Genetic Background Characterization and Stabilization in Genetically Modified Models

Taconic

Smart Solutions To Improve Human Health

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Outlay



- What is Genetic Background
- Phenotype variation due to Genetic Background
- How to stabilize the genetic background
- What are Single Nucleotide Polymorphisms (SNPs)
- What are speed congenics

What is Genetic Background?

Genetic Background are all the genes and all of the alleles present in a particular strain or stock of a mouse or rat not including the introduction of a mutation through molecular biology techniques.

Homogenous Genetic Background

- **Inbred mouse**
- **Brother x sister mating for 20 generations**
- **At any one locus/gene—homozygosity: H/H or h/h**
- **No (or minimal) segregation over whole genome, so next generation is equivalent to previous one**
- **Each strain approximates multiple copies of a single individual, but are not clones. Thus, each strain has certain characteristics**
  - C57BL/6, FVB, 129S6, etc

Inbred Genetic Background



C57BL/6NTac
aaBBCCDDPP

DBA/2JBomTac
aabbCCddPP

129S6/SvEvTac
$A^wA^wBBCCDDPP$

A/a = Agouti locus (*a;* Chr 2) (banded black/yellow [A] vs. black [a] vs. white-bellied [A^w])

B/b = brown locus (*Tyrp1;* Chr 4) (black vs. brown pigment)

C/c = albino locus (*Tyr;* Chr 7) (pigment vs. no pigment)

D/d = dilute locus (*Myo5a;* Chr 9) (intense pigment vs. diluted pigment)

P/p = pinked-eyed dilution locus (*p;* Chr 7)

Heterogenous Genetic Background



- **Outbred Stocks-Mixed backgrounds**
  - Original genetic modification in a 129 background bred to C57BL/6
- **Maximize genetic diversity**
  - Desirable sizes, weights, fecundity, etc.
  - Sometimes reveal phenotypes not observed on inbred backgrounds
  - Often allows maintenance of mutations that have severe effects on inbred strains
- **Maximal heterozygosity at each locus**
  - Allele segregeation
  - Small colony of outbreds, replace frequently
  - Rederivations, genetic bottlenecks
  - Genetic monitoring checking allele frequencies *

What is Phenotype?



Phenotype is the result obtained from the simultaneous expression of all of those genes from a particular strain or stock

It could be observable by the naked eye or detectable by biochemical reactions

What Phenotype Color are these Genotypes?

