

# Production & Screening of Mouse Neurological Mutants: The Jackson Laboratory's Neuroscience Mutagenesis Facility



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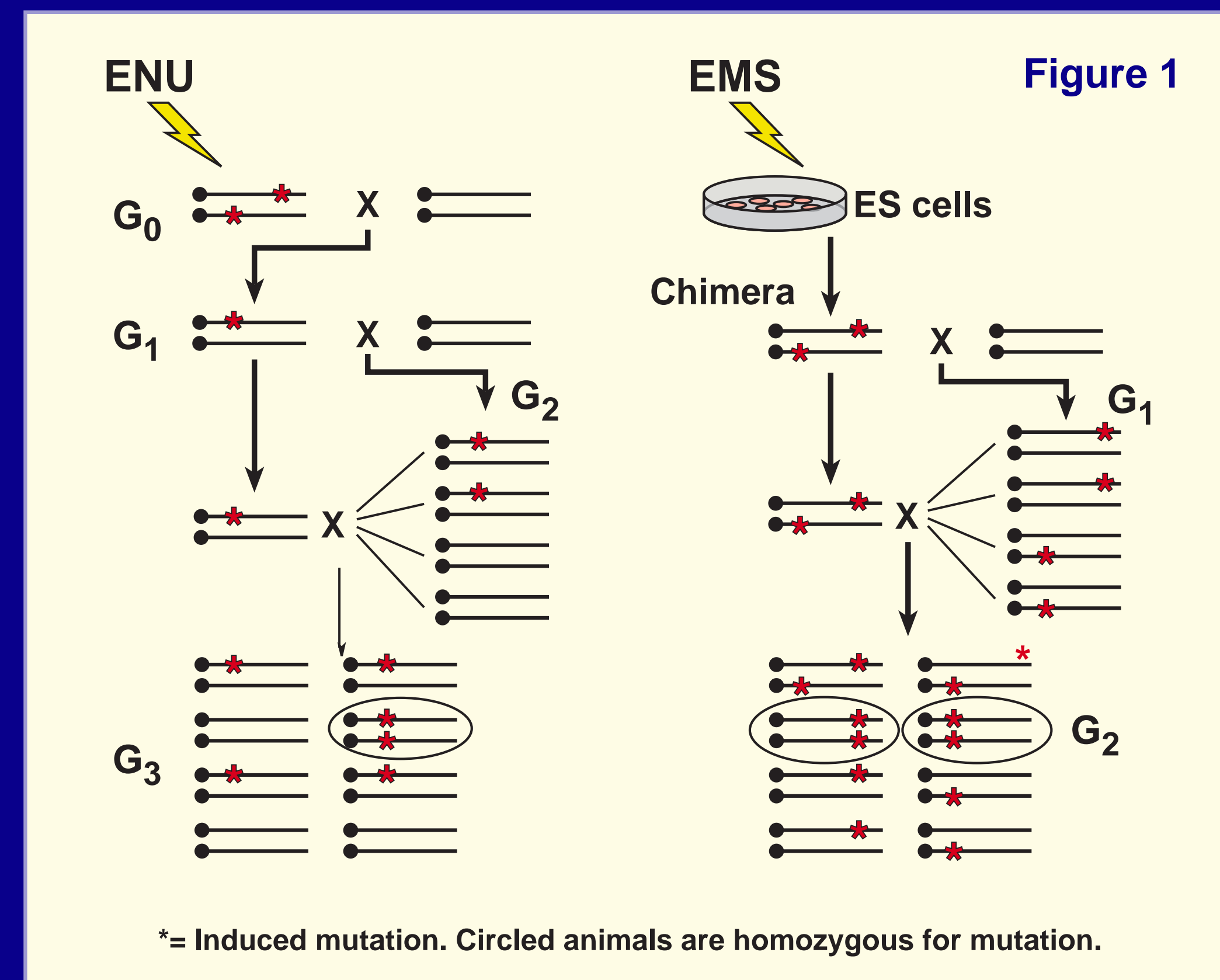
## Introduction

The mouse has emerged as the premiere experimental mammal because of the ability to analyze known genes and for the variety of new heritable phenotypic variants available for study. Recent progress in gene cloning, expression and gene targeting promise to further our knowledge, but genotype-based approaches alone are insufficient due to the complexity and redundancy of CNS functions. The next step is to systematically collect a large number of mouse mutations for specific neurological disorders and mechanisms. Large-scale mutagenesis and phenotypic screens offer a powerful approach towards this end.

