A Mouse Is Not A Mouse Is Not A Mouse

Taconic

Smart Solutions To Improve Human Health

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Acknowledgements

Taconic Genetics Group:

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Taconic R&D

Jan Gray

Michael Young

Taconic Molecular Analysis Group

Kim Mulinex

Rebecca Farinacci

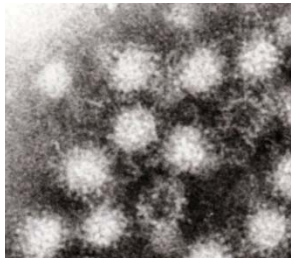
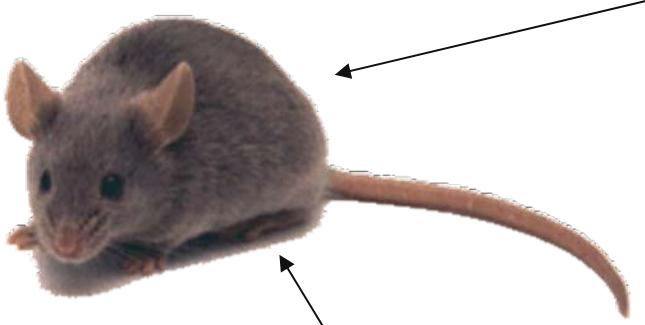
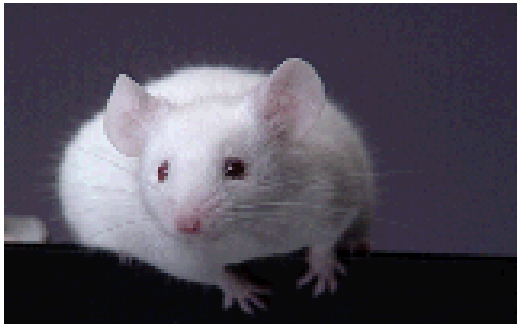
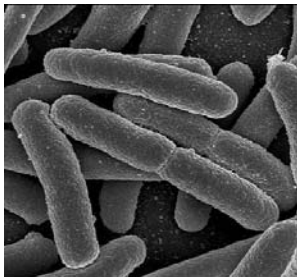
Jamie Rusconi

Steve Festin

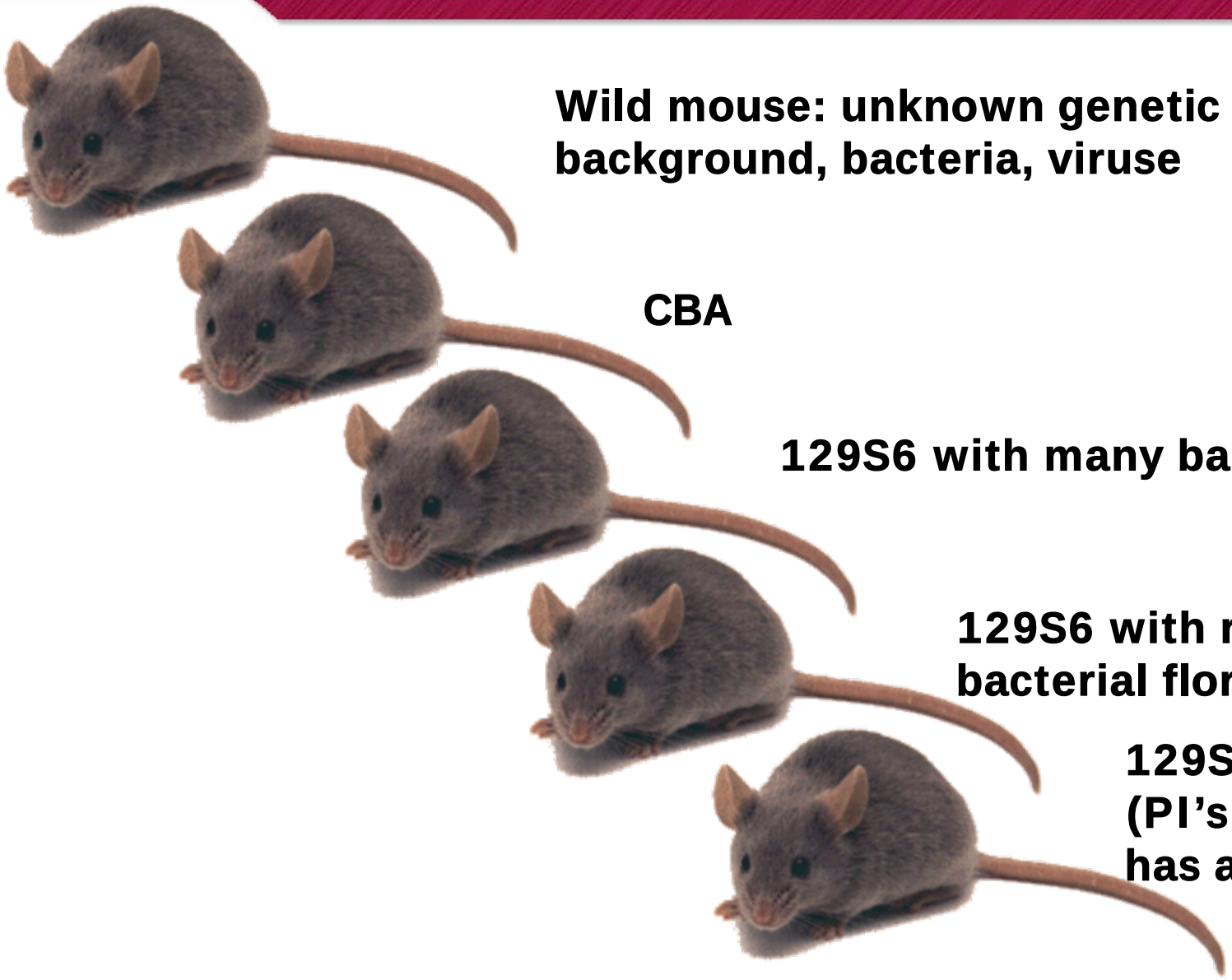
Genetics in Life Science Experiments



- **Genetic variation is of great importance in life science**
  - Basis of experiment or
  - Confounding factor
- **A Mouse Is Not A Mouse Is Not A Mouse**
- **Bacteria also have genes!**


