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# Mouse Genotyping

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## Abstract

Even though the human genome was mapped over 10 years ago, scientists are still far from understanding the function of every human gene! One way to evaluate how a gene functions is to disrupt the sequence encoding it and then evaluate the impact of this change (the phenotype) on the animal's biology. This approach is commonly used in the mouse (*Mus musculus*), since it shares a high degree of genetic similarity with humans. To track the animals bearing genetic changes over several generations, it is necessary to screen the DNA of each mouse in a process known as genotyping.

This video provides an overview of the theory and practice behind genotyping mice. The discussion begins with the basic principles of mouse genetics, including a review of the terms homozygote, heterozygote, wildtype, mutant, and transgenic. Next, step-by-step instructions are supplied for extracting and purifying genomic DNA from mouse tissue. Examples are provided demonstrating how to interpret genotyping results, as well as how to keep track of mice with the desired genotype. Finally, some representative applications of the genotyping procedure will be presented in order to demonstrate why this common technique is so essential to mouse research.

## Transcript

Genotyping is the process of detecting the presence, or absence, of specific DNA sequences in a particular organism's genome.

Since genes can influence a mouse's phenotype, being able to probe an individual mouse's genetic make-up, or "genotype," is critical for attributing a phenotype to a specific gene. This video will explore mouse genetics, demonstrate key steps of the genotyping procedure, and explain how to interpret PCR-based genotyping results.

In order to understand what researchers look for when they genotype mice, let's review some common manipulations to mouse genetics.

To study a gene, scientists frequently disrupt its function by altering its genetic sequence. However, mice are diploid, so they have two copies of any given gene. Since most genes need only one normal copy to function, the mice must be bred to produce an animal with both genes disrupted prior to studying phenotype.

This genotype is called a "homozygous knockout," or more commonly just "knockout" for short. Conversely, a normal mouse with two functional copies is called a "homozygous wildtype," or just "wildtype." Finally, mice with just one functional copy are referred to as "heterozygous," or "hets."

Instead of removing genetic material, some experiments require the introduction of DNA sequences into the mouse genome. These inserted DNA fragments are called "transgenes," and the mice that carry them are "transgenic." The most common "transgene" introduced into mice is one that drives the expression of green fluorescent protein, or GFP, from jellyfish. By using a tissue-specific promoter (a regulatory sequence that "promotes" the activity of a gene) to drive GFP production, cells from a specific tissue type can be easily identified by green fluorescence.

Because many genetic changes do not lead to a readily observable phenotype, like GFP expression, mice need to be genotyped to determine which specific animal should be used in experiments. Prior to genotyping, mice should be carefully labeled so that they can be identified again later. No, you'll need something more permanent than that. One common method is to make notches in the ear, such that the position and number of notches corresponds to an ID number.

Once the mouse is labeled, collect a small piece of tissue (typically 2 — 5 mm of tail, using a razorblade or scissors) from which to extract DNA. If you're collecting tissue from more than one mouse, mark the used segments of the blade to ensure you won't use the same part on the next animal, which could lead to cross-contamination in your samples.

To begin the process of separating the genetic material from other components of the tissue, the sample is digested in lysis buffer containing the enzyme proteinase K. After an overnight digestion, the sample is centrifuged to pellet hair and any other undigested material. To isolate DNA from the digested lysate, a simple and effective method is to add alcohol to precipitate nucleic acids. After a brief incubation, the sample is centrifuged again to collect the DNA in a pellet.

After washing with 70% ethanol to remove excess salts, the DNA pellet is resuspended in water or buffer and is ready for genotyping.

There are many strategies to determine whether a specific DNA sequence is present in your mice. Most of them require you to first amplify the genetic region of interest using the polymerase chain reaction, or PCR. For more information on how to set up PCR, please check out the JoVE Science Education "Guide to PCR."

One method to distinguish genotypes is to detect changes in the size of the PCR-amplified fragment. Let's say that you're screening for mice with a genetic insertion of 700 base pairs. After PCR, wildtype bands are 200 base pairs, and transgene bands should be 900 base pairs.

After running your PCR, separate the reaction products on an appropriate percentage agarose gel, so you can distinguish the sizes of your fragments. The wildtype control should only have the 200 base pair wildtype band; the transgenic control should only have the one 900 base pair, transgene band; and the het control should have both bands. Finally, a "no template" control is included to be sure the reagents do not contain any contaminating DNA, and should therefore not generate any bands.

Now that we are confident that the controls worked as expected, let's check out the unknown mice. Since mice 1 and 2 each have only one, wildtype band, they are homozygous wildtype. Mice 4 and 6 each have only one, transgene band, and are therefore homozygous transgenic.

And mice 3 and 5 each have two bands, and are therefore hets.

Finally, when you go back to find the mice with the genotype you want, you just need to match up the pattern of ear notches with the mouse's ID number.

After gaining an understanding of what genotyping is and how it's done, let's look at some examples of why it's useful.

In some cases, more complex genetic modifications are required to produce the desired phenotype. For example, to establish this model of skin cancer in mice, two transgenes are required. One carries an inducible "oncogene," or a gene that can cause cancer, and the second carries the enzyme Cre recombinase, which is only expressed in skin cells and excises a sequence that prevents oncogene transcription, thereby allowing oncogene expression. To produce offspring with both transgenes, mice heterozygous for each are mated. Genotyping is then used to identify the desired offspring.

Sometimes it may not be possible to genotype mice before an experiment. For example, in this study of embryonic heart rate, it is not feasible to collect tissue for genotyping from the embryos without disrupting their behavior. Therefore, the embryo positions within the mother are first carefully labeled, then ultrasound measurements are recorded. Finally, tail biopsies are collected to determine the effect of the genotype on heart rate.

This video has demonstrated one method of DNA extraction, but there are many variations. For example, the Direct PCR system requires less than five minutes of tissue digestion. Additionally, after spinning down undigested material, the supernatant is ready for PCR, eliminating the need for DNA purification.

You've just watched JoVE's guide to genotyping mice. In this video, we've reviewed the basics of mouse genetics, how to prepare and analyze mouse DNA samples, as well as some practical applications of this technique. Thanks for watching!