

Mixed Panel Development for Genetic Integrity Testing of Rodent Animal Models

Taconic
Smart Solutions To Improve Human Health



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Company:

“Smart Solutions to Improve Human Health”

Molecular Analysis:

“Increasing the quality of research animals through innovative technology and molecular services.”

Which of these is a 129 mouse?





- **Genetic Monitoring: 96 SNP Panel Development**
 - The problem
 - Potential solutions
 - The Taconic/Illumina 80+16 idea
 - Custom 96 panel development with Illumina and Implementation
- **Speed Congenics and Background Characterization using Illumina Panels**

Genetic Monitoring (GenMon)



- **The problem: 170,000 mouse cages in US (Germantown, NY, Oxnard, CA, Rockville, MD, Cambridge City, IN)**
- Potential solutions
- The Taconic/Illumina 80+16 idea
- Custom 96 panel development with Illumina and Implementation

The Problem: 170,000 Mouse Cages in the US



- **Traditional Models**
 - C57Bl/6
 - 129
- **Spontaneous Mutants**
 - Ncr nude (T-cell deficient)
 - CBA (retinal degeneration model)
- **Induced Models**
 - Aged B6 Mice
 - Superovulated Mice
- **Taconic Transgenic Models™ (TTMs), Knockout Repository, and Emerging Models**
 - ApoE knockout
 - HRN™ (Hepatic Reductase Null)
- **Contract Breeding Services**
- **TaconicArtemis Custom Model Generation**
 - Custom RNAi Knock Down Model Generation
 - Traditional or constitutive KO
 - Humanization



The Problem: What do we need to do about it?



- **Keep track of animals**
- **Keep track of the genetic background of the animals to prevent contamination and assure our animals what we say they are!**
 - Quality Control: periodic monitoring
 - Must be ready to test if there is an issue



Taconic Genetic Monitoring Report



Production Site: Germantown, NY

Test Frequency: Bi-Weekly

Testing Facility: Taconic Albany/CRL^a

Year: 2009

Model Name	Strain Name	Number of Tested Animals								
		Total to Date	First Quarter		Second Quarter		Third Quarter		Fourth Quarter	
			Transfer ^b	Production ^c	Transfer	Production	Transfer	Production	Transfer	Production
1325	BALB/cByJjic@ Tac	24	9	15						
1326	C57BL/6Jjic@ Tac	24	10	14						
1393	C57BL/6JcITac-TgN(RasH2)	13	7	6						
4007	B6.SJL-Ptprc ^o /BoyAi Tac	32	4	28						
Black 10	C57BL/10SgSnAi Tac	4	4	0						
B10.A	B10.A-H2 ^o H2-T18 ^o /SgSnAi Tac	17	3	14						
129S6	129S6/SvEv Tac	110	21	89						
A strain	A/JCr Tac	10	10	0						
BALB/c	BALB/cAnNTac	4	4	0						
Black 6	C57BL/6NTac	625	85	540						
Black 6 J Bom	C57BL/6JBom Tac	44	2	42						
B6129F1	B6129F1/Tac	16	0	16						
B6C3F1	B6C3F1/Tac	10	0	10						
C3H	C3H/HeNTac	36	10	26						
C.B-17	C.B-Igh1 ^b /lcr Tac	7	7	0						
C.B-17 scid	C.B-Igh-1 ^b /lcr Tac-Prkdc ^{scid}	34	34	0						
scid-beige	C.B-Igh-1b/Gbms Tac-Prkdc scid-Lystbg N7	13	13	0						
CBA	CBA/JBom Tac	16	7	9						
DBA/1	DBA/1JBom Tac	9	9	0						
DBA/2	DBA/2NTac	15	15	0						
FVB	FVB/NTac	13	6	7						
NOD	NOD/Mrk Tac	94	6	88						
NOD scid	NOD/MrkBom Tac-Prkdc ^{scid}	9	9	0						
SJL	SJL/JCrNTac	25	7	18						
Fisher	F344/NTac	21	21	0						
Lewis	LEW/Mol Tac	5	5	0						
Goto-Kakizaki	GK/Mol Tac	2	2	0						
Total		1232	310	922	0	0	0	0	0	0

^a Routine Genetic Monitoring for 2008 on mice was performed at Taconic Albany. Routine Testing on rats was performed at Charles River Laboratories (CRL).

^b Moving animals from one location to another creates a potential risk of strain mix-up. To monitor these risk points in the production process, one animal from each transfer is tested. All types of transfer monitoring are included in these totals.

^c Genetic Monitoring made necessary because of raising different strains with the same coat color in Isolated Barrier Units (IBUs) and of raising F1 hybrids that are the same color as one or both inbred parent.



- The problem: 170,000 mouse cages in US (Germantown, NY, Oxnard, CA, Rockville, MD, Cambridge City, IN)
- **Potential solutions**
- The Taconic/Illumina 80+16 Project
- Custom 96 panel development with Illumina and Implementation



Microsatellites (SSRs): short repeated sequences in non-coding regions

- Problems with analysis due to PCR reaction or sample issues
- Not enough numbers to evaluate all of our different strains
- Have to pay to use them!

SNPs: conserved single nucleotide polymorphisms that can be located anywhere in the genome.

- Can test for individual SNPs
- Combine these to make mini-panels
- ALWAYS binary!



- The problem: 170,000 mouse cages in US (Germantown, NY, Oxnard, CA, Rockville, MD, Cambridge City, IN)
- Potential solutions
- **The Taconic/Illumina 80+16 idea**
- Custom 96 panel development with Illumina and Implementation