They all look the same



Wild mouse: unknown genetic background, bacteria, viruse

CBA

129S6 with many bacteria

129S6 with restricted bacterial flora

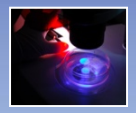
129S1 with virus (PI's child has a hamster)

Natural Populations Are Messy



- Varying, undefined symbionts and pathogens
- Sub-populations and sub-sub populations
- Selective pressure
- Random drift
- Migration
- New mutations
- Overdominance (heterozygote fitter than either homozygote)
- Heterozygotes abundant, and fitness varies according to circumstances
- But: this is life, and human populations are just like this
- Therefore, need for a variety of research models that have either eliminated or controlled variation depending on experiment

Steps To Good Experiments With Animals



- **How can we set up a “good” experiment involving laboratory animals?**
- **Control pathogens**
- **Control symbionts**
- **Elimate or control genetic variation of the experimental animal itself**
- **Examples for controlling genetic variation**
  - Make transgenics or knockouts
  - Move mutations to the desired genetic background
  - Hybrid strains
  - Mutagenesis
  - All controlled by genetic testing

Step One: Control Pathogens

- Barrier Concept (Taconic was the first to develop Barrier Units for lab animals in the 1980's): enclose the animals in a secure unit that has filtered air, and where all supplies are autoclaved or irradiated
- Humans accessing the facility have to shower and change clothes, or are completely kept out of small units (glove boxes aka isolators)
- People from dirty environments have to take a clean-up time-out of five days before they can enter a facility
- No rodent pets allowed for employees
- For higher health status animals, technicians have to use space-suit like Dryden suits that filter the exhaust air
- Health Status: a system of tests and definitions that makes sure a class of pathogens is excluded
- Gnotobiotics: germ-free rearing of animals

Taconic Barrier Concept

- Each one is a barrier
- Larger buildings have “Biobubble” isolators or are central autoclaving, supply, office facilities
- Any employee can work only in one barrier on any given day
- All supplies autoclaved or irradiated

Health Testing

- Periodic health tests at 2, 6, 13, 26 and 53 weeks intervals form the International Health Monitoring System (IHMS™)
- IHMS exceeds the European FELASA guidelines
- Common pathogens are tested every 2 weeks, rare ones every year
- Should a pathogen break occur in a facility, it is eliminated via a test and cull procedure
- Taconic production facilities had no virus break for 30 years
- Bacteria have occurred infrequently in the past. In that case, customers are informed immediately by e-mail and web posting

Microbial Testing

How To Get Clean “Starting Material”



- This system works only if an initial supply of clean animals is available.
- All mice are embryo re-derived before they are allowed to enter a Taconic barrier
- All rats are caesarian re-derived
- Absence of all known pathogens is verified by testing
- Initial result: germ-free (GF) animal

Separate Re-Derivation Facility



Gnotobiotics: Handling the GF Mice

Step 2: Control Symbionts



- Germ-free animals are not healthy
- Intestinal problems, food not completely digested, some vitamins may be in short supply as they are not provided by intestinal flora
- Mice: enlarged cecum - often resulting in cecal volvulus or so-called “intestinal strangulation”, up to 15% death rate.
- Animals that leave the germ-free environment are extremely susceptible to infection. For example, LD-50 for Salmonella is reduced 100,00fold due to the loss of colonization resistance
- Under-stimulated immune system
- Solution: add carefully selected symbionts to germfree mice

Altered Schaedler Flora

- To all Taconic animal lines, a “cocktail” of eight symbiotic bacteria is added after re-derivation
- This is the Altered Schaedler Flora
- All species were selected to fill important ecological niches in the intestinal “habitat”, and also to be recognizable by microscopy and culture
- All species grow anaerobically
- Two can grow also in air (microaerophiles)
- Two anearobes
- Four extremely oxygen sensitive (EOS) anaerobes

Other Bacteria

- In Defined Flora (DF) animals, no other bacteria are allowed
- In Restricted Flora (RF) animals, the opportunists Klebsiella oxytoca, Kl. pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus are excluded, and viruses and other pathogens are excluded as well
- In Murine Pathogen Free (MPF) animals, all viruses and pathogens are excluded

Step 3: Control Genetic Variation

- After pathogens have been eliminated and variation in symbionts has been dealt with, we can turn to the animal itself
- Many questions can be investigated using genetically homogeneous lab animals
- Such strains are inbred and related strains, which are produced by inbreeding brother-to-sister over 20 generations or more
- A wide variety of inbred strains exist, the most common are 129S6, BALB/c, and most importantly, C57BL/6
- Models with controlled/stabilized genetic variation are needed for some experiments

Inbred Strains

- Inbred Strains are produced by brother-sister mating over at least 20 generations
- All traditional inbred strains are derived from pet mice (“fancy mice”) of the early 1900s
- After 20 generations, 98.7% of all loci are homozygous, or 1.3% of original heterozygosity remains
- After 80 generations, 3.9×10^{-8} of original heterozygosity remains
- Each animal will carry approximately 60 new mutations of which 15 will become fixed (homozygous) in the germline and there will be around 120 loci that are heterozygous due to recent mutations (depends on mutation rate, details of which are still debated)

Major Inbred Strains

- C57BL/6 (short: B6): the ‘standard model’
- About 150 generations ago, B6 split up into B6J (C57BL/6J) and B6N (e.g. C57BL/6NTac), these substrains have some differences, e.g. C57BL/6J (but not C57BL6/JBomTac) carries the Nnt deletion which changes the response to high-fat diet and glucose metabolism
- 129 strains, e.g. 129S6/SvEvTac, are used to produce knockout mice
- BALB/c
- DBA/2

Genetic Background

- Each inbred strain carries a unique combination of genes
- This “genetic background” is the same for every mouse of this strain
- The genetic background influences the phenotype of any transgene that is produced using this strain
- Some transgenes are lethal on certain genetic backgrounds

Example: APP-SWE Transgene

- A transgenic mouse line carrying the swedish mutation of the Amyloid Precurson Protein (APP) was made in mice that had a mixed genetic background consisting of B6 and SJL
- When these mice were crossed to B6 mice, rising lethality was observed
- In mice with a mixed B6-SJL background, the mortality of transgene carriers was not higher than that of non-carriers.
- Currently, the transgene is either propagated on a mixed B6-SJL background by mating to B6SJLF1 in each generation, or it is propagated on a 129S6 background.