The Jackson Laboratory has established a Neuroscience Mutagenesis Facility (JAX-NMF) to produce new mouse models of human neurological disease. Following mutagenesis and a three-generation backcross breeding scheme, a broad phenotyping protocol has been applied focusing on areas of motor function, epilepsy, vision, hearing, and gustation. The overall goal of the facility is to maximize the number of neurological mutant mice that become important research tools.

## Methods

**Mutagenesis:** Our facility is generating new mutations using ENU mutagenesis. A three-generation screen is being used to uncover dominant, semi-dominant and recessive mutations. Both C57BL/6J and A.B6-tyr+ mice third generation offspring (G3) are produced from a G1 founder, which represents the equivalent of one mutagenized gamete (genome). To increase efficiency, ES cell mutagenesis using other mutagens is also being employed (Fig.1).



**Phenotyping:** A major challenge in large-scale mutagenesis is balancing the need to gather sufficient information on each mutation against the need to screen large numbers of animals to gain broad coverage of the genome. Proper balance of these factors maximizes the eventual recovery of interesting new mutations. Our approach uses a sequence of simple and efficient primary screens, multiple domain screens where possible, for a variety of phenotypic domains (Fig. 2), and then relies on more sophisticated secondary characterization of potential deviants (Table 1).

## Phenotyping Strategy

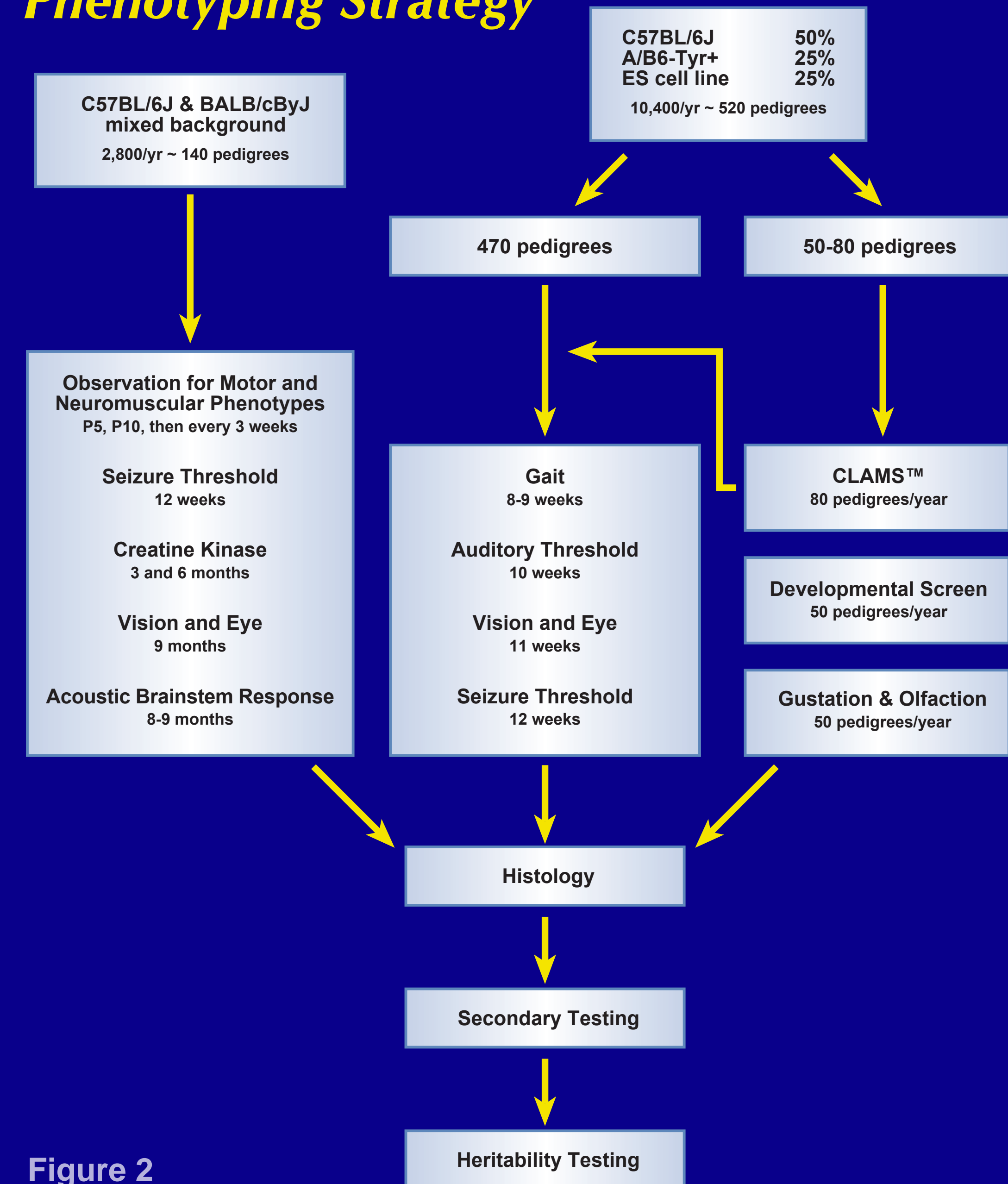


Figure 2

## Phenotyping - 1° and 2° Characterization

Domain (detection screen)	Secondary characterization
Motor and Neuromuscular Function (Gait, grip, CK)	Rotarod, contractile properties
Epilepsy/Seizure threshold (ECT +/- AED)	PTZ, EEG, AED Profile, IEG
Vision & Eye (eye exam, ERG)	ERG, FLA, PHO, IOP
Auditory & Vestibular - Acoustic Startle (ASR)	Acoustic Brainstem Response (ABR)
Gustation (two-bottle)	Two-bottle (diff. concs.), lickometer
Developmental Screen (E18.5 observation)	Histological work-up
Multi-Domain (CLAMS™-neurological, metabolic, ingestive and behavioral)	Body composition, blood analysis, hole board, zero-maze, wheel running

Table 1

## Mouse Model Development

Once a phenotypic deviant is identified, a process is launched to confirm and further characterize the phenotype and to establish and preserve the new mutant line. Once heritability is confirmed and appropriate phenotypic testing is completed, the new NMF mutant lines are made available through the NeuroMice distribution portal ([www.neuromice.org](http://www.neuromice.org)).

