Deep sequencing and systems biology: steps on the way to an individualised treatment of cancer patients

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Life is the translation of the information in the genome into the phenotype of the organism:

The organism ‚computes‘ this phenotype from its genotype, given a specific environment

World wide ~11 million new cancer cases/year

Cure rates for most common forms of cancer have hardly changed over the last decades

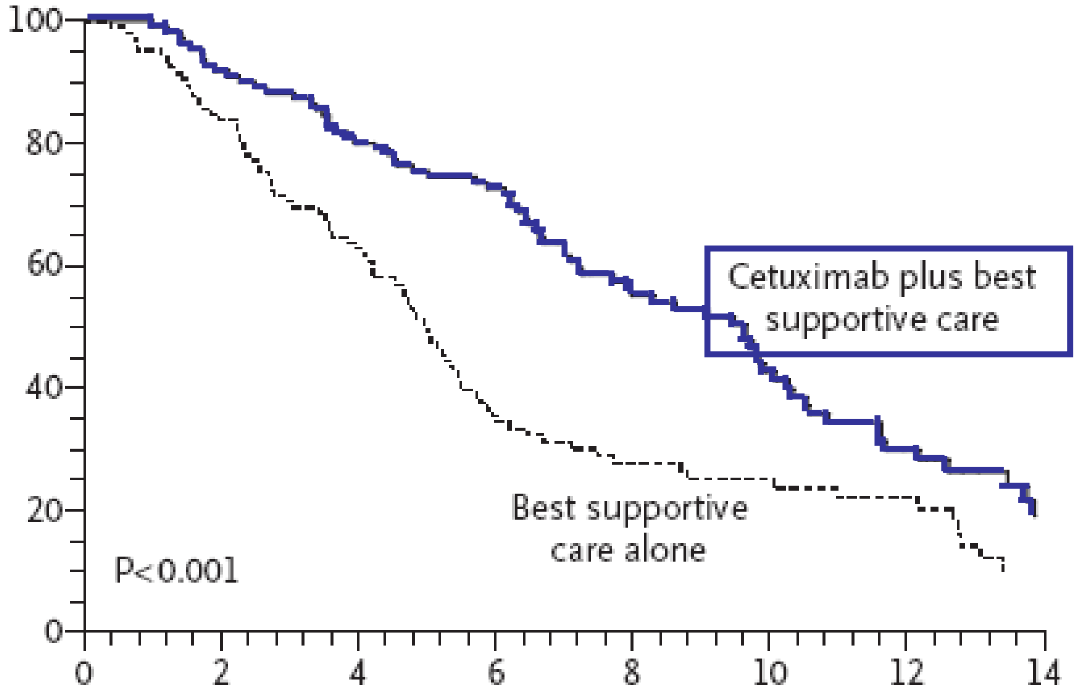
Even the most advanced targeted therapies are typically only effective for a small fraction of the patients

Pharma development costs have dramatically increased, while the number of new drugs keeps dropping

Intestinal cancer

(K-ras wild type)

S
U
R
VI
V
A
L

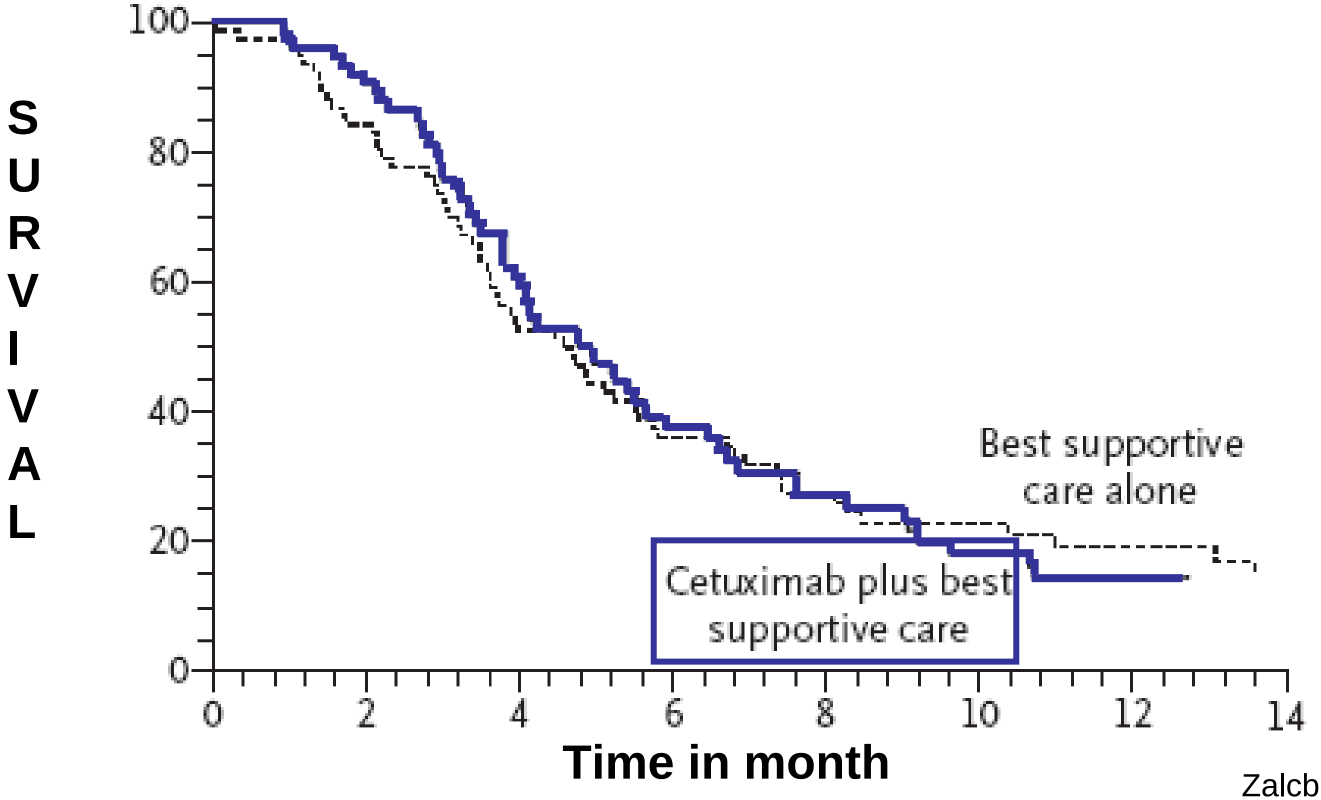


Time in month

Zalcberg et al, NEJM 2008

Intestinal cancer

Mutated K-ras



Zalcberg et al, NEJM 2008

Der Anfang: 1973

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 12, Part I, pp. 3581–3584, December 1973

The Nucleotide Sequence of the *lac* Operator

(regulation/protein–nucleic acid interaction/DNA–RNA sequencing/oligonucleotide priming)

WALTER GILBERT AND ALLAN MAXAM

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

Communicated by J. D. Watson, August 9, 1973

ABSTRACT The *lac* repressor protects the *lac* operator against digestion with deoxyribonuclease. The protected fragment is double-stranded and about 27 base-pairs long. We determined the sequence of RNA transcription copies of this fragment and present a sequence for 24 base pairs. It is:

5′--T G G A A T T G T G A G C G G A T A A C A A T T 3′
3′--A C C T T A A C A C T C G C C T A T T G T T A A 5′

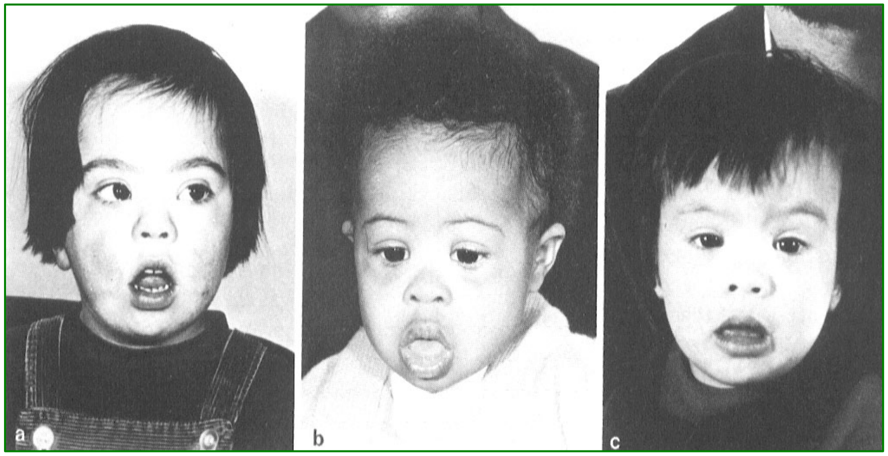
The sequence has 2-fold symmetry regions; the two longest are separated by one turn of the DNA double helix.

The lactose repressor selects one out of six million nucleotide sequences in the *Escherichia coli* genome and binds to it to prevent the expression of the genes for lactose metabolism.

bind again to the repressor, and is about 27 base-pairs long. Here we shall describe its sequence.

METHODS

Sonicated DNA Fragments. Sonicated [^{32}P]DNA fragments were made by growing a temperature-inducible lysogen of λc1857p*lac*5S7 at 34° in a glucose–50 mM Tris·HCl or TES (pH 7.4) medium in 3 mM phosphate, heating at 42° for 15 min at a cell density of 4 × 10^8/ml, then washing and resuspending the cells at a density of 8 × 10^8/ml in the same medium with 0.1 mM phosphate. 100 mCi of neutralized $H_3{}^{32}PO_4$ was added to 10 ml of cells, and the incorporation was continued for 2 hr at 34°. The cells were washed, suspended in 2



- clinical hallmarks:
- facial characteristics

brain abnormalities and mental retardation

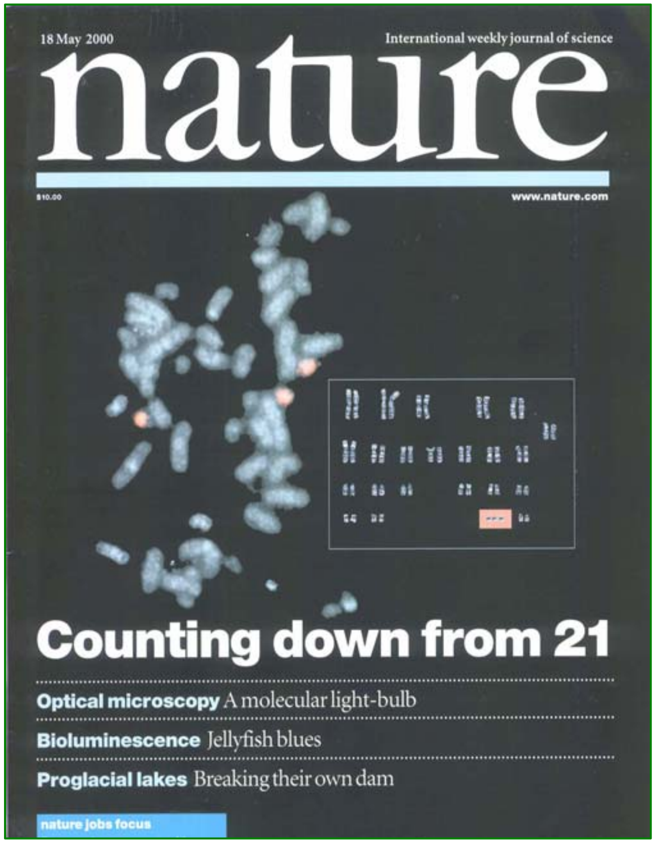
Alzheimer’s-like pathology

metabolic dysfunctions

premature aging

congenital heart disease

increased risk of leukemia



18 May 2000

International weekly journal of science

nature

$10.00

www.nature.com

Counting down from 21

Optical microscopy A molecular light-bulb

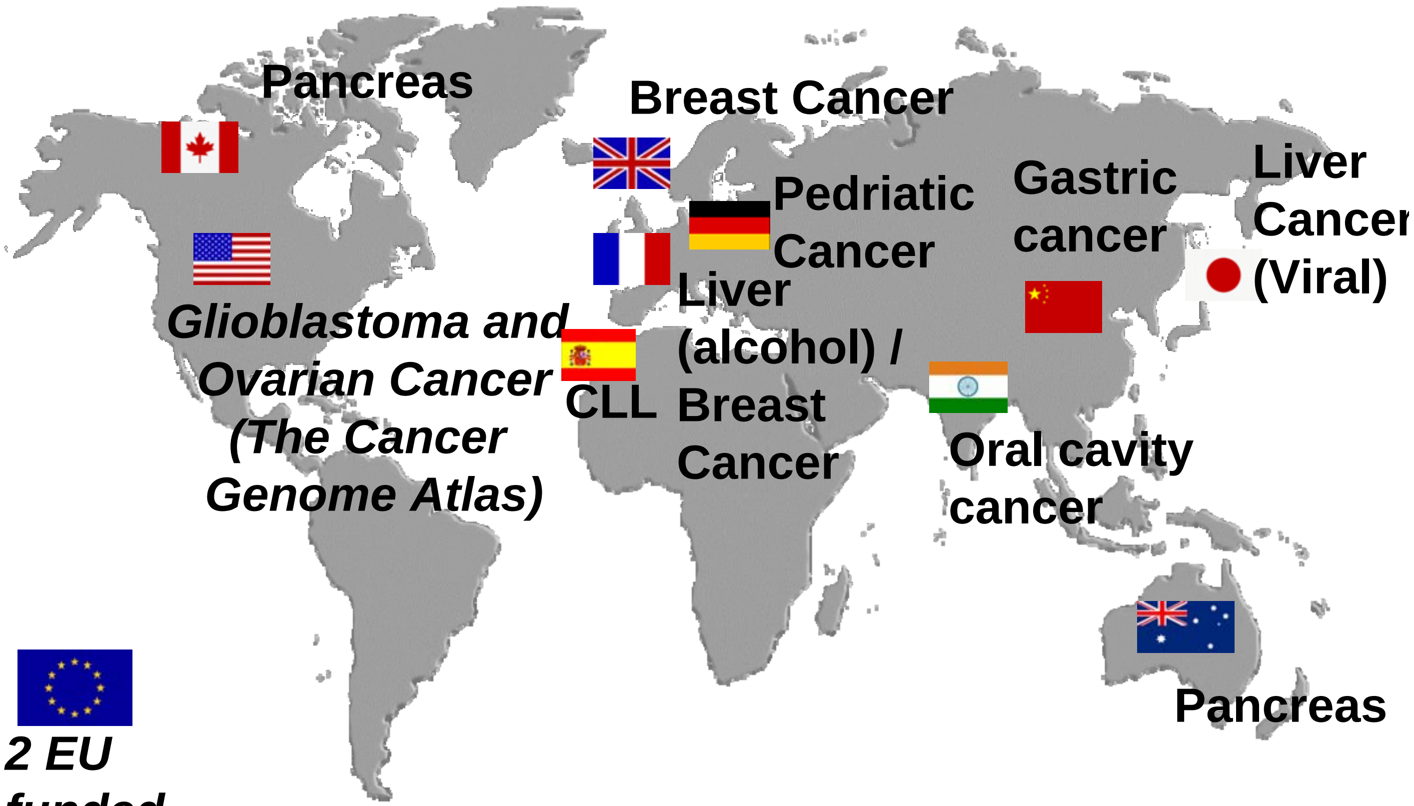
Bioluminescence Jellyfish blues

Proglacial lakes Breaking their own dam

nature jobs focus

The 1000 genomes project:
a catalogue of human polymorphism
created using next generation sequencing

International Cancer Genome Consortium (ICGC)



Pancreas

Breast Cancer

Pedriatic Cancer

Gastric cancer

Liver Cancer (Viral)

Glioblastoma and Ovarian Cancer (The Cancer Genome Atlas)

Liver (alcohol) / Breast Cancer

CLL

Oral cavity cancer

Pancreas

2 EU funded consortia

Chromosomal coverage in snap frozen vs. FFPE tissue

Figure 1A Chromosomal coverage: different FFPE preparation methods

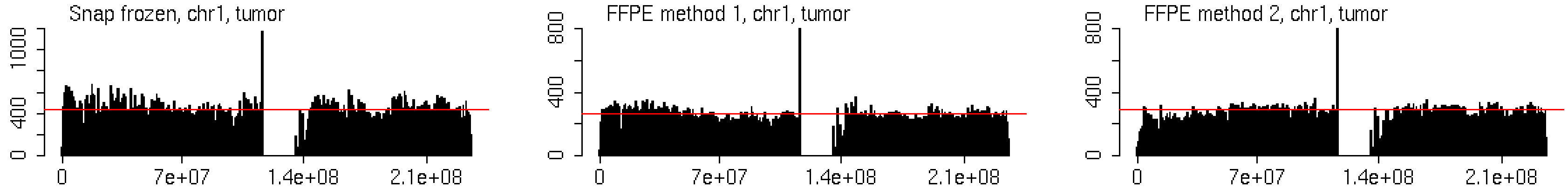
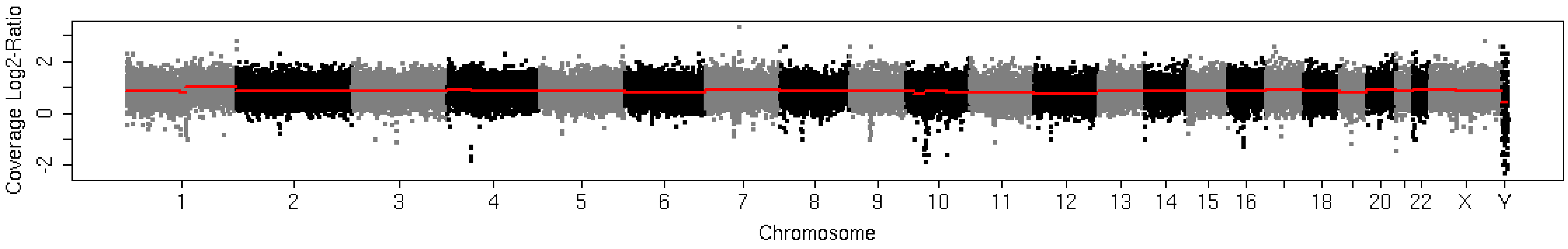
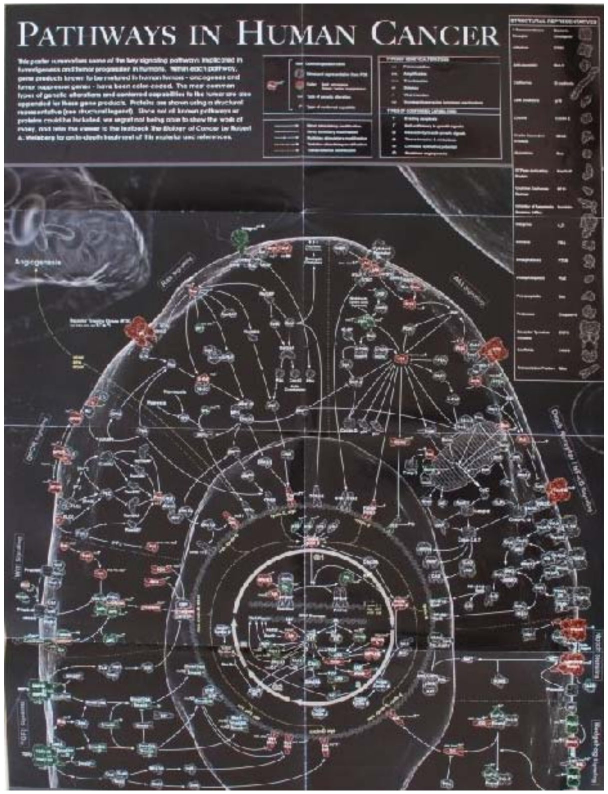


Figure 1B Chromosomal coverage ratio: Snap frozen / FFPE preparation

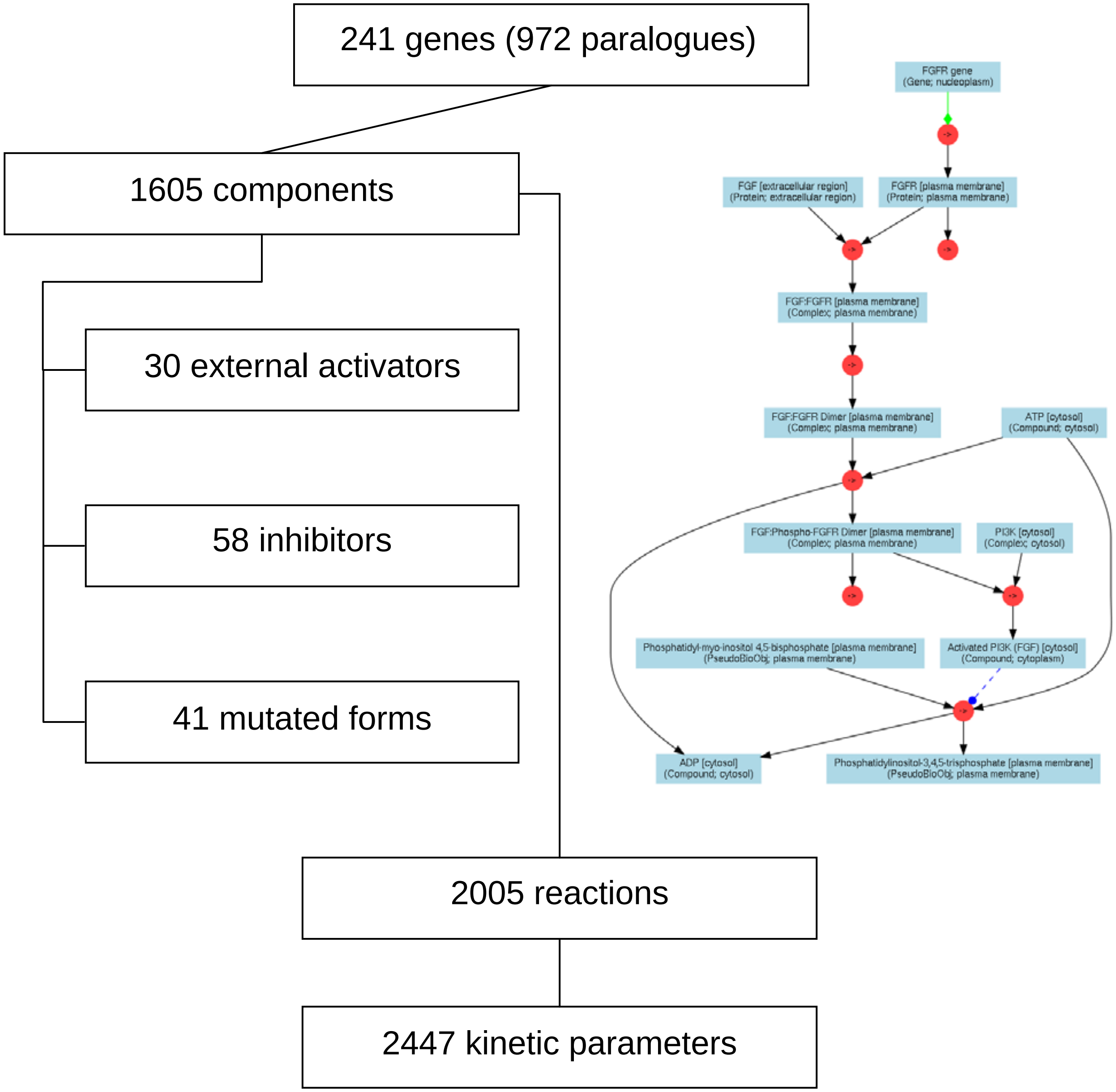


Systems Biology:
the virtual patient

Hannahan & Weinberg Cancer Modell



Cytokine signaling
Death receptor signaling
(Fas, TNFa, TRAIL)
DNA repair
GCPR/Hormone signaling
(Glucagon, Insulin, Testosterone)
Hedgehog signaling
Notch signaling
RTK signaling
(bNGF, EGF, FGF, IGF, PDGF)
TGFb signaling
(BMP, TGFb)
Wnt signaling



