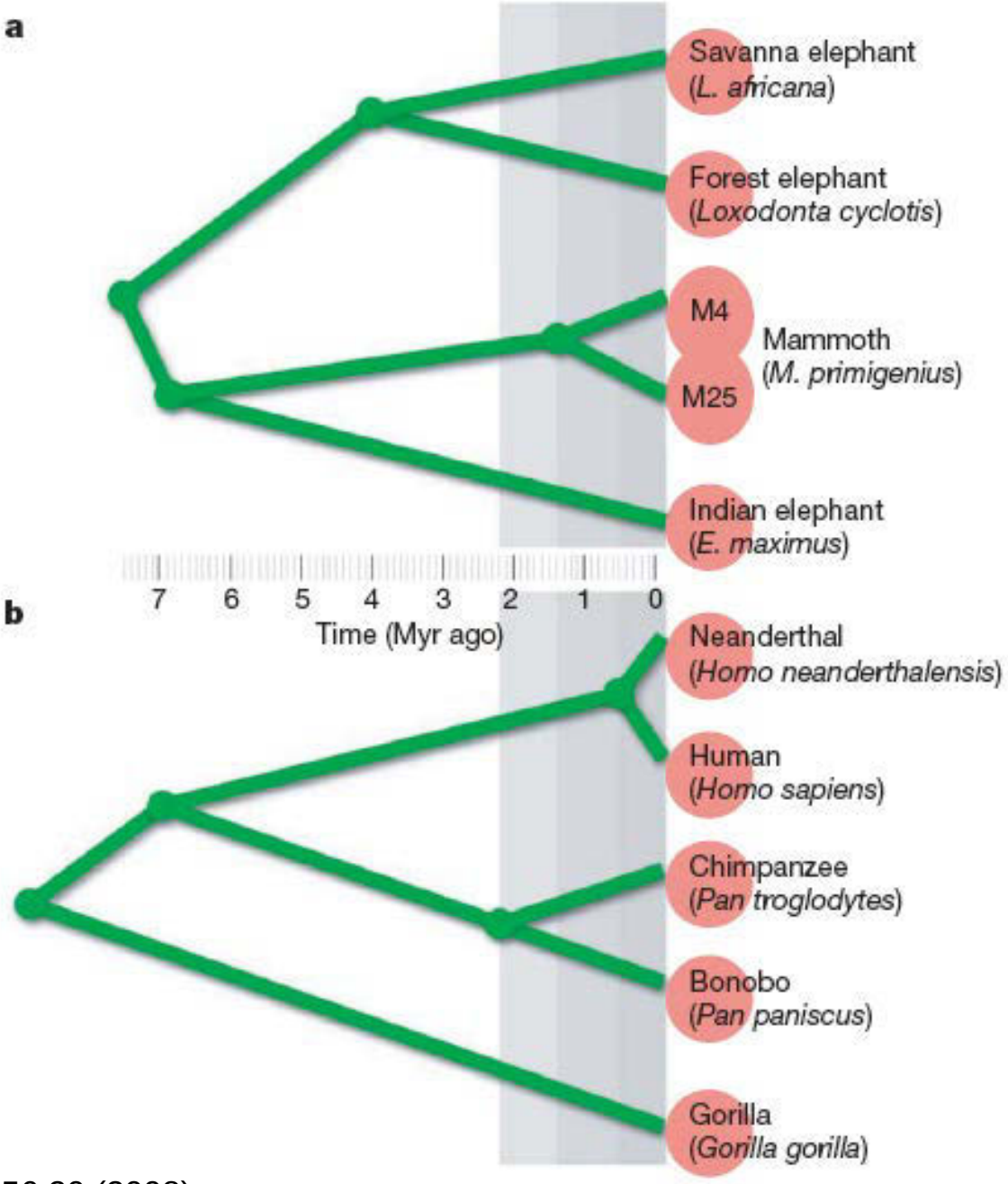
Whole Genome Sequencing: Ancient DNA

Neandertal

Svante Paabo has completed about 60% of the genome using 454 technology. The previously published the sequence of 1million bases of sequence in 2007. Over 4 billion bases with GAII and 454 reads

Wooly Mammoth

4.17Gb of individual reads from two wooly mammoth species.



