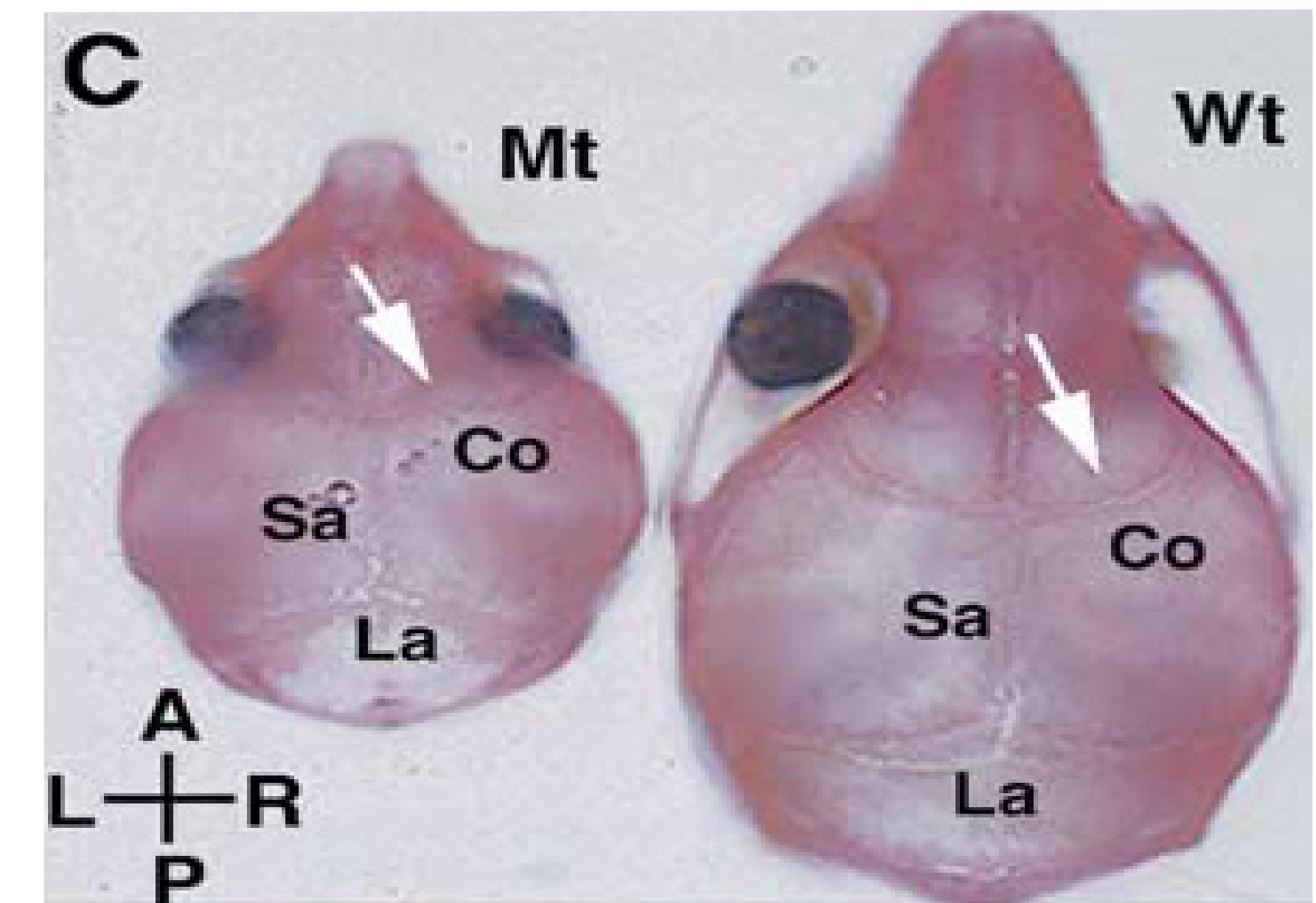
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The craniosynostoses are craniofacial skeletal disorders involving temporally inappropriate fusion of the cranial sutures. The syndromes originate from mutations in the membrane-bound tyrosine kinase receptor, fibroblast growth factor receptors (FGFR). Point mutations in the linker between Ig-like domains 2 and 3 of human FGFR2 are known sources of five craniosynostoses syndromes: Apert, Crouzon, Jackson-Weiss, Beare-Stevenson, and Pfeiffer. Apert syndrome is a rare, autosomal-dominant disorder characterized by premature fusion of the coronal suture, mental deficiency, and brain malformations. The Apert etiological defect has been traced to one of two point mutations on chromosome 10q in humans (7q in the mouse).

A transgenic mouse model of Apert syndrome was studied. MALDI mass spectrometry was used to screen for transgene expression. Mass spectrometry provided the same data as the validated Polymerase Chain Reaction (PCR) conventional approach, and provides a rapid throughput, high-specificity method for determining construct expression.