Goal: Create a panel to differentiate both Genetic Background and Genotype



Create a core panel then mix and match sub-panels

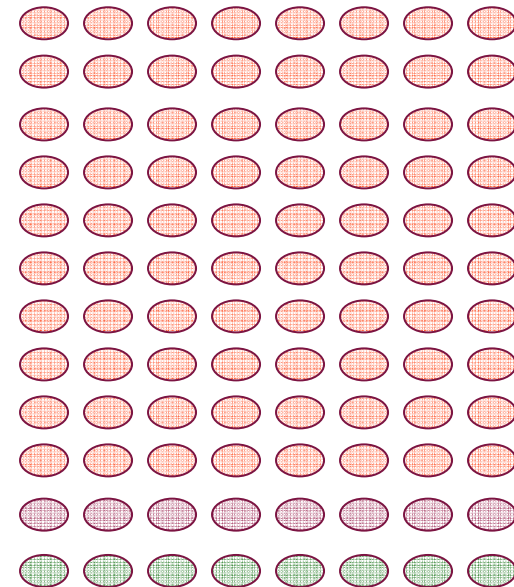
Background Strain

80

Functional Genes

Genotype: Tg or TM

16



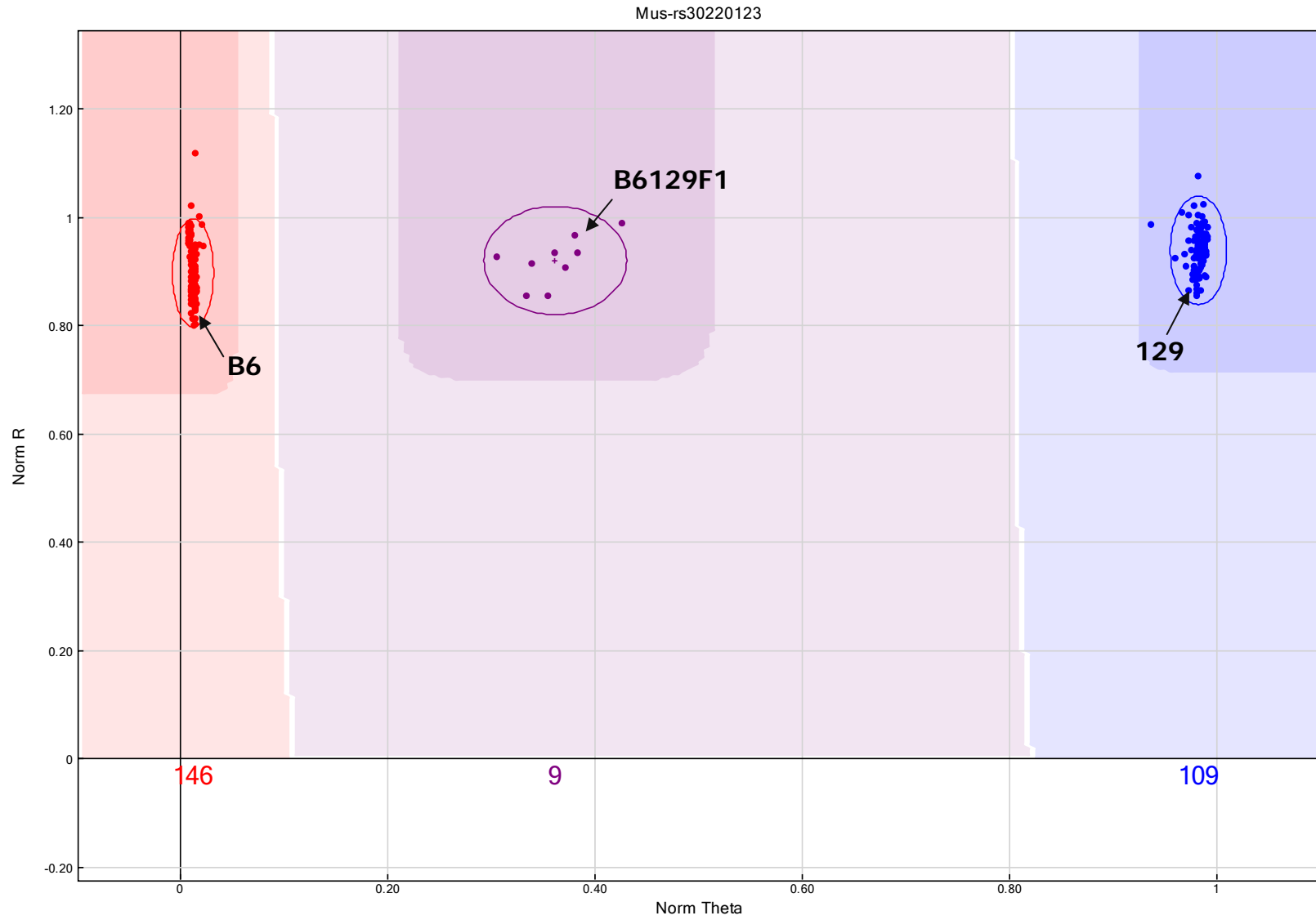


- The problem: 170,000 mouse cages in US (Germantown, NY, Oxnard, CA, Rockville, MD, Cambridge City, IN)
- Potential solutions
- The Taconic/Illumina 80+16 idea
- **Custom 96 panel development with Illumina and Implementation**



- **Develop a 96 SNP Panel with Illumina that allows us to characterize 30+ genetic backgrounds.**
 - 129S6, AJTAC, APOE, B10, B10.A-1, B6129F1, B6C3F1, B6JBOM, B6TAC, BALB, BLBANU, BALBOM, BALJBO, C3H-1, CB17-1, CB17SC-2, CBA-1, CBSCBG, DB-1, DBA1-1, DBA2-1, FVB-1, NOD-1, NODSC, OB M-1, OB T, OB W, SJL-1
 - Adaptability of system: can do even more
- **Bead Studio Software—user friendly!**

Standard GenMon



Standard GenMon



Resulting Data

Sample Log #	Compare to	# SNPS	Call
0900005845	C57Bl/6Tac	92	Correct
0900005846	CB17SC	93	Correct
0900005185	B6JBOM	94	Correct
0900005852	129S6	93	Correct
0900005859	SJL-1	94	Correct
0900005861	BALJBO	93	Correct
0900005863	C3H	93	Correct

96 GenMon Panel Differences



Strain Differences detected by the 96 SNP Veracode GenMon Panel	129S6/SvEvTac	A/JcRTac	BALB/cAnNTac	BALB/cABomTac	BALB/cJBomTac	C3H/HeNTac-MTV	C57BL/10SgSnAiTac	C57BL/6NTac	C57BL6/J	C57BL6/JBomTac	CBA/JBomTac (s.6)	DBA/1BomTac	DBA/2 JBom Tac	DBA/2NTac
129S6/SvEvTac		37	35	35	34	32	48	49	55	52	34	41	43	43
A/JcRTac	37		19	20	23	33	55	60	64	63	35	42	44	44
BALB/cAnNTac	35	19		1	4	32	48	49	53	52	38	37	39	39
BALB/cABomTac	35	20	1		5	33	49	50	54	53	39	38	40	40
BALB/cJBomTac	34	23	4	5		32	52	53	56	56	38	37	37	37
C3H/HeNTac-MTV	32	33	32	33	32		50	49	55	52	8	25	29	29
C57BL/10SgSnAiTac	48	55	48	49	52	50		5	12	8	46	43	45	45
C57BL/6NTac	49	60	49	50	53	49	5		7	3	47	44	44	44
C57BL6/J	55	64	53	54	56	55	12	7		4	53	49	49	49
C57BL6/JBomTac	52	63	52	53	56	52	8	3	4		50	47	47	47
CBA/JBomTac (s.6)	34	35	38	39	38	8	46	47	53	50		25	29	29
DBA/1BomTac	41	42	37	38	37	25	43	44	49	47	25		4	4
DBA/2 JBom Tac	43	44	39	40	37	29	45	44	49	47	29	4		0
DBA/2NTac	43	44	39	40	37	29	45	44	49	47	29	4	0	

96 SNP Panel Development Conclusions



- The problem: 170,000 mouse cages in US (Germantown, NY, Oxnard, CA, Rockville, MD, Cambridge City, IN)
- Potential solutions
- The Taconic/Illumina 80+16 idea
- **Custom 96 panel development with Illumina and Implementation**
- **Set out to create at a super-flexible panel**
 - Might get there
- **Developed a system to keep track of more than 30 genetic backgrounds**
 - Flexibility as we need it
- **Can go from Post-hybridization to data analysis complete in about an 1 hr for 250+ samples!!!!!!!!!!!!!!!!!!!!!!**

Outline



- **Genetic Monitoring: 96 SNP Panel Development**
 - The problem
 - Potential solutions
 - The Taconic/Illumina 80+16 idea
 - Custom 96 panel development with Illumina and Implementation
- **Speed Congenics and Background Characterization using Illumina LDL and MDL Panels**