241 genes (972 paralogues)

1605 components

30 external activators

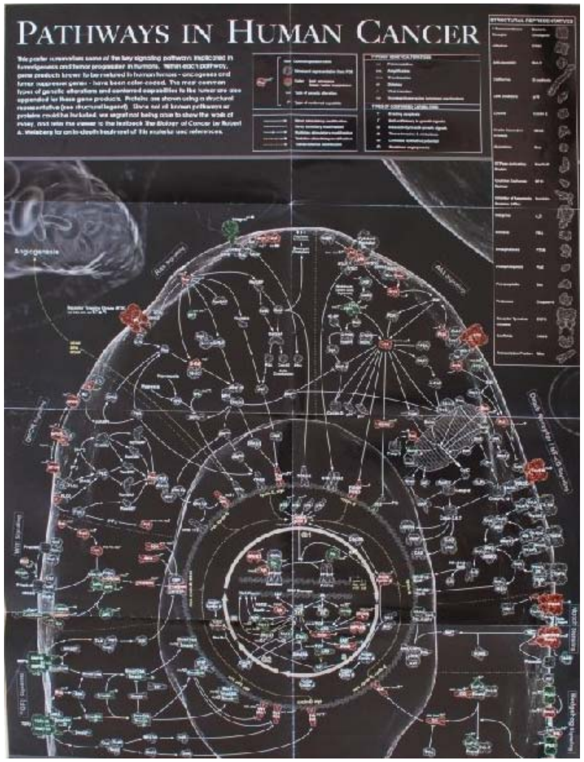
58 inhibitors

41 mutated forms

2005 reactions

2447 kinetic parameters

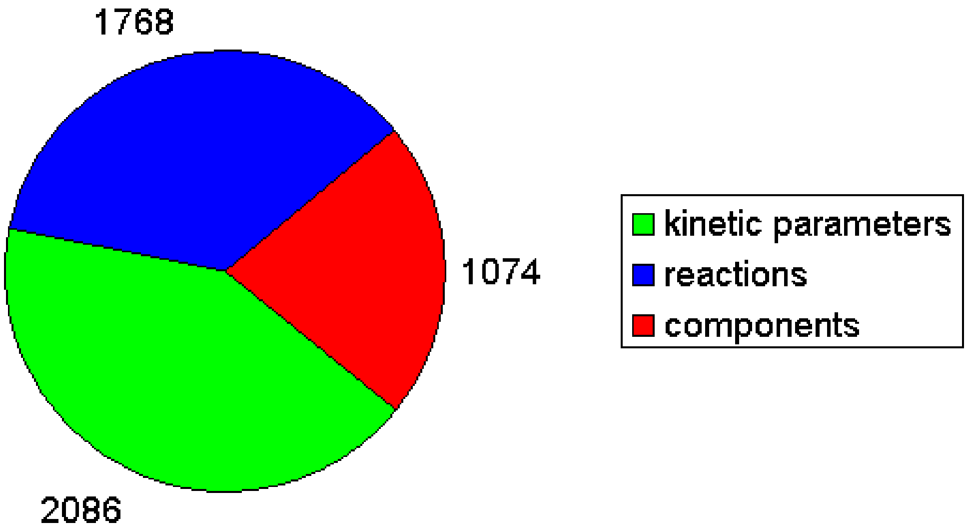
Hanahan / Weinberg Cancer Model

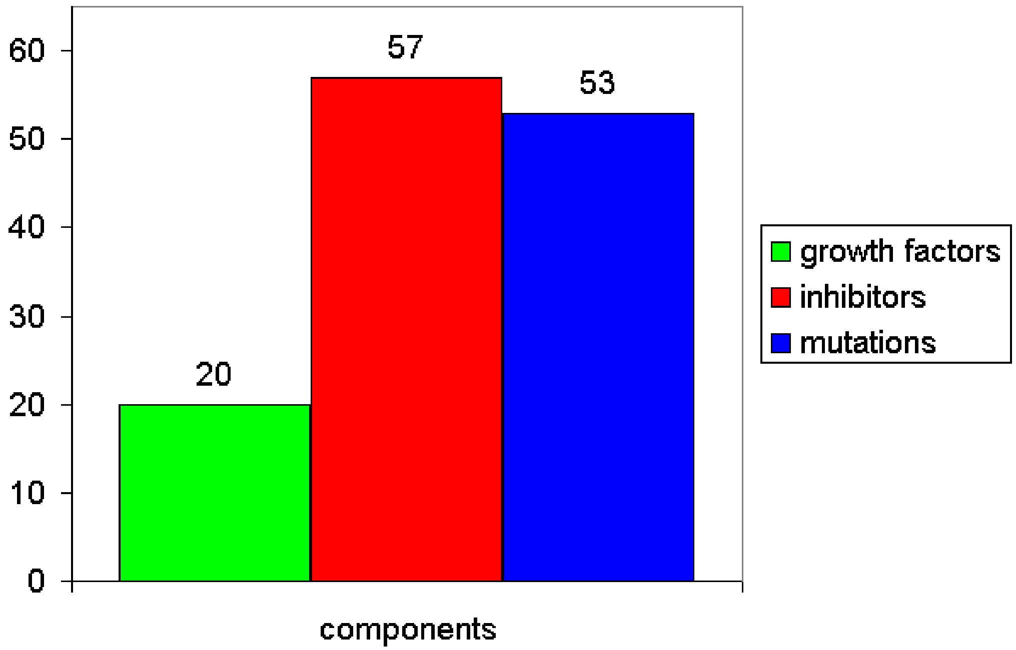


Weinberg, The Biology of Cancer (2007) Garland Science

Pathways of the model

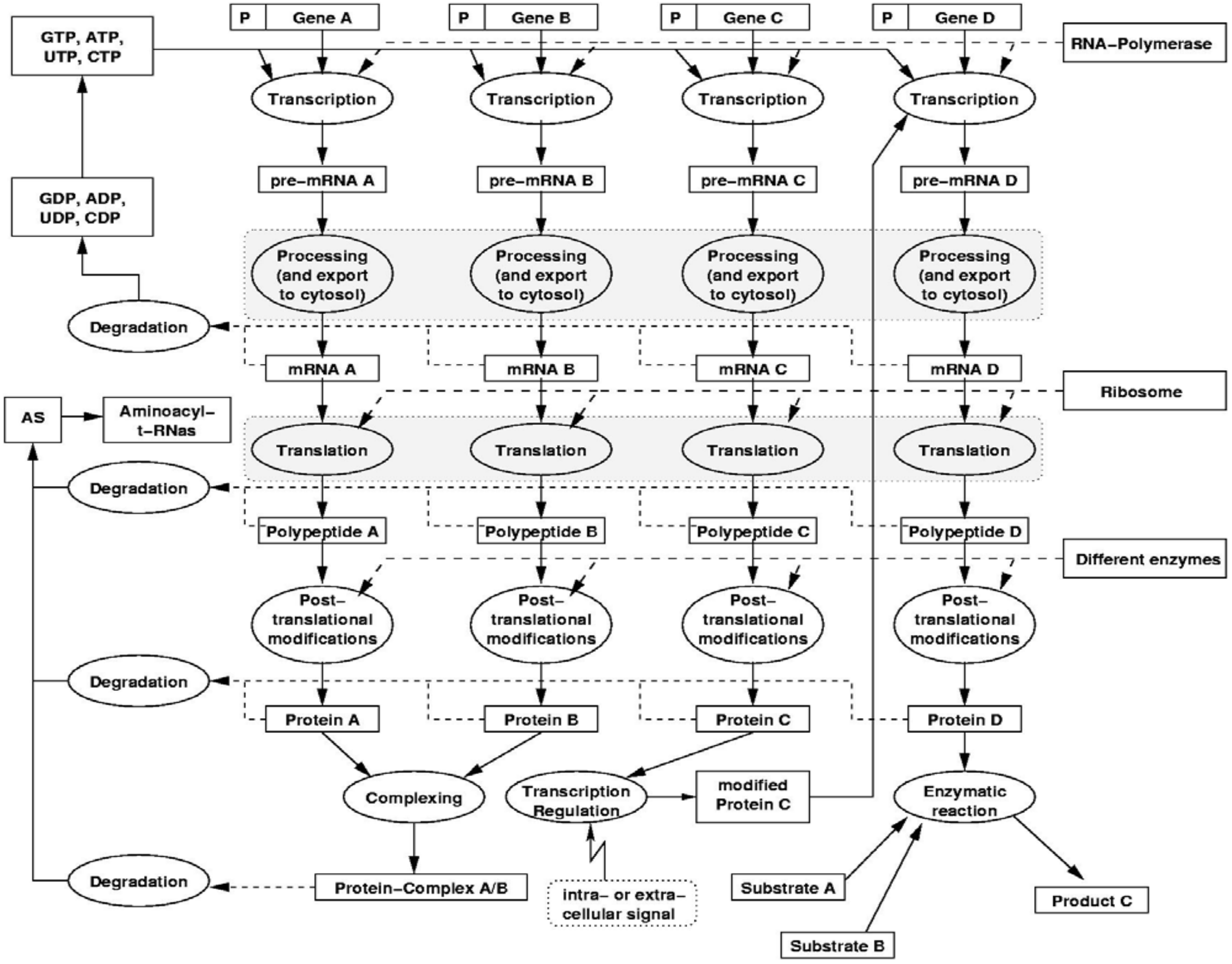
- IGF-1 signaling
- Cytokine signaling
- BMP signaling
- TGFbeta signaling
- Hedgehog signaling
- Notch signaling
- TNF-alpha signaling
- Fas signaling
- TRAIL signaling
- E-cadherin pathway
- Wnt signaling
- PLC signaling
- EGF signaling
- TLR3/TLR10 signaling
- GPCR pathway
- NGFR signaling
- Rb/E2F pathway
- DNA repair



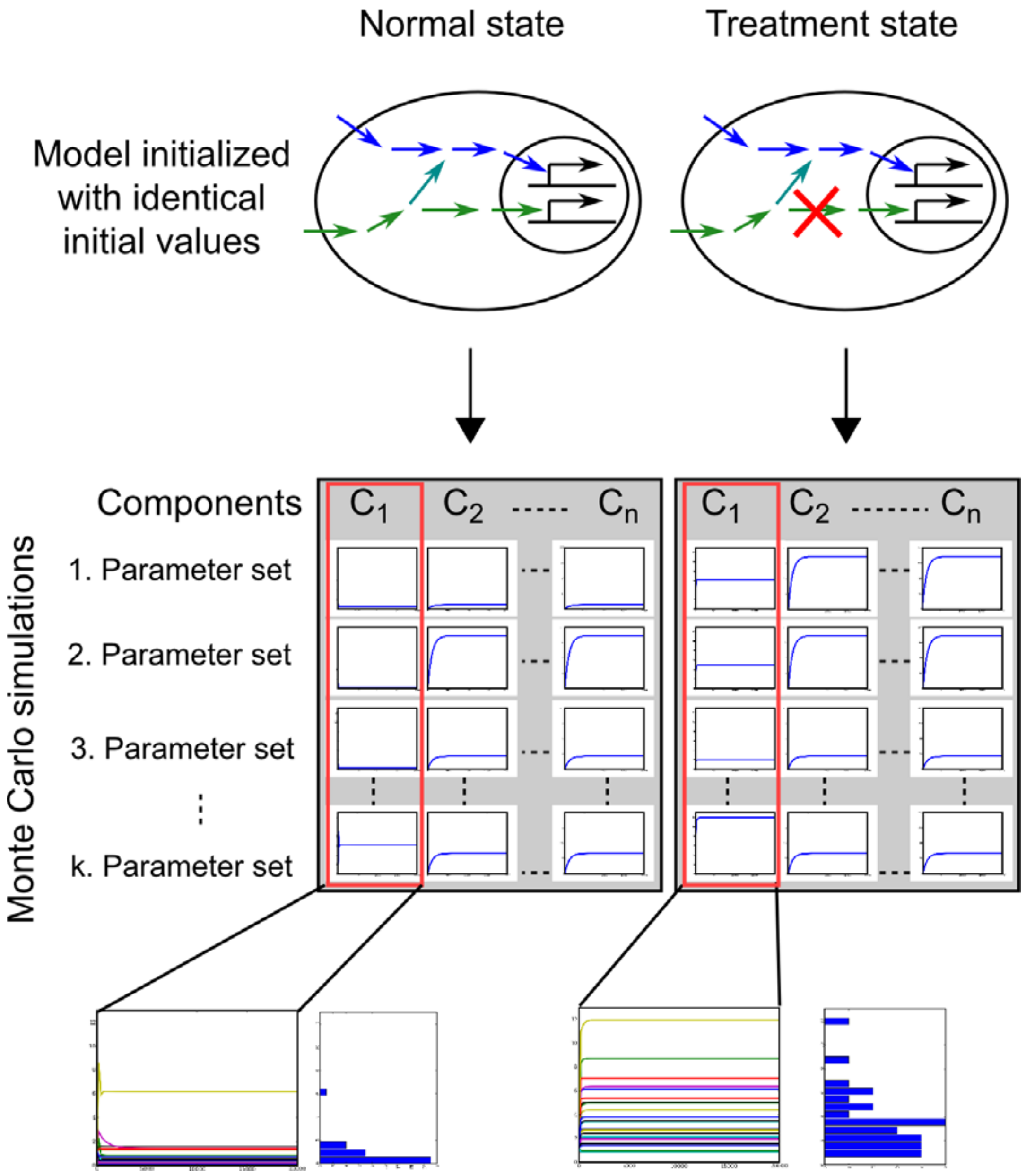


Systems biology – modelling platform

MAX-PLANCK-GESELLSCHAFT



Monte Carlo Approach – Simulation Pipeline



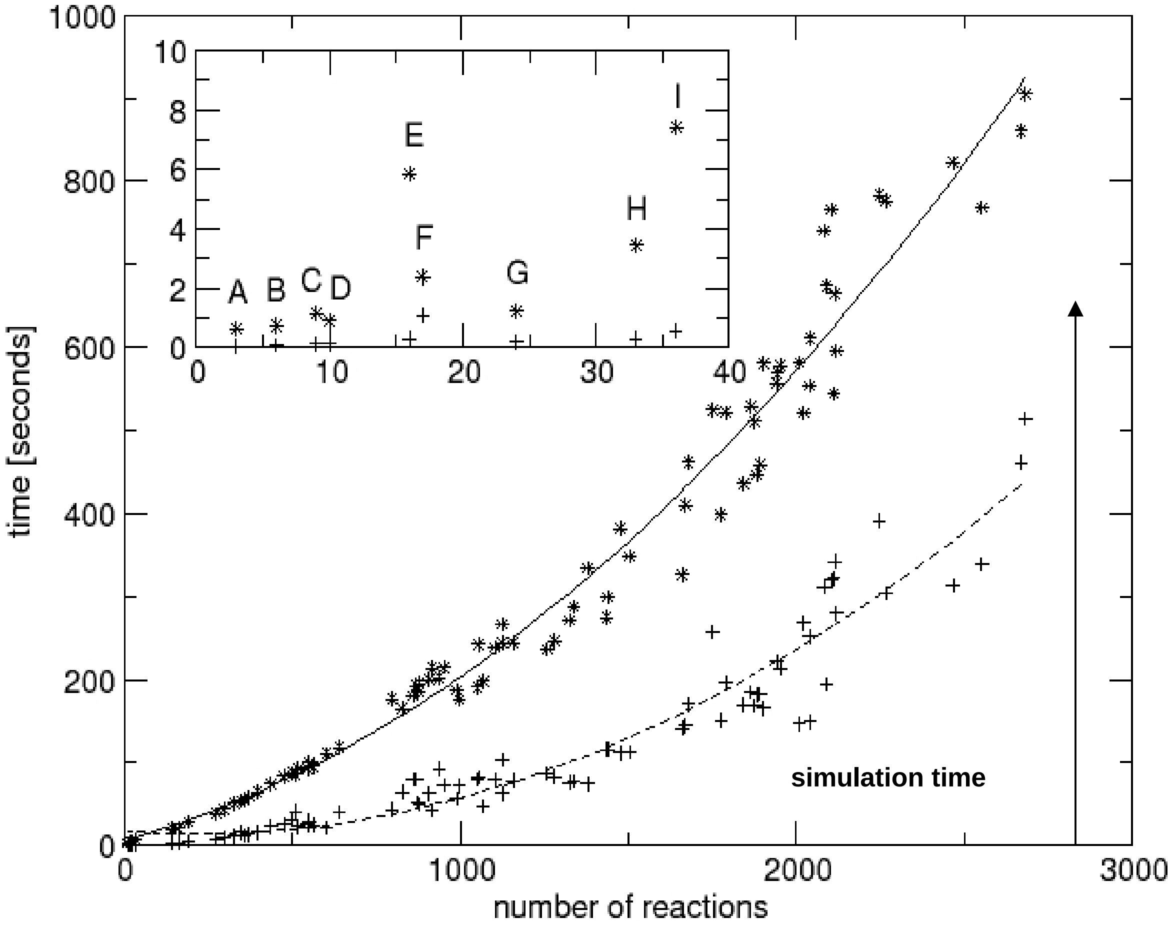
Implementation of an automatic pipeline for the simulation of individual studies (e.g. mutation, drug treatment, etc.).

This includes:

1. state definition
2. simulation
3. evaluation

Systems biology – modelling platform

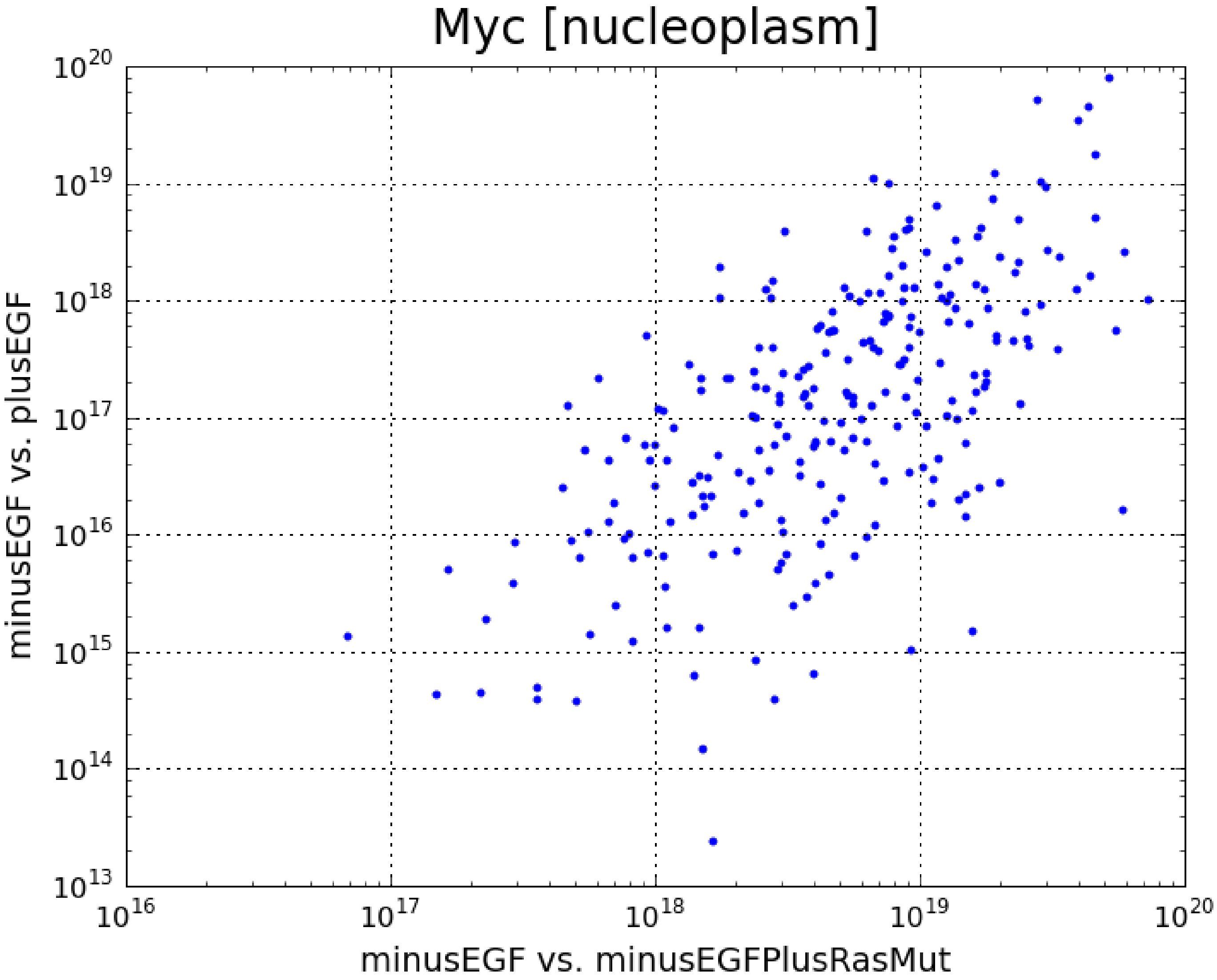
MAX-PLANCK-GESELLSCHAFT



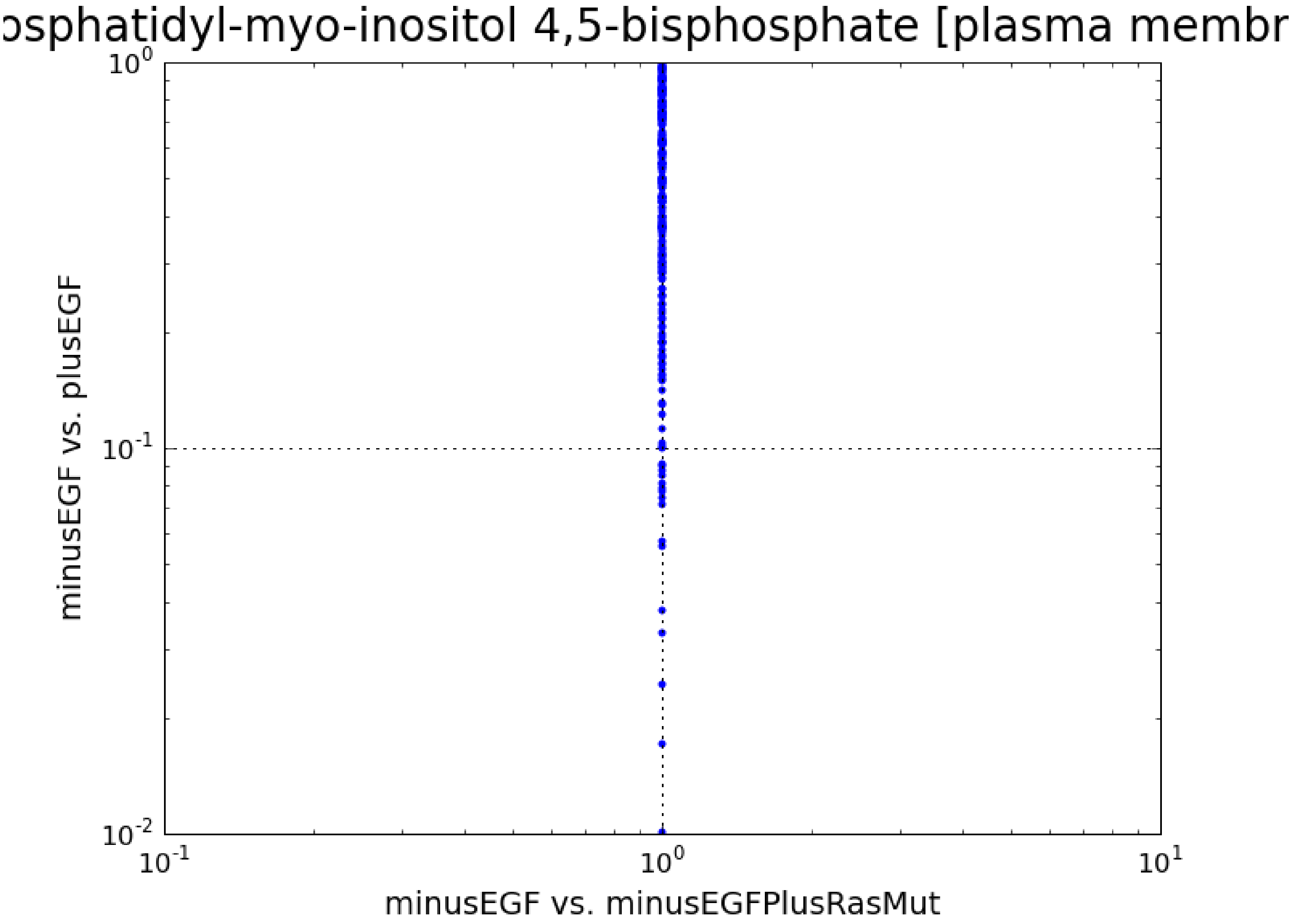
System performance and scaling

- quadratic scaling behaviour
- simulations can handle systems with thousands of components and reactions