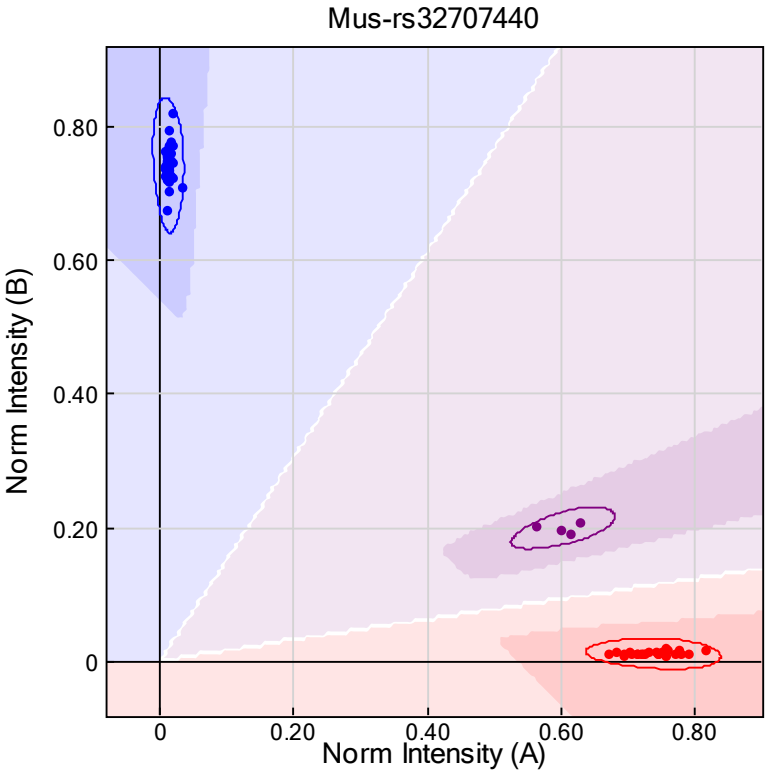
Telling them apart

- To avoid accidental (unwanted) crosses, there must be a way to tell strains apart
- Historically, this was done by biochemical markers (isoenzymes) and microsatellite markers
- Today, the best way to identify strains is through single nucleotide polymorphisms
- Example:
  actggatcccattatttagtgta[g/t]caaccgtaatacgattactga

SNP Panels

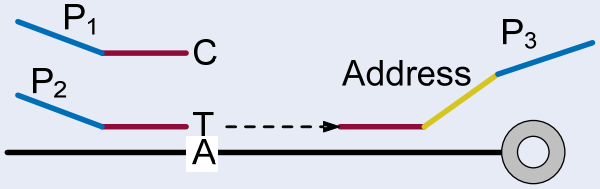
- To tell strains/genetic background apart or localize mutations, at least 90 SNP are needed (96 for practical purposes/96-well plate)
- 96 marker panel: general genetic monitoring. Not suitable for following backcrossing or localizing genes (too low resolution)
- Low resolution: e.g. 377-SNP panel for accelerated backcrossing and low-resolution mapping
- Mid-resolution: e.g. 1449 SNP panel for backcrossing, mapping of mendelian mutations and Quantitative trait analysis

Technology

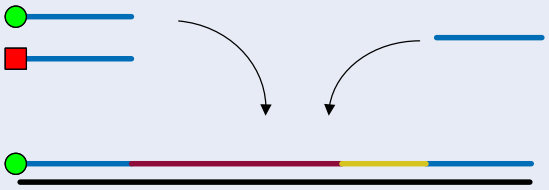


Genomic DNA (on solid support)

