WHITE PAPER

Mouse tail genotyping: A LabChip application to assess the genetic modifications on mutant mouse populations.

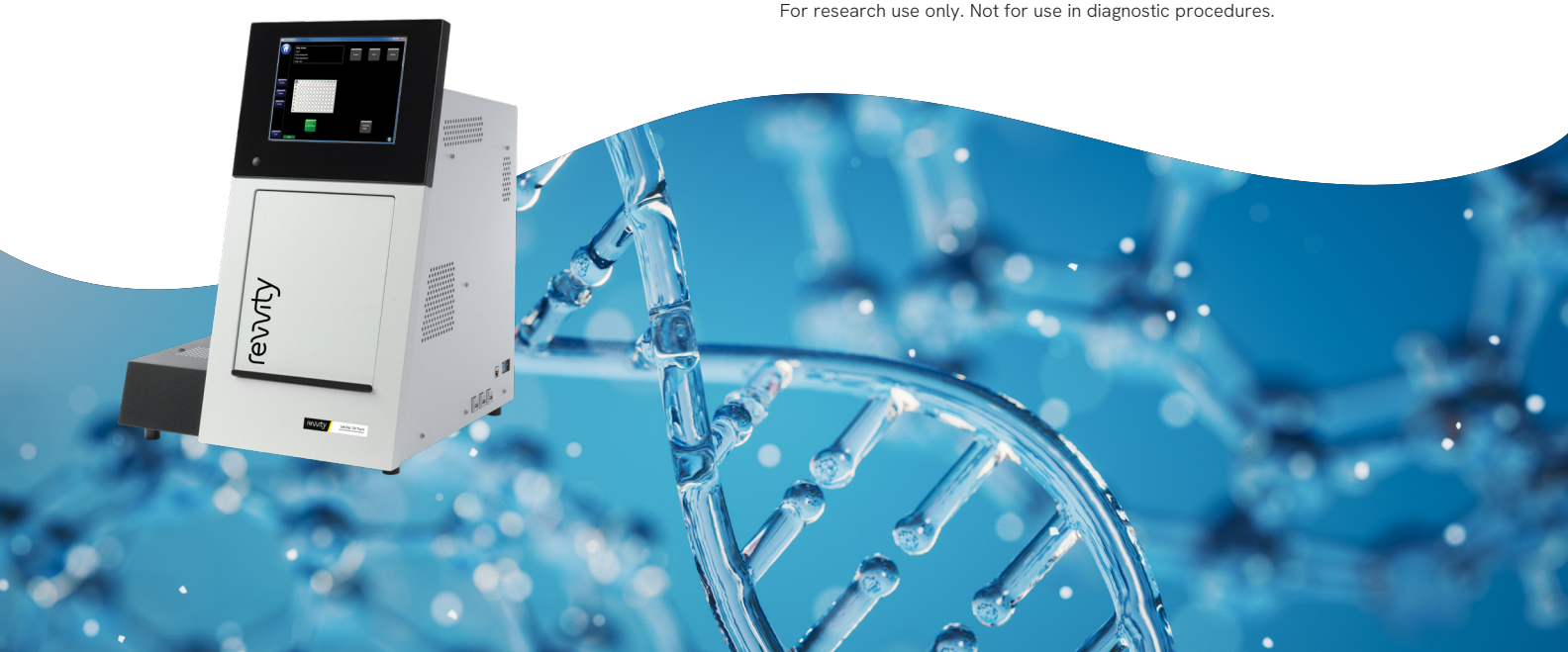
Introduction

Mouse tail genotyping in biomedical research is a technique commonly used to assess the effects of genetic modifications on transgenic and mutant mouse populations. Expanding mouse models have dictated that these time-intensive experiments be performed in a more rapid, high-throughput manner, leaving scientists constantly searching for more efficient laboratory workflows and generation of higher quality data in order to keep up.

Typical mouse genotyping workflows include the isolation and purification of DNA fragments from samples followed by PCR-amplification, electrophoretic separation and analysis. The process can include multiple, manual and labor- intensive steps, and becomes extremely time-prohibitive when working with large mouse colonies as they not only require a greater number of samples but also many different genotypes to be processed and analyzed. Depending on the genotyping analysis required, different PCR primer pair combinations may also be needed, further increasing the complexity of experimental design, setup and data interpretation.

Here we will highlight how researchers at two organizations utilized robotic liquid handling for genotyping reaction setup and microfluidic-based CE for PCR fragment analysis. This combination of automated solutions dramatically improved their experimental turn-around times and data quality while also reducing hands-on time and labor costs.