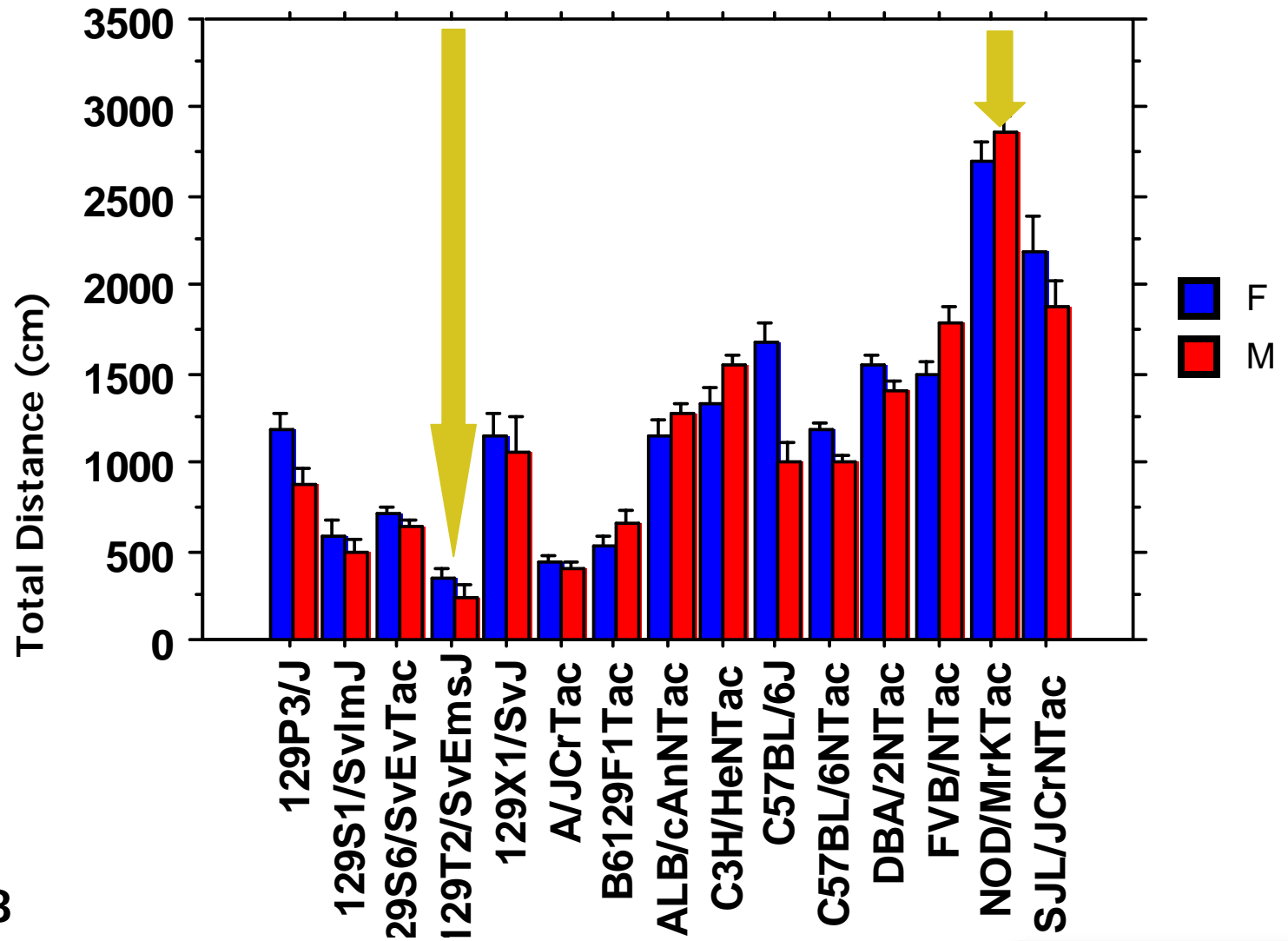
Do you know your mouse?



Once you have inserted a transgene or created a knockout, how do you know what else has happened in the genome or how the background could effect your results?

- Genetic Drift: GenMon
 - **Move to the Illumina LDL and MDL SNP Panels: Lose flexibility but gain density and therefore, sensitivity for drift (yearly)**
- Genetic Contamination
- Genetic Variability
 - **examples**

Open Field Activity (first exposure)



G. Bothe 2008

Genetic Background Controls Tumor Development in *Pten*-Deficient Mice



Table 1. *Pten* heterozygous tumor models: a comparison

Freeman, et. al. Cancer Res 2006; 66: (13). July 1, 2006

Features	Pandolfi	Mak	Parsons	Wu
Deletion	Exons 4-5	Exons 3-5	Exon 5	Exon 5
Background	129/C57	129/CD1 or 129/C57	129/C57	129/BALB/c
Homozygotes die at:	<E7.5	E9.5	E6.5	E9.5
Tumors in heterozygotes				
Earliest detection	1.5 mo (6)	2 mo (9)	1.5 mo (7)	6 mo
Tumor types and frequencies				
Gastrointestinal hyperplasia	All? (6)	All? (9)	90% (lymphoid)	2% (inflammation)
Lymphoid hyperplasia	100% female (20) and 83% male (20)	88% (9) (T-cell lymphoma)	100% female and 45% male	33% female and 20% male
Adrenal medullary tumor	100% (21)	23% (8)	NR	None
Endometrial hyperplasia	70% (21)	80% (8)	100%	9% (45% hemangioma)
Breast	NR	49% (8)	NR	37%
Prostate	50% (21)	44% (8)	75%	90%
Thyroid	60% (21)	None (9)	30%	None



- **Background can has effects on both the neurobiology and cancer biology (and everything else!) of animals**
- **What's a researcher to do?**
 - Know the genetic background of your mice
 - Check the background of your mice
 - Do not mix backgrounds for experiments
- **How do we help?**
 - Background Characterization
 - Speed Congenics



96 GenMon Panel Differences



Strain Differences detected by the 96 SNP Veracode GenMon Panel	129S6/SvEvTac	A/JcRTac	BALB/cAnNTac	BALB/cABomTac	BALB/cJBomTac	C3H/HeNTac-MTV	C57BL/10SgSnAiTac	C57BL/6NTac	C57BL6/J	C57BL6/JBomTac	CBA/JBomTac (s.6)	DBA/1BomTac	DBA/2 JBom Tac	DBA/2NTac
129S6/SvEvTac		37	35	35	34	32	48	49	55	52	34	41	43	43
A/JcRTac	37		19	20	23	33	55	60	64	63	35	42	44	44
BALB/cAnNTac	35	19		1	4	32	48	49	53	52	38	37	39	39
BALB/cABomTac	35	20	1		5	33	49	50	54	53	39	38	40	40
BALB/cJBomTac	34	23	4	5		32	52	53	56	56	38	37	37	37
C3H/HeNTac-MTV	32	33	32	33	32		50	49	55	52	8	25	29	29
C57BL/10SgSnAiTac	48	55	48	49	52	50		5	12	8	46	43	45	45
C57BL/6NTac	49	60	49	50	53	49	5		7	3	47	44	44	44
C57BL6/J	55	64	53	54	56	55	12	7		4	53	49	49	49
C57BL6/JBomTac	52	63	52	53	56	52	8	3	4		50	47	47	47
CBA/JBomTac (s.6)	34	35	38	39	38	8	46	47	53	50		25	29	29
DBA/1BomTac	41	42	37	38	37	25	43	44	49	47	25		4	4
DBA/2 JBom Tac	43	44	39	40	37	29	45	44	49	47	29	4		0
DBA/2NTac	43	44	39	40	37	29	45	44	49	47	29	4	0	

LDL (377) SNP Panel Differences



	129S2/SvHsd	129S4/SvJae	129S6/SvEvTac	A/Jtac	BALB/cAnNTac	BALB/cJBomTac	BTBR/J	C3H/HeNTac	C57BL/10SgSnAiTac	C57BL/6J	C57BL/6JBomTac	C57BL/6NTac	CBA/JBomTac	DBA/1JBomTac
129S2/SvHsd		1	6	160	166	166	108	160	225	241	235	230	153	152
129S4/SvJae	1		5	161	167	167	109	161	224	240	234	229	154	153
129S6/SvEvTac	6	5		160	162	162	114	158	227	243	237	232	153	152
A/Jtac	160	161	160		76	76	166	116	251	271	265	260	129	159
BALB/cAnNTac	166	167	162	76		0	166	106	225	245	239	234	129	165
BALB/cJBomTac	166	167	162	76	0		166	106	225	245	239	234	129	165
BTBR/J	108	109	114	166	166	166		164	177	193	187	182	155	159
C3H/HeNTac	160	161	158	116	106	106	164		239	259	253	248	55	131
C57BL/10SgSnAiTac	225	224	227	251	225	225	177	239		28	22	17	226	222
C57BL/6J	241	240	243	271	245	245	193	259	28		6	11	248	242
C57BL/6JBomTac	235	234	237	265	239	239	187	253	22	6		5	242	236
C57BL/6NTac	230	229	232	260	234	234	182	248	17	11	5		237	231
CBA/JBomTac	153	154	153	129	129	129	155	55	226	248	242	237		118
DBA/1JBomTac	152	153	152	159	165	165	159	131	222	242	236	231	118	
DBA/2NTac	167	168	167	152	162	162	162	124	231	251	245	240	121	23