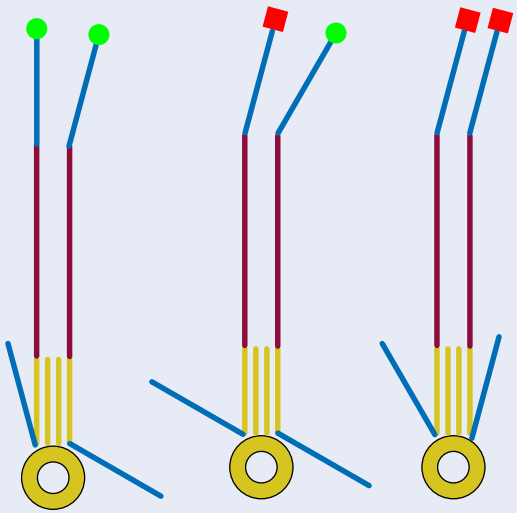
Allele-specific extension, ligation



PCR with universal primers



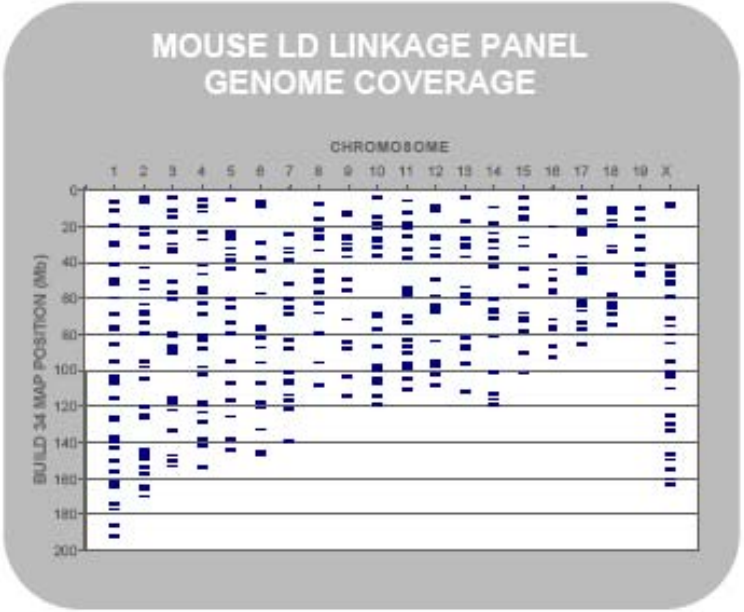
Hybridization

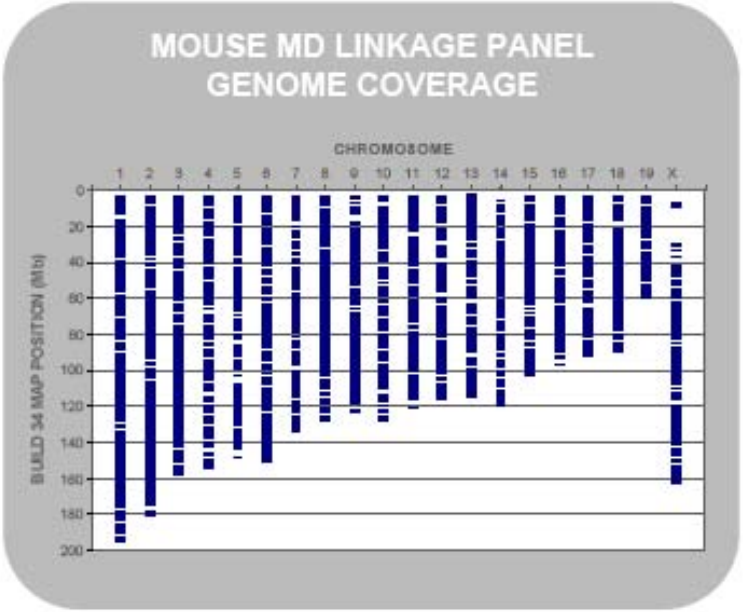


377- and 1449-SNP Panels



Mouse Linkage Genome Coverage





illumina

Taconic

1449 SNP Panel

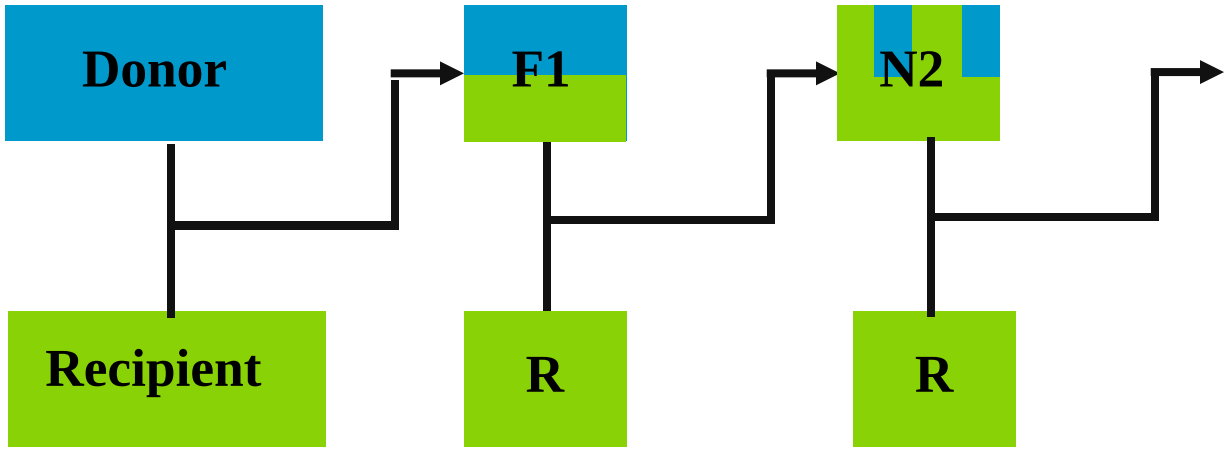
	129P1	129S6	129X1	A/JCrTac	BALB/cAnNTac	BTBR T<+>	C3H	C57BL/10	C57BL/6J	B6NTac	B6JBom	CBA	DBA/1	DBA/2	FVB	NOD	SJL
129P1/REJ		41	115	713	664	451	680	848	873	861	870	694	692	721	622	639	593
129S6/SvEvTac	41		150	704	652	447	659	854	879	867	876	675	678	707	620	630	595
129X1/SvJ	115	150		691	610	498	664	835	866	854	863	675	718	749	639	651	630
A/JCrTac	713	704	691		326	650	413	904	953	941	950	477	638	650	519	546	567
BALB/cAnNTac	664	652	610	326		644	452	795	832	820	829	486	612	629	579	561	562
BTBR T<+>	451	447	498	650	644		668	690	715	703	712	673	666	688	598	617	639
C3H	680	659	664	413	452	668		880	923	911	920	202	482	476	593	610	606
C57BL/10SgSnAiTac (B10)	848	854	835	904	795	690	880		68	56	65	856	838	856	797	793	766
C57BL/6J (B6J)	873	879	866	953	832	715	923	68		12	3	901	877	892	822	820	795
C57BL/6NTAC (B6NTac)	861	867	854	941	820	703	911	56	12		9	889	865	880	810	808	783
C57BL6/JBomTac (B6JBom)	870	876	863	950	829	712	920	65	3	9		898	874	889	819	817	792
CBA/JBomTac	694	675	675	477	486	673	202	856	901	889	898		439	454	597	618	632
DBA/1JBOMTAC	692	678	718	638	612	666	482	838	877	865	874	439		99	641	634	629
DBA/2NTac	721	707	749	650	629	688	476	856	892	880	889	454	99		646	616	630
FVB	622	620	639	519	579	598	593	797	822	810	819	597	641	646		491	382
NOD/Tac	639	630	651	546	561	617	610	793	820	808	817	618	634	616	491		521
SJL	593	595	630	567	562	639	606	766	795	783	792	632	629	630	382	521	

Application: Backcrossing

- Often, a mutation or a transgene has to be transferred from one genetic background to another, for example to study genetic interactions, or to get a model that is viable into adulthood, or to standardize the genetic background to B6
- To transfer a gene to another mouse line, a carrier has to be crossed to a mouse of the desired background, and the process has to be repeated several times
- If this has been repeated 10 generations, the strain is called congenic