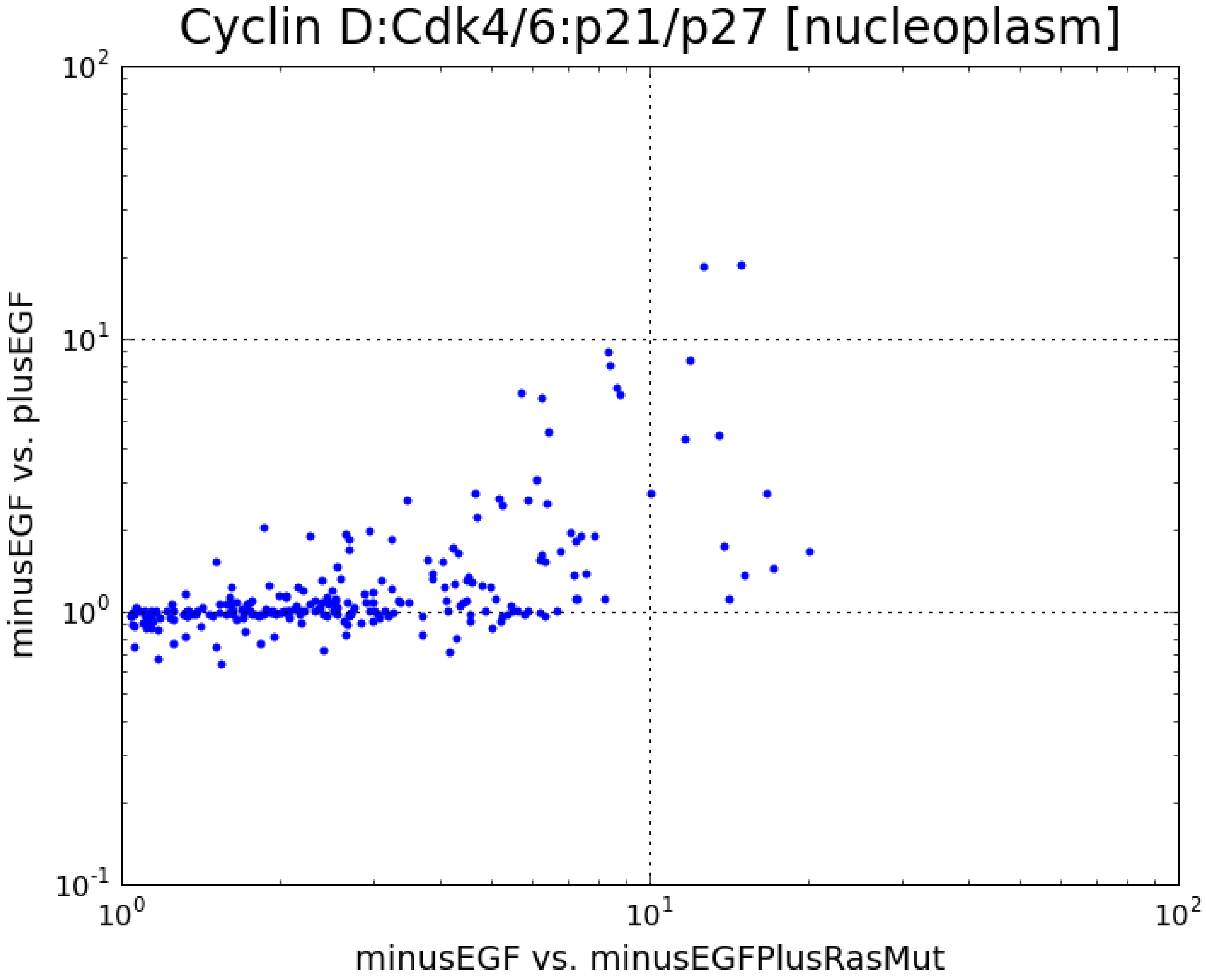
- numeric ODE solver is stable



Myc [nucleoplasm]
minusEGF vs. plusEGF
minusEGF vs. minusEGFPlusRasMut
10^{20}
10^{19}
10^{18}
10^{17}
10^{16}
10^{15}
10^{14}
10^{13}
10^{16}
10^{17}
10^{18}
10^{19}
10^{20}



ɔsphatidyl-myo-inositol 4,5-bisphosphate [plasma membr
10^0
10^{-1}
10^{-2}
minusEGF vs. plusEGF
10^{-1}
10^0
10^1
minusEGF vs. minusEGFPlusRasMut


Cyclin D:Cdk4/6:p21/p27 [nucleoplasm]
10^2
10^1
10^0
10^{-1}
minusEGF vs. plusEGF
10^0
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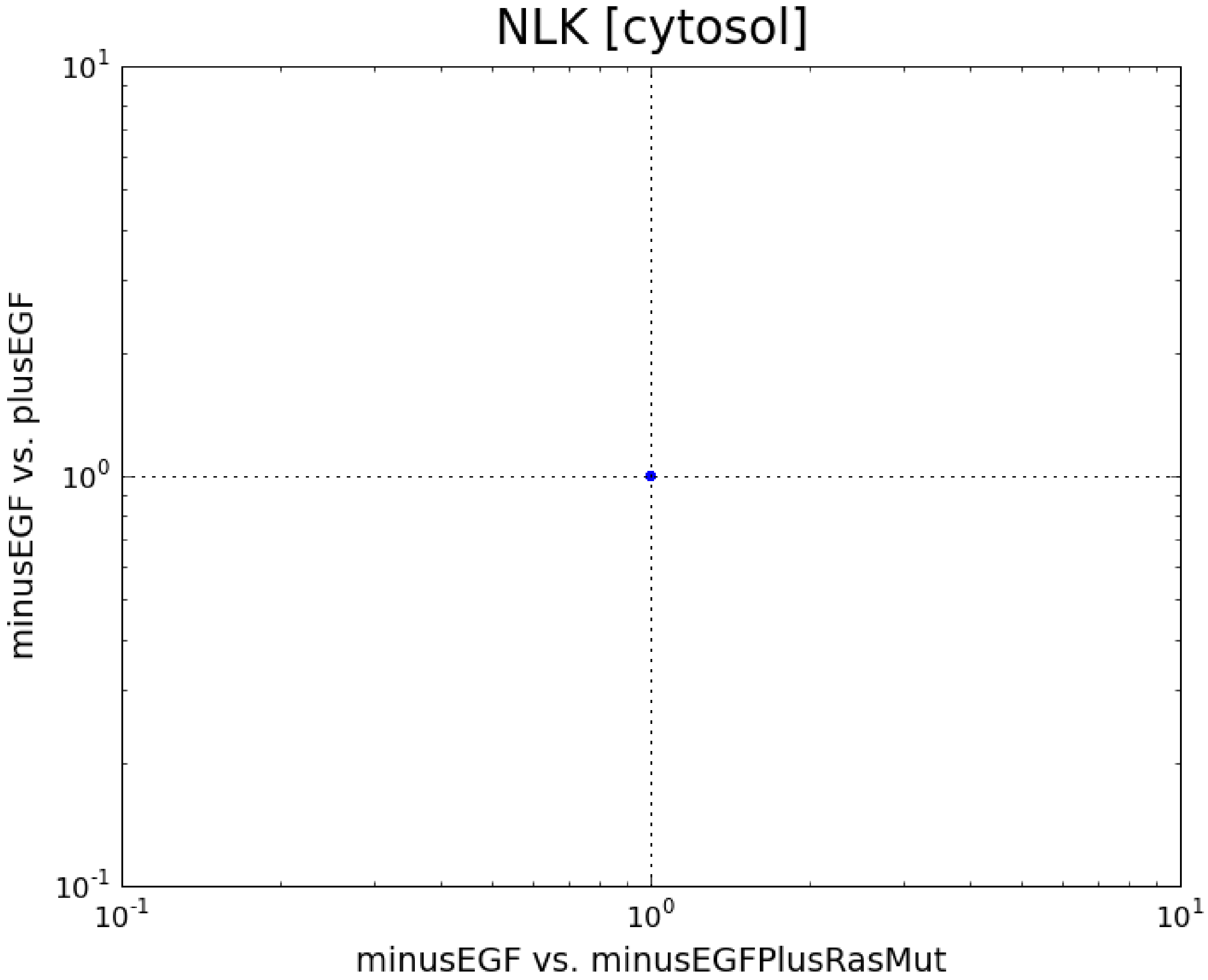
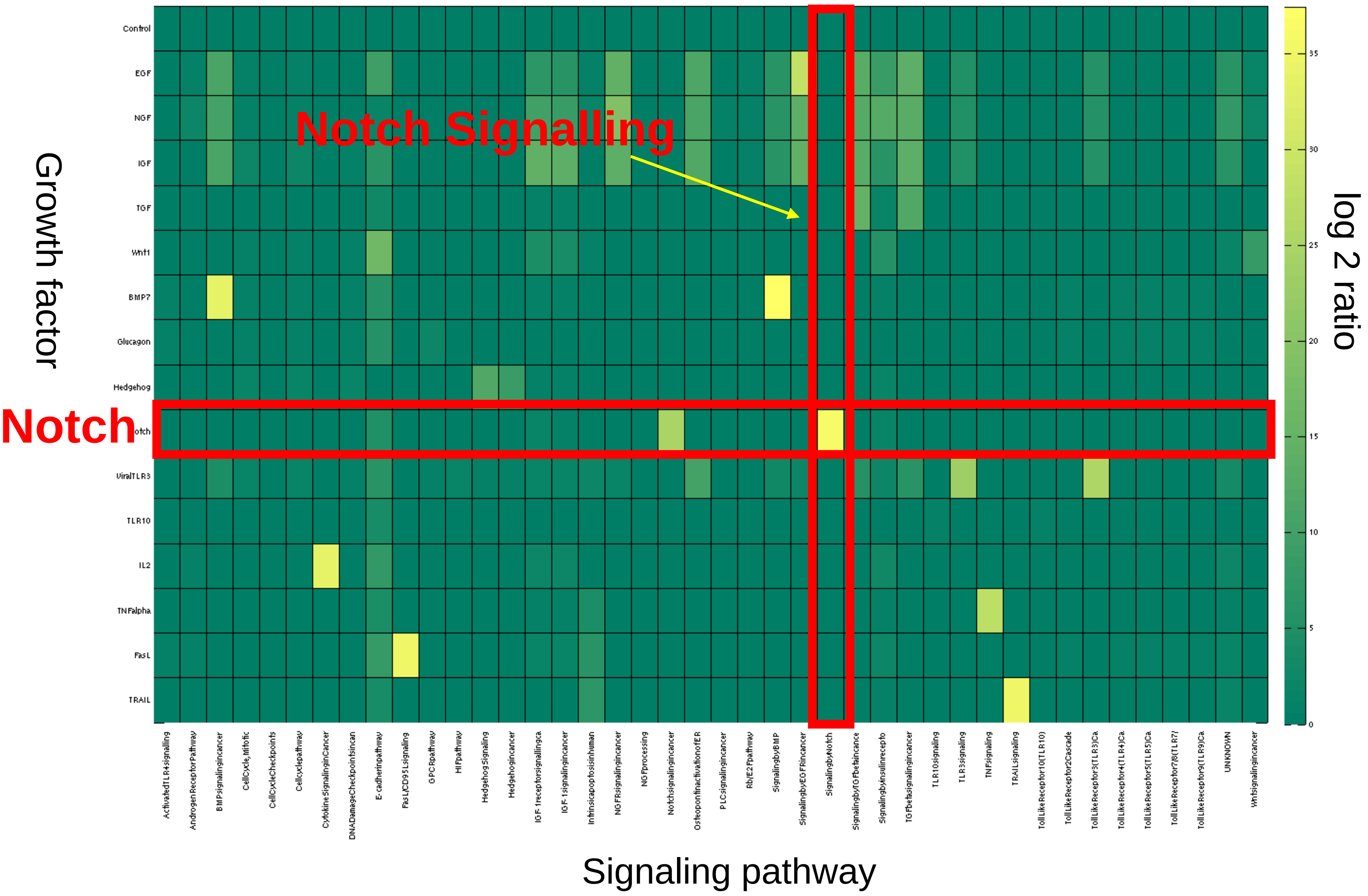
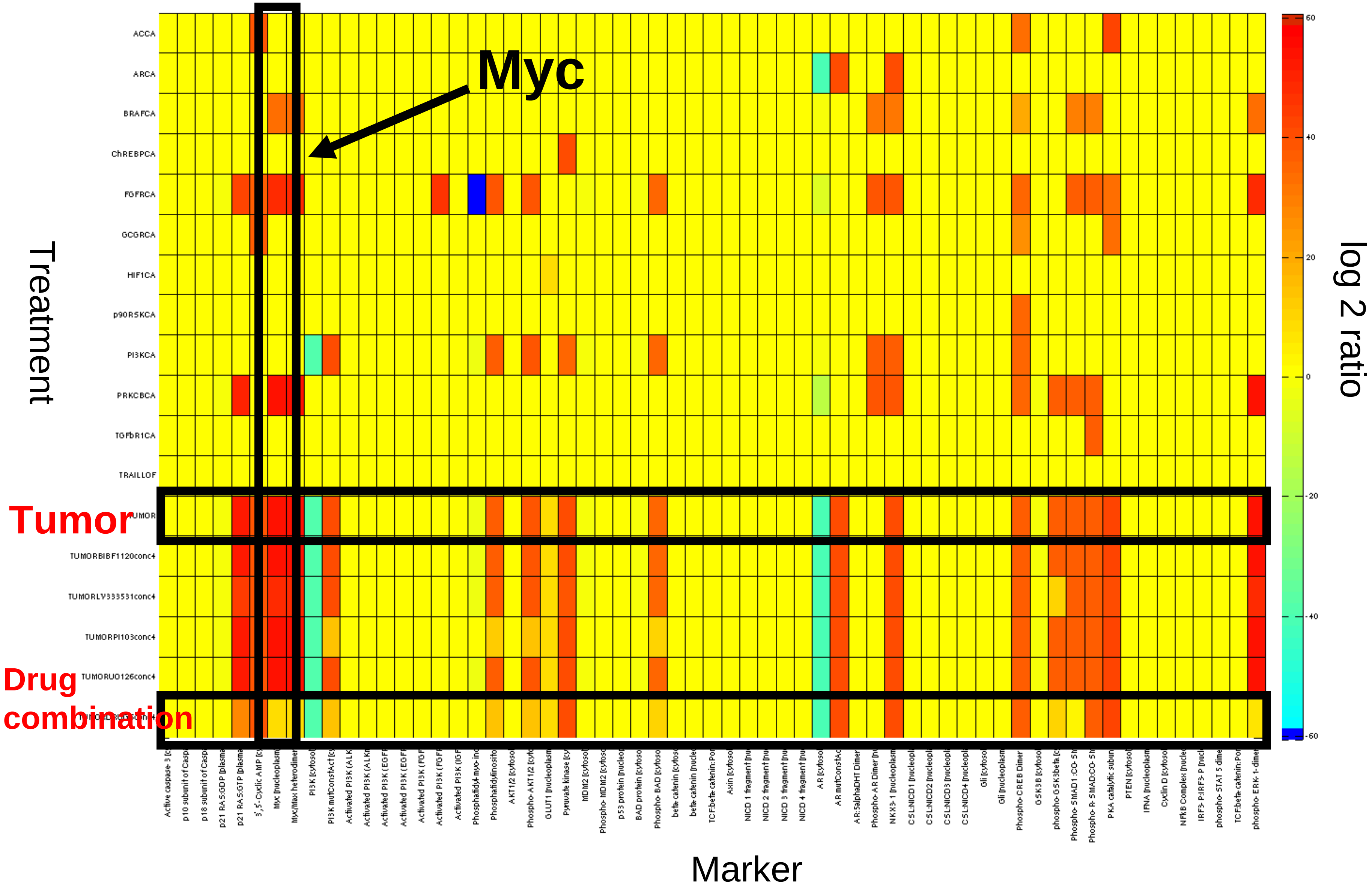
10^2
minusEGF vs. minusEGFPlusRasMut


NLK [cytosol]
10^1
10^0
10^{-1}
minusEGF vs. plusEGF
10^{-1}
10^0
10^1
minusEGF vs. minusEGFPlusRasMut

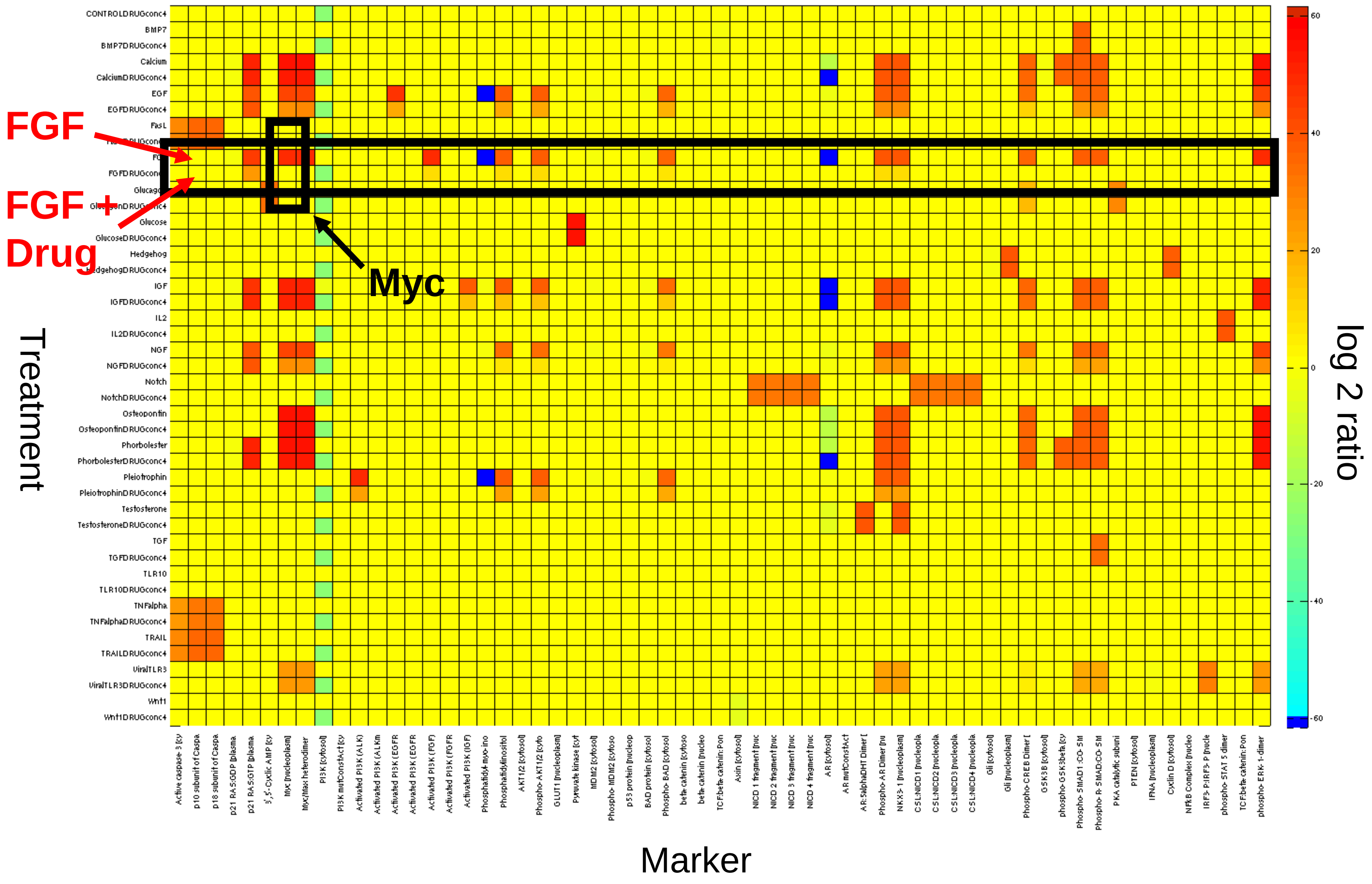
Validation of the Mathematical Model of Cancer Relevant Signalling Pathways



Modelling Effects of Patient Specific Genetic Variations to Drug Response



Modelling Side Effects of Drugs



FGF

FGF + Drug

Myc

The TREAT 1000 project

Making tomorrow's treatment available today

WELCOME TO THE TREAT 1000 WEBSITE!

TREAT1000 is an innovative project with the aim of bringing the benefits of genomic medicine to the cancer care of 1000 patients now. TREAT1000 was founded by top researchers and doctors from the Max Planck Institute of Molecular Genetics in Berlin, Harvard Medical School, the Charité Universitätsmedizin Berlin, Alacris Pharmaceuticals GmbH and CollabRx Inc. The project aim is to use a hybrid combination of funding sources, including patient and donor funding to fund applied research in patient treatment, research which will lead both to medical advances and to direct benefit for the patients involved in the project.

Every patient and every tumor is unique. Sequencing each patient's genome and their tumor genome will help their oncologists understand the specific mechanisms of tumor resistance and susceptibility for each patient's specific disease.

All data and conclusions generated in the project will be made publicly available through collaborations with the Personal Genome Project and with Health Commons.