C57BL/6NTac

Genotype
aaBBCCDDPP

Phenotype
BLACK

DBA/2JBomTac

Genotype
aabbCCddPP

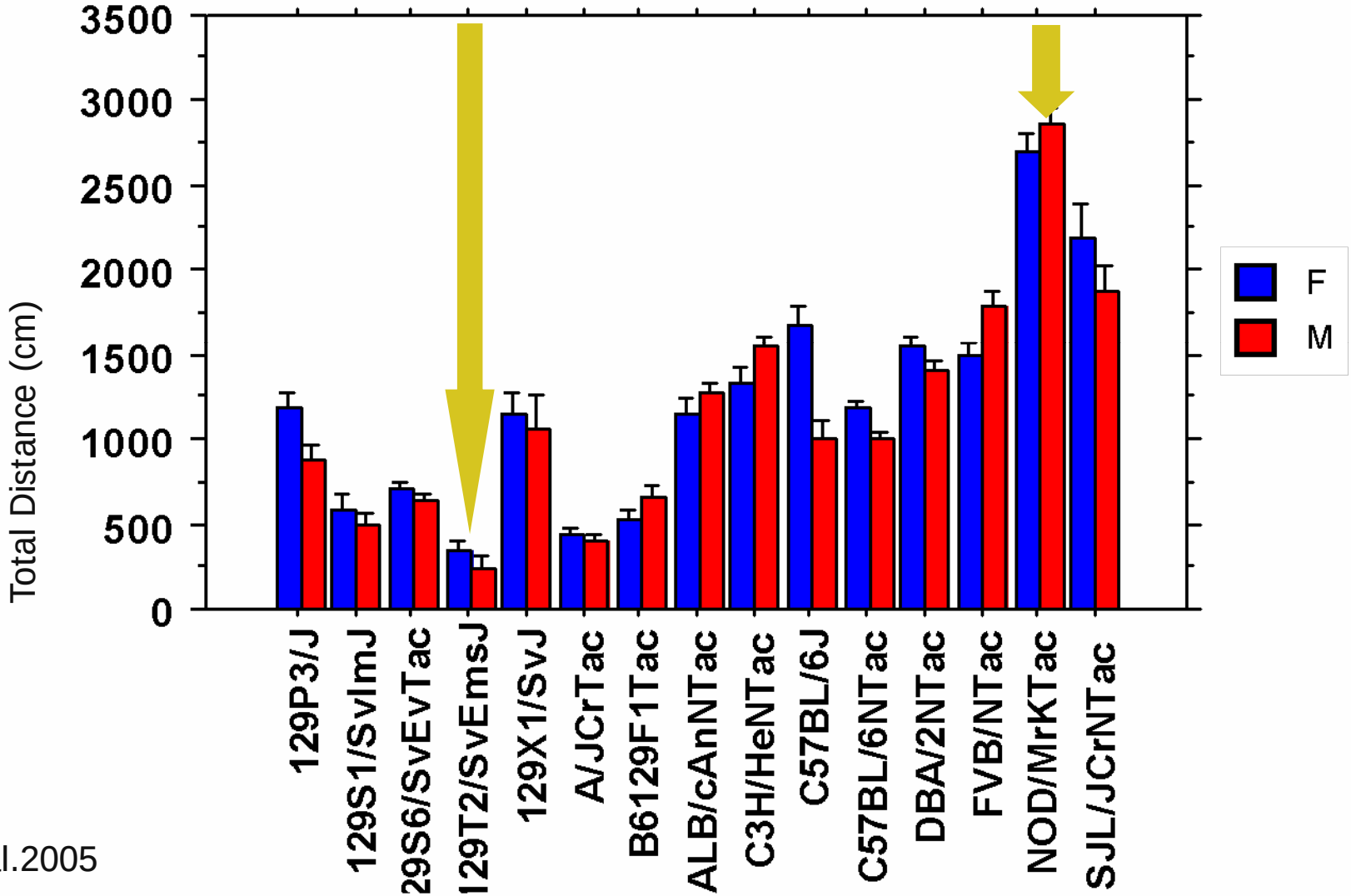
Phenotype
DILUTE BROWN

129S6/SvEvTac

Genotype
$A^wA^wBBCCDDPP$

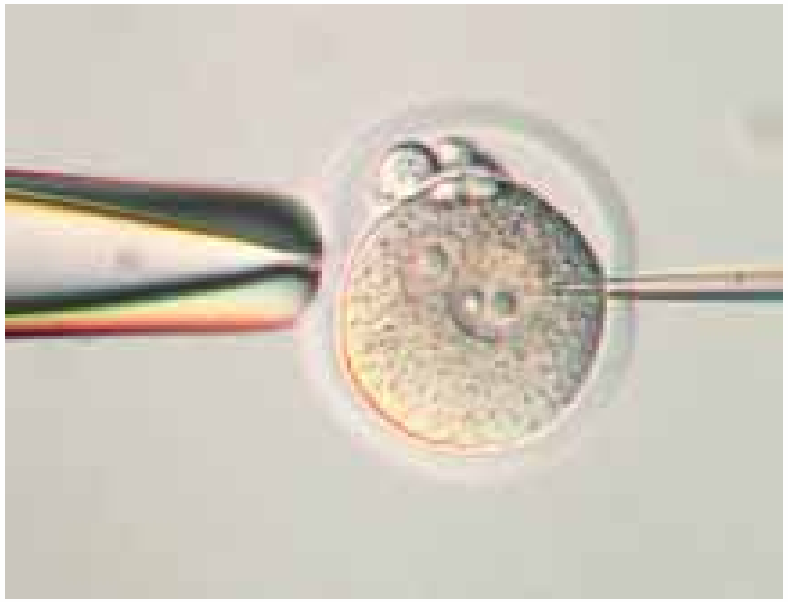
Phenotype
AGOUTI

Open Field Activity (first exposure)



Taconic -G. Bothe et al.2005

Genetically Modified Models



Pronuclear Injections

Embryonic Stem Cells

Importance of Genetic Background when introducing a mutation

C57BL/6NTac
aaBBCCDDPP
BLACK
Tg(APPSWE)

129S6/SvEvTac
$A^wA^wBBCCDDPP$
AGOUTI
Tg(APPSWE)

APPSWE Transgenic mouse model



- **Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by progressive cognitive decline and memory loss**
- **Accumulation of beta amyloid protein is believed to be an important pathological feature**
- **APPSWE is known as the amyloid precursor protein that contains the Swedish mutation which is an allele that encodes a double amino acid substitution and it is associated with heritable susceptibility to AD**
- **It does not completely recapitulate the human AD, it can be use to investigate specific intracellular processing pathways**

Origin of the APPSWE Model



- The APPSWE Model was developed by Karen Hsiao Ashe at the Department of Neurology and Neuroscience, University of Minnesota.
- A construct was created that carried the Swedish mutation form of the human APPSWE gene, which produces a 695-amino acid protein with two substitutions (Lys670→ Asn and Met671→ Leu).
- The construct was inserted into a hamster prion protein cosmid vector in which the reading frame was replaced with the variant APPSWE open reading frame.
- The transgene originally was developed in FVB/N mice, but they were poor breeders and died prematurely.
- Therefore, the vector was introduced by microinjection into B6SJL F2 single-cell embryos, producing transgenic founders.

Origin of APPSWE Model (con't)

- **Taconic's colony was established by transfer of embryos resulting from breeding a hemizygous transgenic male to a C57BL/6NTac female. The resultant male progeny were bred to SJL/JcrNTac females. The model 001349 colony is now maintained by breeding hemizygous transgenic male mice with female B6SJLF1/Tac mice.**

- **To generate model 002789, mice from Founder Line 2576 were backcrossed sixteen generations (N16) to 129S6. Taconic received stock in September 2003.**

- **The mice were derived by embryo transfer and are maintained by backcrossing hemizygous male mice with 129S6/SvEvTac female mice.**

Characteristics APPSWE Transgenic mouse model



- Early and progressive accumulation of beta amyloid and development of plaques
- Behavioral deficits that correlate with degree of amyloid deposition
- Neuritic dystrophy and altered synaptic efficacy of plaque-associated neurons
- No evidence of neuronal loss
- Expression of inflammatory mediators by plaque-associated microglial cells and astrocytes
- Sex differences in some aspects of physiology and behavior
- Age-correlated elevation in brain levels of apoE and cholesterol
- Deposition of amyloid in cerebral blood vessel walls

Genetic Background Differences in APPSWE models

- **Model 1349 is a on a mixed B6;SJL background**
- **Model 002789 is on an inbred 129S6 background**
- **In a C57BL/6 background the mutation is lethal**
- **Pink eyed animals, associated with certain coat colors, and the *Pde6b*$_{rd1}$ *retinal* degeneration mutation can cause light sensitivity and/or blindness in some animals. This may impact the results of behavioral testing.**
- **The mixed genetic background of model 001349 can result in pink eyed animals or homozygosity for the *Pde6b*^{rd1} *retinal degeneration mutation.***
- **The 129S6 background of model 002789 does not carry the *Pde6b*^{rd1} retinal degeneration mutation, and this strain has pigmented eyes.**