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	



- **What is Genetic Characterization?**

- Accurately characterizes the background of animal model prior to starting that expensive breeding program.
- Provides differentiation among some mouse strains

- **What can Speed Congenics do?**

- Ultimately reduces the total number of backcrossing steps necessary to make a congenic (*pure*) line
- Realize 100% of your targeted background as early as N5! instead of N10



Report Example



Projects

1. Project Submission: 0800012345

Project Type: Speed Congenics
Panel Type: 377LD Linkage

Operator: SNP1
Date:12-21-2008

Generation: N2
Reference or Target Strain:C57BL/6
Potential Background Strains: TBD

Animal ID	Sex	% C57	Gen* Number	Select Breeder	Comment
1	M	87.1%	N3		
2	M	87.3%	N3	Y-3	
3	M	86.1%	N3		
4	M	85.8%	N3		
5	M	88.4%	N3	Y-1	
6	M	87.3%	N3	Y-4	
7	M	86.9%	N3		
8	M	88.1%	N3	Y-2	

* See Taconic Guidelines Tab for determination.

According to 377 SNP analysis data samples range from 85.8%-88.4% of C57BL/6 recipient genome. Animal 5 received the highest score of 88.4% and animal 8 recieved the second highest score of 88.1% for C57BL/6 and should be selected for breeding. Animals 2 and 6 are the next best breeders with scores of 87.3% C57BL/6.

SNP Report – Raw Data



SNP Report Example - G11 and G1174

Index	Name	Chr	Position	Genotyping Results								Congenic Results							
				B6-1_C57BL/6NTac_CHIP 11_12	1_CHIP 11_12/17/2008.GType	2_CHIP 11_12/17/2008.GType2	3_CHIP 11_12/17/2008.GType	4_CHIP 11_12/17/2008.GType	5_CHIP 11_12/17/2008.GType	6_CHIP 11_12/17/2008.GType	7_CHIP 11_12/17/2008.GType	8_CHIP 11_12/17/2008.GType	1_CHIP 11_12/17/2008.GType2	2_CHIP 11_12/17/2008.GType23	3_CHIP 11_12/17/2008.GType4	4_CHIP 11_12/17/2008.GType5	5_CHIP 11_12/17/2008.GType6	6_CHIP 11_12/17/2008.GType7	7_CHIP 11_12/17/2008.GType8
66	rs13475706	1	5917284	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
342	rs6259073	1	11106394	BB	AB	BB	AB	BB	AB	AB	AB	AB	0.5	1	0.5	1	0.5	0.5	0.5
203	rs3658044	1	19498836	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
67	rs13475783	1	28549433	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
68	rs13475788	1	30024577	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
29	gnf01.037.906	1	41423174	BB	AB	AB	AB	AB	AB	AB	AB	AB	0.5	0.5	0.5	0.5	0.5	0.5	0.5
1	CEL-1_49807741	1	49807741	BB	AB	AB	AB	AB	AB	AB	AB	AB	0.5	0.5	0.5	0.5	0.5	0.5	0.5
69	rs13475870	1	52159056	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
329	rs6206420	1	59624742	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
70	rs13475909	1	69117243	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
357	rs6356603	1	75362320	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
55	mCV24115911	1	76984491	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
30	gnf01.085.746	1	85664769	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
320	rs6157345	1	95497940	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
71	rs13476024	1	103459296	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
212	rs3664662	1	104472398	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
72	rs13476036	1	106831730	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
369	rs6408002	1	115462613	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
73	rs13476098	1	126400694	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
356	rs6354736	1	128165659	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
339	rs6250257	1	137529520	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
74	rs13476137	1	139114404	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
75	rs13476148	1	142911628	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
76	rs13476177	1	150586221	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
226	rs3677638	1	156461112	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
235	rs3685643	1	162261064	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
269	rs3707322	1	164408862	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
77	rs13476248	1	174277920	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
78	rs13476259	1	177399967	AA	AA	AA	AB	AA	AA	AA	AB	AA	1	1	0.5	1	1	1	0.5
51	mCV22849619	1	186531848	AA	AA	AB	AB	AA	AA	AA	AB	AA	1	0.5	0.5	1	1	1	0.5
242	rs3689947	1	192397145	AA	AB	AB	AB	AB	AB	AB	AB	AB	0.5	0.5	0.5	0.5	0.5	0.5	0.5
31	gnf02.001.197	2	4196507	AA	AB	AB	AB	AB	AB	AB	AB	AB	0.5	0.5	0.5	0.5	0.5	0.5	0.5
79	rs13476331	2	6048930	BB	AB	AB	AB	AB	NC	AB	AB	AB	0.5	0.5	0.5	0.5	NA	0.5	0.5
323	rs6165425	2	20814394	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
11	CEL-2_23847726	2	23847726	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
53	mCV23209429	2	31240017	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
347	rs6295520	2	43034728	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
80	rs13476490	2	50749857	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1
81	rs13476507	2	54765962	AA	AA	AA	AA	AA	AA	AA	AA	AA	1	1	1	1	1	1	1
82	rs13476540	2	63511489	BB	AB	AB	AB	AB	AB	AB	AB	AB	0.5	0.5	0.5	0.5	0.5	0.5	0.5
83	rs13476554	2	67180899	BB	BB	BB	BB	BB	BB	BB	BB	BB	1	1	1	1	1	1	1

Traditional Backcross Generation Table



The following theoretical analysis (based on Markel et al. [1997] Nature Genetics 17:280.) was used to predict the % recipient genome:

Table 1. Traditional Backcross Generation Table

Generation	Average % heterozygous D/R segments +/- SD	% Recipient Genome
P	0	0
F1	100	50
N2	50 +/- 7.07	75 (67.9-82.4)
N3	25 +/- 5.00	87.50 (82.5-90.1)
N4	12.5 +/- 3.54	93.75 (90.2-95.5)
N5	6.25 +/- 2.5	96.88 (95.6-98.0)
N6	3.13 +/- 1.76	98.44 (98.1-99.1)
N7	1.56 +/- 1.25	99.2-99.5
N8	0.78 +/- 0.88	99.6-99.80
N9	0.39 +/- 0.63	99.81-99.89
N10	0.2 +/- 0.44	99.90-100.00

Summary



- **Genetic Monitoring: 96 SNP Panel Development**
- **Speed Congenics and Background Characterization using Illumina LDL and MDL Panels**

Acknowledgements



- **Stephen Festin, PhD**
- **Gerald Bothe, PhD**
- **Ana Perez, PhD**
- **Rebecca D. Farinacci, MS**
- **Kim Mullinax, MS**

Genetic Background Problems



- **Both physiological and behavioral phenotypes depend on genetic background.**
- **Targeted mutations are mostly produced in 129 mice, which are genetically heterogeneous.**
- **129 mice are not well suited for some experiments. Therefore, mutations are often moved to another strain.**