Miller et al., Nature 456:20 (2008)

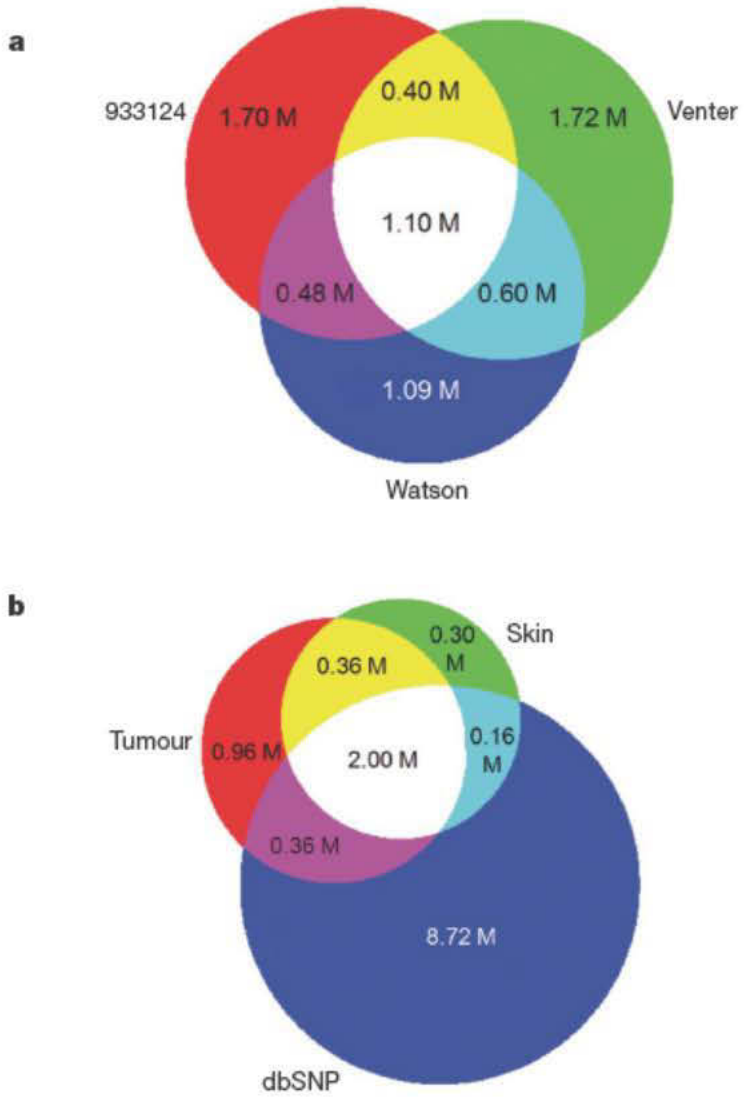
Genomic Sequence of the AML Genome: The Numbers