How to test the Genetic Background

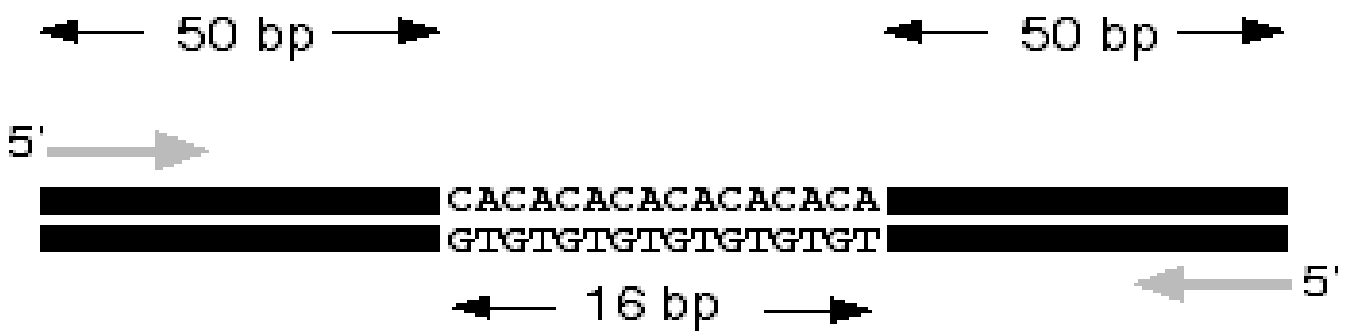


- **Biochemical testing- tests for proteins /genetic polymorphisms**
- **DNA Microsatellites**
- **Single Nucleotide Polymorphisms or SNPs**

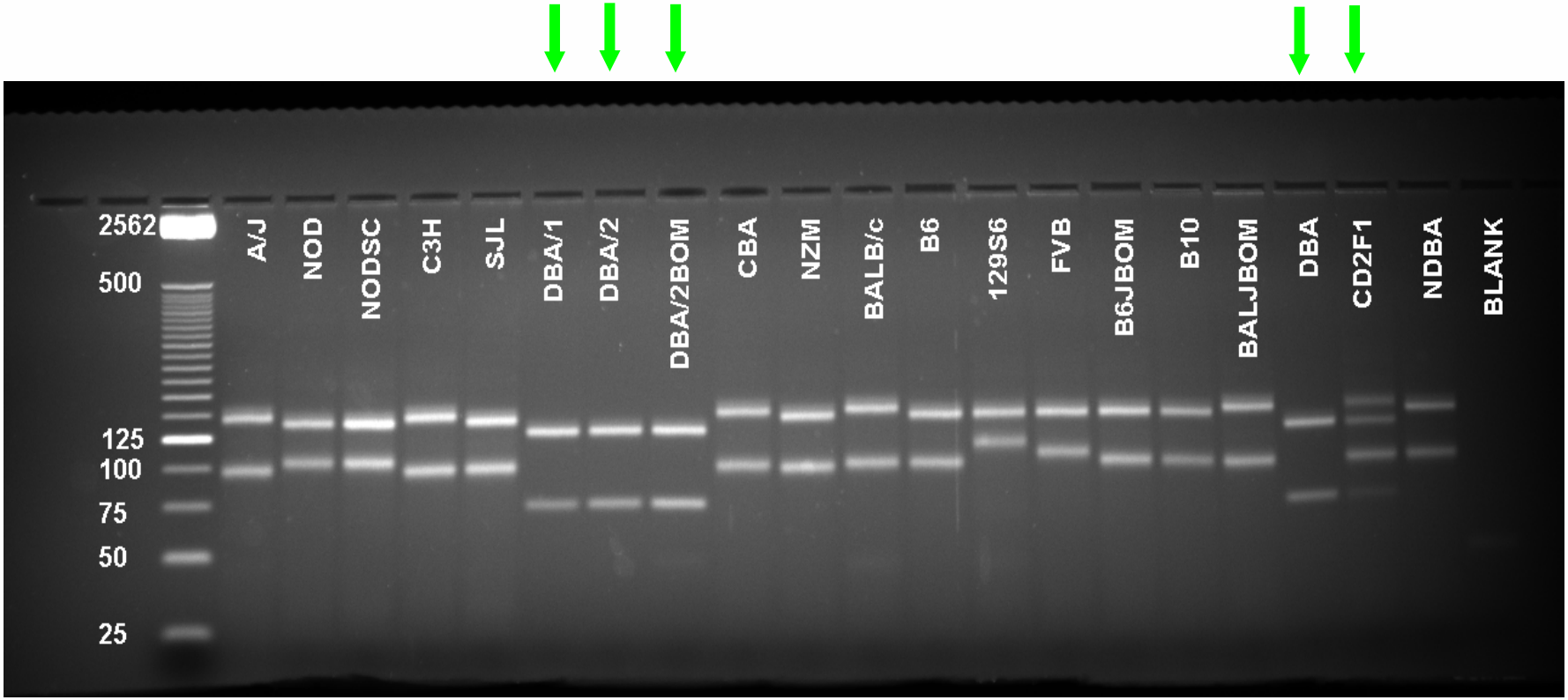
Genetic Background Testing



- **DNA microsatellites**
  - Short segments of DNA that have a repeated sequence
  - The length of the sequence repeat varies from species to species and from strain to strain
  - It is somewhat unstable, higher mutation rate
  - Artifact/Technical problems
  - It is difficult to perform in a high-throughput manner



