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Development of a PCR assay to genotype aromatase knockout mice
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The aromatase knockout mouse lacks the gene sequence coding for functional aromatase, a protein that converts androgens into estrogens. Previously, to genotype an aromatase mouse, two separate PCRs were run: #1) Neomycin primers to detect the KO insertion and #2) Aromatase primers to detect the wild type aromatase. When we attempted to combine the neomycin and aromatase primers into one PCR, the expected band patterns were not attained. Some possible factors were the high difference in melting temperatures of the primers, and a GC dinucleotide at the end of the primer set which resulted in strong primer dimer bands on the gel. To resolve these and other factors, we searched the aromatase deleted DNA sequence for possible new primers. Three pairs of aromatase primers (1882/2270, 2285/2652, and 1190/1525) and a new neomycin primer pair were chosen. Five PCRs of 15 aromatase wild type, heterozygous and knockout samples were run using the new and old primers. Following analysis, 1190/1525 and 1882/2270 showed multiple bands and fuzzy bands respectively; they nonspecifically amplified other sites in the DNA sequence; hence 2285/2652 was chosen as the best to for further experiments. A new PCR assay was developed by combining the new and old neomycin primers with the new 2285/2652 aromatase primers ensuring the size between the two sets of primers was at least five percent different and the last few 3 primer bases of the primers did not base pair with one another. We combined the new and old neomycin primers with 2285/2652 primers and ran two PCRs. The heterozygous showed two bands on an agarose gel at 368 bp and 515 bp with the old neomycin and 368 bp and 571 bp with the new neomycin. The wild type showed a band at 368 bp with both primers. The neomycin knockout showed one band at 515 bp with the old neomycin and 571 bp with the new neomycin. Since none of the 15 samples showed three or more bands, we deduced that the two sets of primers did not base pair with one another nor did they amplify another site non-specific in the whole mouse genome; therefore, they are compatible to run simultaneously. A confirmatory PCR using 15 DNA samples from additional true aromatase knockouts, heterozygous and wild type mice is being run now.