Congenic Strains

- **Mutations are moved to another genetic background through backcrossing:**



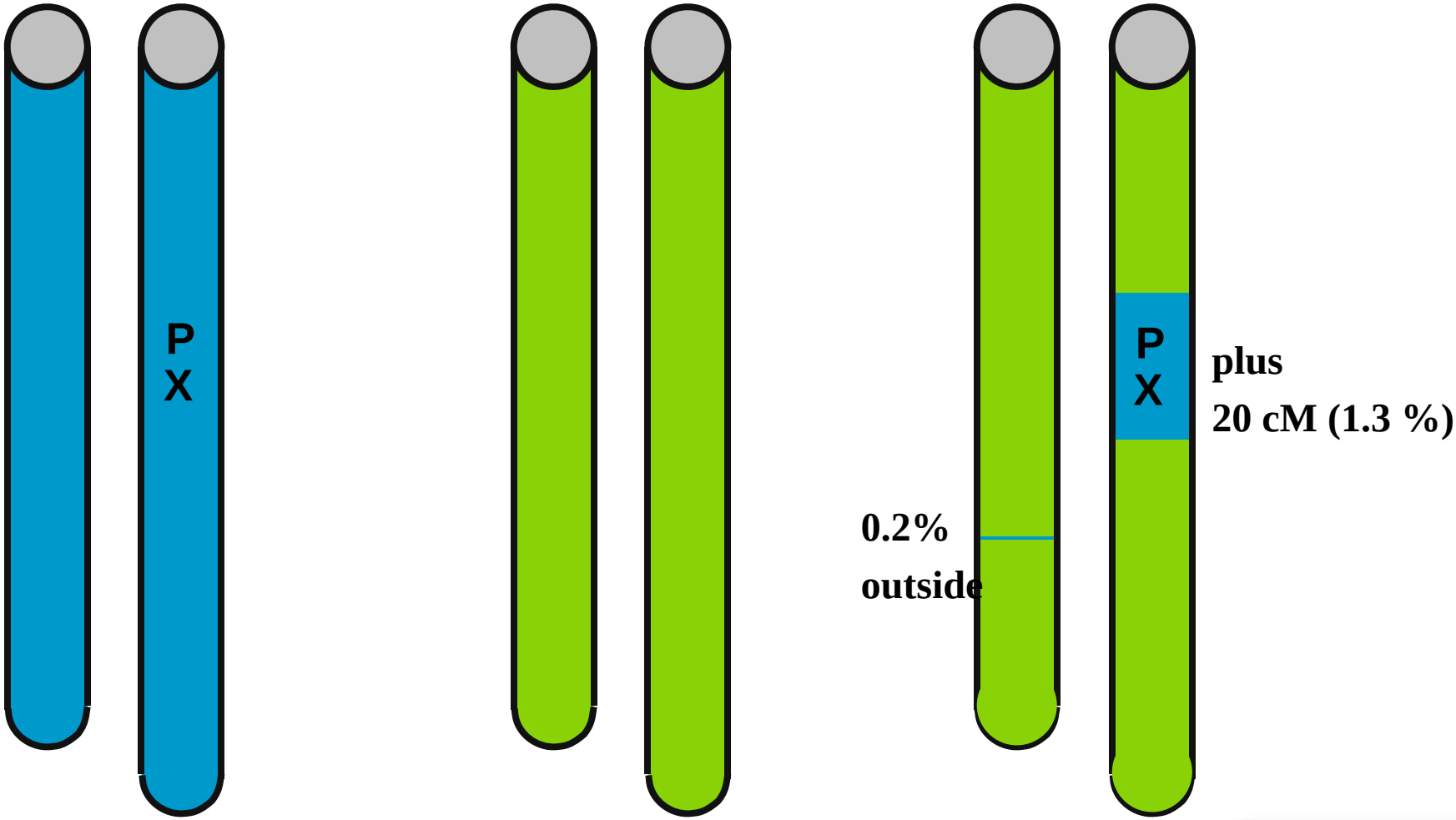
Passenger Loci



- **Rapid elimination of donor genes occurs only in regions of the genome that are not linked to the donor allele**
- **Region around the donor allele (e.g., knockout) is slowly and randomly reduced by crossover**

Congenic Strain

Donor (129) **Recipient (B6)** **Congenic (N10)**

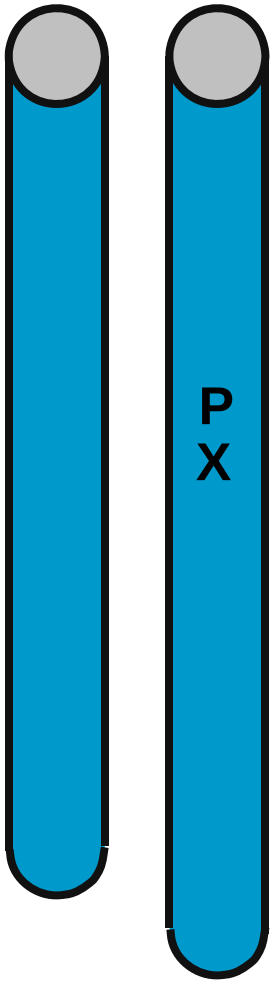


Speeding Things Up

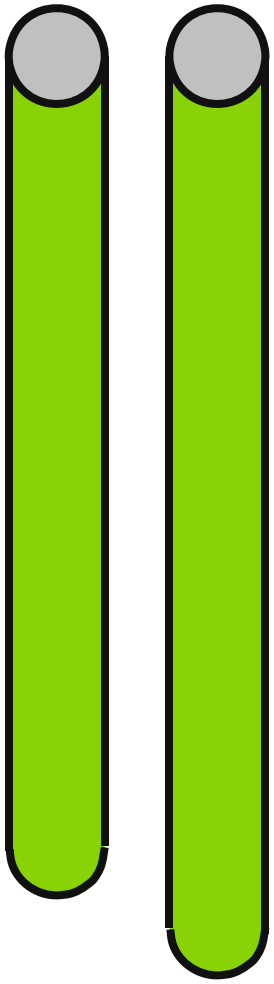
- The production of congenic strains is a time-consuming process: 2.5 years to N10
- In each generation, there is a random distribution of offspring: some have more donor genes than others
- SNP markers can be used to select the “best” offspring and accelerate backcrossing
- SNP markers can also be used to reduce the size of the donor segment, which improves the quality of the congenic line
- Optimal results achieved with 20 male carriers tested per generation, but this can be reduced to 5 if cost is a problem