More information about TREAT1000:

TREAT1000 workshop at Harvard Medical School, January 21st-22nd

Dr. Schlag with the first patient’s tumor

Genome and Transcriptome Landscapes of a Human Metastatic Melanoma

Genome SOLiD Sequence reads (50 bp) for melanoma and blood

- Blood:

Total reads 3,460,825,879

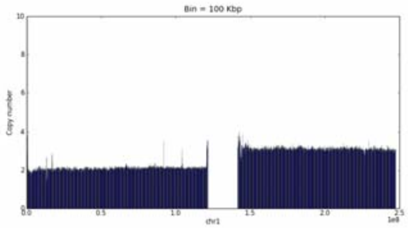
Uniquely mapped: 1,681,606,439 (coverage 28 fold)

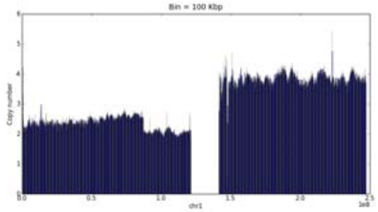
- Melanoma:

Total reads: 4,666,542,88

Uniquely mapped: 2,233,230,202 (coverage 37 fold)

l	g	changes	es	gname	desc
1	ENSG0000013	sp	ENSE0000145	NGF	Beta-nerve growth factor Precursor (Beta-NGF) [Source:UniProtKB/Swiss-Prot;Acc:P01138]
1	ENSG0000017	GAA->AAA,Gl	ENSE0000113	PDGFD	Platelet-derived growth factor D Precursor (PDGF-D)(Iris-expressed growth factor)(Spinal cord-derived growth factor B)(S
1	ENSG0000013	CCA->CTA,Pr	ENSE0000112	PIK3C2G	Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing gamma polypeptide (EC 2.7.1.154)(Phosphoinositide 3-
1	ENSG0000017	TCC->TTC,Ser	ENSE0000119	PDE3A	cGMP-inhibited 3',5'-cyclic phosphodiesterase A (EC 3.1.4.17)(Cyclic GMP-inhibited phosphodiesterase A)(CGI-PDE A) [So
1	ENSG0000012	TTC->CTC,Phe	ENSE0000131	LRP1	Prolow-density lipoprotein receptor-related protein 1 Precursor (LRP)(Alpha-2-macroglobulin receptor)(A2MR)(Apolipoprot
1	ENSG0000012	GTA->ATA,Val	ENSE0000081	APAF1	Apoptotic protease-activating factor 1 (Apaf-1) [Source:UniProtKB/Swiss-Prot;Acc:O14727]
1	ENSG0000016	CGA->CAA,Ar	ENSE0000112	PRKCB	Protein kinase C beta type (PKC-beta)(PKC-B)(EC 2.7.11.13) [Source:UniProtKB/Swiss-Prot;Acc:P05771]
1	ENSG0000011	GAG->AAG,Gl	ENSE0000075	APC2	Adenomatous polyposis coli protein 2 (Adenomatous polyposis coli protein-like)(APC-like) [Source:UniProtKB/Swiss-Prot;
1	ENSG0000014	CTG->CAG,Le	ENSE0000095	CBLC	Signal transduction protein CBL-C (SH3-binding protein CBL-C)(CBL-3)(RING finger protein 57) [Source:UniProtKB/Swiss-
1	ENSG0000012	CTT->TTT,Leu	ENSE0000147	MCM8	DNA replication licensing factor MCM8 (Minichromosome maintenance 8) [Source:UniProtKB/Swiss-Prot;Acc:Q9UJA3]
1	ENSG0000019	sp	ENSE0000150	NKIRAS1	NF-kappa-B inhibitor-interacting Ras-like protein 1 (I-kappa-B-interacting Ras-like protein 1)(Kappa B-Ras protein 1)(Kap
1	ENSG0000019	sp	ENSE0000150	NKIRAS1	NF-kappa-B inhibitor-interacting Ras-like protein 1 (I-kappa-B-interacting Ras-like protein 1)(Kappa B-Ras protein 1)(Kap
1	ENSG0000006	CCT->TCT,Pro	ENSE0000149	FGFR3	Fibroblast growth factor receptor 3 Precursor (FGFR-3)(EC 2.7.10.1)(CD333 antigen) [Source:UniProtKB/Swiss-Prot;Acc:
1	ENSG0000017	sp	ENSE0000152	TLR10	Toll-like receptor 10 Precursor (CD290 antigen) [Source:UniProtKB/Swiss-Prot;Acc:Q9BXR5]
1	ENSG0000018	sp	ENSE0000102	CSF1R	Macrophage colony-stimulating factor 1 receptor Precursor (CSF-1-R)(EC 2.7.10.1)(Fms proto-oncogene)(c-fms)(CD115 a
1	ENSG0000007	AGA->AAA,Ar	ENSE0000115	RPS6KA2	Ribosomal protein S6 kinase alpha-2 (S6K-alpha 2)(EC 2.7.11.1)(90 kDa ribosomal protein S6 kinase 2)(p90-RSK 2)(Ribo
1	ENSG0000015	GTG->GAG,Va	ENSE0000103	BRAF	B-Raf proto-oncogene serine/threonine-protein kinase (EC 2.7.11.1)(p94)(v-Raf murine sarcoma viral oncogene homolog
1	ENSG0000012	sp	ENSE0000133	TNFRSF10B	Tumor necrosis factor receptor superfamily member 10B Precursor (Death receptor 5)(TNF-related apoptosis-inducing liga
1	ENSG0000016	CCA->CTA,Pr	ENSE0000116	AR	Androgen receptor (Dihydrotestosterone receptor)(Nuclear receptor subfamily 3 group C member 4) [Source:UniProtKB/S





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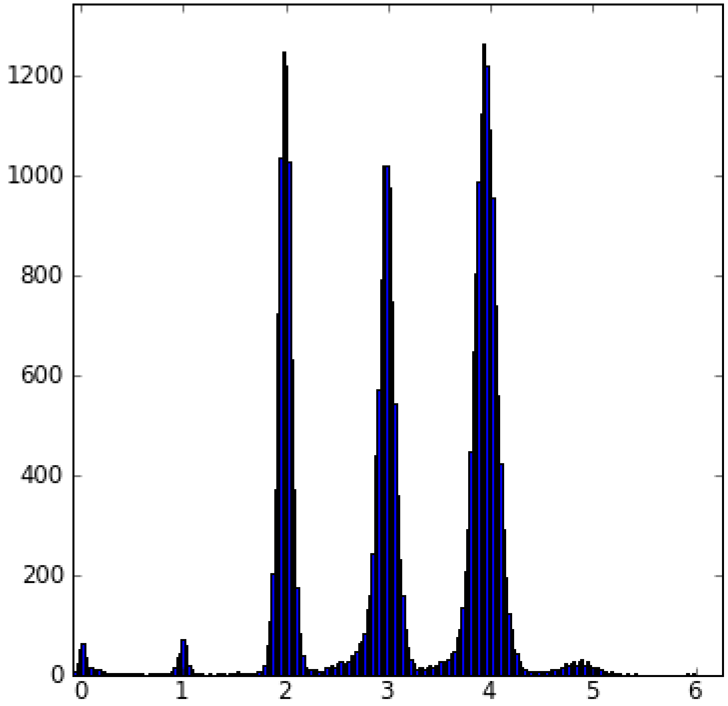
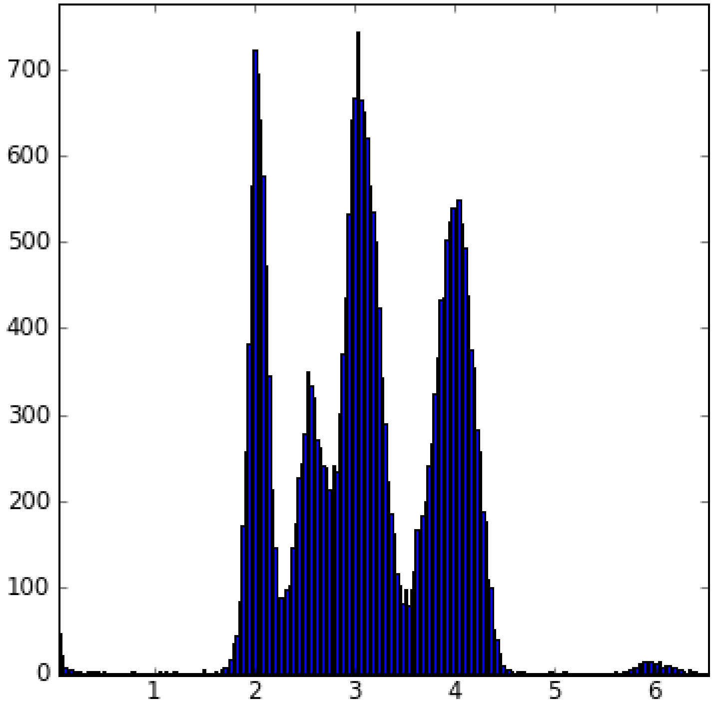
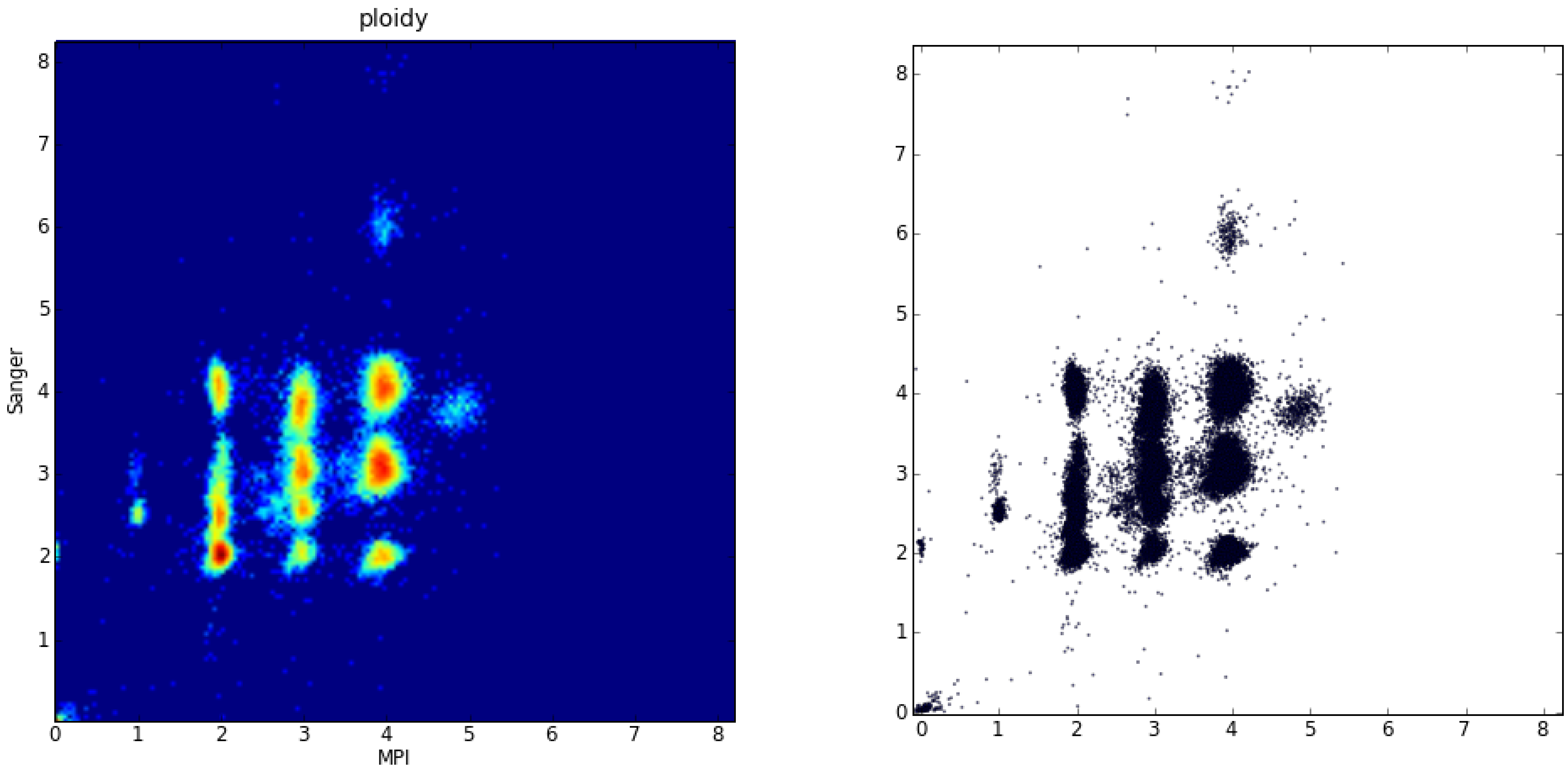


Ploidy Sanger





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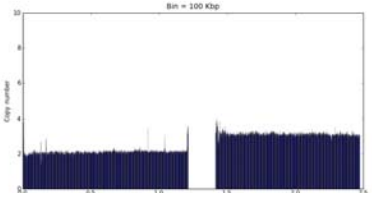
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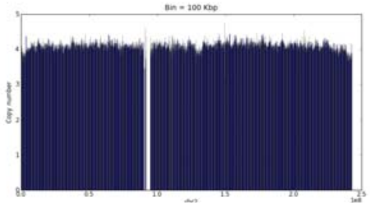
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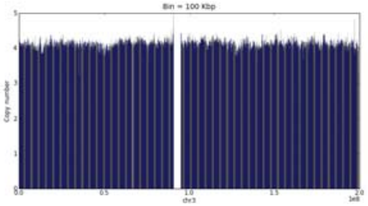
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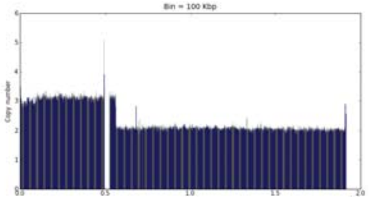
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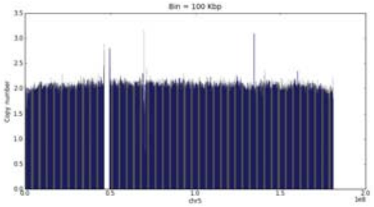
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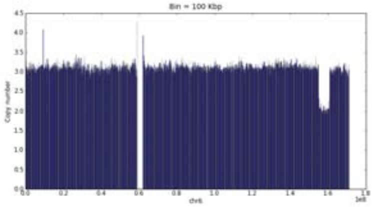
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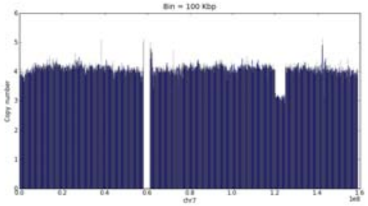
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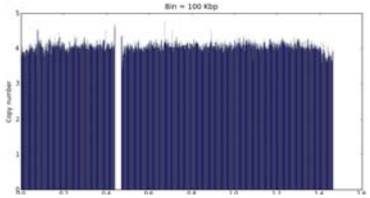
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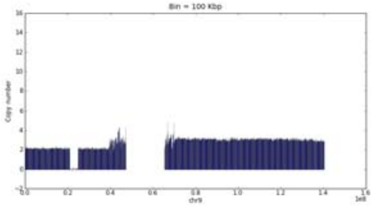
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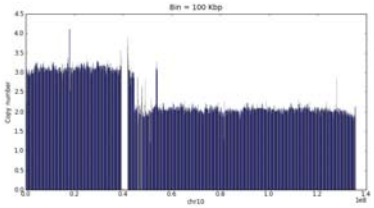
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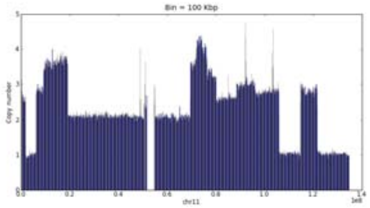
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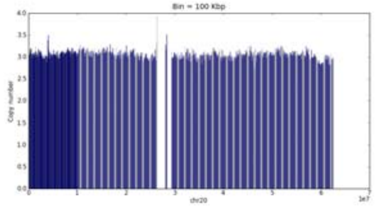
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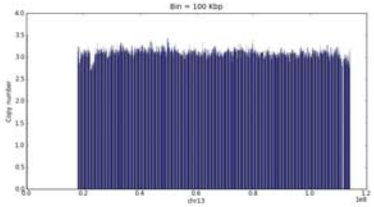
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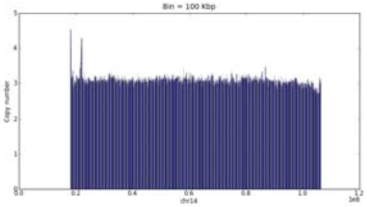
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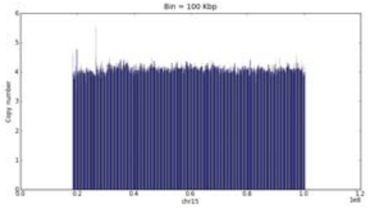
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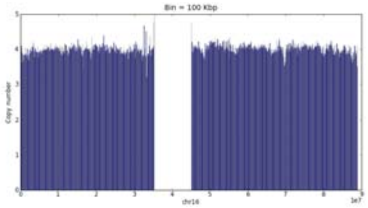
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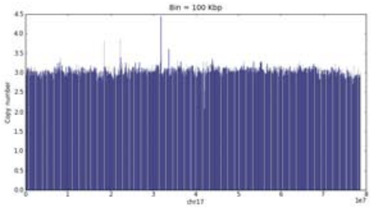
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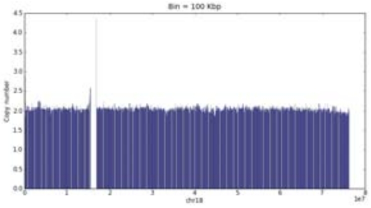
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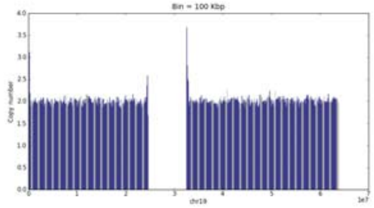
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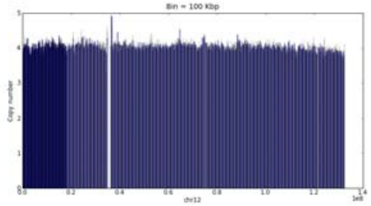
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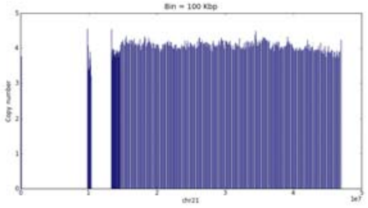
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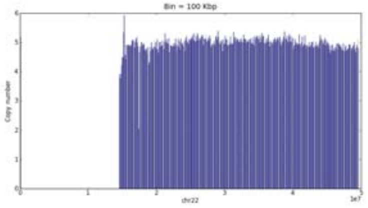
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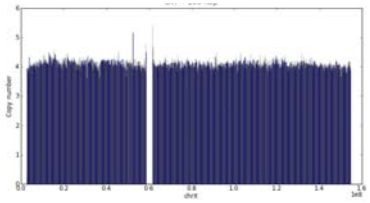
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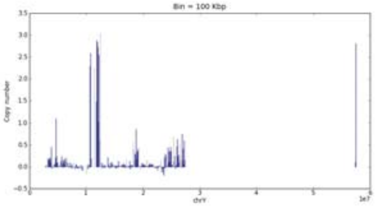
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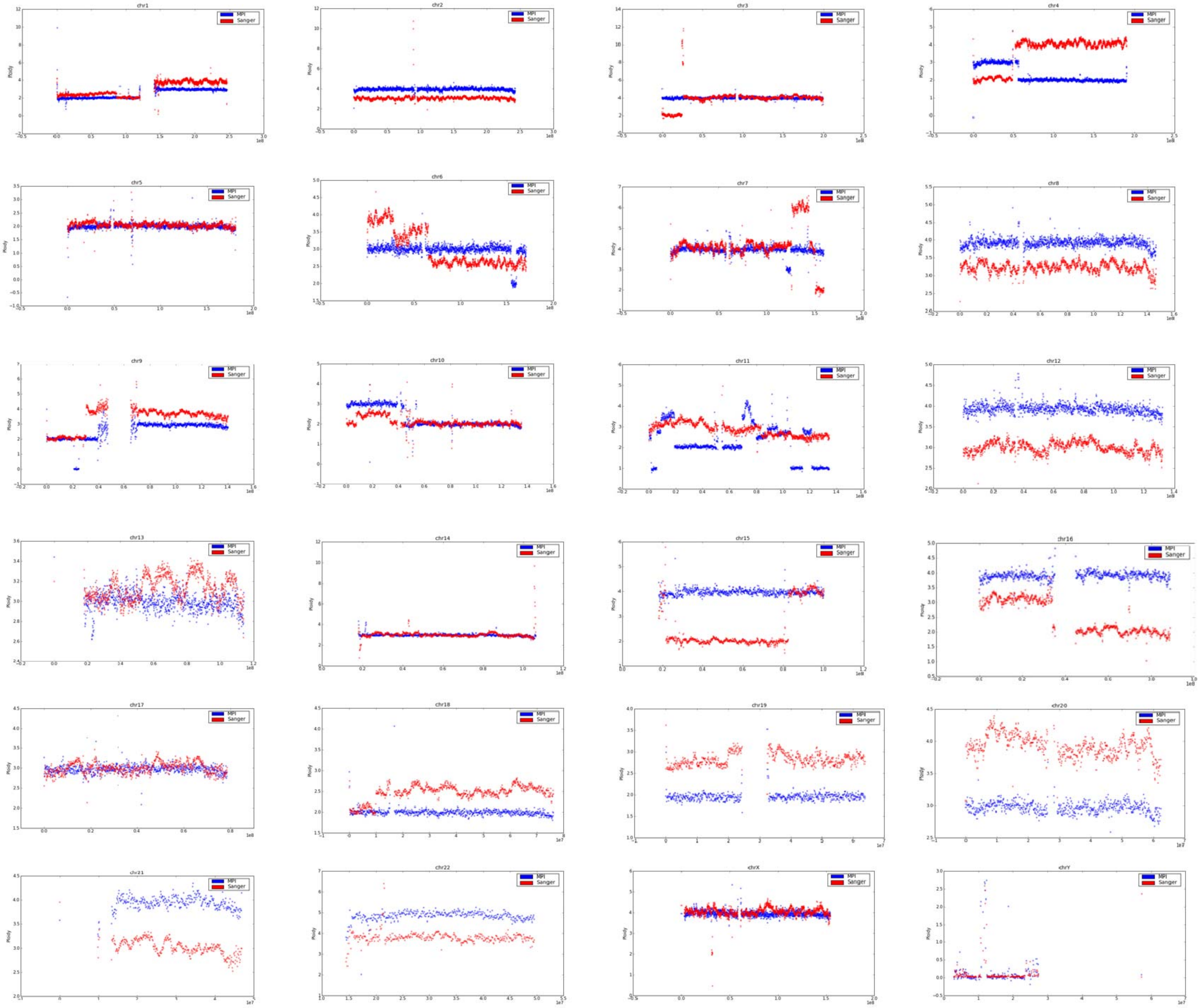
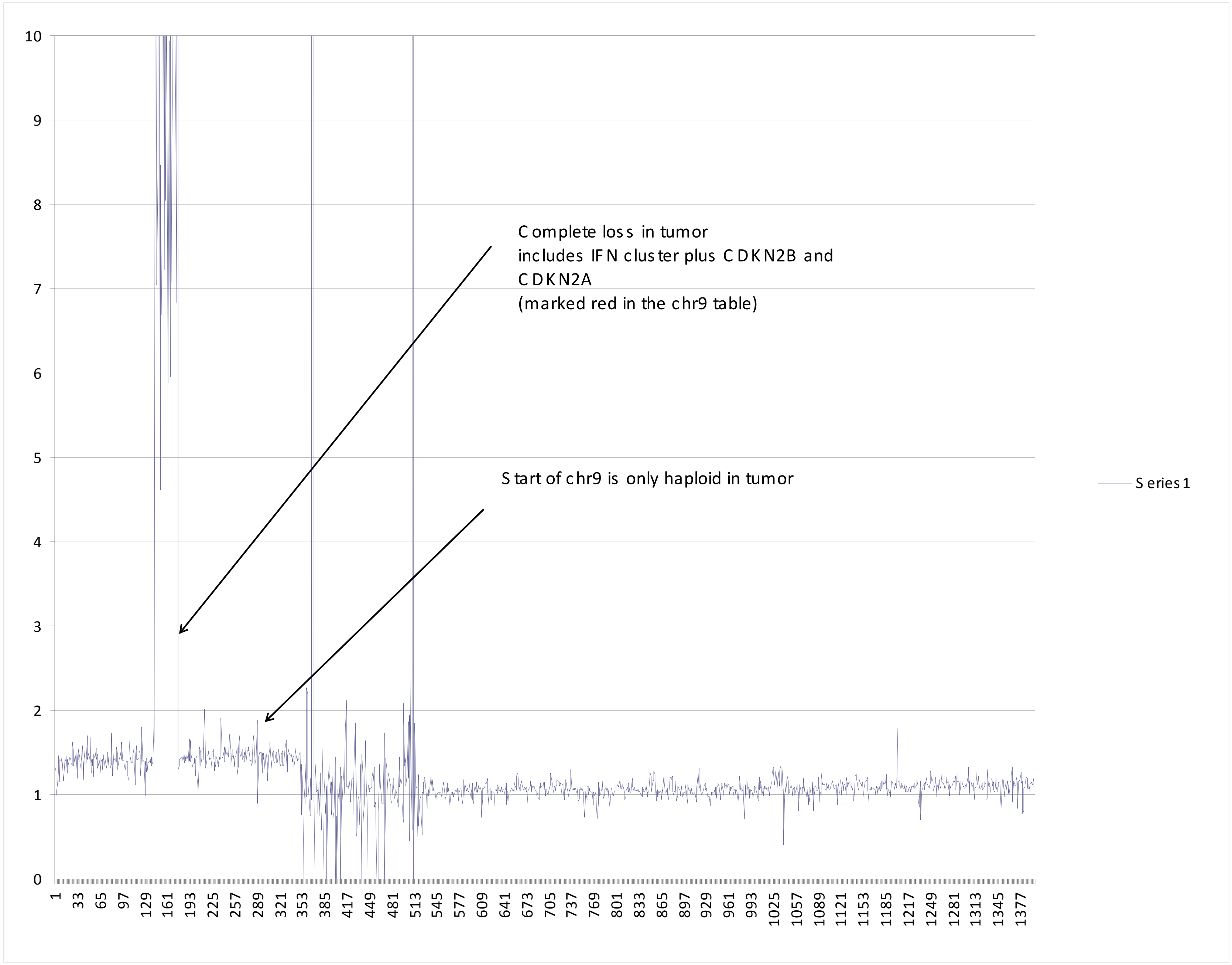
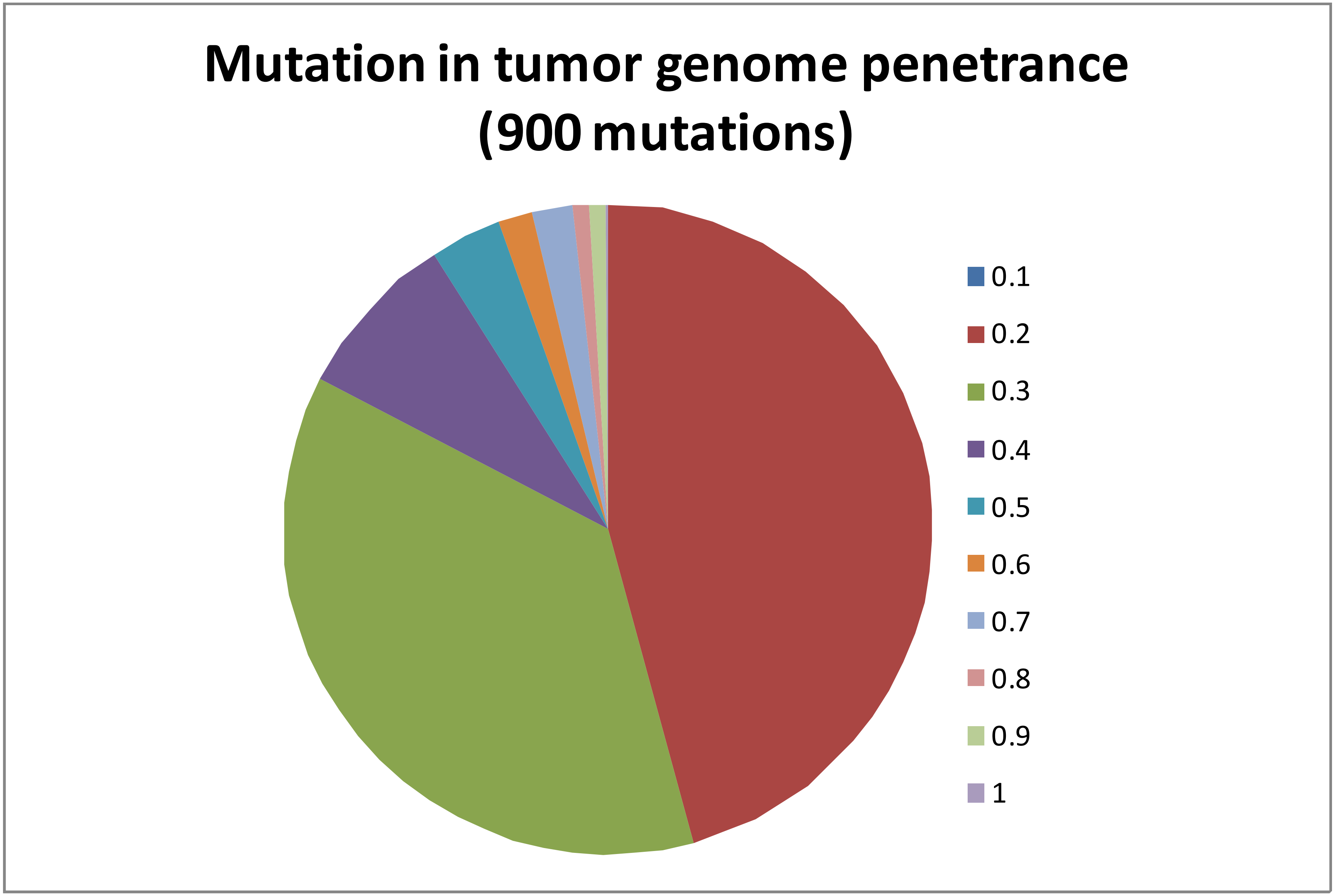

chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
chrX
chrY
MPI
Sanger
Ploidy
1e8
1e7

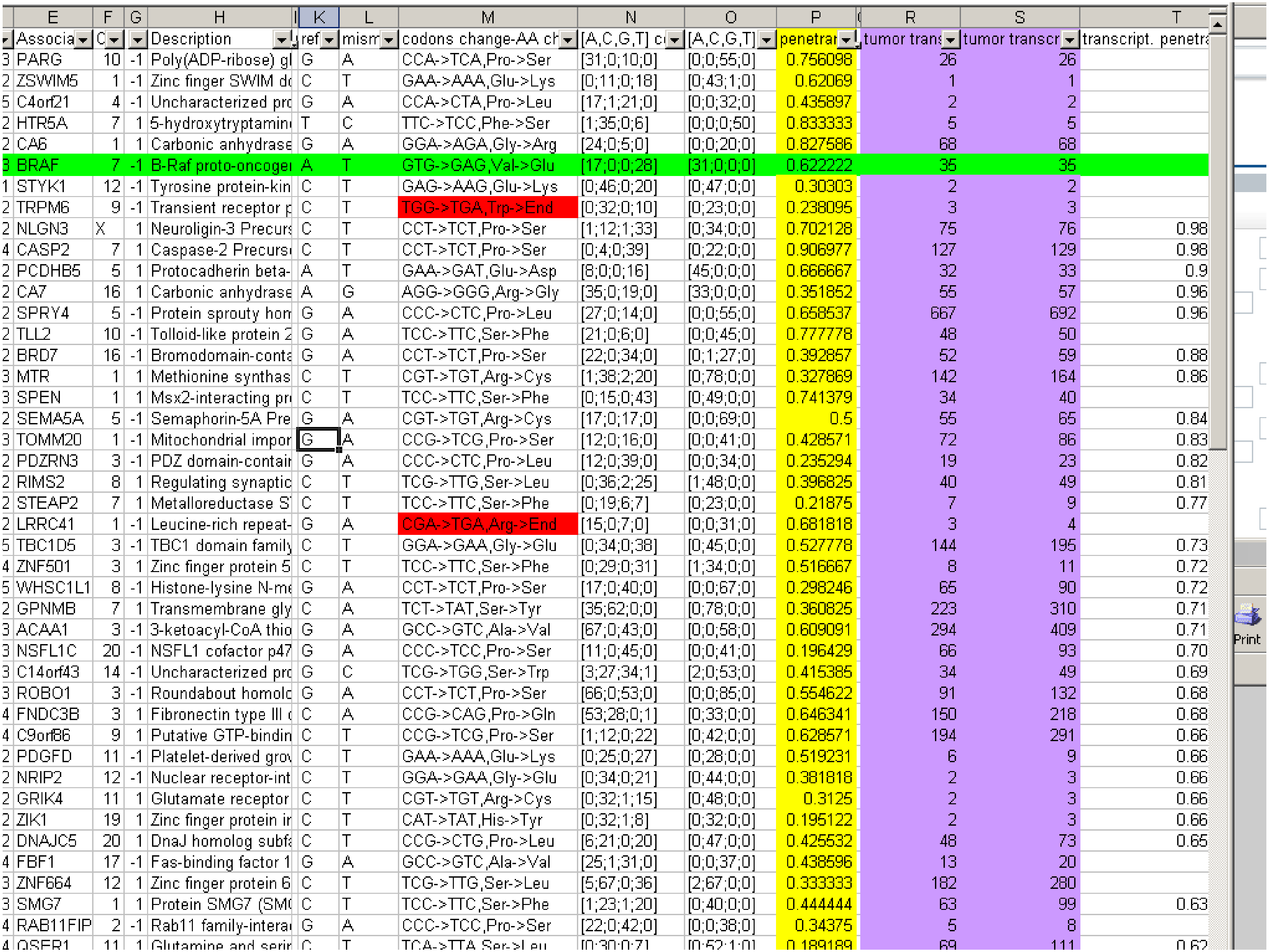


Complete loss in tumor
includes IFN cluster plus CDKN2B and
CDKN2A
(marked red in the chr9 table)

Start of chr9 is only haploid in tumor



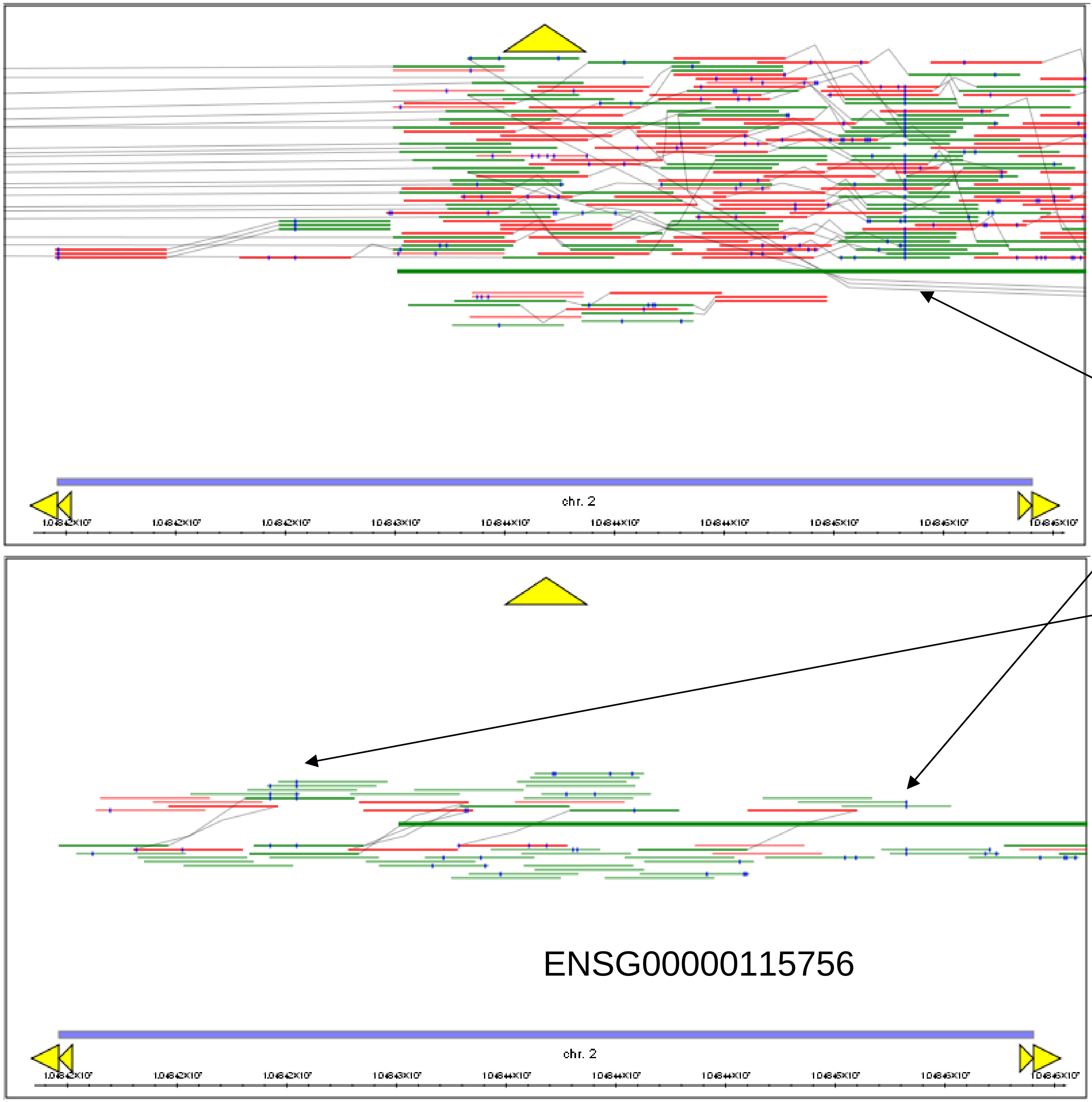
Mutation in tumor genome penetrance (900 mutations)



	E	F	G	H	K	L	M	N	O	P	R	S	T
	Associa	C		Description	ref	mism	codons change-AA ch	[A,C,G,T] c	[A,C,G,T]	penetrar	tumor trans	tumor transcr	transcript. penetra
3	PARG	10	-1	Poly(ADP-ribose) gl	G	A	CCA->TCA,Pro->Ser	[31;0;10;0]	[0;0;55;0]	0.756098	26	26	
2	ZSWIM5	1	-1	Zinc finger SWIM dc	C	T	GAA->AAA,Glu->Lys	[0;11;0;18]	[0;43;1;0]	0.62069	1	1	
5	C4orf21	4	-1	Uncharacterized prc	G	A	CCA->CTA,Pro->Leu	[17;1;21;0]	[0;0;32;0]	0.435897	2	2	
2	HTR5A	7	1	5-hydroxytryptamin	T	C	TTC->TCC,Phe->Ser	[1;35;0;6]	[0;0;0;50]	0.833333	5	5	
2	CA6	1	1	Carbonic anhydrase	G	A	GGA->AGA,Gly->Arg	[24;0;5;0]	[0;0;20;0]	0.827586	68	68	
3	BRAF	7	-1	B-Raf proto-oncoger	A	T	GTG->GAG,Val->Glu	[17;0;0;28]	[31;0;0;0]	0.622222	35	35	
1	STYK1	12	-1	Tyrosine protein-kin	C	T	GAG->AAG,Glu->Lys	[0;46;0;20]	[0;47;0;0]	0.30303	2	2	
2	TRPM6	9	-1	Transient receptor p	C	T	TGG->TGA,Trp->End	[0;32;0;10]	[0;23;0;0]	0.238095	3	3	
2	NLGN3	X	1	Neuroligin-3 Precurs	C	T	CCT->TCT,Pro->Ser	[1;12;1;33]	[0;34;0;0]	0.702128	75	76	0.98
4	CASP2	7	1	Caspase-2 Precurs	C	T	CCT->TCT,Pro->Ser	[0;4;0;39]	[0;22;0;0]	0.906977	127	129	0.98
2	PCDHB5	5	1	Protocadherin beta-	A	T	GAA->GAT,Glu->Asp	[8;0;0;16]	[45;0;0;0]	0.666667	32	33	0.9
2	CA7	16	1	Carbonic anhydrase	A	G	AGG->GGG,Arg->Gly	[35;0;19;0]	[33;0;0;0]	0.351852	55	57	0.96
2	SPRY4	5	-1	Protein sprouty hon	G	A	CCC->CTC,Pro->Leu	[27;0;14;0]	[0;0;55;0]	0.658537	667	692	0.96
2	TLL2	10	-1	Tolloid-like protein 2	G	A	TCC->TTC,Ser->Phe	[21;0;6;0]	[0;0;45;0]	0.777778	48	50	
2	BRD7	16	-1	Bromodomain-conta	G	A	CCT->TCT,Pro->Ser	[22;0;34;0]	[0;1;27;0]	0.392857	52	59	0.88
3	MTR	1	1	Methionine synthas	C	T	CGT->TGT,Arg->Cys	[1;38;2;20]	[0;78;0;0]	0.327869	142	164	0.86
3	SPEN	1	1	Msx2-interacting pr	C	T	TCC->TTC,Ser->Phe	[0;15;0;43]	[0;49;0;0]	0.741379	34	40	
2	SEMA5A	5	-1	Semaphorin-5A Pre	G	A	CGT->TGT,Arg->Cys	[17;0;17;0]	[0;0;69;0]	0.5	55	65	0.84
3	TOMM20	1	-1	Mitochondrial impor	G	A	CCG->TCG,Pro->Ser	[12;0;16;0]	[0;0;41;0]	0.428571	72	86	0.83
2	PDZRN3	3	-1	PDZ domain-contair	G	A	CCC->CTC,Pro->Leu	[12;0;39;0]	[0;0;34;0]	0.235294	19	23	0.82
2	RIMS2	8	1	Regulating synaptic	C	T	TCG->TTG,Ser->Leu	[0;36;2;25]	[1;48;0;0]	0.396825	40	49	0.81
2	STEAP2	7	1	Metalloreductase S	C	T	TCC->TTC,Ser->Phe	[0;19;6;7]	[0;23;0;0]	0.21875	7	9	0.77
2	LRRC41	1	-1	Leucine-rich repeat-	G	A	CGA->TGA,Arg->End	[15;0;7;0]	[0;0;31;0]	0.681818	3	4	
5	TBC1D5	3	-1	TBC1 domain family	C	T	GGA->GAA,Gly->Glu	[0;34;0;38]	[0;45;0;0]	0.527778	144	195	0.73
4	ZNF501	3	1	Zinc finger protein 5	C	T	TCC->TTC,Ser->Phe	[0;29;0;31]	[1;34;0;0]	0.516667	8	11	0.72
5	WHSC1L1	8	-1	Histone-lysine N-me	G	A	CCT->TCT,Pro->Ser	[17;0;40;0]	[0;0;67;0]	0.298246	65	90	0.72
2	GPNMB	7	1	Transmembrane gly	C	A	TCT->TAT,Ser->Tyr	[35;62;0;0]	[0;78;0;0]	0.360825	223	310	0.71
3	ACAA1	3	-1	3-ketoacyl-CoA thio	G	A	GCC->GTC,Ala->Val	[67;0;43;0]	[0;0;58;0]	0.609091	294	409	0.71
3	NSFL1C	20	-1	NSFL1 cofactor p47	G	A	CCC->TCC,Pro->Ser	[11;0;45;0]	[0;0;41;0]	0.196429	66	93	0.70
3	C14orf43	14	-1	Uncharacterized prc	G	C	TCG->TGG,Ser->Trp	[3;27;34;1]	[2;0;53;0]	0.415385	34	49	0.69
3	ROBO1	3	-1	Roundabout homolc	G	A	CCT->TCT,Pro->Ser	[66;0;53;0]	[0;0;85;0]	0.554622	91	132	0.68
4	FNDC3B	3	1	Fibronectin type III	C	A	CCG->CAG,Pro->Gln	[53;28;0;1]	[0;33;0;0]	0.646341	150	218	0.68
4	C9orf86	9	1	Putative GTP-bindin	C	T	CCG->TCG,Pro->Ser	[1;12;0;22]	[0;42;0;0]	0.628571	194	291	0.66
2	PDGFD	11	-1	Platelet-derived grov	C	T	GAA->AAA,Glu->Lys	[0;25;0;27]	[0;28;0;0]	0.519231	6	9	0.66
2	NRIP2	12	-1	Nuclear receptor-int	C	T	GGA->GAA,Gly->Glu	[0;34;0;21]	[0;44;0;0]	0.381818	2	3	0.66
2	GRIK4	11	1	Glutamate receptor	C	T	CGT->TGT,Arg->Cys	[0;32;1;15]	[0;48;0;0]	0.3125	2	3	0.66
2	ZIK1	19	1	Zinc finger protein ir	C	T	CAT->TAT,His->Tyr	[0;32;1;8]	[0;32;0;0]	0.195122	2	3	0.66
2	DNAJC5	20	1	DnaJ homolog subfa	C	T	CCG->CTG,Pro->Leu	[6;21;0;20]	[0;47;0;0]	0.425532	48	73	0.65
4	FBF1	17	-1	Fas-binding factor 1	G	A	GCC->GTC,Ala->Val	[25;1;31;0]	[0;0;37;0]	0.438596	13	20	
3	ZNF664	12	1	Zinc finger protein 6	C	T	TCG->TTG,Ser->Leu	[5;67;0;36]	[2;67;0;0]	0.333333	182	280	
3	SMG7	1	1	Protein SMG7 (SM	C	T	TCC->TTC,Ser->Phe	[1;23;1;20]	[0;40;0;0]	0.444444	63	99	0.63
4	RAB11FIP	2	-1	Rab11 family-intera	G	A	CCC->TCC,Pro->Ser	[22;0;42;0]	[0;0;38;0]	0.34375	5	8	
4	QSER1	11	1	Glutamine and serir	C	T	TCA->TTA,Ser->Leu	[0;30;0;7]	[0;52;1;0]	0.189189	69	111	0.62

RNAseq transcriptome patient 1: 51 bp reads, with strand information

New protocol with RNA fragmentation		
Run/lane nb	Sample	Total.number_unique_hits
091211_EAS451_1	Tumor	28 859 047
091211_EAS451_2	Tumor	29 373 327
091211_EAS451_3	Tumor	29 456 853
091211_EAS451_4	Control	30 461 674
091211_EAS451_5	Control	29 622 024
Standard protocol with cDNA fragmentation		
Run/lane nb	Sample	Total.number_unique_hits
091113_EAS451_5	Tumor	21 995 533
091113_EAS451_6	Tumor	21 919 636
090522_EAS451_7	Tumor	11 277 659
090714_EAS451_1	Stem cells-CD133+	16 468 893
090714_EAS451_2	Stem cells-CD133-	21 808 807
091106_EAS451_6	Control	24 766 112