## Confirm Heritability

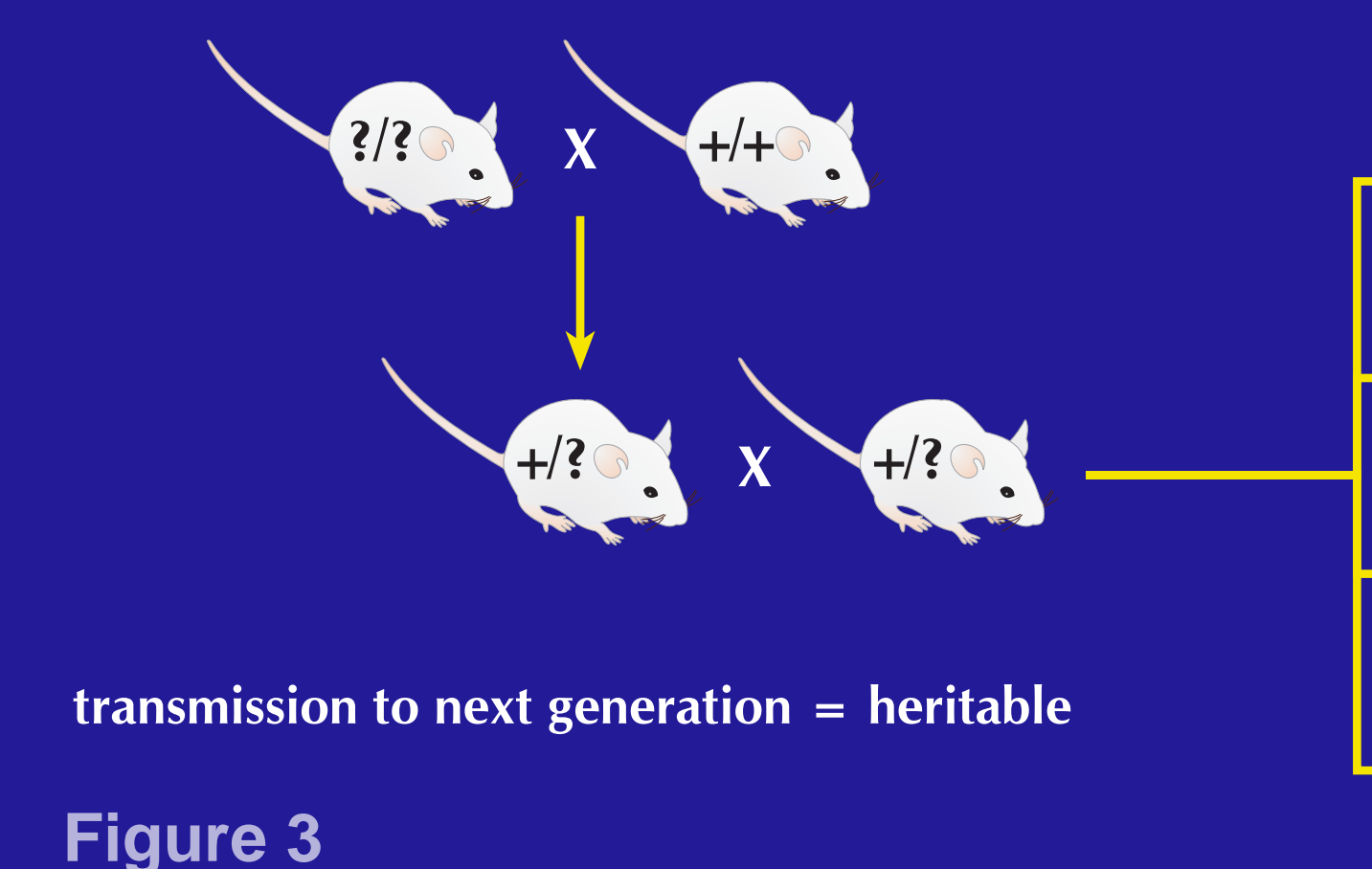


Figure 3

## Heritability Tests - Status

	Mutant Lines			
	Attempted	Abandoned /Failed	Proven	Under Development
Overt	49	26	5	18
Motor and Neuromuscular	21	5	10	6
Epilepsy/Seizure Threshold	32	15	4	13
Vision and Eye	47	20	2	25
Auditory and Vestibular	24	19	3	2
Gustation and Olfaction	11	5	0	6
Developmental	3	0	0	3
FPS/PPI	45	45	0	0
Other (DNA Scan)	2	0	0	0
Total	234	135	24	73

## Mapping

A founder mouse, carrying the NMF mutation (genotype n/n) is outcrossed to an inbred mouse strain of choice (genotype +/+) and the resultant F1 heterozygotes are intercrossed to generate F2 progeny (Figure 4). A genome scan is carried out on pooled DNA from 20 affected and 20 unaffected F2 mice using microsatellite markers. PCR reactions are performed and analyzed by electrophoresis, and subsequently map distance and gene order are determined using the MAPMAKER computer program. To date over 75% of our heritable mutations have been mapped, corresponding to regions on chromosomes 1, 2, 5, 10, 11, 15, and 16 (Figure 5).