Genetic Markers - Microsatellites



Genetic Background Testing



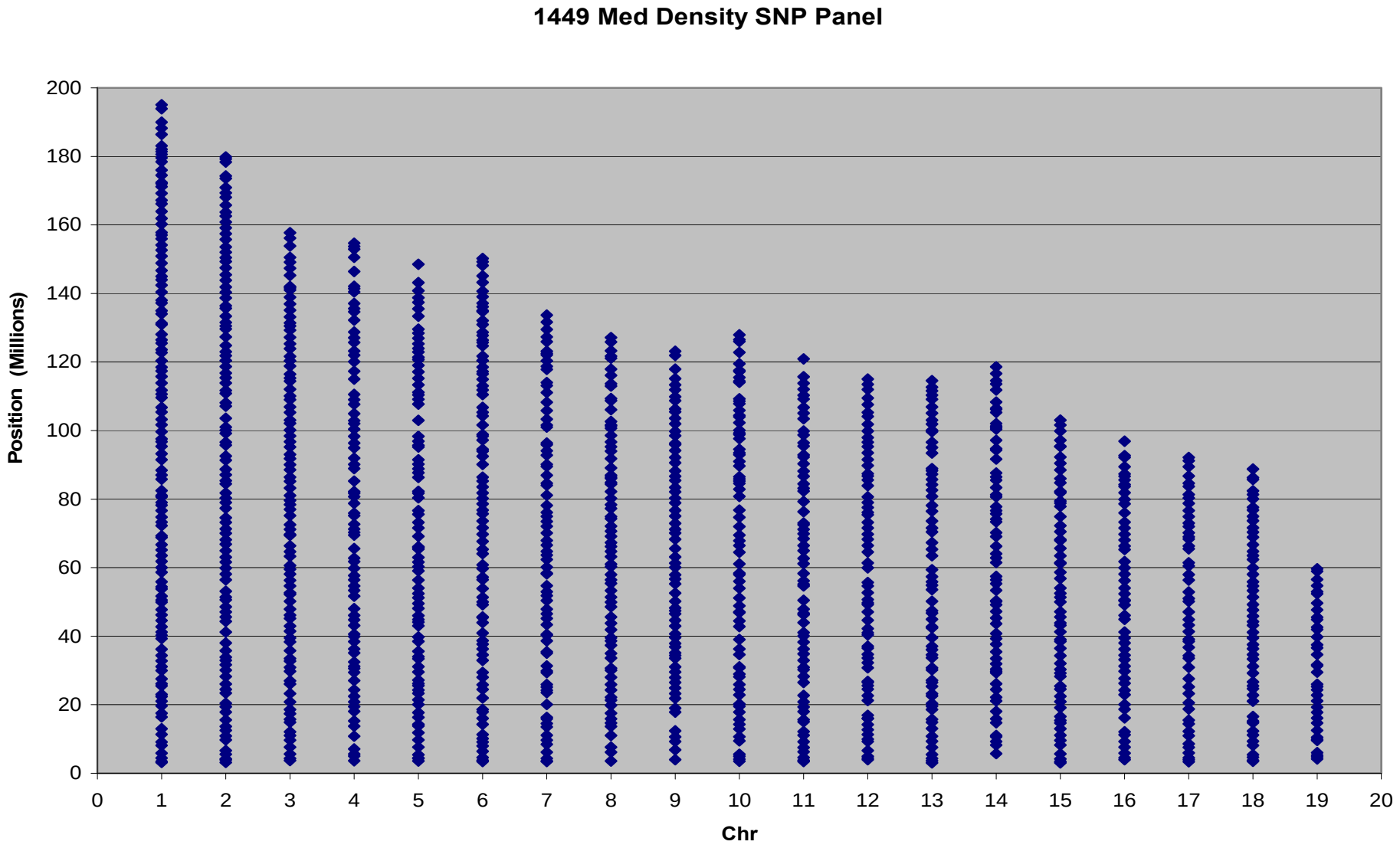
- **Single Nucleotide Polymorphisms or SNPs**
  - DNA sequence variations that occur when a single nucleotide (A,T,C,or G) in the genome sequence is altered
  - More stable than microsatellites
  - Scalable for high-throughput, tested in the order of thousands

    AAGGCTAA to
    ACGGCTAA

  - SNPs allow us to detect strain differences

Genomic Profiling Illumina 1449 SNP Panel





MDL (1449) SNP Panel Differences



Table 2: Strain Differences in 1449 Marker Panel

	129P3/J	129S4/SvJae	129S6/SvEvTac	A/JCrTac	BALB/cAnNTac	BALB/cJBomTac	BTBR T<+>	C3H/HeNTac	C57BL/10SgSnAiTac	C57BL/6J	C57BL/6NTac	C57BL6/JBomTac	CBA/JBomTac	DBA/1JBomTac	DBA/2NTac	FVB/NTac	NOD/Tac	SJL/JCrNTac
129P3/J		19	39	716	666	683	451	685	844	869	857	866	699	696	725	619	638	592
129S4/SvJae	19		22	713	662	678	443	667	847	872	860	869	683	687	716	621	638	599
129S6/SvEvTac	39	22		704	652	669	447	659	854	879	867	876	675	678	707	620	630	595
A/JCrTac	716	713	704		326	337	650	413	904	953	941	950	477	638	650	519	546	567
BALB/cAnNTac	666	662	652	326		0	644	452	795	832	820	829	486	612	629	579	561	562
BALB/cJBomTac	683	678	669	337	0		664	464	828	864	852	861	497	628	647	600	578	584
BTBR T<+>	451	443	447	650	644	664		668	690	715	703	712	673	666	688	598	617	639
C3H	685	667	659	413	452	464	668		880	923	911	920	202	482	476	593	610	606
C57BL/10SgSnAiTac	844	847	854	904	795	828	690	880		68	56	65	856	838	856	797	793	766
C57BL/6J	869	872	879	953	832	864	715	923	68		12	3	901	877	892	822	820	795
C57BL/6NTAC	857	860	867	941	820	852	703	911	56	12		9	889	865	880	810	808	783
C57BL6/JBomTac	866	869	876	950	829	861	712	920	65	3	9		898	874	889	819	817	792
CBA/JBomTac	699	683	675	477	486	497	673	202	856	901	889	898		439	454	597	618	632
DBA/1JBOMTAC	696	687	678	638	612	628	666	482	838	877	865	874	439		99	641	634	629
DBA/2NTac	725	716	707	650	629	647	688	476	856	892	880	889	454	99		646	616	630
FVB	619	621	620	519	579	600	598	593	797	822	810	819	597	641	646		491	382
NOD/Tac	638	638	630	546	561	578	617	610	793	820	808	817	618	634	616	491		521
SJL	592	599	595	567	562	584	639	606	766	795	783	792	632	629	630	382	521	