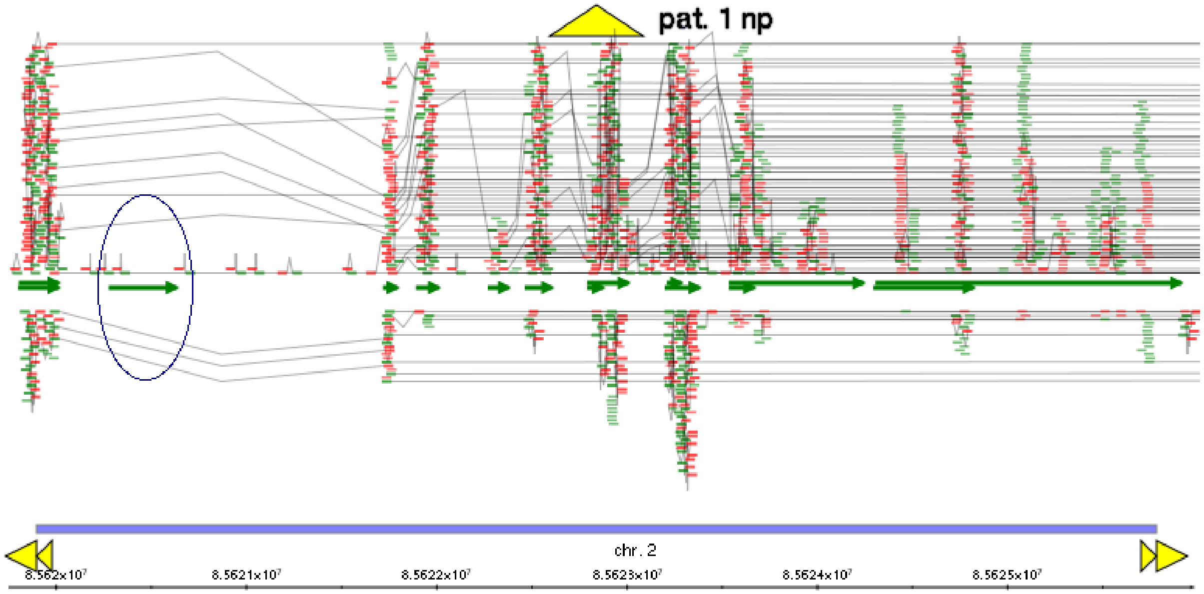
Cross-validated homozygous SNP

Heterozygous exome SNP (in splicing region) looks as homozygous in transcriptome

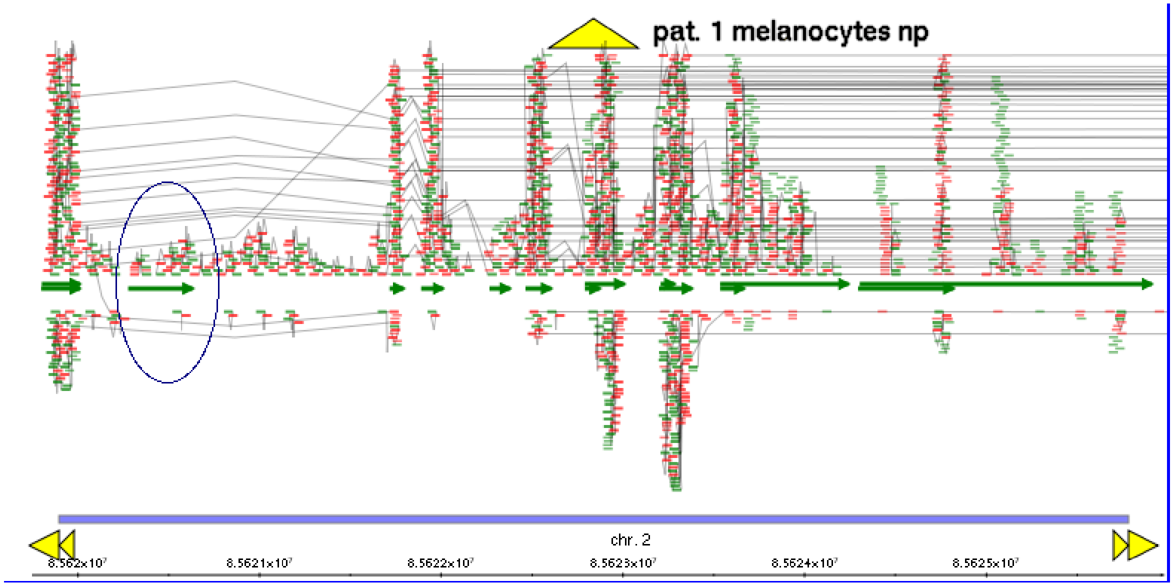
- **1021 induced** (activated and/or ectopic) genes in melanomas
- 845 genes were strongly induced (< 25 reads in at least one of the melanoma lanes, < 5 reads in the control lane)
- 176 showed a more modest expression (up to 25 reads in melanoma, and < 5 reads in controls).

MAT2A

Pat1

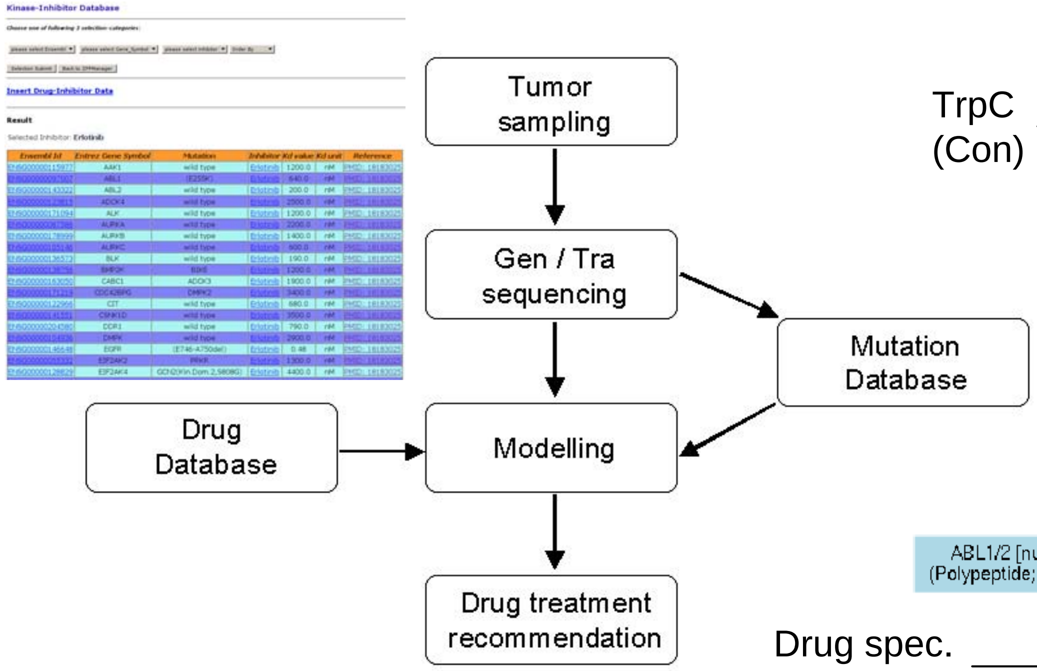


Melanocytes



Modelling a Virtual Patient using NGS Technologies

LoF →	0.0	0.0
WT →	1.0	0.0
Mut (mut/mut) →	0.0	1.0
Mut (mut/wt) →	1.0	1.0



Kinase-Inhibitor Database

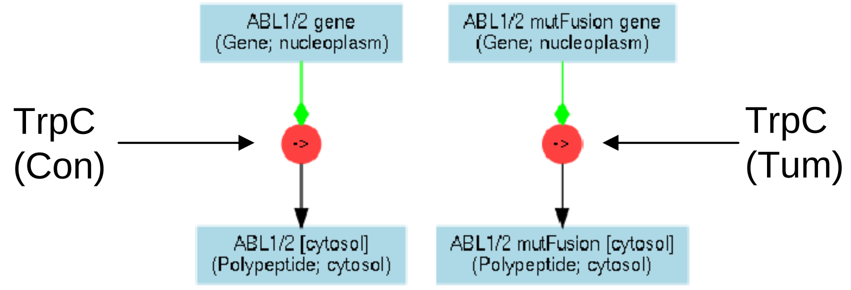
Choose one of following 3 selection categories:

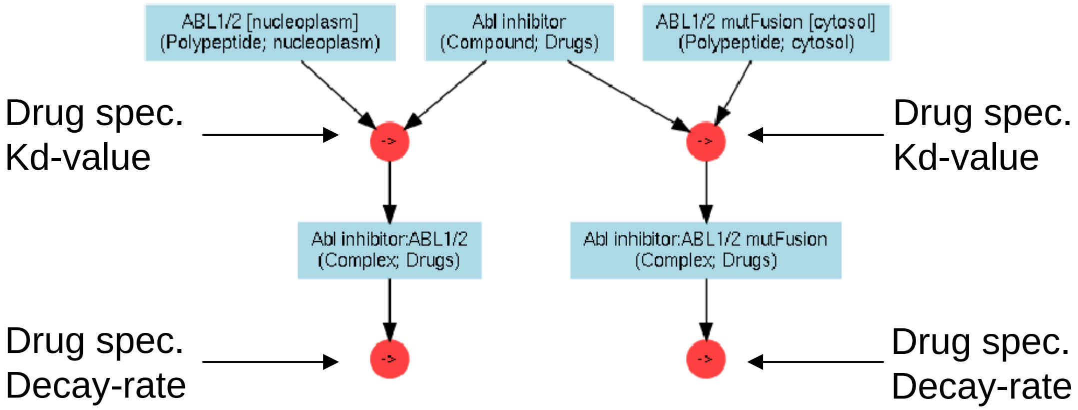
Insert Drug-Inhibitor Data

Result

Selected Inhibitor: **Erlotinib**

Ensembl Id	Entrez Gene Symbol	Mutation	Inhibitor	Kd value	Kd unit	Reference
ENSG00000115977	AAK1	wild type	Erlotinib	1200.0	nM	PMID: 18183025
ENSG00000097007	ABL1	(E255K)	Erlotinib	640.0	nM	PMID: 18183025
ENSG00000143322	ABL2	wild type	Erlotinib	200.0	nM	PMID: 18183025
ENSG00000123815	ADCK4	wild type	Erlotinib	2500.0	nM	PMID: 18183025
ENSG00000171094	ALK	wild type	Erlotinib	1200.0	nM	PMID: 18183025
ENSG00000087586	AURKA	wild type	Erlotinib	2200.0	nM	PMID: 18183025
ENSG00000178999	AURKB	wild type	Erlotinib	1400.0	nM	PMID: 18183025
ENSG00000105146	AURKC	wild type	Erlotinib	600.0	nM	PMID: 18183025
ENSG00000136573	BLK	wild type	Erlotinib	190.0	nM	PMID: 18183025
ENSG00000138756	BMP2K	BIKE	Erlotinib	1200.0	nM	PMID: 18183025
ENSG00000163050	CABC1	ADCK3	Erlotinib	1900.0	nM	PMID: 18183025
ENSG00000171219	CDC42BPG	DMPK2	Erlotinib	3400.0	nM	PMID: 18183025
ENSG00000122966	CIT	wild type	Erlotinib	680.0	nM	PMID: 18183025
ENSG00000141551	CSNK1D	wild type	Erlotinib	3500.0	nM	PMID: 18183025
ENSG00000204580	DDR1	wild type	Erlotinib	790.0	nM	PMID: 18183025
ENSG00000104936	DMPK	wild type	Erlotinib	2900.0	nM	PMID: 18183025
ENSG00000146648	EGFR	(E746-A750del)	Erlotinib	0.48	nM	PMID: 18183025
ENSG00000055332	EIF2AK2	PRKR	Erlotinib	1300.0	nM	PMID: 18183025
ENSG00000128829	EIF2AK4	GCN2(Kin.Dom.2,S808G)	Erlotinib	4400.0	nM	PMID: 18183025