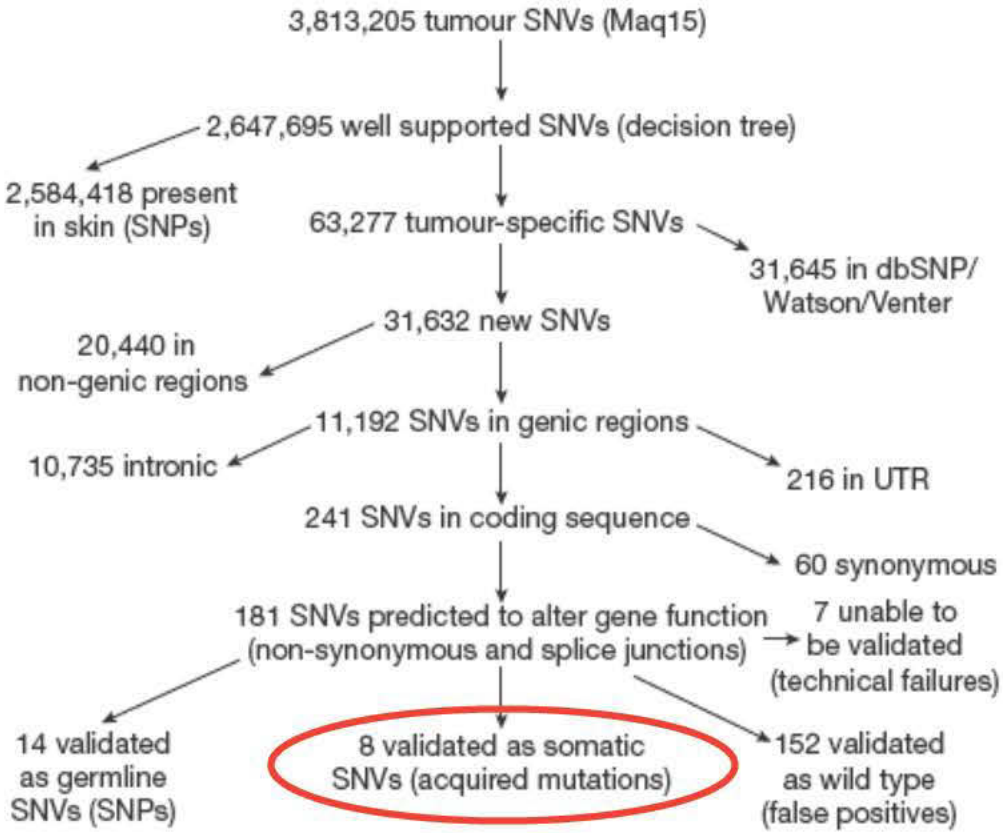
Table 1 | Tumour and skin genome coverage from patient 933124

	Tumour	Skin
Libraries	4	3
Runs	98	34
Reads obtained	5,858,992,064	2,122,836,148
Reads passing quality filter	3,025,923,365	1,228,177,690
Bases passing quality filter	98,184,511,523	41,783,794,834
Reads aligned by Maq	2,729,957,053	1,080,576,680
Reads unaligned by Maq	295,966,312	138,276,594
SNVs detected with respect to hg18 (no Y)	3,811,115	2,918,446
SNVs (chr 1–22) detected with respect to hg18	3,681,968 (100.0%)	2,830,292 (100.0%)
SNVs also present in dbSNP	2,368,458 (64.3%)	2,161,695 (76.4%)
SNVs also present in Venter genome	1,499,010 (40.7%)	1,383,431 (48.9%)
SNVs also present in Watson genome	1,573,435 (42.7%)	1,456,822 (51.5%)
SNVs not in dbSNP/Venter/Watson	1,223,830 (33.2%)	591,131 (20.9%)
SNVs not in dbSNP/Venter/Watson/skin	925,200 (25.1%)	–
HQ SNPs	46,494 (100.0%)	46,572 (100.0%)
HQ SNPs where reference allele is detected	42,419 (91.2%)	38,454 (82.6%)
HQ SNPs where variant allele is detected	43,164 (92.9%)	39,220 (84.2%)
HQ SNPs where both alleles are detected	42,415 (91.2%)	38,454 (82.6%)

Assessments are shown of the haploid and diploid coverage of the tumour and skin genomes from AML patient 933124.
Chr, chromosome; hg18, human genome version 18; HQ, high quality.