## Mapping Strategy

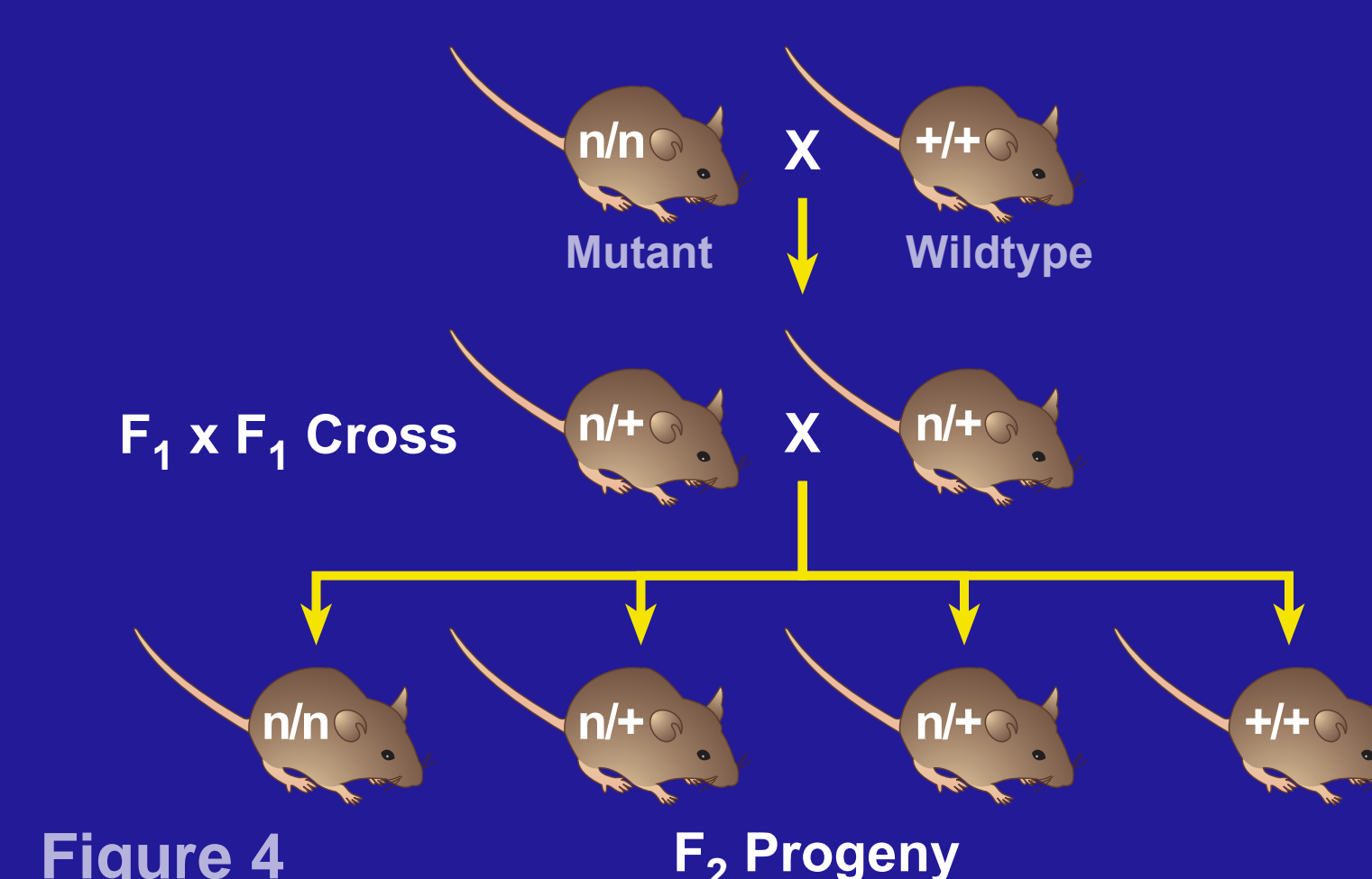