Modelling a Patient 1 Specific Tumor

667 non-silent mutations

461 mutated genes

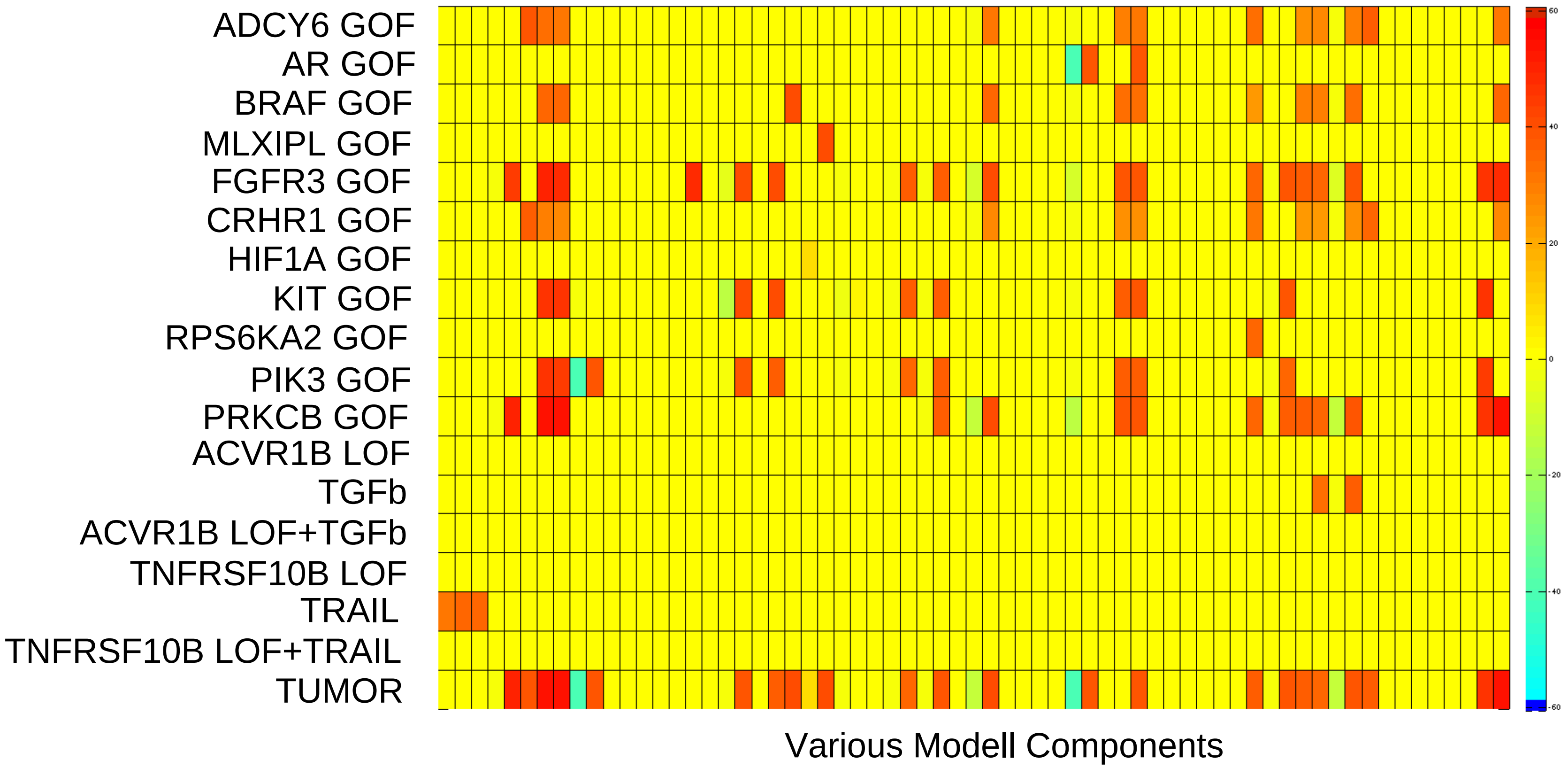
17275 expressed genes

15460 genes expressed
in control sample

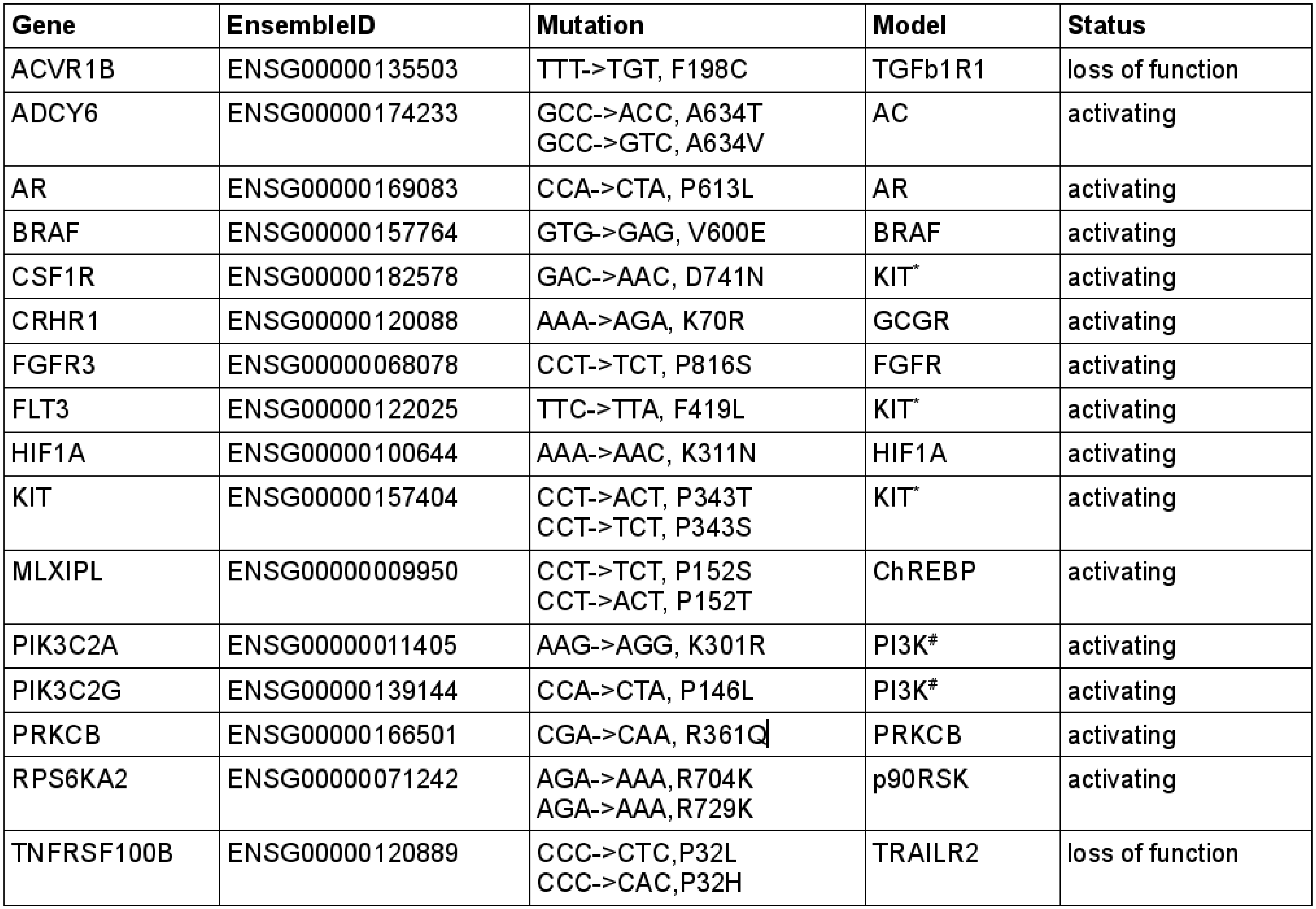
From ~0.01 to ~6100 TrpC

15929 genes expressed
in tumor sample

From ~0.01 to ~9000 TrpC

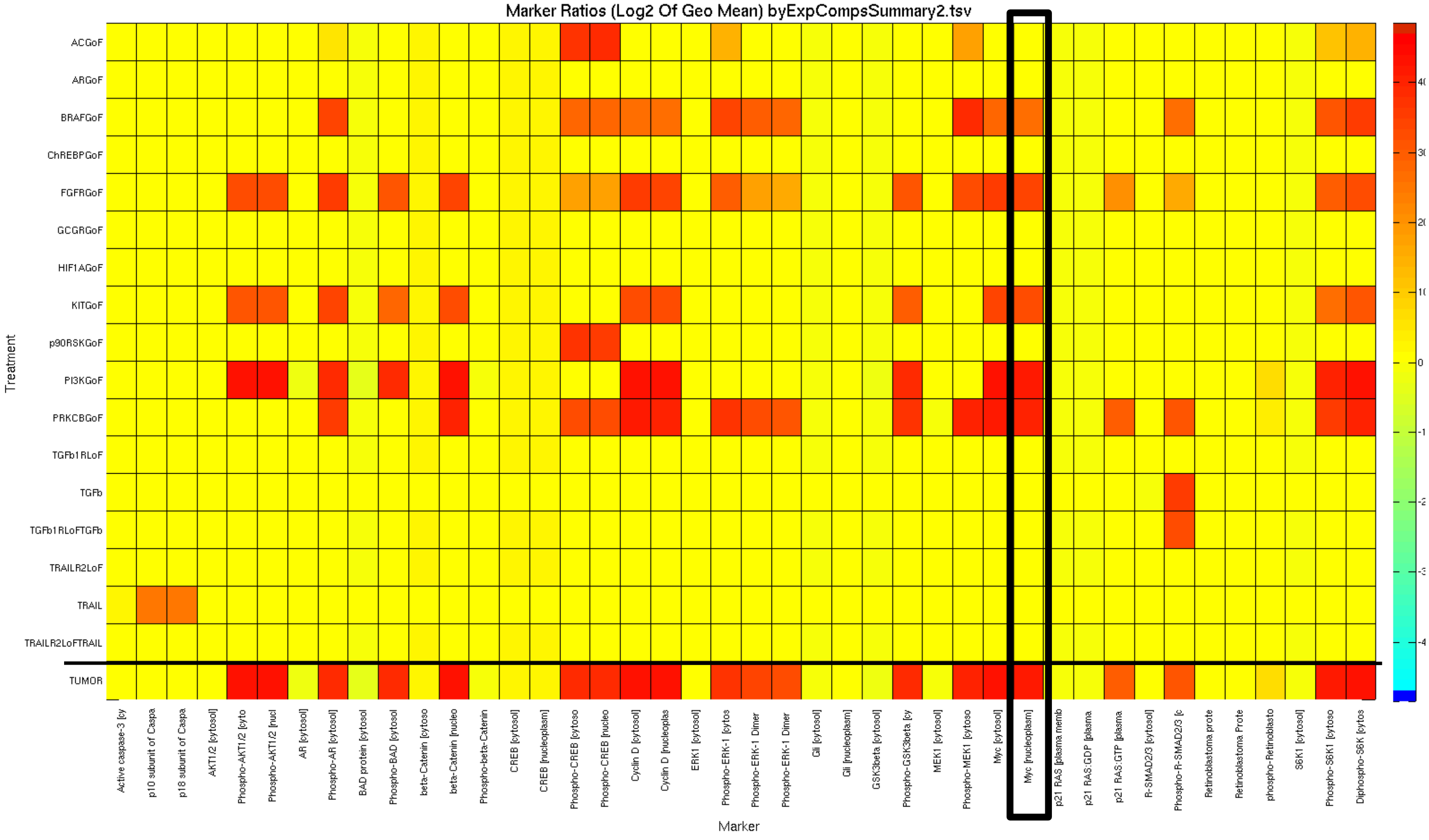


Relevant changes introduced into the model of the tumor of patient 1

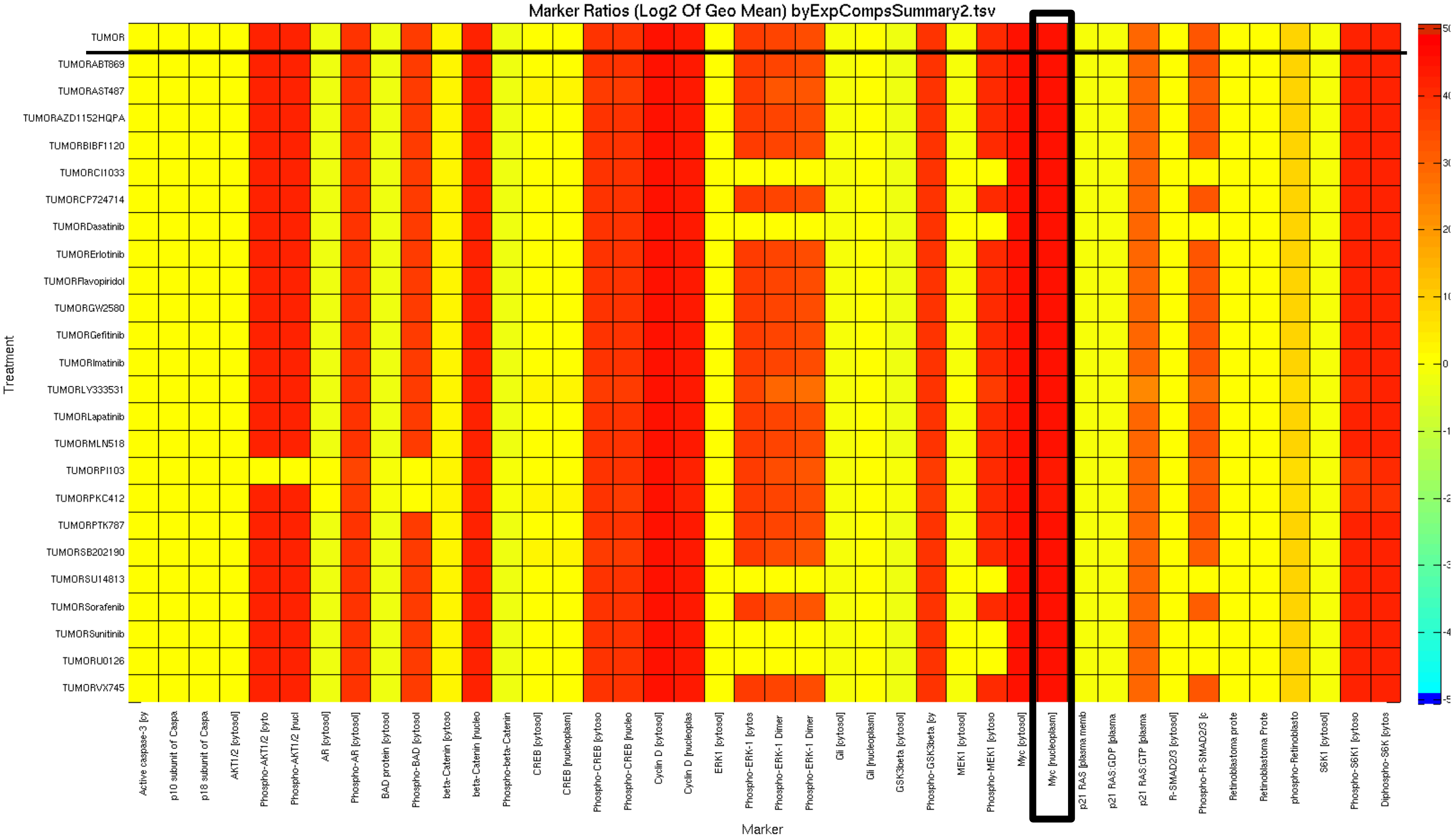


Gene	EnsembleID	Mutation	Model	Status
ACVR1B	ENSG00000135503	TTT->TGT, F198C	TGFb1R1	loss of function
ADCY6	ENSG00000174233	GCC->ACC, A634T GCC->GTC, A634V	AC	activating
AR	ENSG00000169083	CCA->CTA, P613L	AR	activating
BRAF	ENSG00000157764	GTG->GAG, V600E	BRAF	activating
CSF1R	ENSG00000182578	GAC->AAC, D741N	KIT*	activating
CRHR1	ENSG00000120088	AAA->AGA, K70R	GCGR	activating
FGFR3	ENSG00000068078	CCT->TCT, P816S	FGFR	activating
FLT3	ENSG00000122025	TTC->TTA, F419L	KIT*	activating
HIF1A	ENSG00000100644	AAA->AAC, K311N	HIF1A	activating
KIT	ENSG00000157404	CCT->ACT, P343T CCT->TCT, P343S	KIT*	activating
MLXIPL	ENSG00000009950	CCT->TCT, P152S CCT->ACT, P152T	ChREBP	activating
PIK3C2A	ENSG00000011405	AAG->AGG, K301R	PI3K#	activating
PIK3C2G	ENSG00000139144	CCA->CTA, P146L	PI3K#	activating
PRKCB	ENSG00000166501	CGA->CAA, R361Q	PRKCB	activating
RPS6KA2	ENSG00000071242	AGA->AAA,R704K AGA->AAA,R729K	p90RSK	activating
TNFRSF100B	ENSG00000120889	CCC->CTC,P32L CCC->CAC,P32H	TRAILR2	loss of function

Modelling a patient 1 specific tumor

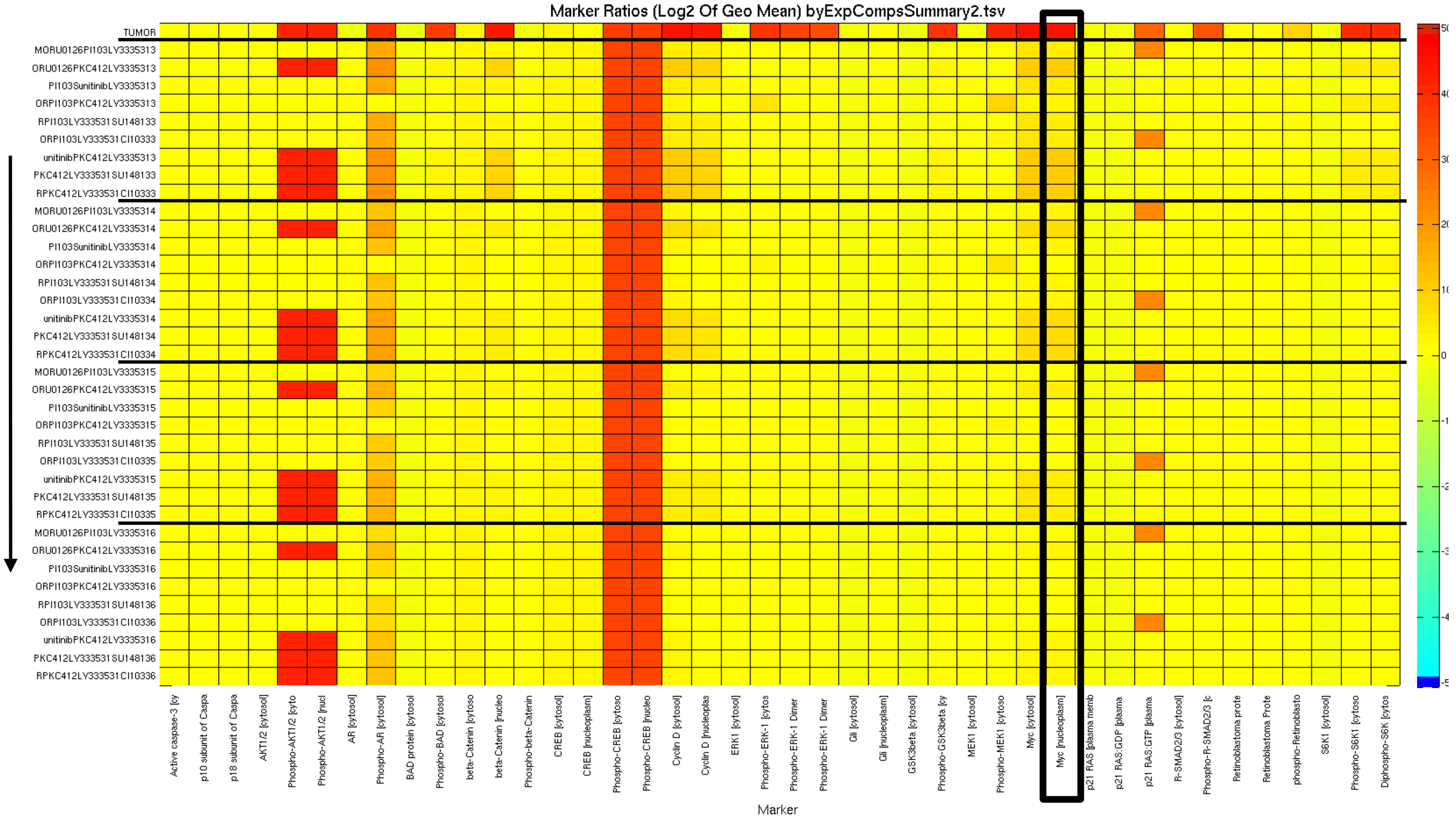


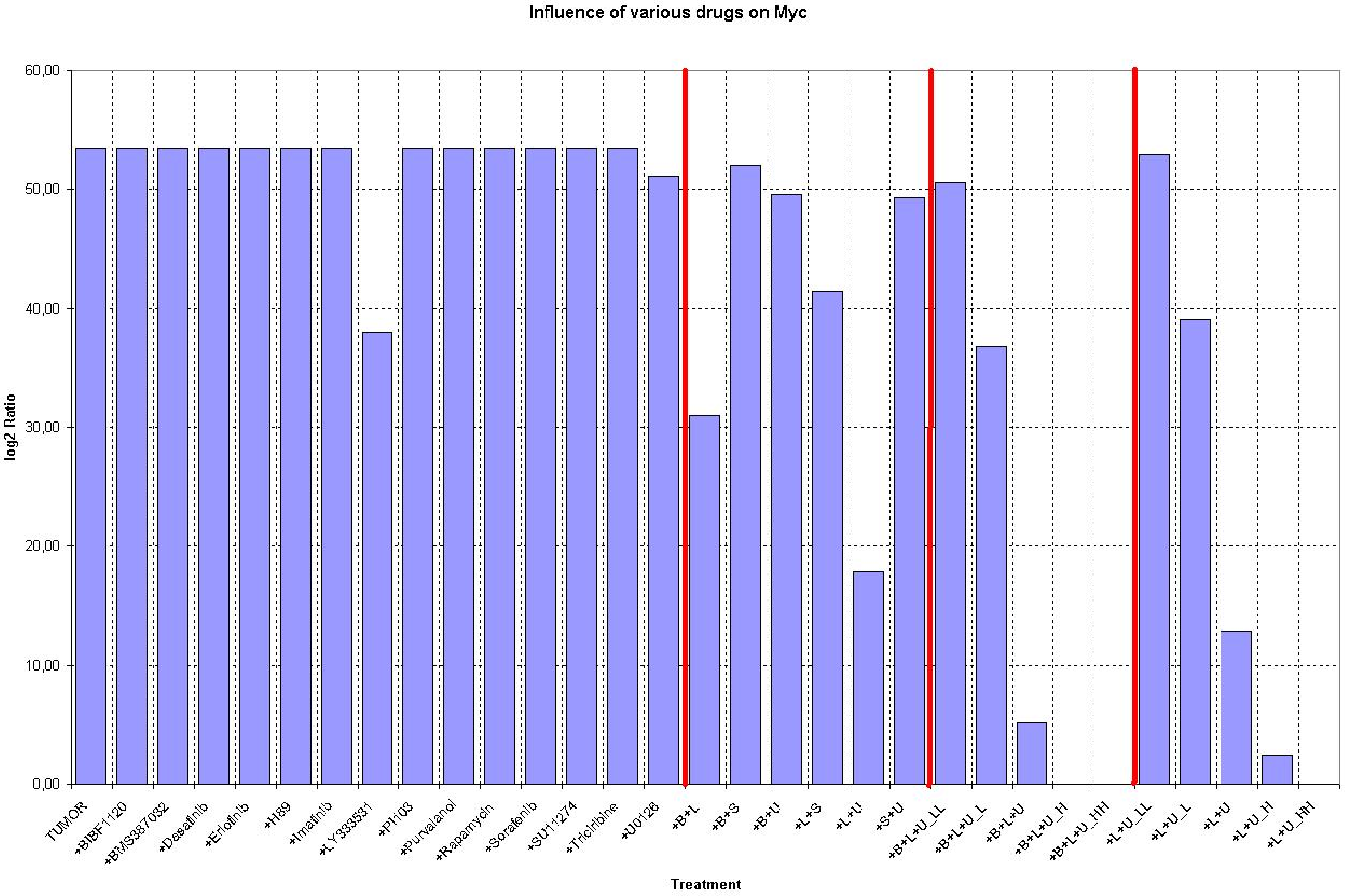
Finding a patient 1 specific optimal drug treatment



Optimization of drug concentration

Increasing concentration




Influence of various drugs on Myc
log2 Ratio
60,00
50,00
40,00
30,00
20,00
10,00
0,00
TUMOR
+BIBF1120
+BMS387032
+Dasatinib
+Erlotinib
+H89
+Imatinib
+LY333531
+PI103
+Purvalanol
+Rapamycin
+Sorafenib
+SU11274
+Triciribine
+U0126
+B+L
+B+S
+B+U
+L+S
+L+U
+S+U
+B+L+U_LL
+B+L+U_L
+B+L+U
+B+L+U_H
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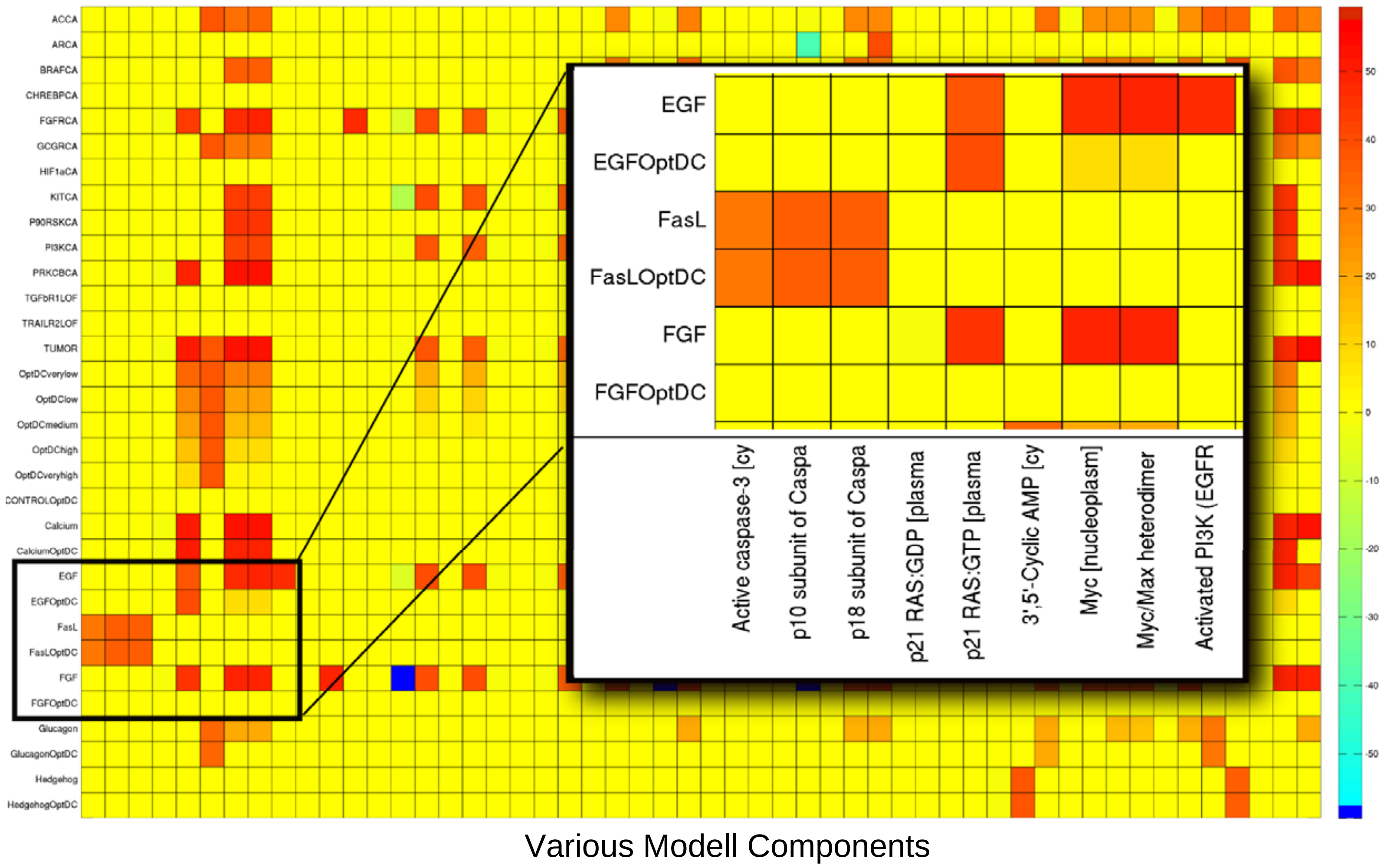
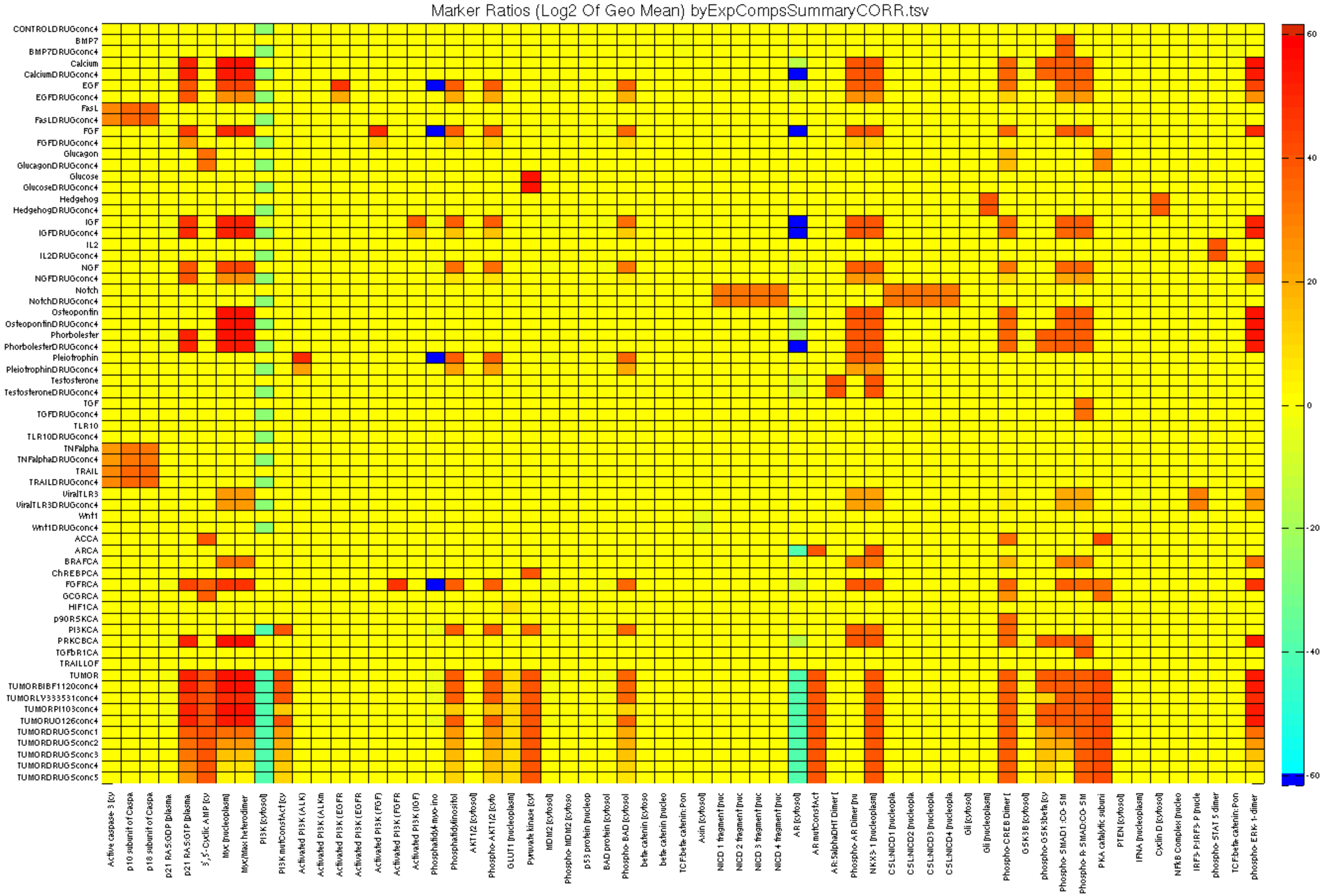
Treatment

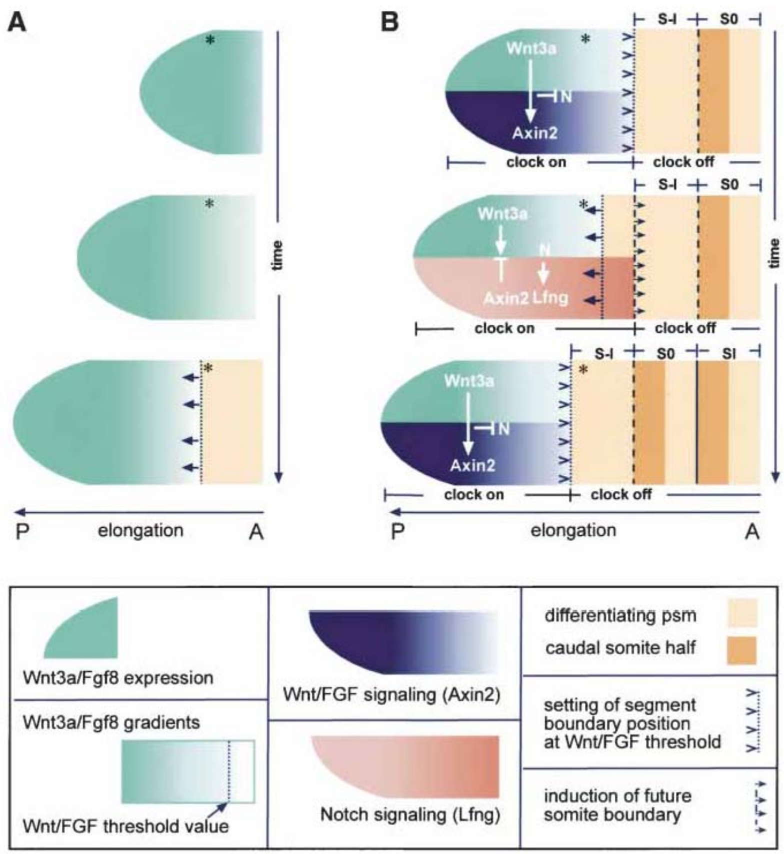
Modelling Side Effects of Drugs




Marker Ratios (Log2 Of Geo Mean) byExpCompsSummaryCORR.tsv

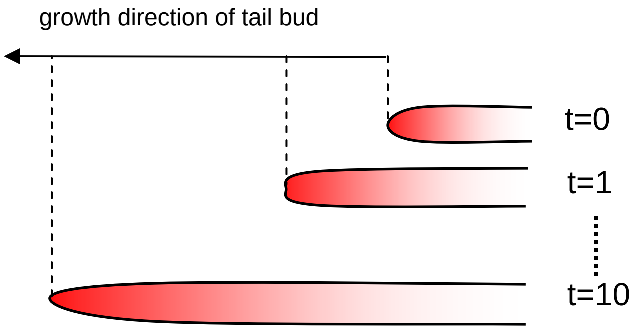
Model on mouse somitogenesis (I)

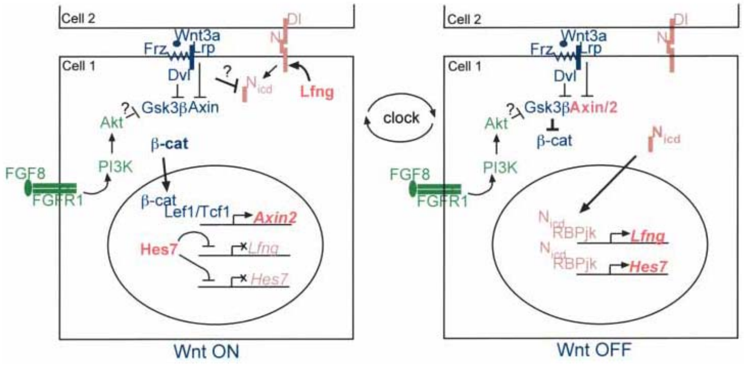
Segmentation clock of vertebral somitogenesis



Aulehla and Herrmann (2004) *GENES & DEVELOPMENT* **18:**2060–2067.