The molecular weight of the endogenous FGFR2 protein was calculated at 91.9 kD and the addition of the myc-his tag (approximately 25 amino acids) increased the construct's mass to approximately 94.9 kD. Both proteins were identified with MALDI mass spectrometry from global protein extractions of murine tail tissue.

This study provides proof of principle for MALDI protein identification in transgenic mice. Thus, mass spectrometry may allow replacement of classical PCR in the study of protein expression in genetically engineered mice. This would provide large scale, rapid, robotic protein analysis in virtually any genetically engineered animal model.

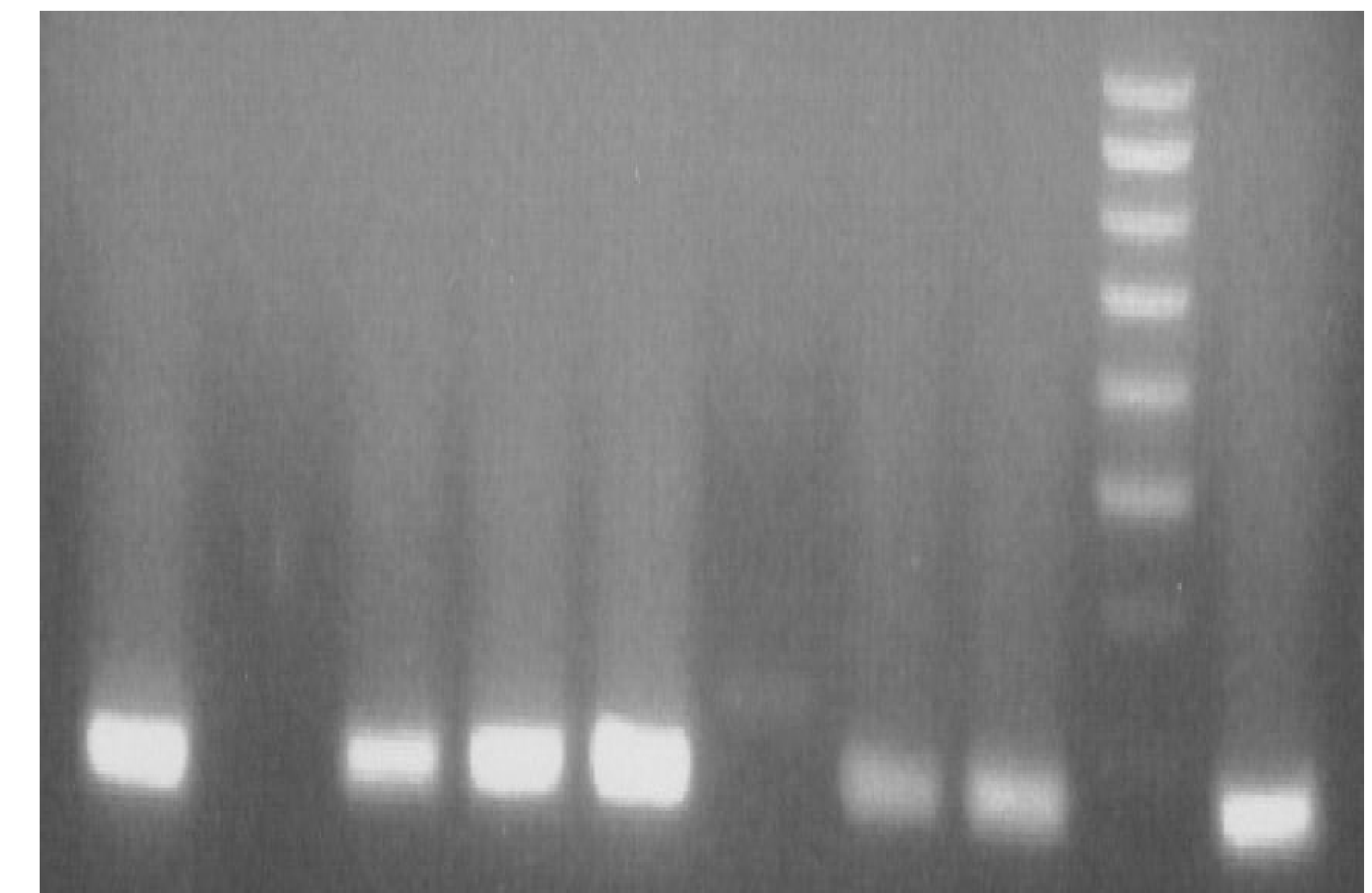


Top view of P20 wild type (Wt) and mutant (Mt) mice; notice the mutant mice have a significantly shortened head and premature fused coronal suture.