Figure 4

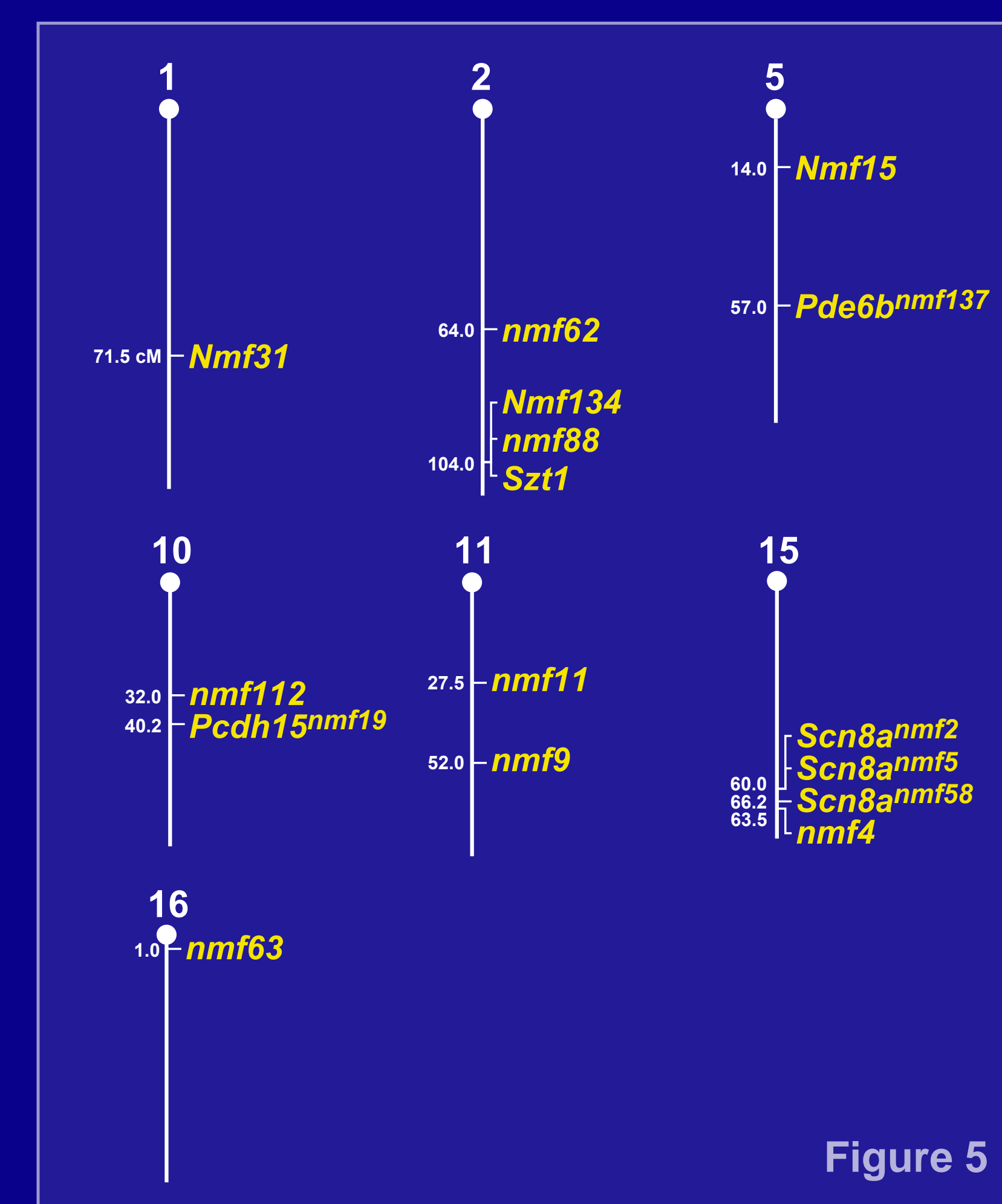