Wnt3a gradient



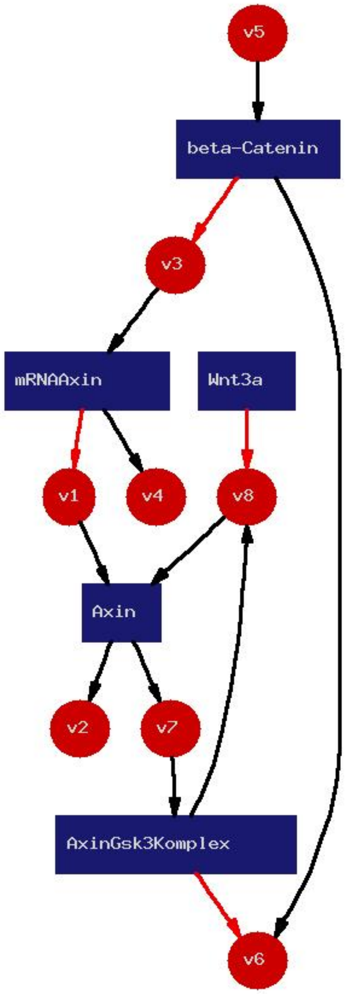


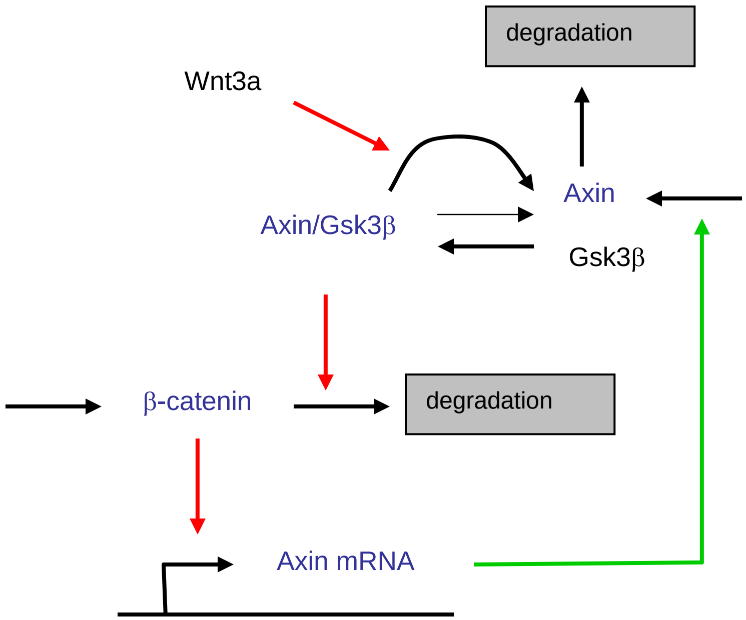
Aulehla and Herrmann (2004) *GENES & DEVELOPMENT* **18:**2060–2067.

Model on mouse somitogenesis (II)

MAX-PLANCK-GESELLSCHAFT

Model of the Wnt signaling pathway





Selected drugs for therapy optimization

Compound	**Target protein(s)**	**k_D value(s) (nM)**
ABT-869	ALK, KIT, MET, PDGFR, TRKR	1600, 2, 1300, 1.9, 3100
AST-784	ABL, ALK, BRAF, EGFR, FGFR, KIT, MET, PDGFR, PKA, SRC, TRKR	5.6, 1400, 1700, 520, 620, 5.4, 4400, 8.1, 260, 340, 320
AZD-1152HQPA	EGFR, KIT, PDGFR	450, 17, 41
BIBF-1120	FGFR	69
CI-1033	ABL, EGFR, KIT, MEK, MET, PDGFR, SRC	1400, 0.19, 7800, 1800, 5600, 7500, 1100,
CP-724714	EGFR	42
Dasatinib	ABL, BRAF, EGFR, FGFR, KIT, MEK, PDGFR, SRC	0.53, 500, 120, 3700, 0.62, 1000, 0.63, 0.21
EKB-569	ALK, ABL, EGFR, MEK, MET, SRC	7100, 560, 0.44, 360, 6200, 280
Erlotinib	ALK, ABL, EGFR, MET, PDGFR, SRC	1200, 310, 0.67, 3800, 1400, 700
Flavopiridol	ALK	670
GW-2580	TRKR	630
Gefitinib	EGFR, SRC	1, 3800
Imatinib	ABL, KIT, PDGFR	12, 14, 14
LY-333531	PKA, PRKCB	3200, 5
Lapatinib	EGFR	2.4
MLN-518	EGFR, KIT, PDGFR, TRKR	410, 2.7, 4.5, 450
PI-103	BRAF, PI3K	2900, 1.5
PKC-412	ALK, AKT, EGFR, FGFR, KIT, MET, PDGFR, PKA, SRC, TRKR	270, 950, 1300, 1600, 220, 110, 690, 240, 1200, 380
PTK-787	KIT, PDGFR	5.1, 25
SB-202190	BRAF, EGFR, PKA	4800, 2600, 530
Sorafenib	ABL, BRAF, FGFR, KIT, PDGFR, TRKR	680, 540, 2800, 31, 37, 6300
SU-14813	ABL, ALK, FGFR, INSR, KIT, MEK, PDGFR, SRC, TRKR	1500, 490, 1900, 1200, 0.68, 77, 0.29, 4900, 480
Sunitinib	ABL, ALK, FGFR, IGFR, INSR, KIT, MEK, PDGFR, SRC, TRKR	830, 170, 520, 2600, 500, 0.37, 130, 0.08, 2100, 100
U0126	MEK	72
VX-745	ABL, PDGFR, SRC	730, 8400, 5500

Drug Optimisation Patient 1

ROUND1

	MYC	PIP3
TUMOR	1,06609E+016	5,41213E+011
BIBF1120	1,06320E+016	5,41213E+011
BMS387032	1,06609E+016	5,41213E+011
Dasatinib	1,06609E+016	5,41213E+011
Erlotinib	1,06609E+016	5,41213E+011
H89	1,06609E+016	5,41213E+011
Imatinib	1,06609E+016	5,41213E+011
LY333531	3,13429E+014	5,41213E+011
PI103	1,06609E+016	1,36000E+006
Purvalanol	1,06609E+016	5,41213E+011
Rapamycin	1,06609E+016	5,41213E+011
Sorafenib	1,06608E+016	5,41213E+011
SU11274	1,06609E+016	5,41213E+011
U0126	7,10811E+014	5,41213E+011

ROUND2

	MYC	PIP3
TUMOR	1,06081E+016	3,01787E+011
B+L	3,25483E+009	3,01787E+011
B+P	1,04455E+016	7,26606E+005
B+S	1,04455E+016	3,01787E+011
B+U	7,43861E+014	3,01787E+011
L+P	6,11556E+014	7,26606E+005
L+S	6,11492E+014	3,01787E+011
L+U	1,44048E+011	3,01787E+011
P+S	1,06081E+016	7,26606E+005
P+U	7,82296E+014	7,26606E+005
S+U	7,82211E+014	3,01787E+011

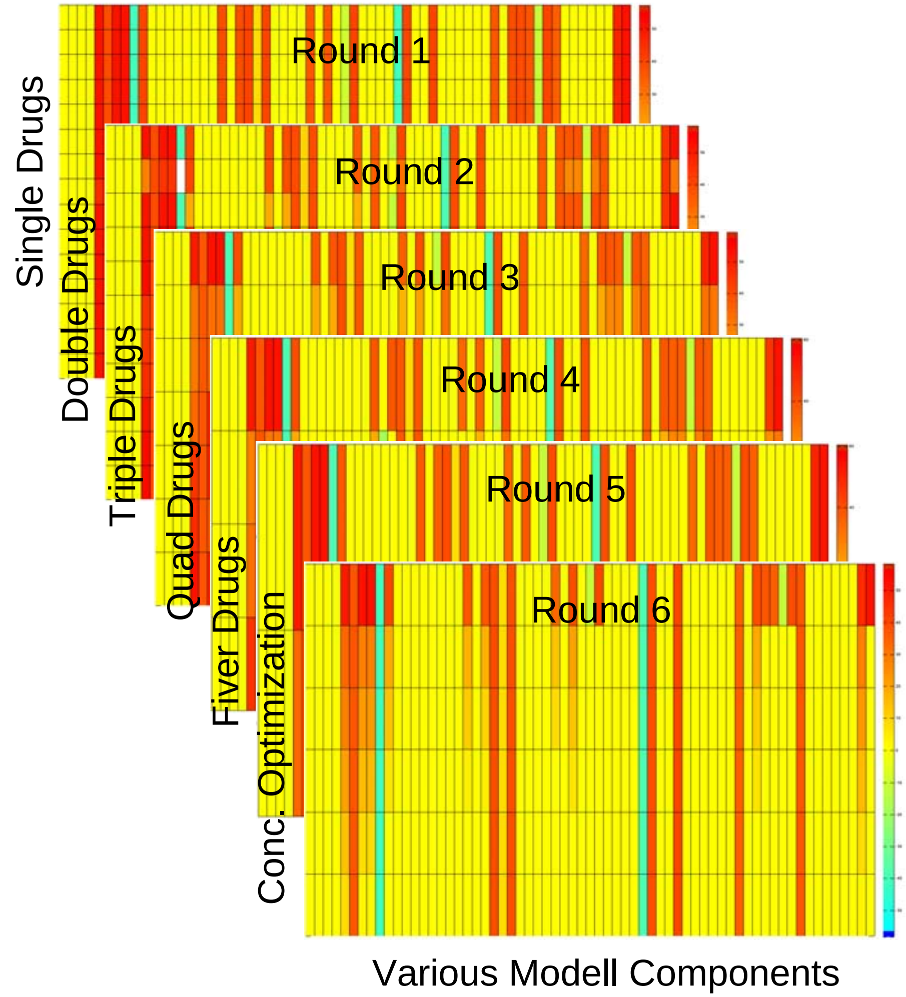
ROUND3

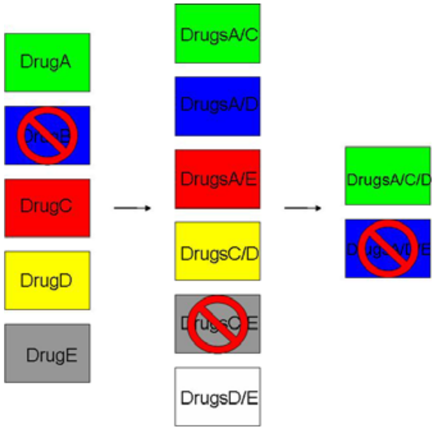
	MYC	PIP3
TUMOR	1,04904E+016	2,84198E+011
B+L+P	4,21090E+009	6,61147E+005
B+L+U	2,45951E+000	2,84198E+011
B+P+U	4,92797E+014	6,61147E+005
L+P+U	6,44934E+008	6,61147E+005

ROUND4

	MYC	PIP3
TUMOR	1,31681E+016	4,29230E+011
B+L+P+U	1,55283E+000	9,06212E+005

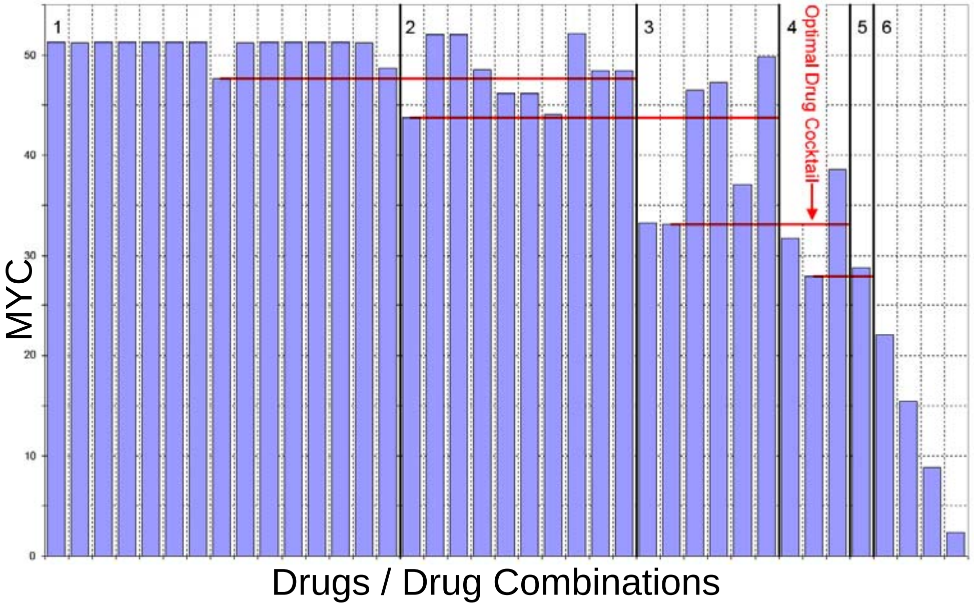
Finding a Patient 1 Specific Optimal Drug Treatment




DrugA
DrugB
DrugC
DrugD
DrugE

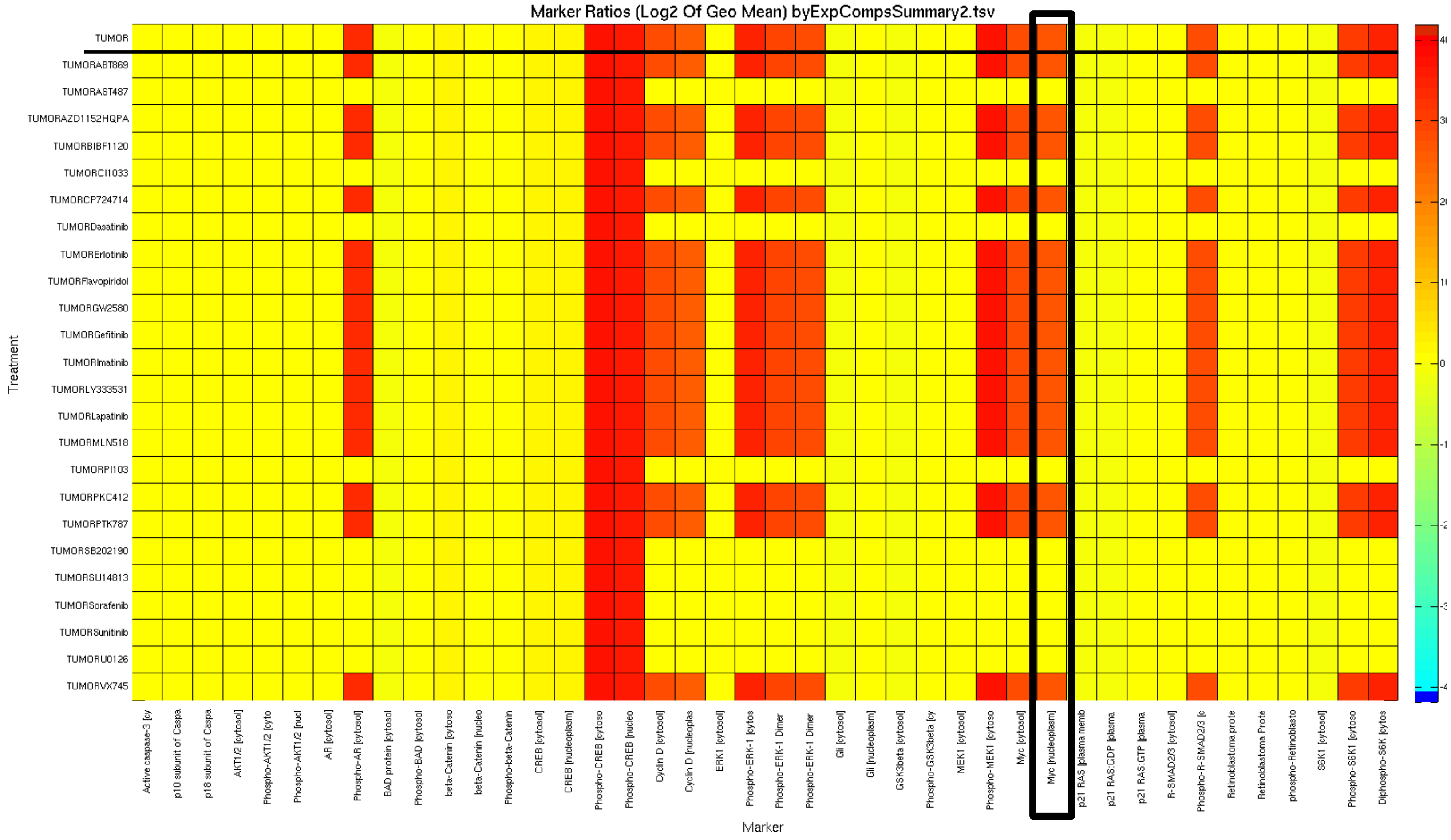
DrugsA/C
DrugsA/D
DrugsA/E
DrugsC/D
DrugsC/E
DrugsD/E

DrugsA/C/D
DrugsA/D/E



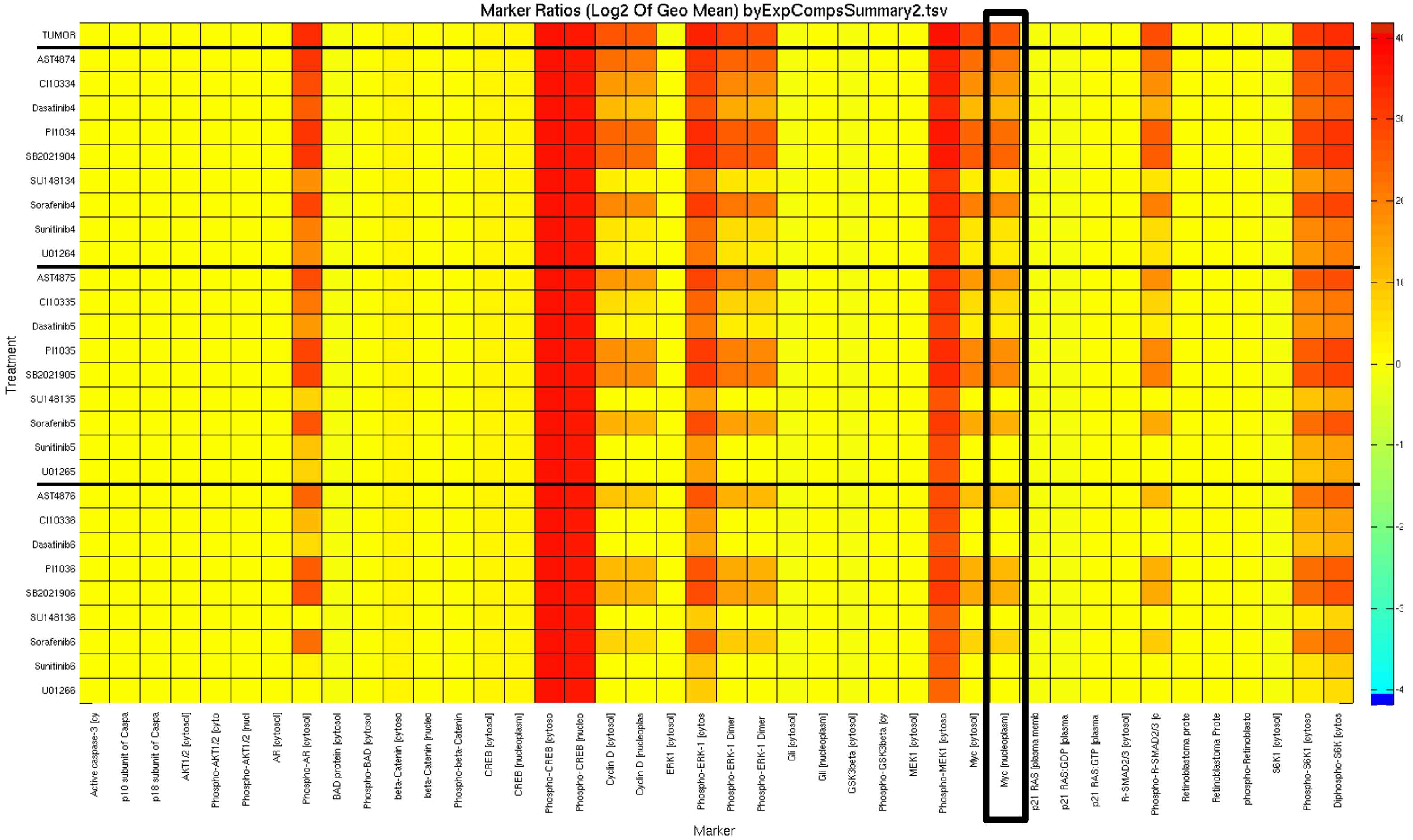
Modelling a melanoma cell line & finding an optimal drug treatment

Gene	**EnsembleID**	**Mutation**	**Model**	**Status**
BRAF	ENSG00000157764	GTG->GAG, Val->Glu	BRAF	activating
GLI1	ENSG00000111087	TCC->TTC, Ser->Phe	GLI	activating
RPS6KA2	ENSG00000071242	GAA->AAA, Glu->Lys	p90RSK	activating



Optimization of drug concentration

Increasing concentration



	Transcriptome Sequencing	**Genome Sequencing**	**Exome Sequencing**
Patient 2	Tumor tissue (2*PE lanes GAII lanes)	Tumor Tissue (3*Fragment FC + 1*Mate Pair FC on SOLiD3) Blood (1*fragment on SOLiD3)	Tumor tissue (1*Fragment lane on GAII) Blood 81*Fragment lane on GAII)
Patient 3			
Patient 4	Tumor tissue (1*PE lanes GAII lanes) Control tissue (1*PE lanes GAII lanes)		Libraries of Tumor tissue and Blood are ready for Sequencing
Patient 5	Tumor tissue (1*PE lanes GAII lanes)		Libraries of Tumor tissue and Blood are ready for Sequencing
Patient 6	Tumor tissue (1*PE lanes GAII lanes)		Libraries of Tumor tissue and Blood are ready for Sequencing
Patient 7			

DNA frorm all Patient have been sent for SNP analysis on Seqenom's MassArray system

- **PacBio has been quoted that, by 2013, their technology will be able to give a ‘raw’ human genome sequence in less than 3 min, and a complete high-quality sequence in just 15 min**

Modelling the response of cancer stem cells?

Cancer, a curable disease?

The ‚red Queen‘ approach to cancer treatment: Modelling cancer progression.

Cancer, a chronic disease‘

Marie-Laure Yaspo
Marc Sultan
Aleksey Soldatov
Tatjana Borodina
Bernd Timmermann
Andreas Dahl
Michal Schweiger
Wolfgang Lehrach
Dmitri Parkhomchuk
Andrei Grigorieff
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Andriani Daskalaki
Elisabeth Maschke-Dutz

Bernhard Herrmann

- Charitè
  - Peter Schlag
  - Christian Regenbrecht
  - Reinhold Schäfer
  - Manfred Dietel
- Genomatix
  - Matthias Scherf
  - Andreas Klingenhoff
  - Martin Seifert
- DKFZ
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  - Stefan Wiemann

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