Optimizing PCR for mouse genotyping: recommendations for reliable, rapid, cost effective and high-throughput genotyping protocol for any mutation

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INTRODUCTION

Many rodent genotyping protocols are based on polymerase chain reaction (PCR) amplification of genes or genetic markers, as PCR is easy, fast, sensitive, and cost effective. Wrongly, PCR genotyping is often considered as a straightforward and easy step; in reality, providing robust, accurate, and fast results is frequently more challenging that it seems.

In Jacquot *et al.* 2019 protocol, we describe polymerase chain reaction (PCR) genotyping protocols for fast, sensitive, easy, and cost-effective characterization of mouse genotype. We discuss optimization of parameters to improve the reliability of each assay and propose recommendations for enhancing reproducibility and reducing the occurrence of inconclusive genotyping. Here, we present examples of recommendations to ensure reproducible and easy PCR genotyping.

GENOTYPING ERRORS CAN RESULT IN IRREPRODUCIBLE RESULTS, GENETIC CONTAMINATION OR LOSS OF A MOUSE LINE

Genotyping accuracy

Evaluation over a 5 years period

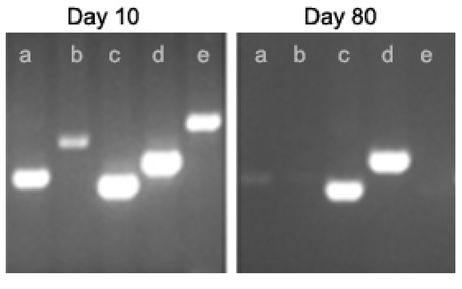
Year	Total animals analyzed	Animals genotyped with two independent biopsies	Comparison between both results		
			Confirmed genotypes	Distinct genotypes	% inconclusive genotypes
2013	56 331	4 140	3 867	273	6.6
2014	51 309	4 588	4 328	260	5.7
2015	58 250	7 166	6 673	493	6.9
2016	50 267	4 411	4 152	259	5.9
2017	51 272	6 900	6 576	324	4.7

Even in a highly standardized process, inconclusive genotypes are observed.

Error rate

A wrong genotype can be explained by different errors like:

- during tissue biopsy:
  - the wrong mouse was sampled because of misreading the identification code
  - tubes or samples were inverted
- during PCR: a sample was inverted
- during analysis: the wrong line was labeled



The same animal was sampled at newborn stage) and at its shipping date. The first biopsy leads to a transgenic animal genotype, the second biopsy only amplified the WT PCRs (c & d) corresponding to a WT animal genotype.

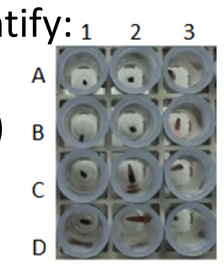
All key animals need to be genotyped twice to ensure a correct identification

TISSUES SAMPLING- AN UNDERESTIMATED SOURCE OF CONTAMINATION AND PCR FAILURE

Proposed quality check point for each tube to be processed

A visual verification of each tube can be done to identify:

- Absence or presence of additional biopsy
- Calibrated sample (e.g. C2 & D2: Oversize biopsy)
- Expected type of biopsy (ear notch, tail, …)

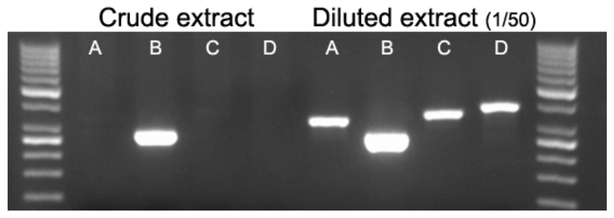


Uncalibrated biopsy

Calibrated sample size reduces the risk of inconclusive or incorrect genotyping.

In case of oversize biopsy the lysis buffer volume has to be adapted.

If it was not done, it is still possible to dilute the crude extract:

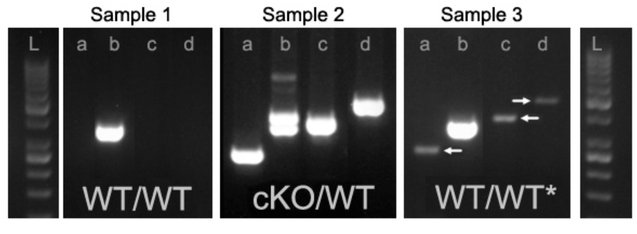


An oversize sample was analysed with or without dilution. A wrong or inconclusive genotype can be obtain due to inhibitors in crude extract

A, B, C and D are different primers pairs

Properly cleaned instruments

When collecting tissue samples, instruments must be cleaned between individual animals to avoid cross-contamination of genetic material.



The sample 3 shows a contamination during tissue collection that clearly impact the genotype determination.

IMPROVING GENOTYPING STRATEGY AND MOVING TO HIGH THROUGHPUT

In our protocol, we also describe:

- Recommendations to establish a robust genotyping strategy with reliable assays
- Standardized genotyping conditions that can be used for any mutant line
- Troubleshooting for each PCR genotyping steps
- Key steps required for transition to high-throughput automatized PCR, including mix miniaturization and automation

Jacquot, S., Chartoire, N., Piguet, F., Hérault, Y., & Pavlovic, G. (2019). **Current Protocols in Mouse Biology**
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EXCELLENCE IN MOUSE PHENOGENOMICS