LabChip® GX Touch™ platform



High-throughput genotyping challenges

A general overview of the genotyping process is outlined in Figure 1. A small portion of the distal tail is removed from young mouse pups. Typically, biopsied samples are then subject to overnight lysis, followed by purification of the DNA to remove salts and detergents. The purified DNA is then PCR-amplified using the appropriate primers and analyzed by gel electrophoresis.[1]

The sheer number of samples to be analyzed combined with the complexity of experimental setup in genotyping studies creates a processing bottleneck in many laboratories, and lends itself to many inefficiencies and sources of error. When manual protocols are used, DNA isolation and PCR amplification (often with multiple primer combinations) are subject to human error during reaction setup and vulnerable to lower data quality. The manual preparation of slab gels for electrophoretic separation of PCR fragments as well as the interpretation of results during data analysis can also be inconsistent. In addition, this tedious and time-consuming experimental process from start to final result can take days to complete, limiting availability of already costly animal holding space. Some low throughput, semi-automated, instrumented systems are available that can partially resolve the ongoing issues manual workflows create in mouse tail genotyping experiments. However, they do not relieve the processing bottleneck, as manual pipetting is still required to initiate the analysis.

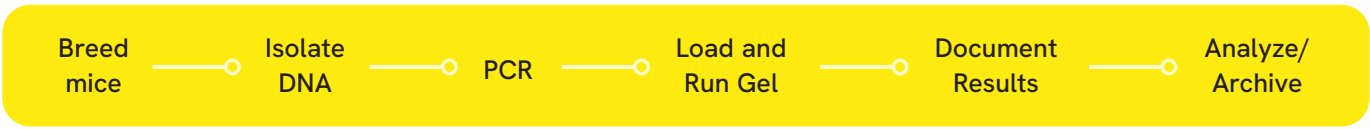


Figure 1. Typical workflow process in a mouse tail genotyping experiment.

Overcoming the challenges

Replacing manual processes with automated solutions can have a dramatic impact on the efficiency of genotyping operations. Incorporation of liquid handlers designed specifically for genomic applications from Revvity can automate genotyping reaction setup for DNA isolation and PCR amplification, eliminating manual hands-on time and potential for human error. In addition, the LabChip® GX system, an automated alternative to slab gels, provides direct sampling from 96- and 384-well plates and electrophoretic separation and quantitative analysis of PCR fragments, drastically improving the consistency and accuracy of results. Integration of either or both platforms can assist in overcoming laboratory inefficiencies and bottlenecks, effectively transitioning any genotyping facility into a more efficient, higher-throughput operation.

TaconicArtemis

Scientists at TaconicArtemis in Cologne, Germany eliminated multiple manual processes in their laboratory workflow that ultimately lead to both an increase in genotyping capacity and a reduction in FTE requirements over the course of two years.[2] Phase 1 included the integration of a liquid handler for automated reaction setup. Master mix recipes and pipetting instructions were transferred to the liquid handler via a custom designed LIMS, which both drastically reduced hands-on time and ensured preparation consistency. Phase 2 incorporated the LabChip GX system for direct analysis of genotyping PCR products (Figure 2). Plate layout files including sample names and expected DNA fragments were automatically exported to the instrument from the in-house information system and upon completion of analysis, the LabChip GX system then transferred results directly into the main laboratory database. This automated analysis and data transfer reduced labor costs even further by eliminating manual slab gel processing and data entry.

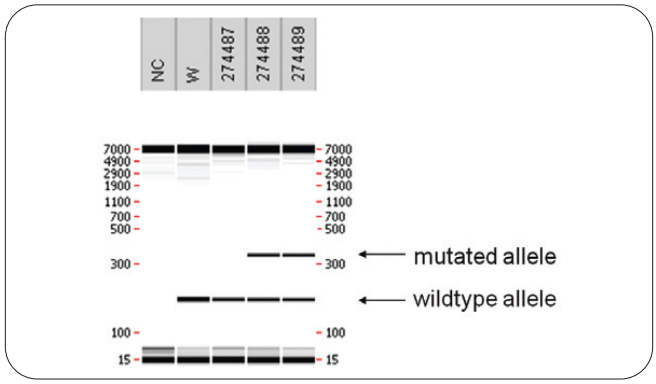


Figure 2. LabChip GX DNA fragment analysis for TaconicArtemis genotyping experiments. The data distinguishes mutants and wildtype mice by size differences of PCR-products. Reproduced with permission of TaconicArtemis.

By incorporating automated solutions into their genotyping program, TaconicArtemis increased their genotyping throughput from 44,000 genotypes in 2007 to 63,000 in 2009 (a 143% improvement) and significantly reduced the hands-on time compared to their previous manual methods. The scientists in the genotyping program presented the following key values that automation brought to their operation:

- **Automatic data exchange between the in-house** information system, liquid handler and LabChip GX decreases pipetting and data entry errors
- **Manual calculation of master mixes no longer needed,** recipes are just one click away
- **A consistency check could be added during PCR setup,** ensuring use of the right controls at all times
- **Not having to use ethidium bromide in manual slab gel** casting procedures improves researcher safety
- **Automated information flow eliminates paperwork for** laboratory staff and reduces error rates
- **Results are automatically archived, simple to find, and** database reports are easily generated