Speed Congenic Strain, N10 Equivalent

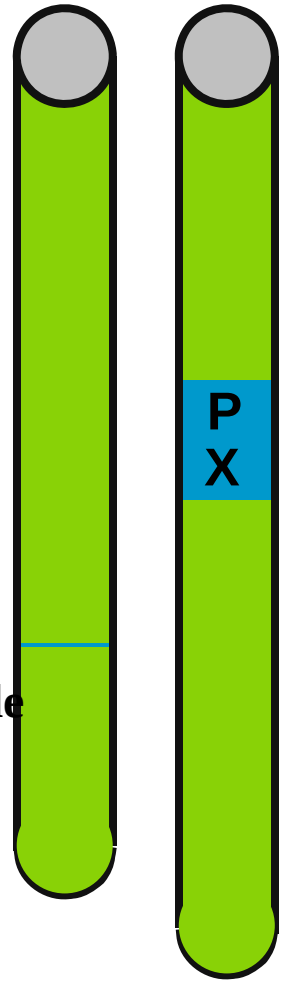
Donor (129)



Recipient (B6)



Congenic (N5, equivalent to N10 or better)



plus

15 cM (1.0 %)

0.3%

outside

Application: Mapping/QTL analysis

- Genetic markers can be used to find out where mutations are localized on a chromosome: mapping
- The position of multiple genes that each only have a small influence on phenotype, can be found by quantitative trait loci (QTL) analysis
- Approach: create a cross between a mouse line that carries the gene and the phenotype, i.e. gene and genetic background together produce the phenotype
- Analyze F1: is it uniform (or only males show the phenotype), does it matter whether the mother of the F1 is strain 1 or strain 2? Distribution of phenotype?
- Produce F2. Distribution of phenotype? Measure phenotype and determine SNP marker status for at least 100 individuals
- Perform QTL analysis (programs available on the web)
- Result: Position of gene of interest (may be natural or transgene) and of modifier loci

Outbred Stocks As Model Of Nature

- **Some questions cannot be studied in inbred strains: all those that need genetic diversity in a starting population**
- **Example: try to select for alcohol-preferring phenotype**
- **Start with a genetically diverse population of mice**
- **Maintain genetic diversity by rotational breeding of hundreds of animals**
- **Avoid genetic drift by:**
  - Large population size
  - No selection (selection for fertility is unavoidable, but avoid all other)
- **Resulting population will maintain diversity and multitude of haplotypes that is typical of natural populations while avoiding the “chaos”**
- **Uses: toxicology and other drug research, selection of interesting phenotypes**
- **Less stringent rules for stocks where only breeding performance is needed**

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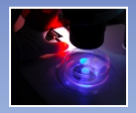
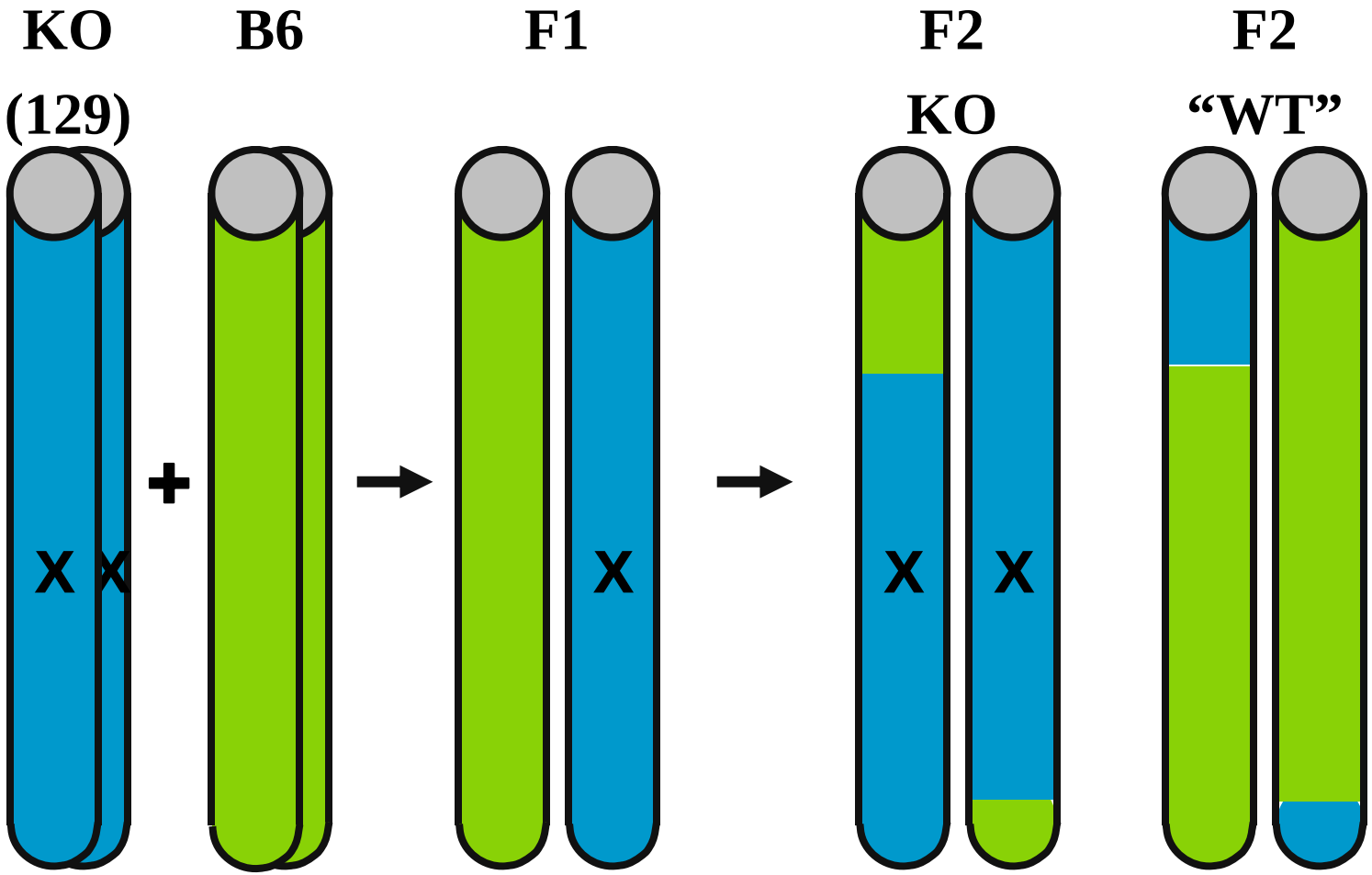
Health Testing Technologies