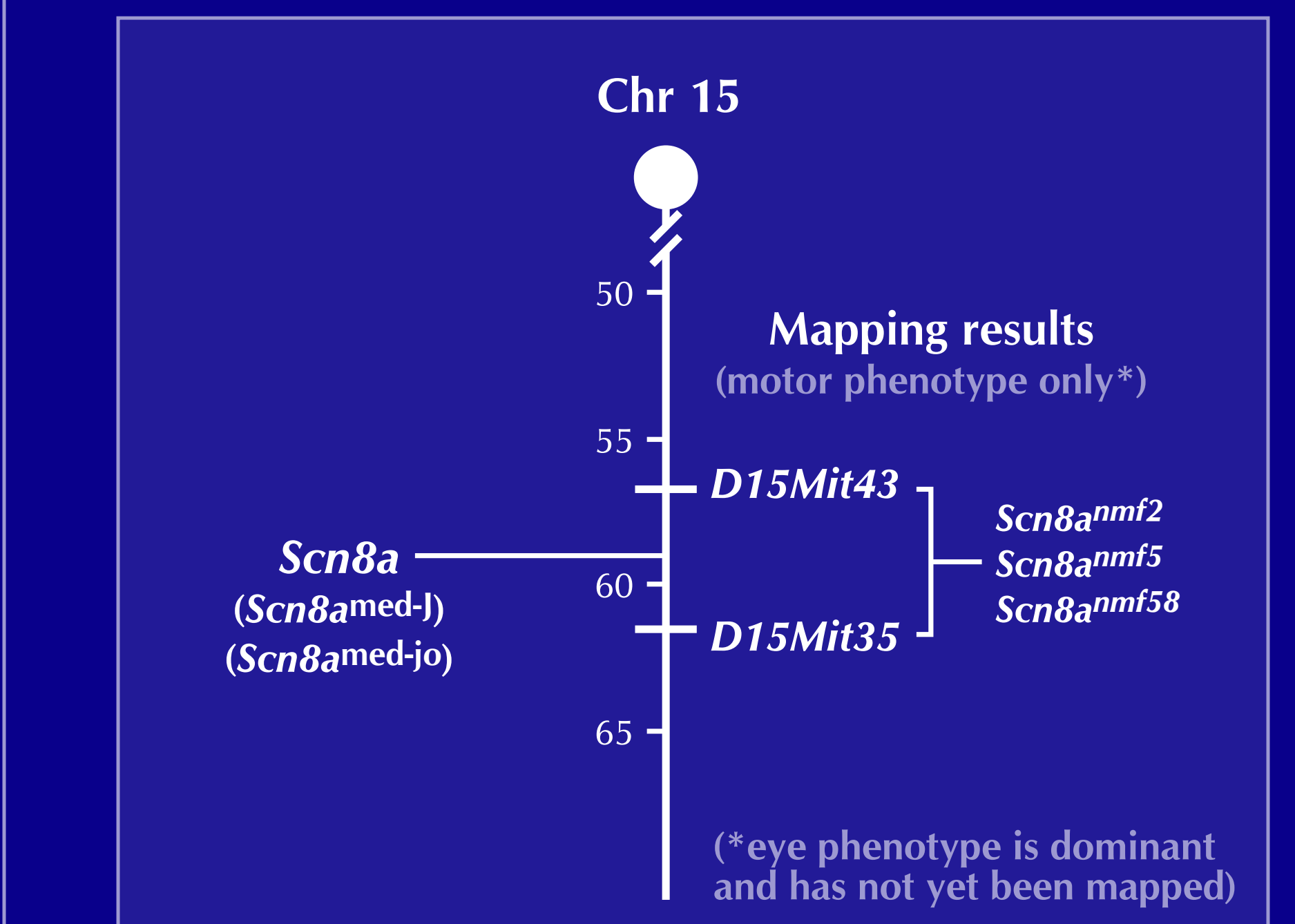


