JAX Mouse Genotyping Assay Troubleshooting Guide

The Jackson Laboratory

Genotyping mouse strains requires dedicated time and resources. Having a genotyping assay not working correctly can create headaches for you and possible delays in setting up new matings and your experiments. This guide is designed to guide you in troubleshooting common problems with genotyping mice.

CONTROLS

Consistent genotyping of mouse strains and effective troubleshooting of genotyping assays requires that you use appropriate controls every time you genotype your mice. The following table lists which controls you need to use.

Control (Mutant / Transgene)	Homozygous mutant/transgene	Heterozygote/Hemizygote**	Homozygous Wild Type/Non-carrier	No DNA Template (water)
When Needed:	When distinguishing between homozygotes and heterozygotes/ hemizygotes*	Always	Always	Always (tests for contamination)

** If homozygotes are not produced in a mouse colony, then homozygous controls are not required. If homozygotes are embryonic lethal for example.*
***If a colony is maintained as homozygous and no heterozygous/hemizygous controls are available, you can create a pseudo heterozygote/hemizygote control by mixing DNA for a homozygote and a wild type together in a 1:1 ratio.*

FOUR COMMON GENOTYPING PROBLEMS

- *No bands*
- *One band not amplifying (mutant/Tg/WT/IPC)*
- *Artifact band*
- *Contamination*

NO BANDS

Troubleshooting genotyping assays when you get no bands can be challenging. The underlying problem can be any part of the PCR including the primers or other reagents, the DNA (quality and/or quantity), or the thermal cycling parameters. Determining where the problem lies requires a methodical approach. Following this approach will help you identify the problem so that it can be fixed.

First, check your primers.

Solution	Rationale
Check Sequence	Wrong sequence may not amplify.
Check Dilution	If primers are too dilute, they will not amplify.

If the primer sequences and dilutions are confirmed to be correct, the next steps are to look at the DNA. If not, then you may need to re-order primers with the correct sequence, or optimize the primer concentration.

Start by genotyping the DNAs for strain giving you no bands with a genotyping assay for another strain that is currently working for you, along with the controls for that other strain that have worked previously.

If you get no bands, then your problem is most likely with the DNA.

Solution	Rationale
Quantify DNA	Too much or too little DNA can lead to no bands. Use approximately 0.5 ng – 0.5 µg of total genomic DNA per 25 µl reaction.
Check Dilution	If the DNA quality is poor, it may not amplify bands.
Try diluting DNA	To reduce contaminates that may interfere with amplification.

If you do get bands, then the problem is not directly with the DNA. The problem could be the thermal cycling parameters or the DNA extraction protocol.

Solution	Rationale
Test an annealing temperature gradient	Find the optimal annealing temperature. If your annealing temp is too high, you may not amplify bands.
Increase the number of cycles	Too few cycles can lead to no amplification.
Try re-extracting DNA using a different protocol	Some primers may not work well with your normal DNA extraction protocol.

If the assay previously worked, and now does not, make sure you are including controls that have previously worked. If not, re-genotype with such controls.

If the previous controls work as expected but new unknown samples give no bands, the problem is likely with the new DNAs. Follow the same solutions as the previous section for resolving DNA quality/quantity issues.

If you get no bands for any DNAs, you likely have a problem with your primers.

Solution	Rationale
Make a new working dilution from your concentrated stock	Primers can degrade when maintained at lower working concentrations or following repeat freeze/thaws.
Re-order primers	

ONE BAND NOT AMPLIFYING

Having one of the expected bands in a genotyping assay fail to amplify well or at all can be frustrating. If that band is the mutant or transgene band, it is easy to assume that the mice are wild type and not carrying the mutation or transgene as expected. However, you will want to work through potential solutions to make sure you are not jumping to the wrong conclusion.

If this is a new assay to you, start by checking your order and your primer(s) specific for the band that does not amplify.

Solution	Rationale
Review your order confirmation	Make sure you recieved the correct strain and genotype that you expected.
Check primer sequence	Wrong sequence may not amplify.
Check primer dilution	If primers are too dilute, they will not amplify.

If your order and primers check out, then re-genotype using two separate reactions; one for the mutant allele or transgene, and one for the wild type allele or internal positive control.

If both bands amplify when separated but not when multiplexed, try altering the primer concentration for the primer or primers specific to the band that does not amplify when multiplexed.

If the band fails to amplify even when separated, the problem could be the thermal cycling parameters or the DNA extraction protocol.

Solution	Rationale
Increase amount of primers specific for band that does not amplify	When multiplexing, one band can be preferentially amplified.
Decrease amount of primers specific for band that does amplify	
Both above at same time	

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Solution	Rationale
Make a new working dilution from your concentrated stock	Find the optimal anneal temperature. If your annealing temp is too high, you may not amplify bands.
Re-order primers	Primers can degrade when maintained at lower working concentrations or following repeat freeze/thaws.
Re-extract DNA from the parents and re-genotype	If the parents are not the expected genotype, then the mice may not be the expected genotype. The assay may actually be working correctly.

ARTIFACT BANDS

Artifact or non-specific bands are bands that do not correlate to the expected mutant, transgene, or wild type bands. They are the results of primers annealing non-specifically. The presence of such bands can be disconcerting. However, as long as you can still reliable score your results you do not need to be concerned about the presence of the artifacts.

If the presence of artifact bands does impact your ability to accurately score your results as the artifact is too close in size to an expected band or by making the other bands weak or absent, then you will need to address the situation. Some solutions to eliminate or reduce the artifact bands are....

If this is a new assay to you, start by checking your order and your primer(s) specific for the band that does not amplify.

Solution	Rationale
Increase annealing temperature	Increases specificity for primer annealing
Test an annealing temperature gradient	Find the optimal anneal temperature that amplifies expected bands but not artifacts
Decrease primer concentration	Excess primer can lead to nonspecific amplification.
Use a Hotstart Taq polymerase	If primers anneal non-specifically at low temperature as cycle is ramping up, Taq can extend. Inactivating Taq until temperature is high can limit non-specific amplification
Use Touchdown PCR	Starting with a high annealing temperature allows specific bands to amplify initially.

CONTAMINATION

DNA can easily aerosolize, contaminating reagents, plastic ware, and DNA samples. This contamination leads to problems with band(s) of expected size amplifying from samples they should not amplify from. For example a wild type band amplifying from a homozygous mutant. This is why including a no template control is so important to include, because it can detect DNA contamination and prevent you from make incorrect conclusions about the genotype of your mice.

If you are amplifying bands of expected sizes in the no template control and samples...

Solution	Rationale
Quantify DNA	Too much or too little DNA can lead to no bands. Use approximately 0.5 ng – 0.5 µg of total genomic DNA per 25 µl reaction.
Check Dilution	If the DNA quality is poor, it may not amplify bands.
Try diluting DNA	To reduce contaminates that may interfere with amplification.

If you are amplifying no band in the no template control, but are in your samples, it is likely the DNAs are contaminated.

Solution	Rationale
Test an annealing temperature gradient	Find the optimal annealing temperature. If your annealing temp is too high, you may not amplify bands.
Increase the number of cycles	Too few cycles can lead to no amplification.
Try re-extracting DNA using a different protocol	Some primers may not work well with your normal DNA extraction protocol.

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