APPSWE Transgenic Models



- **Genetic Background Testing**
  - Biochemical testing- tests for proteins /genetic polymorphisms
  - DNA Microsatellites
  - Single Nucleotide Polymorphisms or SNPs

- **Genotyping**
  - Southern Blot
  - Polymerase Chain Reaction or PCR
  - Quantitative PCR
  - Phenotyping, i.e. Flow Cytometry

Some Phenotypic Differences between C57BL/6NTac and C57BL/6J



- A behavioral study comparing C57BL/6NTac and C57BL/6J showed that there is a significant difference between both strains in Open field behavior test (Bothe et al. 2005)
- Metabolic parameters are different in DIO (Diabetes Induced Obesity) mice from C57BL/6NTac and C57BL/6J mice
- C57BL/6J carries Nnt deletion and C57BL/6NTac and C57BL/6JBomTac do not
- Weight gain and adiposity levels are higher in C57BL/6NTac than C57BL/6J

Sub-strain Differences in Genetic Background



- ***Nnt*- N**icotinamide **n**ucleotide **t**ransferase, associated with impair glucose homeostasis and reduced insulin secretion. Reported as ~17 Kbp deletion

- ***Snca*- S**y**n**u**c**lein **a**lpha, associated with resistance to the effects of MPTP (1-methyl-4 phenyl -1,2,3,6-tetrahydropyridine, used as prototypical toxin) on dopamine levels. Reported as 365 Kbp deletion

- ***Crbp1*^{rd8} –** is a single base pair deletion in the *Crumbs homolog 1* gene that causes a mild form of retinal degeneration

Differences between C57BL/6 sub-strains

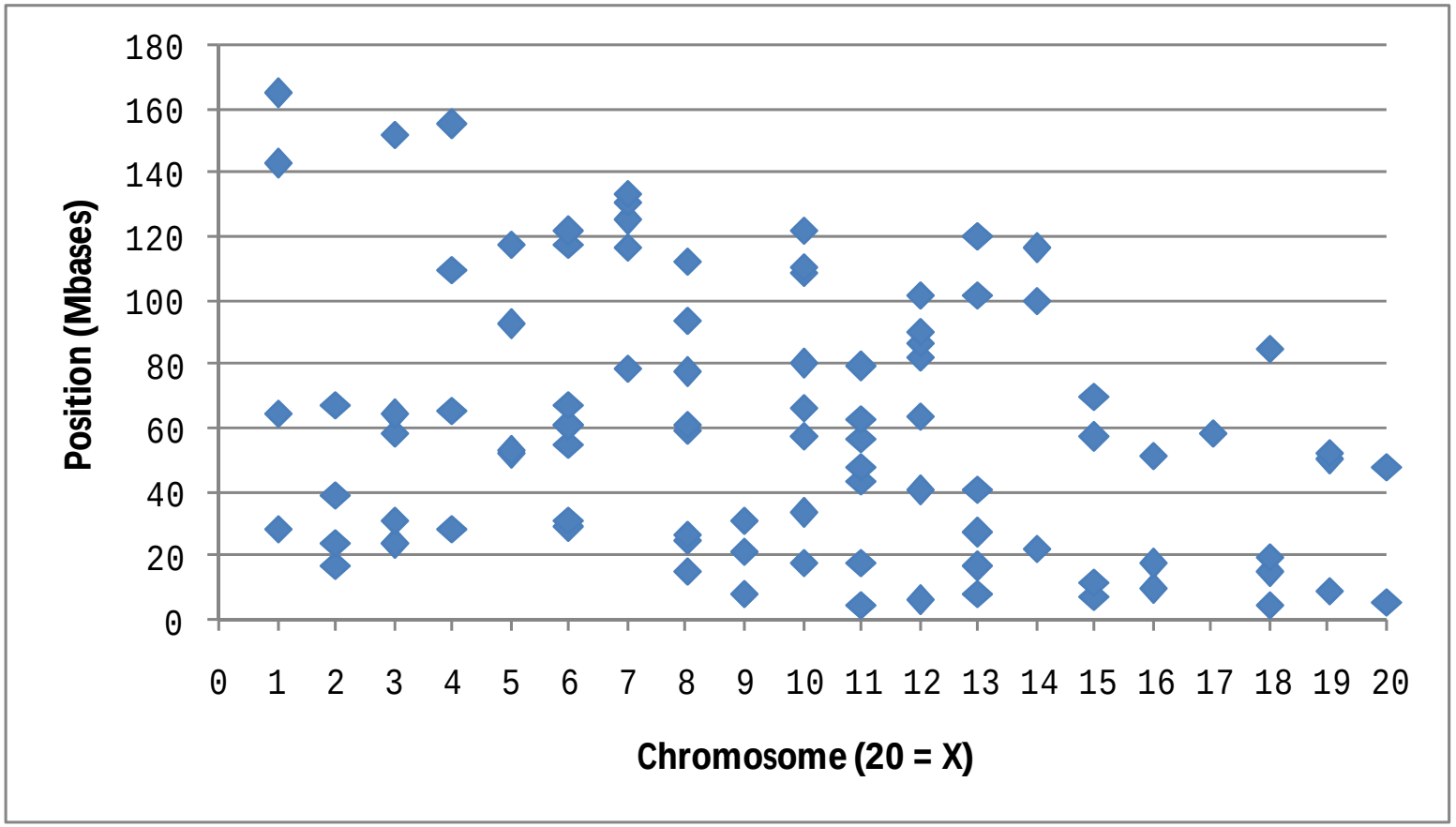


- Different Flavors of C57BL/6: C57BL/6J, C57BL/6NTac, C57BL/6JBomTac, C57BL/6NCrl