Meiosis

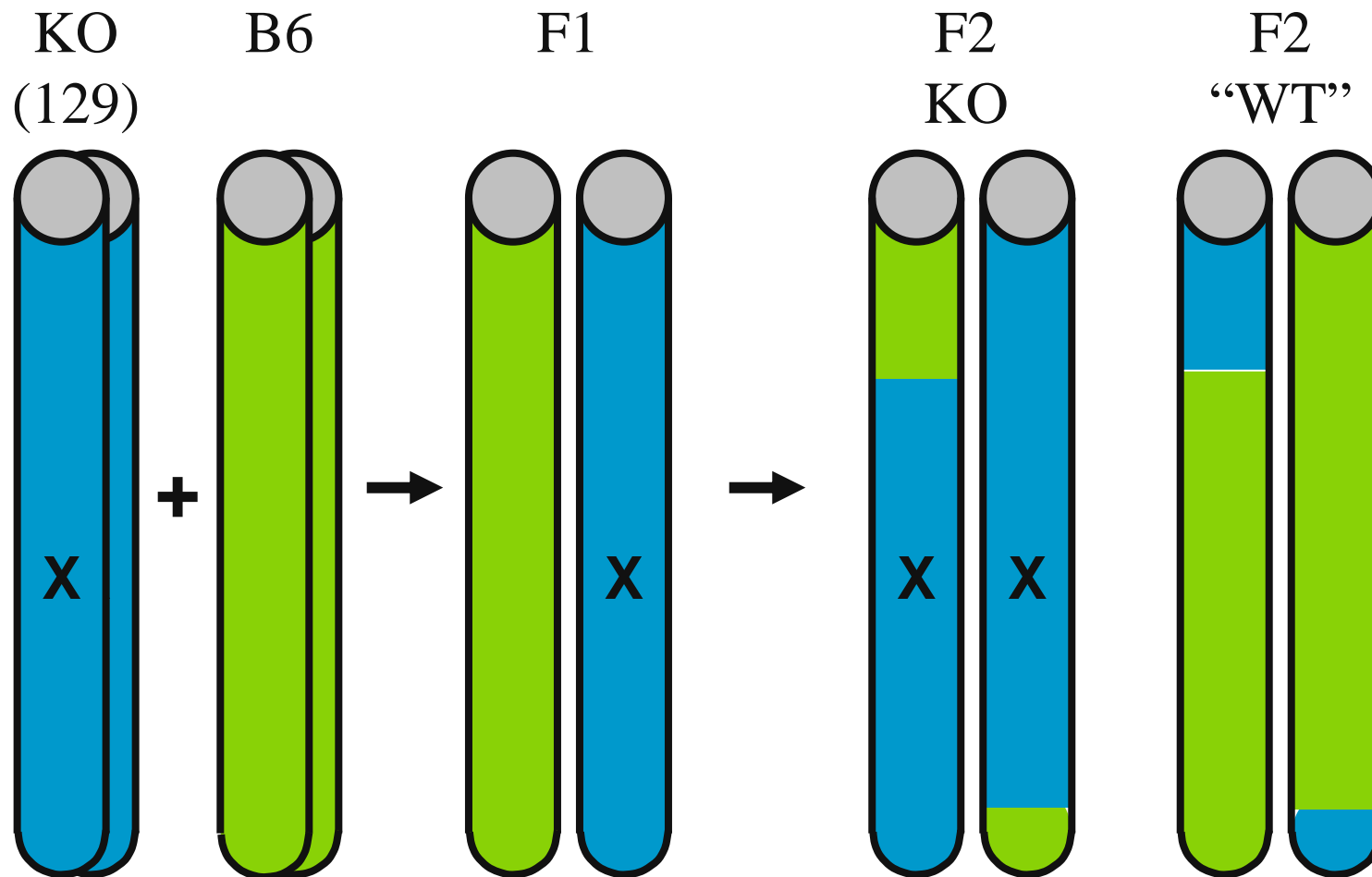


- **Once each generation, the genome is reshuffled during Meiosis**
- **In the zygotene and pachytene of prophase I, homologous chromosomes align and crossovers occurs**
- **Resulting chromosomes are randomly distributed to daughter cells in metaphase I and anaphase I**

Result of a cross



Knockout and “WT” control have different genetic backgrounds



Backcrossing

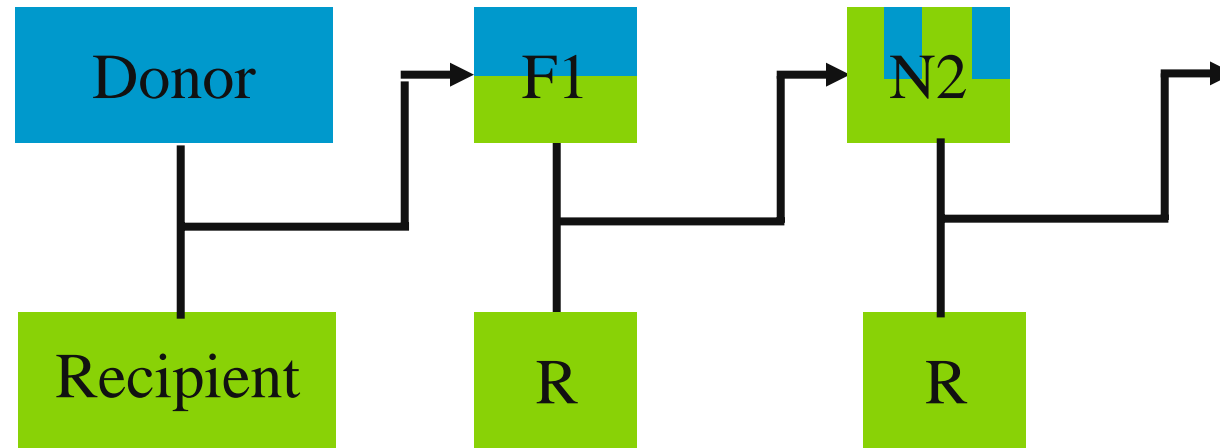


- **If a knockout or mutation-carrying mouse is crossed with a mouse from a different strain, a mixed genetic background results that interferes with the experiments**
- **If a mutation-carrying F1 mouse is crossed to the “new” (recipient) strain again and again, the influence of the original genetic background is reduced.**

Making Congenic Strains



- Mutations are moved to another genetic background through backcrossing:



- Nomenclature:

Parental generation:	P	
First filial generation:	F1	
Second generation:		N2
Third generation:		N3

Linked Loci



- Genes on different chromosomes are distributed randomly to sperm/oocytes.
- Genes that are close neighbors on the same chromosome are “linked”
- These genes tend to remain together

Non-Linked Loci



- Reduced by 50% each generation
- Remaining heterozygosity:

$$p = \frac{1}{2}^{N-1}$$

- Remaining donor genome:

$$p = \frac{1}{2}^N$$

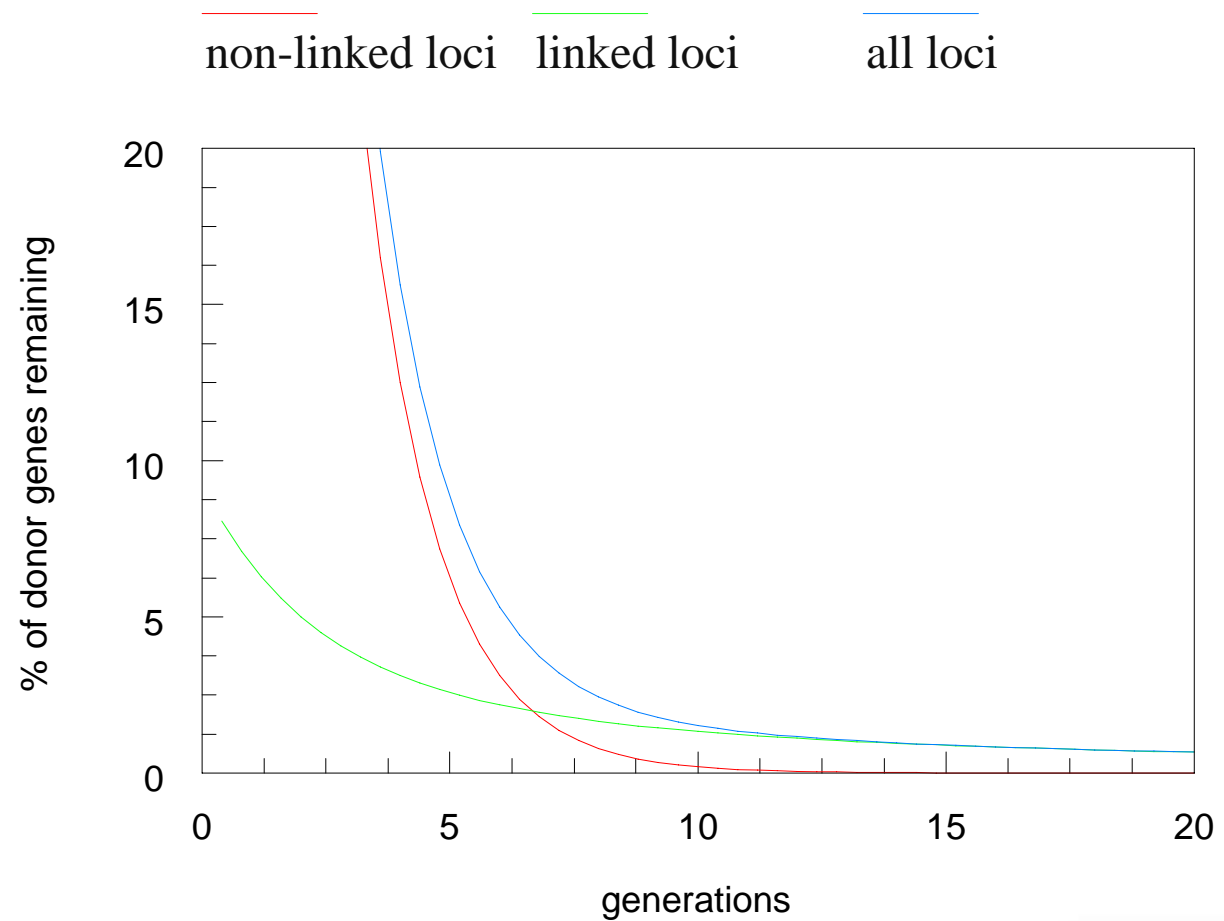


- Reduced at a much slower rate due to linkage/selection
- Size of remaining donor fragment:
$$s = 200 (1 - 2^{-N}) / N \quad [\text{cM}]$$
- From $N6$, simplify to:
$$s = 200 / N \quad [\text{cM}]$$

Backcrossing: Elimination of Heterozygosity



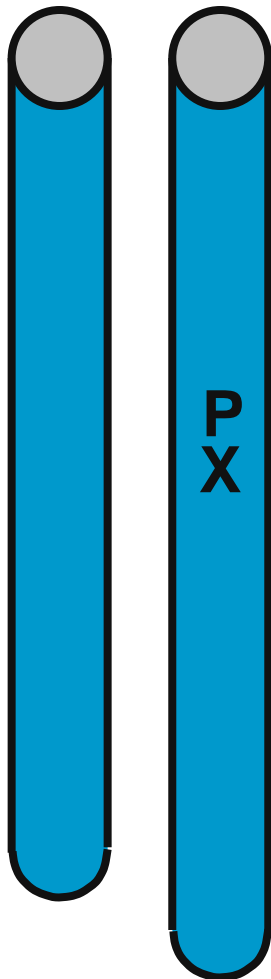
Occurs throughout the genome, but linked loci are less affected



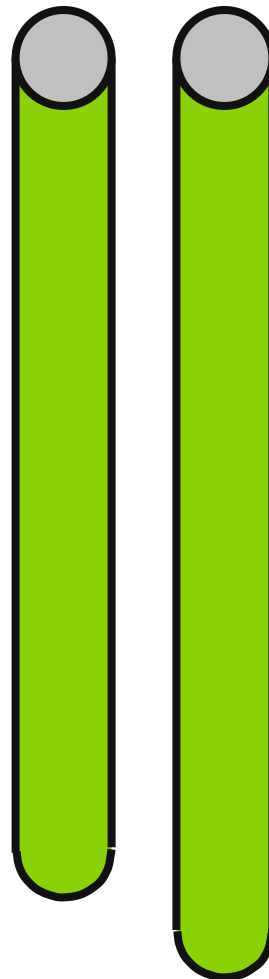
Congenic Strain



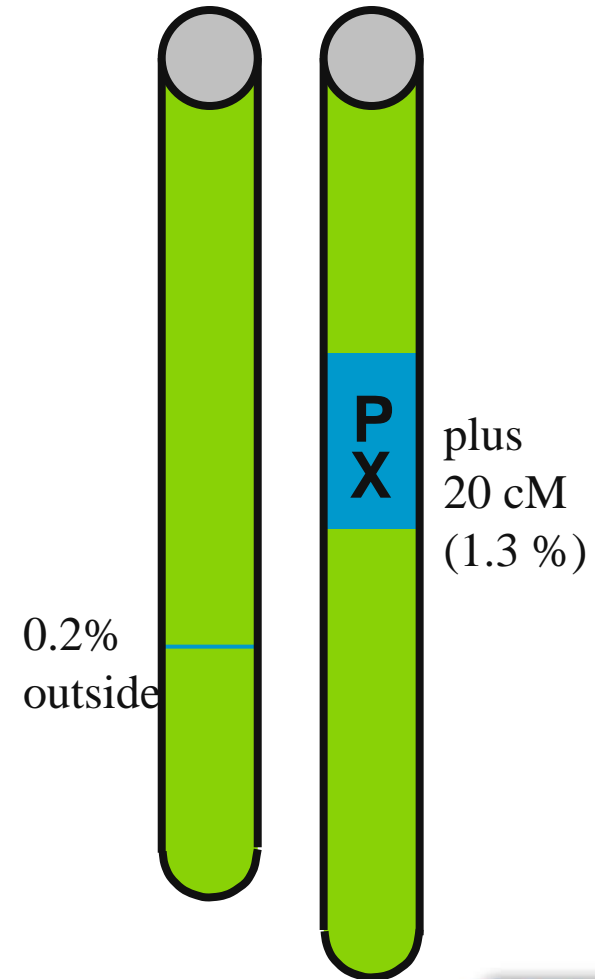
Donor (129)



Recipient (B6)



Congenic
(N10)



More on Backcrossing



- **Backcrossing is a lengthy process: if we receive a pure 129 strain knockout mouse for rederivation, it will take 10 more generations in a barrier to finish - 2 ½ years.**
- **In each generation, there is a random distribution of offspring: some have more donor genes than others**

Speeding Things Up



- **If we select the right offspring, backcrossing can be accelerated.**
- **Need a method to identify from which parent strain any part of a chromosome is inherited.**
- **Select those offspring that inherited most recipient strain material**

