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Mouse genotyping in an hour

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Accurate genotyping is essential for experimental testing and evaluation of mouse models of human disorders. As overall experimental costs increase each year along with mouse colony maintenance costs, the need for quick and accurate mouse genotyping is essential for reducing undesired strain genotypes and for responsible mouse husbandry. A universal polymerase chain reaction (PCR)-based protocol (referred to as 1-HUG) for accurate genotyping of several mouse strains commonly used in neuromuscular experiments has been developed by Joubert and colleagues.[1] The protocol is particularly of value for rapid genotyping of mouse pups for which early postnatal genotyping and sex-determination analysis is desirable.

The protocol by Joubert and colleagues begins with a standard ear punch biopsy of the mouse followed by a rapid incubation in lysis buffer for genomic DNA extraction for approximately 5 min. Modified PCR genotyping for fast amplification of the desired amplicon(s) can be done in just under 40 min with gel products then electrophoretically resolved on agarose gels in under 15 min. The authors first tested their universal PCR protocol on the commonly used Duchenne muscular dystrophy (DMD) *mdx* mouse model, which harbors a C to T transition in exon 23 of the mouse *Dystrophin* (*Dmd*) gene, resulting in a premature stop codon.[2,3] The authors use a four-primer PCR genotyping method adapted from a previously established protocol that allows for efficient detection of the *mdx* point mutation by means of primer competition PCR, resulting in different size amplicons, depending on the presence or absence of the mutant allele.[4] Ear punch, tail biopsy, finger/phalanx removal, and ventral skin swab were compared for genotyping accuracy using this

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CONFLICT OF INTEREST

None of the authors have any conflicts of interest to disclose.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in the ethical publication and affirm that this report is consistent with those guidelines.

universal 1-h genotyping protocol, with ear punch being the most accurate at detecting the presence of the *mdx* mutant or wild type alleles.

The authors adapted their 1-HUG protocol for use in the differential amplicon size amplification of the *Rbm31* gene to distinguish male and female chromosomes as a means of rapid sex determination in mice by means of PCR.[5] The authors then further demonstrated the use of their protocol to genotype an inducible mouse model of facioscapulohumeral muscular dystrophy (FSHD), the *FLExDUX4* mouse strain that overexpresses a human *DUX4*-full-length transgene stably integrated at the *Rosa26* locus upon cassette activation by Cre recombinase.[6] After mating the *FLExDUX4* mice with the *HSA-MerCreMer* Cre recombinase mouse strain, expression of the *DUX4-fl* transgene in skeletal muscle fibers occurs upon exposure to tamoxifen.[7] The authors were able to use 1-HUG to successfully detect both the presence or absence of the *FLExDUX4* cassette, along with the Cre recombinase transgene, in a multiplex PCR reaction.

Notably, there are several limitations that might occur in the widespread adaptation of this protocol, suggesting this approach may not be universally applicable for all mouse genotyping purposes. In fact, one warning is that there may be some particular PCR protocols not amenable to this genotyping method. One example is when large PCR amplicons are needed that may require long reaction run times that push the overall protocol beyond 1 h. Additionally, some mouse strains might not be amenable to genotyping using ear punch biopsies. For example, the Murphy Roths Large (MRL/MpJ) “superhealing” strain may be problematic as ear punch biopsies heal rapidly in this model, rendering the protocol impractical if the mice are housed together unless an additional method for tagging individual animals is added.[8,9] It is of interest to determine if this genotyping protocol could be expanded to other commonly used research animal models, such as fruit flies, zebrafish, or other rodent models. Nevertheless, this universal 1-h PCR genotyping protocol will likely save valuable laboratory time and financial resources while increasing the efficiency of mouse colony maintenance for researchers on a tight budget.

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