Abstract
Most mouse genotyping protocols are tedious and time consuming, starting with overnight digestion and DNA purification, and ending with gel detection. Demonstrated here is a simple, coupled system for rapid extraction, amplification and quantification of genomic DNA from mouse tissue. This can be used in genotyping studies that do not require DNA purification or gel analysis. This system, Extract-N-Amp™, uses a novel extraction treatment that releases sufficient quantities of DNA from tissue (for example, a mouse tail clip) in 3 minutes at room temperature for quantitative PCR analysis. After a 3-minute incubation at 95 °C and the addition of a solution that releases substances inhibitory to PCR, extracted DNA is directly added to a PCR ReadyMix™ that is specially formulated to accommodate the extract for SYBR® Green or fluorescent-probe-based real-time qPCR assay. Results show that single-copy gene dosage resolution is possible. This single-copy resolution will be demonstrated in two different systems. In the first system, the Extract-N-Amp procedure is used to distinguish between one and two copies of an allele, and in the second, to differentiate extracts from mice that are wildtype, heterozygous, and homozygous for an X-linked gene. Either SYBR® Green or dual-labeled probes can be used for detecting during real-time PCR. The results with these two systems demonstrate that extensive tissue digestion and DNA purification are not necessary, and that high-resolution genotyping is possible using a simple, rapid tissue extraction and real-time qPCR with Extract-N-Amp.

Materials

Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich (St. Louis, MO). The PCR primers and dual-labeled probes were obtained from Sigma-Aldrich Corporation (St. Louis, MO). Extract-N-Amp™ Tissue PCR Kit (Cat. No. W5000 or 604055) was used for genomic DNA isolation from mouse tissues and PCR setup. GenElute™ Micro Mammalian Genomic DNA Miniprep Kit (Cat. No. G7120) was used for traditional genomic DNA isolation from mouse tails for positive controls and efficiency studies. Reference Dye (Cat. No. 84516) was used for quantifying PCR.

Whole mouse and mouse tails were obtained cryogenically preserved from either Pol-Freem (Bifent, AR) or Jackson Laboratories (Bar Harbor, ME).

Method

Genomic DNA Extraction

The rapid extraction in our system was completed using Extract-N-Amp™ Tissue (see scheme to right). Hair-contaminated tail pieces released sufficient quantities of DNA for quantitative PCR analysis. Tissue was placed in a mixture of 100 μl of Extraction Solution and 25 μl of Tissue Preparation Solution for 10 minutes at room temperature. After a 3-minute incubation at 95 °C and the addition of a solution that releases substances inhibitory to PCR, extracted DNA is ready to be directly added to 2x PCR ReadyMix™ that is specially formulated to accommodate the extract for SYBR® Green or fluorescent-probe-based real-time qPCR assay. Traditional genomic DNA extractions were performed following the supplier’s protocol.

Quantitative PCR Amplification

All real-time product amplification and detection were performed with either dual-labeled or SYBR® Green on a Biorad iCyclerIQ. Quantitative SYBR® Green reactions included 4 μl of mouse DNA extracts generated using Extract-N-Amp™ system, 2x SYBR® Green Extract-N-Amp™ PCR ReadyMix, primers, and Reference Dye for a total reaction volume of 20 μl. Multiplex dual-labeled reactions included 4 μl of mouse DNA extracts generated using Extract-N-Amp™ system, 2x Extract-N-Amp™ PCR ReadyMix, 2 pmol/μl probe sets (specific and normalizer) and Reference Dye for a total reaction volume of 20 μl of PrimerGeneplexes and the concentrations used can be found above. Similar qPCR efficiencies were verified for all reactions being compared using purified genomic DNA from a traditional DNA isolation method.

For negative controls, 2x Extract-N-Amp Solution and 2x Neutralization Solution were used in place of 4 μl of DNA extract.

Conclusion

Demonstrated above is a streamlined genotyping system capable of distinguishing single copy gene dosage differences. Extraction is completed in less than 20 minutes. Simple amplification and quantification reactions can be performed with a 2x ReadyMix formulated with SYBR® Green or ready for dual-labeled probes. The use of real-time detection eliminates the need for agarose gel analysis. The results from these two assay demonstrate that extensive tissue digestion and DNA purification are not necessary and that high-resolution genotyping is possible using a simple, rapid tissue extraction and real-time qPCR with Extract-N-Amp.