C57BL/6 Sub-strain	*Nnt* Mutation	*Snca* Mutation	*Crb1*^{rd8} Mutation
C57BL/6J	Yes	No	No
C57BL/6JOlaHsd	No	Yes	No
C57BL/6JBomTac	No	No	No
C57BL/6NTac	No	No	Yes
C57BL/6NCrl	No	No	Yes

Distribution of Markers for C57BL/6 Panel





C57BL/6 Sub-strain SNP Panel Differences



Mouse Strain	vs. 129SVE	vs. B10	vs. B6	vs. B6JBom	vs. C57BL/6NCrl	vs. C57BL/6NHsd	vs. C57BL/6J -lyst<bg>	vs. C57BL/6J	vs. C57BL/6ByJ	vs. C57BL/6NJ
129SVE (129S6/SvEvTac)		0	1	46	1	1	86	89	0	3
B10 (C57BL/10SgSnAiTac)	0		1	46	1	1	86	89	0	3
B6 (C57BL/6NTac)	1	1		47	0	0	87	94	1	2
B6JBom (C57BL6/JBomTac)	46	46	47		47	47	40	44	46	49
C57BL/6NCrl	1	1	0	47		0	87	90	1	2
C57BL/6NHsd	1	1	0	47	0		87	90	1	2
C57BL/6J-lyst<bg>	86	86	87	40	87	87		10	86	89
C57BL/6J	89	89	90	44	90	90	10		89	92
C57BL/6ByJ	0	0	1	46	1	1	86	89		3
C57BL/6NJ	3	3	2	49	2	2	89	92	3	

Stabilization of a mutation in a mixed background



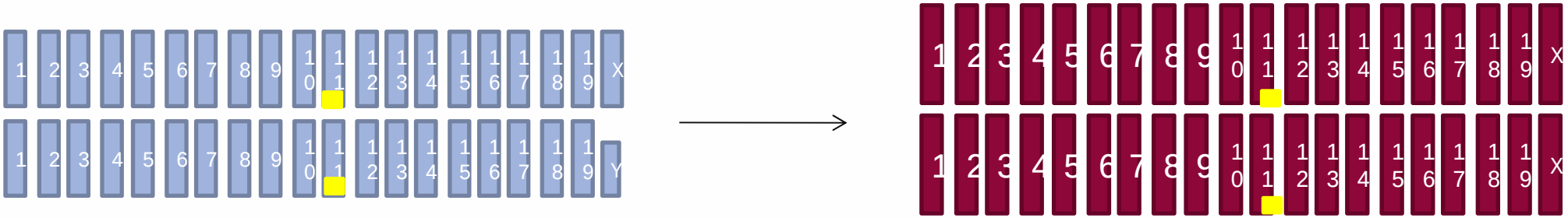
- If we have a mutation in a mixed background it is important to see the effects of such mutation in a uniformed background
- Phenotype varies with genetic background so if we need to compare the data generated by our mutant to other models these should be in the same genetic background
- A way to stabilize a mutation in the genetic background desired is to transfer the mutation to an inbred defined background and develop a congenic strain through backcrossing

Congenic Strain



Balb Nude

B6 Nude



Congenic strain is derived from the transfer of a genetic mutation or knockout region from one genetic background (donor strain) onto another genetic background (recipient strain).

What is backcrossing?