AML:Comparisons





Gorilla Sequencing Stats

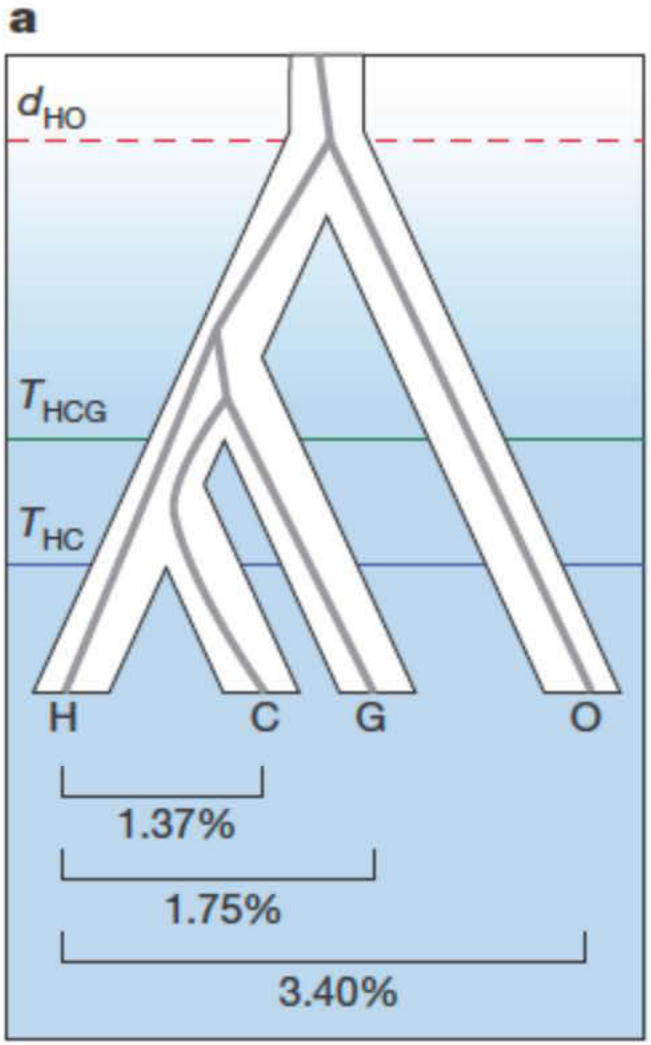
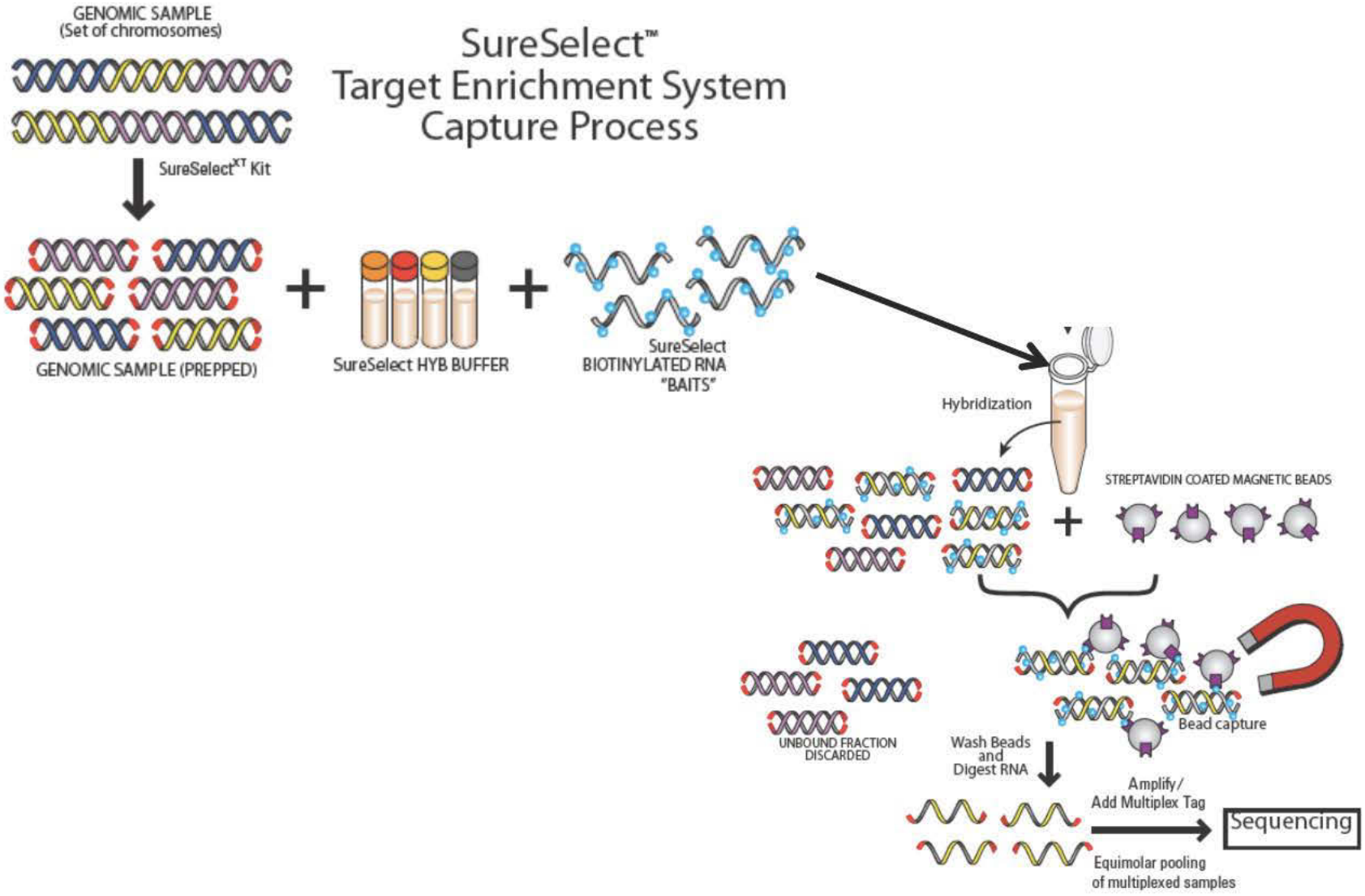