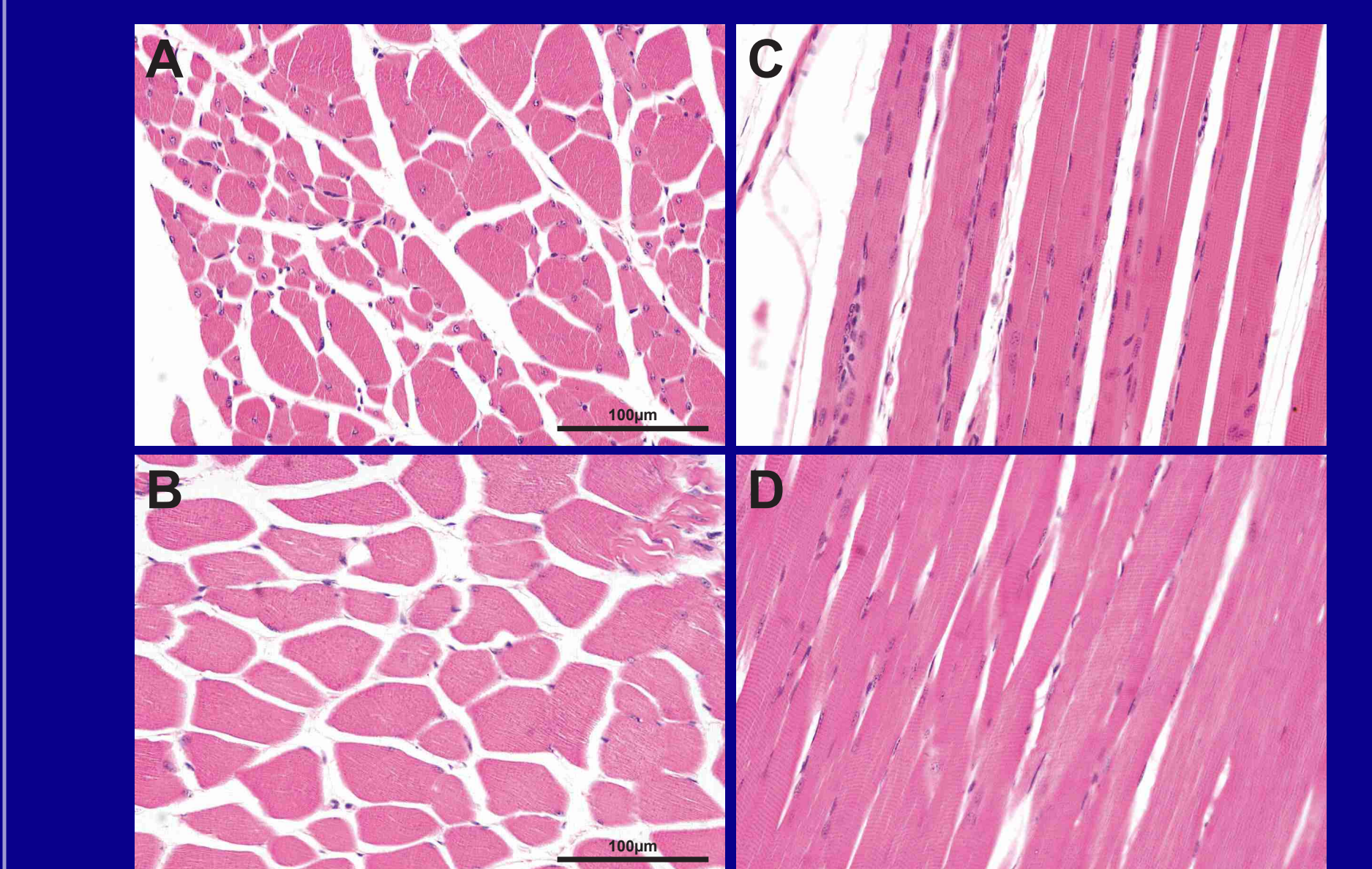
Figure 5

## A Novel Allelic Series

The allelic relationships of the voltage-gated sodium channel VIII, alpha polypeptide (Scn8a) with NMF 2, 5 and 58 was initially determined through complementation tests, and in the case of NMF 2 and 5 confirmed through mapping studies.



The main characteristic that could be observed in all affected mice was a visible hind limb paralysis (see movie clips which can be viewed here upon request) with an average onset of about 15 days of age. In contrast to the observed movement impairment common to all affected mice, pathological examination of muscle tissue revealed abnormalities in only one mutant line, NMF58 which showed atrophic muscle tissue, central nuclei (A), and rowing of nuclei (B) in contrast to normal muscle tissue (C & D) (histology photographs, 40x).



## Summary

The detection and characterization of several alleles of a gene is of particular interest since it provides more comprehensive information about gene function than could be obtained through a single phenotype.

These and all other NMF mutants may be obtained for a low price by any scientific investigator through the NMF (<http://nmf.jax.org>) or Neuromice (<http://www.neuromice.org>) web sites; the latter serves to present available mutants from all three participating consortium centers (University of Tennessee, University of Chicago).

For more information on JAX-NMF  
or to check available mice go to:

<http://nmf.jax.org>

or

<http://neuromice.org>

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