National Institutes of Health

The need for more rapid genotyping of mutant mouse colonies led researchers at NIH in Bethesda, MD to investigate replacing their manual workflow processes with higher-throughput alternatives to reduce time to result and increase experimental accuracy. A Revvity MultiPROBE® II HT robotics workstation was incorporated for automated assembly of the PCR reaction. Software-automated script generation with direct transfer of reaction setup to the liquid handler eliminated manual manipulation errors such as incorrect template and master mix combinations or confusion of wells during manual pipetting. A LabChip 90 system (precursor to the LabChip GX) was integrated to automate the analysis of PCR products (Figure 3), replacing manual slab gel processing and data interpretation. This resulted in a high-throughput genotyping workflow that reduced the total time required to process a typical 96-well plate by 50%, and a reduction in hands-on time of over 80% when compared to their previous, manual processes.[3] In addition, the labor cost savings ultimately reduced NIH's cost per sample by over 60%. A detailed time and cost comparison of the two workflows is shown in Table 2 (reproduced from the original article with permission). The scientists at NIH also stated that use of these automated platforms enabled:

- **Multiple combinations of genotyping reactions to be** assembled simultaneously, allowing even complex genotyping data to be generated rapidly with consistency and accuracy.
- **An important advantage in that the analysis is virtually** error free because human manipulation is minimized.
- **Reduced animal housing costs made possible from more** rapid turnaround of mouse genotype assignments.

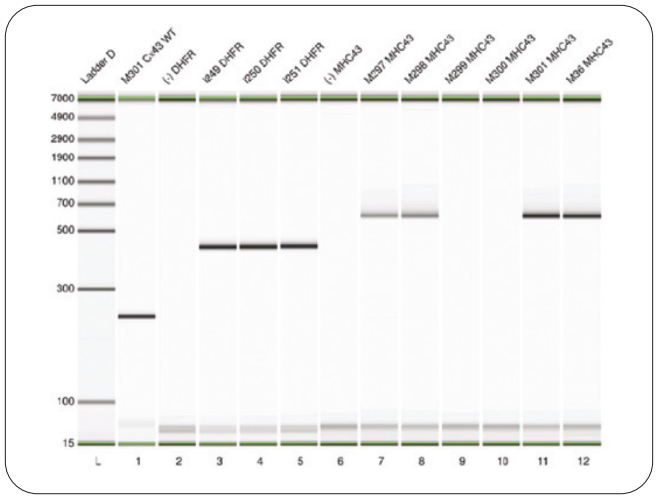


Figure 3. LabChip 90 DNA fragment analysis for NIH genotyping experiments (gel view). Products were generated from a single row from a 96-well plate of PCR samples. Lane labels are imported values that include the mouse Tag identification (ID) and the specific PCR amplifications carried out. Negative controls are indicated by a minus sign (–). The background bands below 50 bp are nonspecific primer products. Reproduced from the original article with permission.

Table 2. NIH cost and time analysis of various genotyping methods (reproduced from the original article with permission).

		Cost per Sample			Hours per 96 Samples	
Method	Start-up Costs	Consumables	Labor	Total	Hands-on	Hands-off
Automation	$160,400.00	$1.06	$0.72	$1.78	1.4	8.9
Manual	$19,200.00	$0.67	$4.13	$4.81	7.9	12.8
Outsource	$28,000.00	N.A	N.A	$27.00	N.A	N.A

Labor costs are based on a $50.00 hourly wage including benefits. Outsourcing start up is based on 40 PCR primer sets requiring sets requiring setup and validation for genotyping. Automation start up includes all equipment and software used in Materials and Methods section. Manual start up includes the thermal cycler, two thermomixers, and a centrifuge. Time values are based on extrapolated averages measured for various counts of samples. N.A. = not applicable.

Summary

Optimizing the mouse tail genotyping process using the automated solutions described here were shown to drastically improve workflows, allowing complex experiments involving large numbers of samples and multiple genotypes to be analyzed rapidly and effectively. Time-intensive and error prone manual processing steps can be completely automated using Revvity's robotic liquid handlers and LabChip GX system to perform DNA isolation and PCR reaction setup as well as PCR fragment analysis. Integration with a local LIMS system further streamlines the process by enabling automatic export of master mix recipes, pipetting instructions and analysis details to the appropriate platform, as well as transfer of fully analyzed results directly into the laboratory database. Automated workflows overcome manual processing bottlenecks and significantly improve data quality by eliminating human interaction in multiple areas – and the associated potential for error. Genotyping experiments can be conducted in a more expedient manner, and the dramatic reduction in time to results also facilitates better utilization of animal holding space.

Labs that integrated Revvity's automated liquid handling and analysis solutions for mouse tail genotyping studies experienced the following:

- **50% reduction in processing time for 96-well plates**
- **>80% reduction in hands-on time compared to manual processes**
- **>60% reduction in overall cost per sample**
- **>140% improvement in number of genotypes processed**
- **Improved lab safety via elimination of ethidium bromide**

References

1. Miller, S.A., Dykes, D.D. and Polesky, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research, 16 (3):1215 (1988).
2. Cziudaj, H. Caliper Owners Group Meeting Presentation (2010).
3. Linask, K.L. and Lo, C.W. High-throughput mouse genotyping using robotics automation. BioTechniques 38 (2): 219-223 (2005)

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com