Table 1 | Assembly and annotation statistics

Assembly		Annotation	
Total length	3,041,976,159 bp	Protein-coding genes	20,962
Contigs	465,847	Pseudogenes	1,553
Total contig length	2,829,670,843 bp	RNA genes	6,701
Placed contig length	2,712,844,129 bp	Gene exons	237,216
Unplaced contig length	116,826,714 bp	Gene transcripts	35,727
Max. contig length	191,556 bp	lincRNA transcripts	498
Contig N50	11.8 kbp		
Scaffolds	22,164		
Max. scaffold length	10,247,101 bp		
Scaffold N50	914 kbp		

N50: 50% of the genome is in fragments of this length or longer; lincRNA: long intergenic non-coding RNA.

SureSelect Exome Capture



Disease Genes Discovered by Direct Whole Exome Sequencing*

Gene Identified	Disease/Syndrome	Reference
MYH3	Freeman-Sheldon Syndrome	Ng SB, et al. 2009. Nature 462
SLC26A3	Bartter Syndrome	Choi M, et al. 2009 PNAS 106(45)
DHODH	Miller Syndrome	Ng SB, et al. 2010 Nat Genet 42(1).
FLVCR2	Fowler Syndrome	Lalonde, E. et al. 2010 Hum Mutat 31(8).
FLNA	Terminal Osseous Dysplasia (TOD)	Sun Y., et al. 2010 Am J. Hum Genet 87(1).
GPSM2	Nonsyndromic Hearling Loss (DFNB82)	Walsh, T. et al. 2010 Am J. Hum Genet 87(1).
HSD17B4	Perrault Syndrome/DBP	Pierce SB, et al. 2010 Am J. Hum Genet 87(2).
MLL2	Kabuki Syndrome	Ng SB, et al. 2010 Nat Genet 42(9).
ABCG5	Hypercholesterolemia	Rios J., et al. 2010 Hum Mol Genet 19(22).
WDR62	Brain Malformations	Bilguvar K, et al. 2010 Nature 467(7312).
PIGV	Hyperphosphatasia Mental Retardation (HPMR)	Krawitz PM, et al. 2010 Nat Genet 42(10)
WDR35	Sensenbrenner Syndrome	Gilissen C, et al. 2010Am J Hum Genet 87(3).
SDCCAG8	Nephromophthisis-related Ciliopathies	Otto EA, et al. 2010 Nat Genet 42(10).
STIM1	Kaposi Sarcoma	Byn M, et al. 2010 J Exp Med 207(11).
SCARF2	Van Den Ende-Gupta Syndrome	Anastasio N. et al. 2010 Am J Hum Genet 87(4).
C20orf54	Brown-Vialetto-Van Laere Syndrome	Green P, et al. 2010 Am J Hum Genet 86(3).
MASP1	Carnevale, Malpuech, OSA and Michels Syndromes	Sirmaci A, at al. 2010 Am J Hum Genet 87(5).
ABCC8	Neonatal Diabetes Mellitus	Bonnefond A, et al. 2010 PLoS One 5(10).
BAP-1	Metastasizing Uveal Melanomas	Harbour JW, et al. 2010 Science Nov 4 Epub.
ACAD9	Complex I Deficiency	Haack TB, et al. 2010 Nat Genet Nov 7 Epub.
DYNC1H1	Mental Retardation	Vissers LELM, et al. 2010 Nat Genet 10.1038/ng.712
RAB39A	Mental Retardation	Vissers LELM, et al. 2010 Nat Genet 10.1038/ng.712
YY1	Mental Retardation	Vissers LELM, et al. 2010 Nat Genet 10.1038/ng.712
DEAF1	Mental Retardation	Vissers LELM, et al. 2010 Nat Genet 10.1038/ng.712