- We use serum ELISAs to detect antibodies from past infections
- PCR assays are available for the detection of current infections of some pathogens
- And classical culture, of course…
- Testing service available
- See www.taconic.com for details

Result Of a Cross

Knockout and “WT” control have different genetic backgrounds



96-SNP Genetic Monitoring Panel



129 Strains: 2-12, 48 diffs. to B6

B6 Substrains: 3-7, 55 to BALB

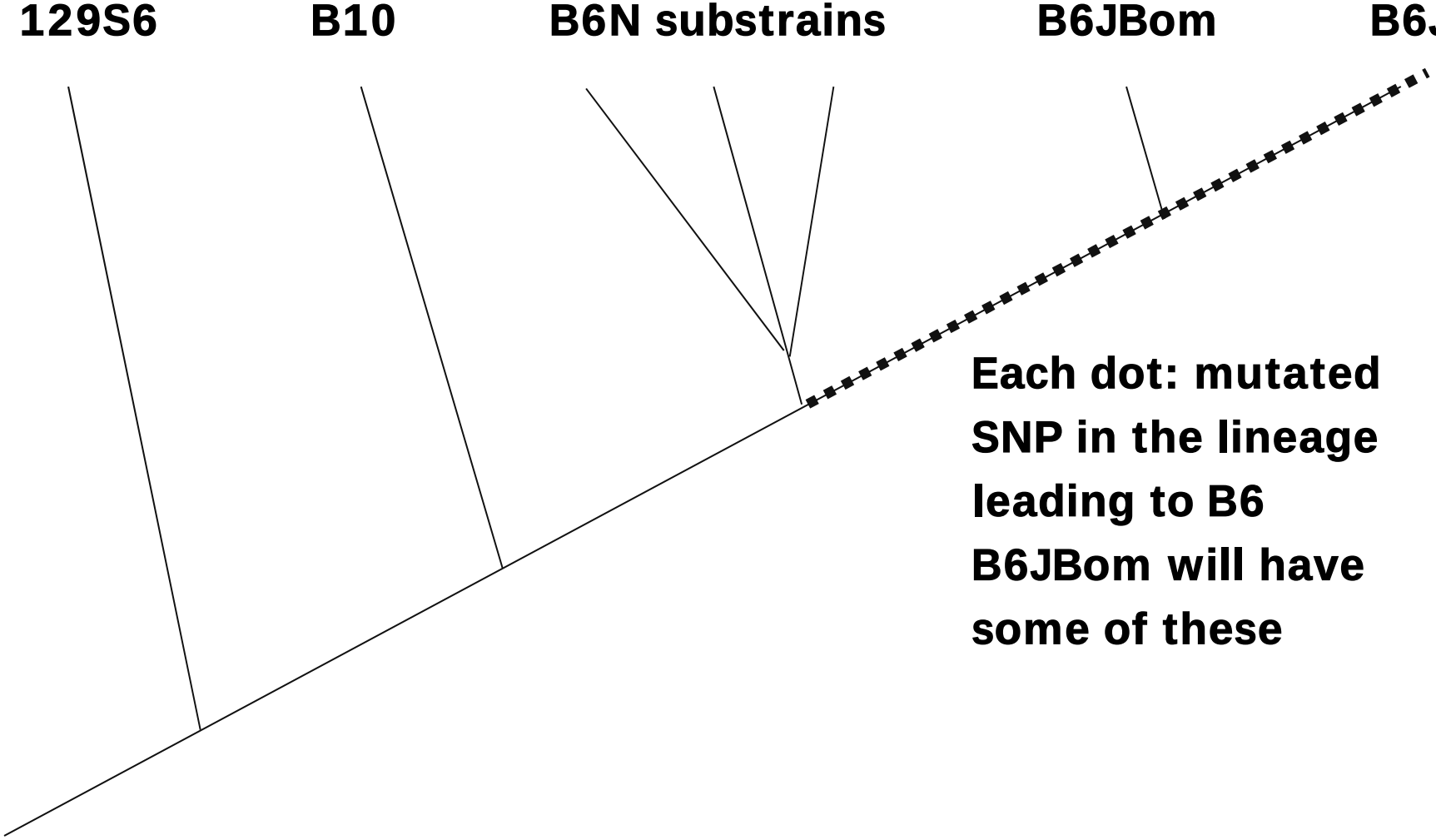
BALB substrains: 1-4, 37 to DBA

Congenics: 1-4 to "mother" strain

	129P2	129P3	129S1	129S2	129S6	129X1	AJTAC	B6	B6J	B6JBOM	B10	BALB	BALB/cJ	BALBOM	BALJBO	C3H	CBA	DBA/1	DBA/2	FVB	NOD	SJL
129P2/OlaHsd		3	7	7	11	12	36	48	55	51	47	33	32	34	33	40	44	42	44	38	43	33
129P3/J	3		6	6	10	10	38	49	56	52	48	35	34	36	35	41	43	41	43	38	43	35
129S1/SvImJ	7	6		2	6	9	37	49	56	52	48	34	33	35	34	35	37	39	41	37	40	36
129S2/SvPasCrl	7	6	2		4	11	37	49	56	52	48	34	33	35	34	35	37	39	41	37	40	36
129S6/SvEvTac	11	10	6	4		15	37	49	56	52	48	34	33	35	34	33	35	41	43	35	40	36
129X1/SvJ	12	10	9	11	15		36	50	57	53	49	33	30	34	31	40	40	44	46	40	43	33
A/JCRTac (AJTAC)	36	38	37	37	37	36		60	67	63	55	19	22	20	23	34	36	42	44	32	29	31
C57BL/6NTac (B6)	48	49	49	49	49	50	60		7	3	5	49	52	50	53	50	48	44	44	52	43	47
C57BL/6J (B6J)	55	56	56	56	56	57	67	7		4	12	56	59	57	60	57	55	51	51	59	50	54
C57BL/6JBomTac (B6JBOM)	51	52	52	52	52	53	63	3	4		8	52	55	53	56	53	51	47	47	55	46	50
C57BL/10SgSnAiTac (B10)	47	48	48	48	48	49	55	5	12	8		48	51	49	52	51	47	43	45	51	44	48
BALB/cAnNTac (BALB)	33	35	34	34	34	33	19	49	56	52	48		3	1	4	33	39	37	39	35	32	26
BALB/cJ	32	34	33	33	33	30	22	52	59	55	51	3		4	1	32	38	36	38	38	35	29
BALB/cABomTac (BALBOM)	34	36	35	35	35	34	20	50	57	53	49	1	4		5	34	40	38	40	36	33	27
BALB/cJBomTac (BALJBO)	33	35	34	34	34	31	23	53	60	56	52	4	1	5		33	39	37	37	37	34	28
C3H/HeNTac (C3H)	40	41	35	35	33	40	34	50	57	53	51	33	32	34	33		8	26	30	34	37	39
CBA/JBomTac (CBA)	44	43	37	37	35	40	36	48	55	51	47	39	38	40	39	8		26	30	32	37	41
DBA1/JBomTac (DBA1BO)	42	41	39	39	41	44	42	44	51	47	43	37	36	38	37	26	26		4	38	31	43
DBA/2NTac (DBA2)	44	43	41	41	43	46	44	44	51	47	45	39	38	40	37	30	30	4		38	31	43