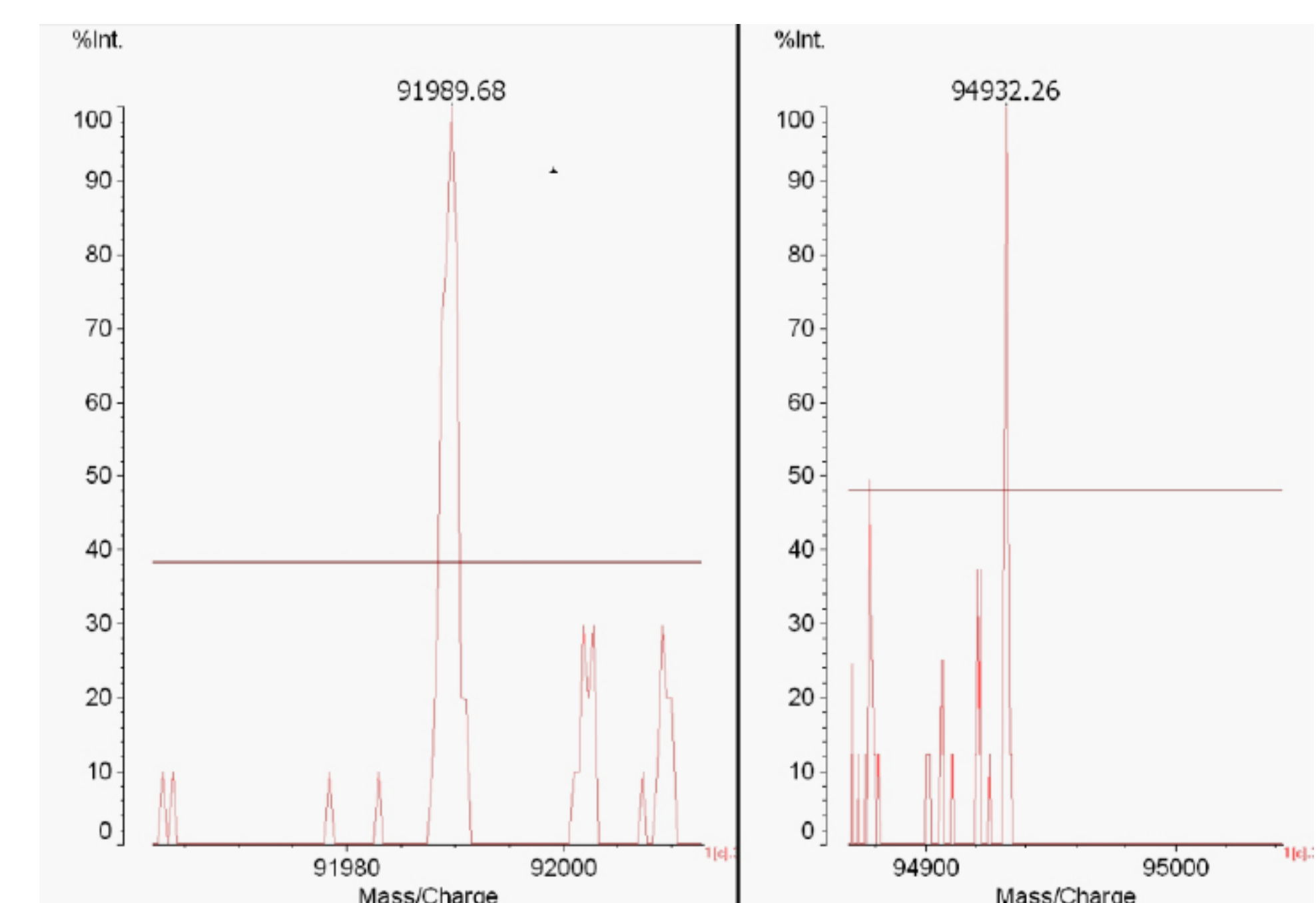
Reproduced from: A Ser252Trp [corrected] substitution in mouse fibroblast growth factor receptor 2 (Fgfr2) results in craniosynostosis.

Authors: Chen,L.; Li,D.; Li,C.; Engel,A.; Deng,C.X.

Source: Bone, 2003, 33, 2, 169-178, United States



Polymerase Chain Reaction (PCR) Cut a piece of tail, digest overnight in proteinase K at 55 degrees, isolate the DNA, set up a PCR on the DNA with the forward primer in the FGFR2 and the reverse primer in the myc-his tag to check if the mouse has the construct in its genome. Expected size is 267 nt. 1st, 3rd, 4th, 5th, 7th, 8th lanes amplify the expected size piece; second and sixth lanes do not. To the right of the marker lane is the positive control.



Both proteins were identified with MALDI mass spectrometry from global protein extractions of murine tail tissue.