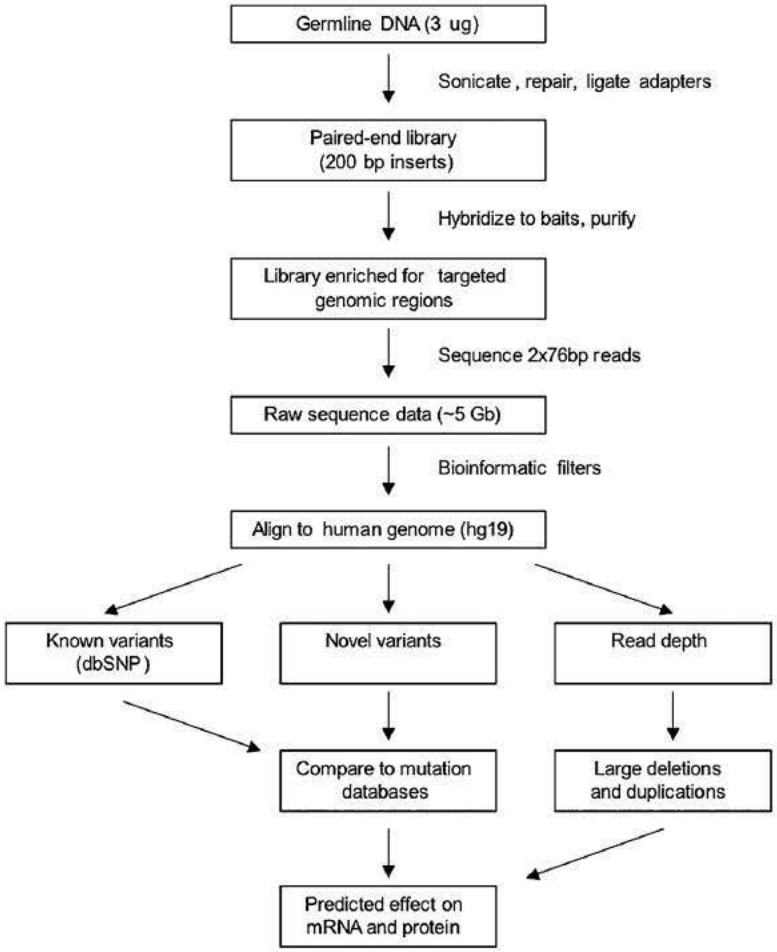
*As of 23 Nov. 2010

Targeted Re-sequencing

The ability to capture specific sequences in the genome

- Microarrays
- Long range PCR
- Solution capture on Biotin labeled oligos
- RainStorm from RainDance

Genomic Capture of Breast Cancer Relevant Genes Followed by Next-Gen Sequencing.