Genetic Background Data

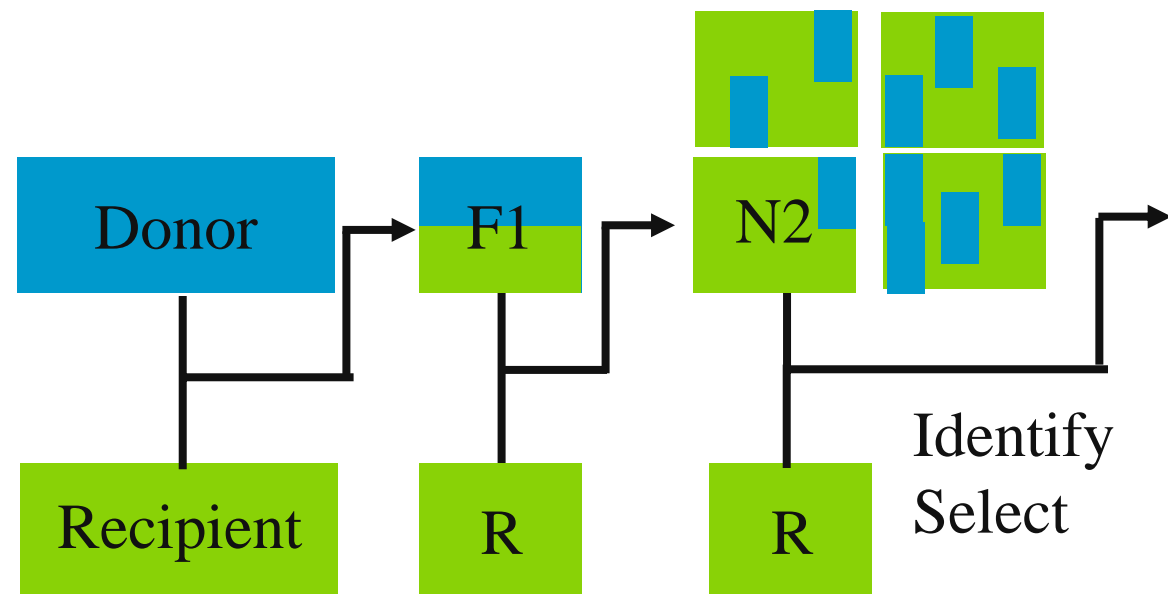


- **Taconic has extensive genetic data:**
 - More than 2000 SNP loci
 - 340 microsatellite markers
- **This information shows that Taconic's B6 strain (C57BL/6NTac) is closely related to other B6 substrains, e. g. C57BL/6J, and that Taconic's 129S6/SvEvTac is quite separate from other 129 strains.**
- **This information can also be used for QC and for speed congenics.**

Making Speed Congenic Strains



- Mutations are moved to another genetic background through backcrossing the “best” animal in each generation:



Marker Density Needed



- **The more resolution the better?**
- **But: economic restrictions**
- **But: very close double crossovers are suppressed in any ONE meiosis (the next generation meiosis is an independent event though)**

Crossover Interference



- Most extensive data are in humans (Kwiatkowski et al., 1993)
- At 20 cM, there were only 10 double crossovers out of 17316 events, or $r = 0.0005$ instead of $r = 0.04$ without interference.
- In a typical speed congenics project, less than 0.4 double crossovers expected if markers are at 20 cM or less.
- Therefore, marker spacing of 20 cM or less is appropriate for speed congenics.
- However, independent events are not included in this estimate.
- Rule of thumb: genome = 3,000 Mbp = 1500 cM
1 cM \approx 2 Mbp

Passenger Loci

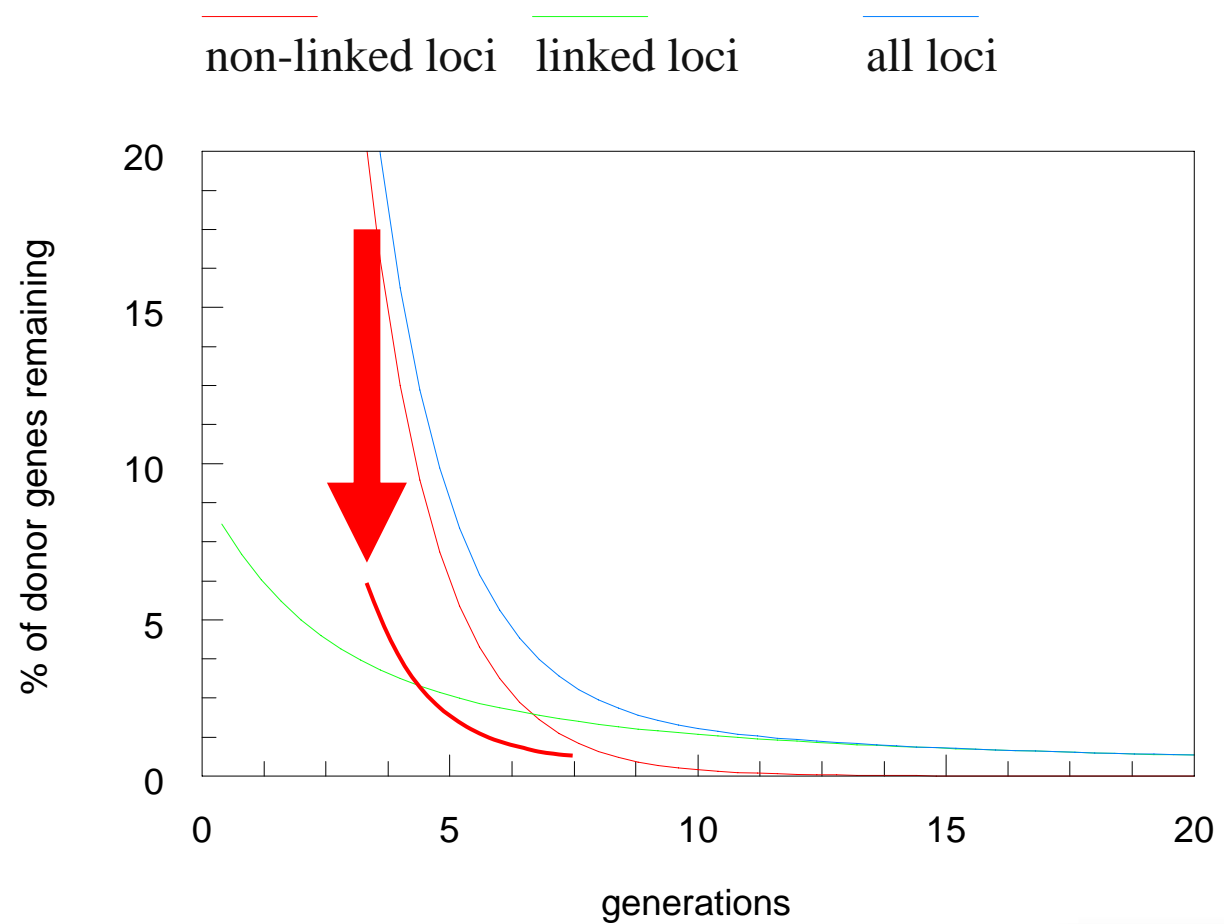


- Rapid elimination of donor genes occurs only in regions of the genome that are *not* linked to the donor allele
- Region around the donor allele (e.g., knockout) is slowly and *randomly* reduced by crossover

Backcrossing: Elimination of Heterozygosity



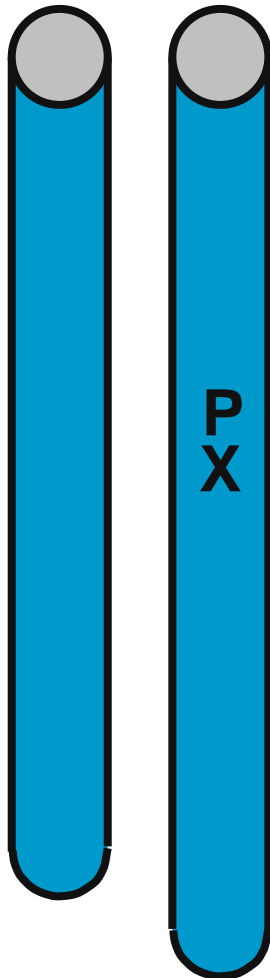
Occurs throughout the genome, but linked loci are less affected