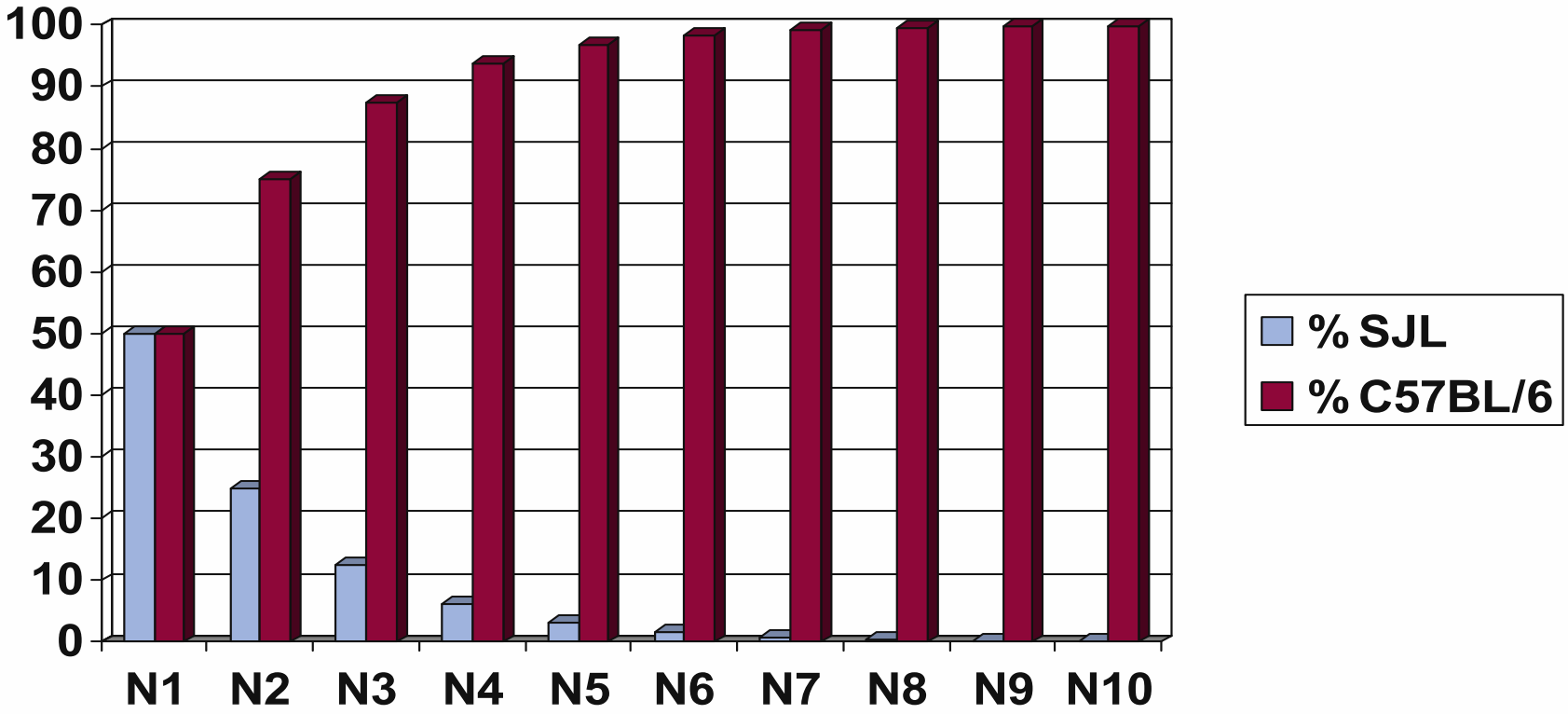


Refers to a mating scheme when an existing mutant line is purposely mated to a different strain for consecutive generations to change the background strain

Two components:

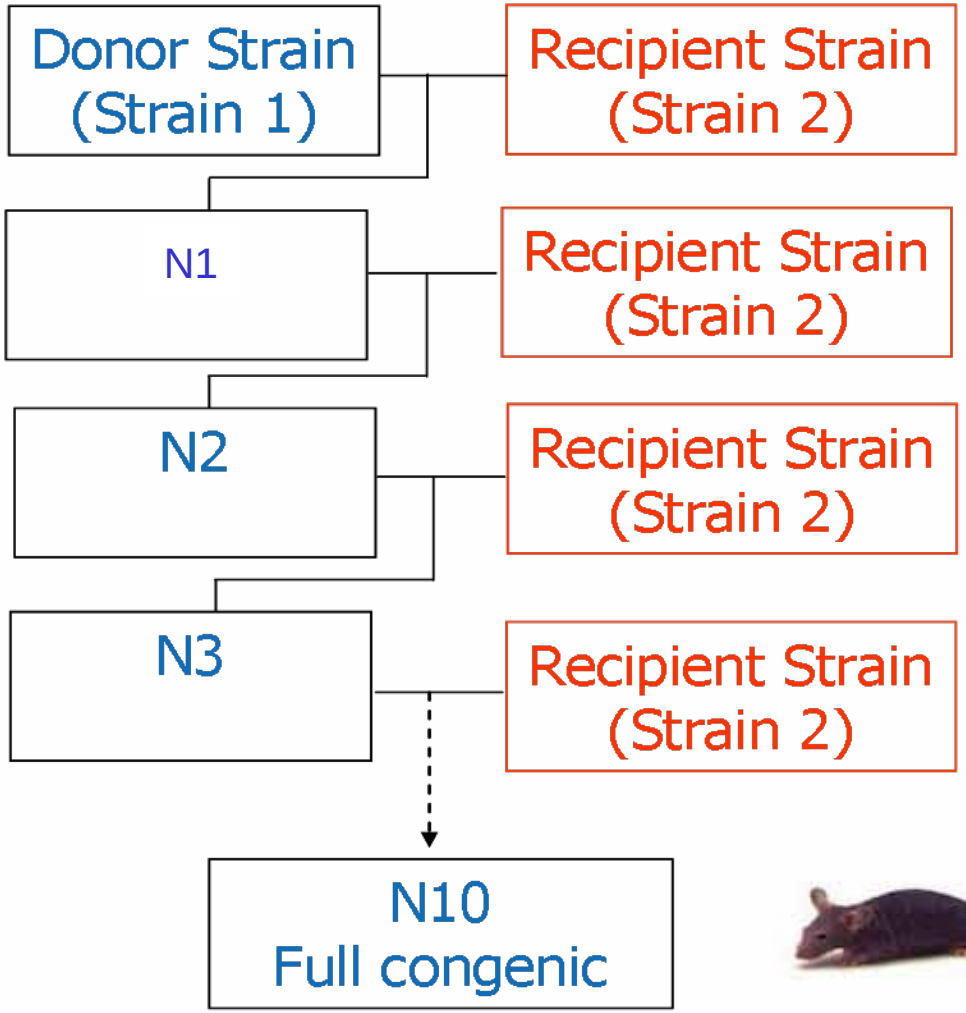
- Donor strain - the line donating the gene of interest
- Recipient strain - the line onto which the gene of interest is being transferred; usually an inbred strain