FVB/NTac (FVB)	38	38	37	37	35	40	32	52	59	55	51	35	38	36	37	34	32	38	38		23	19
NOD/MrkTac (NOD)	43	43	40	40	40	43	29	43	50	46	44	32	35	33	34	37	37	31	31	23		24
SJL/JCrNTac (SJL)	33	35	36	36	36	33	31	47	54	50	48	26	29	27	28	39	41	43	43	19	24	
B10.A-H2^{a}-H2T18^{a}/SgSnAiTac (B10.A)	47	48	48	48	48	49	53	7	14	10	2	48	51	49	52	49	45	41	45	49	42	46
B6.SJL-Ptprc^{a}/BoyAiTac (B6.SJL)	50	51	51	51	51	52	62	4	9	5	9	51	54	52	55	52	50	44	44	50	41	45
C.B-Igh1^{b}/IcrTac (CB17)	35	37	36	36	36	35	21	47	54	50	46	2	5	3	6	33	39	39	41	35	30	24
BKS.Cg-m^{+}Lepr^{db}/BomTac (DB-M)	41	40	40	40	40	47	57	15	22	18	18	48	51	49	52	41	39	37	37	45	38	42
B6.V-Lep^{ob}/JBomTac (OB-M)	53	54	54	54	54	55	63	5	6	2	10	52	55	53	56	53	51	49	49	55	46	52
B6.Cg/NTac-Foxn1^{nu} (B6NU)	48	49	49	49	49	50	60	4	11	7	9	51	54	52	55	50	48	44	44	52	43	49
C.Cg/AnNTac-Foxn1^{nu} (BALBNU)	34	36	35	35	35	34	20	50	57	53	49	1	4	2	5	34	40	38	40	36	33	27
C.Cg/AnBomTac-Foxn1^{nu} (BLBANU)	36	38	37	37	37	36	24	46	53	49	45	7	10	8	9	34	40	38	38	36	35	25
C.B-Igh1^{b}/IcrTac-Prkdc^{scid} (CB17SC)	36	38	37	37	37	36	22	48	55	51	47	3	6	4	7	34	40	40	42	36	31	25
C.B-Igh1^{b}/GbmsTac-Prkdc^{scid}-Lyst^{bg} (CBSCBG)	38	40	39	39	39	38	26	44	51	47	45	7	10	8	11	36	42	40	42	38	29	27
NOD/MrkBomTac-Prkdc^{scid} (NODSC)	44	44	41	41	41	44	30	44	51	47	45	33	36	34	35	38	38	32	32	24	1	25

B6 Substrain Genealogy

< most SNPs discovered by comparing B10 etc. to B6J >



Each dot: mutated SNP in the lineage leading to B6

B6JBom will have some of these

C57BL/6 SNP Panel



B6N vs. B6J:
93 Diffs

B6N all alike,
Except B6NJ

1 new SNP
in B6NTac lineage

B6JBom:
47 vs. B6N,
46 vs. B6J

Mouse	vs. 129SVE	vs. B10	vs. B6	vs. B6JBom	vs. C57BL/6NCrl	C57BL/6NHsd	lyst<bg>	vs. C57BL/6J	vs. C57BL/6ByJ	vs. C57BL/6NJ
129SVE (129S6/SvEvTac)		0	1	46	1	1	85	92	0	3
B10 (C57BL/10SgSnAiTac)	0		1	46	1	1	85	92	0	3
B6 (C57BL/6NTac)	1	1		47	0	0	86	93	1	2
B6JBom (C57BL6/JBomTac)	46	46	47		47	47	39	46	46	49
C57BL/6NCrl	1	1	0	47		0	86	93	1	2
C57BL/6NHsd	1	1	0	47	0		86	93	1	2
C57BL/6J-lyst<bg>	85	85	86	39	86	86		10	85	88
C57BL/6J	92	92	93	46	93	93	10		92	95
C57BL/6ByJ	0	0	1	46	1	1	85	92		3