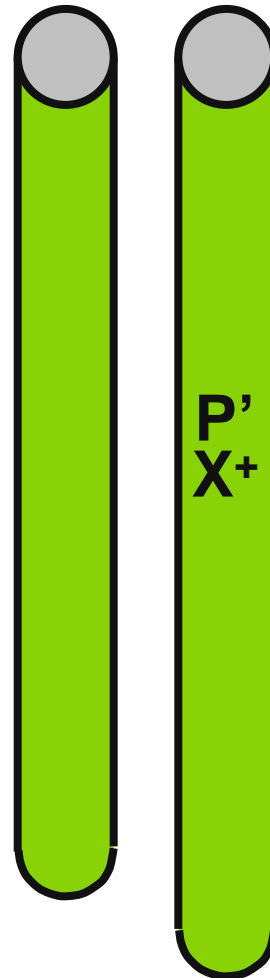
Speed Congenic Strain- no adjustments



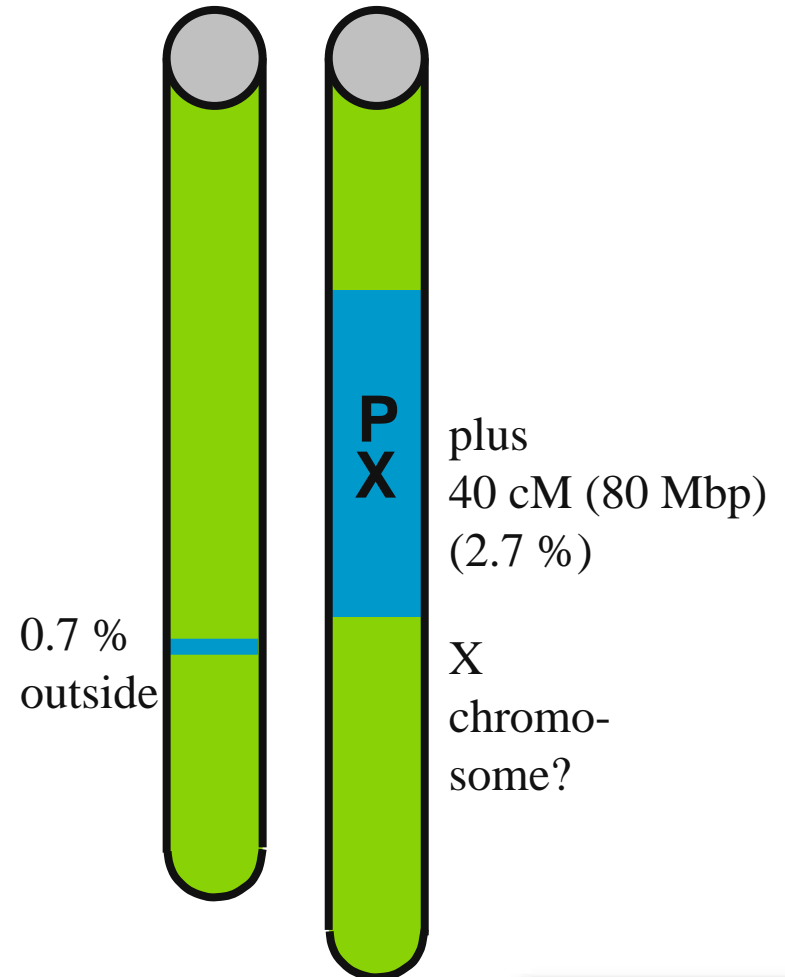
Donor (129)



Recipient (B6)



SpeedCongenic
(N5)





Accelerated backcrossing schemes have been criticized by some scientists:

- fewer backcross generations lead to larger donor segment
- small donor genome regions may be missed in N3 through N5 (independent crossover events)
- X and Y chromosomes sometimes undefined

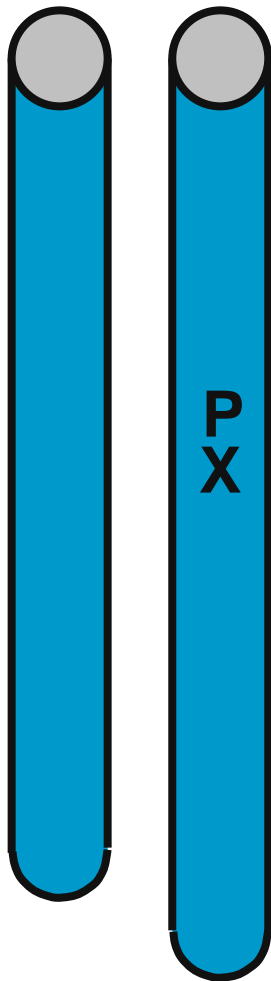


- **Additional testing (“Polishing”) using markers spaced at 20 Mbp or less can be used to reduce the size of the donor segment.**
- **Example: use Taconic’s 377 or 1449 marker panel.**
- **Breeding scheme can be used to assure right X/Y chromosomes – e.g. use a male KO mouse in P and F1 and end with a female N4 mouse that is used for the last step of backcrossing.**

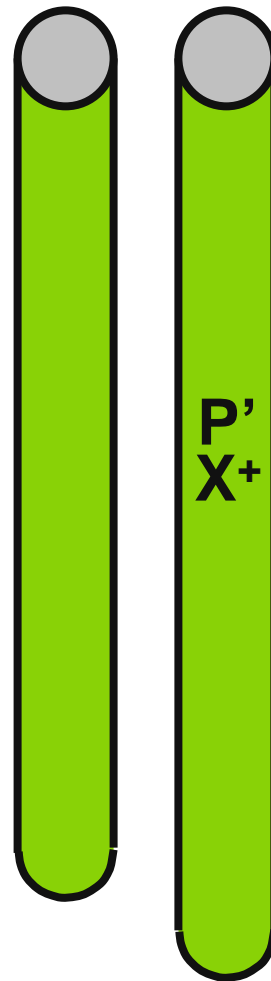
Speed- Congenic Strain - with adjustments



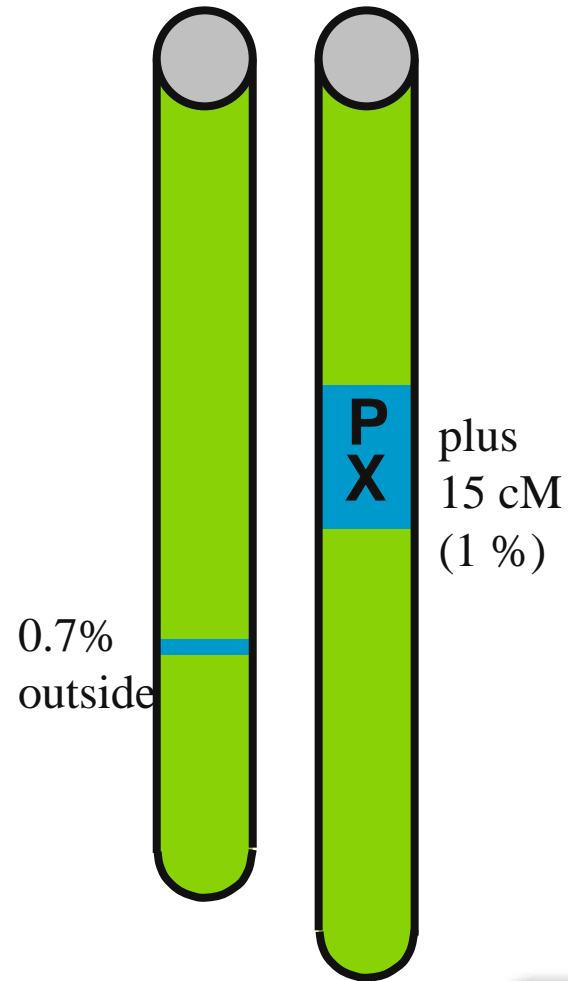
Donor (129)



Recipient (B6)



Speed Congenic
(N5)



Conclusions



- **Definition of genetic background is crucial for the interpretation of experiments with inbred and congenic mouse strains**
- **SNP markers are ideal for definition of genetic background and accelerated backcrossing**
- **Taconic will organize speed congenics for customers and has started to provide in-house services as of January 2009**

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Acknowledgements



Jan Seymour

Scott Tarpinian

Steve Mellor

Robert Kloc

Steve Festin

Jennifer Moran (Broad Institute)

Robert Keefe (Genomics Institute)

MMRRC/NIRR

Experimentals and Controls