Normal Backcrossing-Congenics



Congenic strains are produced by a series of backcrosses

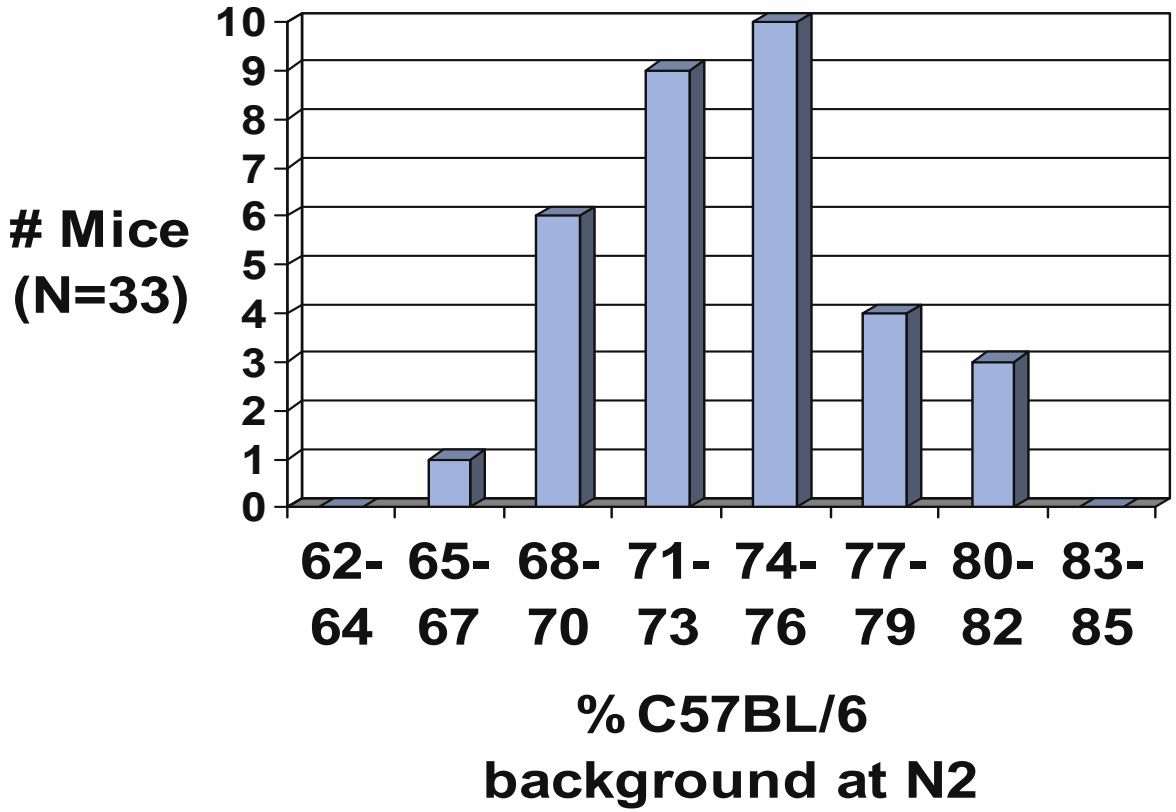
Source of mutation
Often with mixed genetic background



With defined genetic background (an inbred strain)

Generation assignment is N.

Distribution of Normal Backcrossing



SNP Testing of Backcross

				Genotyping Result						Congenic Result			
Index	Chr	Position	SNPs that differentiate between C57BL/6N ar	60_129S6_Ref_Ref_0836 (377).GType	B6N(377).GType2	14A_UNK_BKG_0916.GType	18A_UNK_0916.GType	20A_UNK__0916.GType	21A_UNK___0916.GType	14A_UNK_BKG_0916.GType2	18A_UNK_0916.GType3	20A_UNK__0916.GType4	21A_UNK___0916.GType5
8	17	37478448		AA	AA	AA	AA	AA	AA	1	1	1	1
316	17	41235453		AA	BB	BB	BB	BB	BB	1	1	1	1
376	17	44325625		BB	AA	AA	AA	AA	AA	1	1	1	1
170	17	46772416		BB	BB	BB	BB	BB	BB	1	1	1	1
52	17	61947366		AA	AA	AA	AA	AA	AA	1	1	1	1
63	17	64224877		BB	BB	BB	BB	BB	BB	1	1	1	1
201	17	67483047		BB	AA	AA	AA	AA	AA	1	1	1	1
171	17	73981248		AA	BB	BB	BB	BB	BB	1	1	1	1
218	17	77914489		AA	BB	BB	BB	BB	BB	1	1	1	1
64	17	85857606		AA	BB	BB	BB	BB	BB	1	1	1	1
9	17	86913143		BB	BB	BB	BB	BB	BB	1	1	1	1
45	18	10431432		BB	AA	AA	AA	AA	AA	1	1	1	1
280	18	12221563		BB	BB	BB	BB	BB	BB	1	1	1	1
358	18	16784949		BB	AA	AB	AB	AB	AB	0.5	0.5	0.5	0.5
172	18	19671420	X	BB	BB	BB	BB	BB	BB	1	1	1	1
233	18	30952210		AA	BB	AB	AB	AB	AB	0.5	0.5	0.5	0.5
285	18	33572164		BB	BB	BB	BB	BB	BB	1	1	1	1
253	18	33968545		BB	BB	BB	BB	BB	BB	1	1	1	1
217	18	58570705		AA	BB	BB	BB	BB	BB	1	1	1	1
317	18	63022572		BB	BB	BB	BB	BB	BB	1	1	1	1
351	18	65694361		BB	AA	AA	AA	AA	AA	1	1	1	1

SUMMARY AND CONCLUSIONS



- Reviewed phenotype and genetic background
- The phenotype of a mutation in many cases changes with the genetic background of the strain used
- Aware of sub-strain differences
- To stabilize a mutation we need to put it in a defined inbred background
- Congenic strains are established by repeatedly backcrossing to the recipient strain. There are 2 methods involved: traditional and speed congenics.
- Single Nucleotide Polymorphisms (SNPs) enable identification of mice with highest % recipient strain background in each backcross round.
- Taconic offers these services