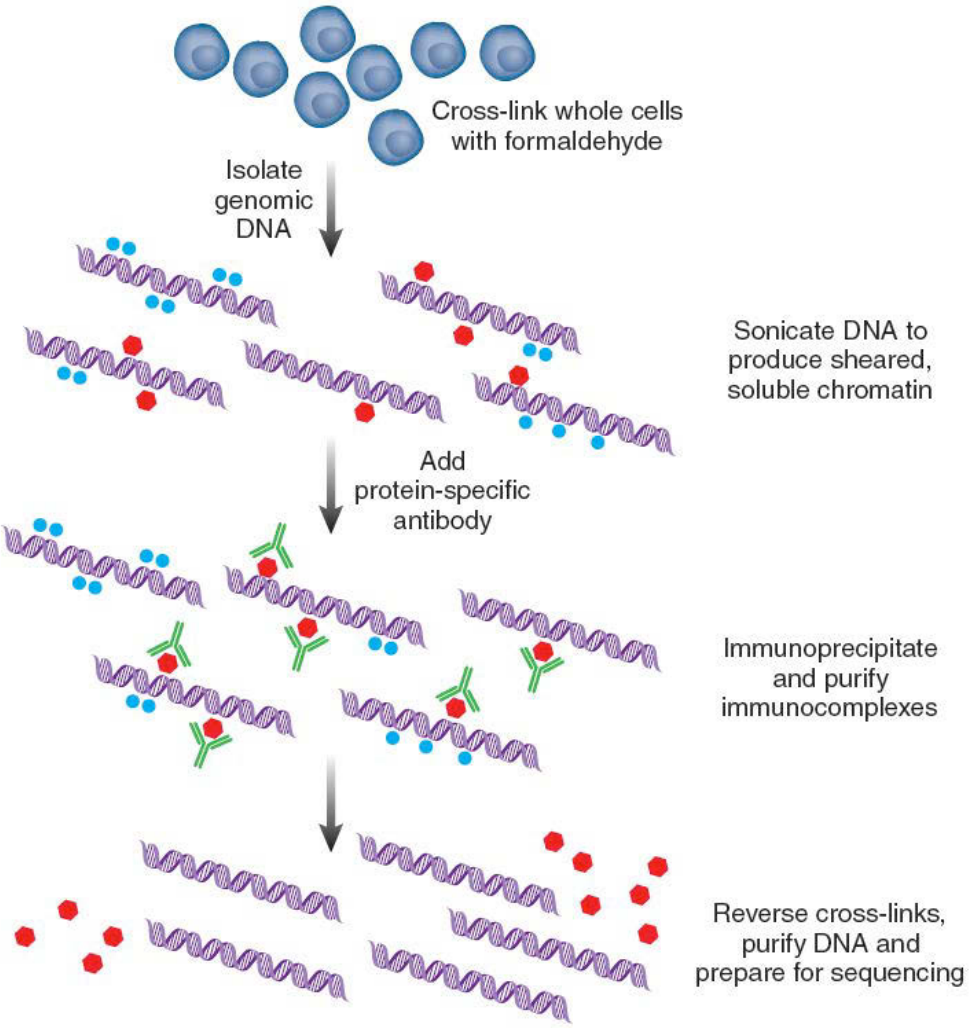
Walsh T et al. PNAS 2010;107:12629-12633

Gene	Chromosome	Start	End
BRCA1	17	41,186,313	41,347,712
BRCA2	13	32,879,617	32,983,809
CHEK2	22	29,073,731	29,147,822
PALB2	16	23,604,483	23,662,678
BRIP1	17	59,759,985	59,940,755
p53	17	7,561,720	7,600,863
PTEN	10	89,613,195	89,738,532
STK11	19	1,195,798	1,238,434
CDH1	16	68,761,195	68,879,444
ATM	11	108,083,559	108,249,826
BARD1	2	215,583,275	215,684,428
MLH1	3	37,024,979	37,102,337
MRE11	11	94,140,467	94,237,040
MSH2	2	47,620,263	47,720,360
MSH6	2	48,000,221	48,044,092
MUTYH	1	45,784,914	45,816,142
NBN	8	90,935,565	91,006,899
PMS1	2	190,638,811	190,752,355
PMS2	7	6,002,870	6,058,737
RAD50	5	131,882,630	131,989,595
RAD51C	17	56,759,963	56,821,692

Something Very Cool.

Johnson et al., Science 316:1497 (2007)

ChIP-Seq



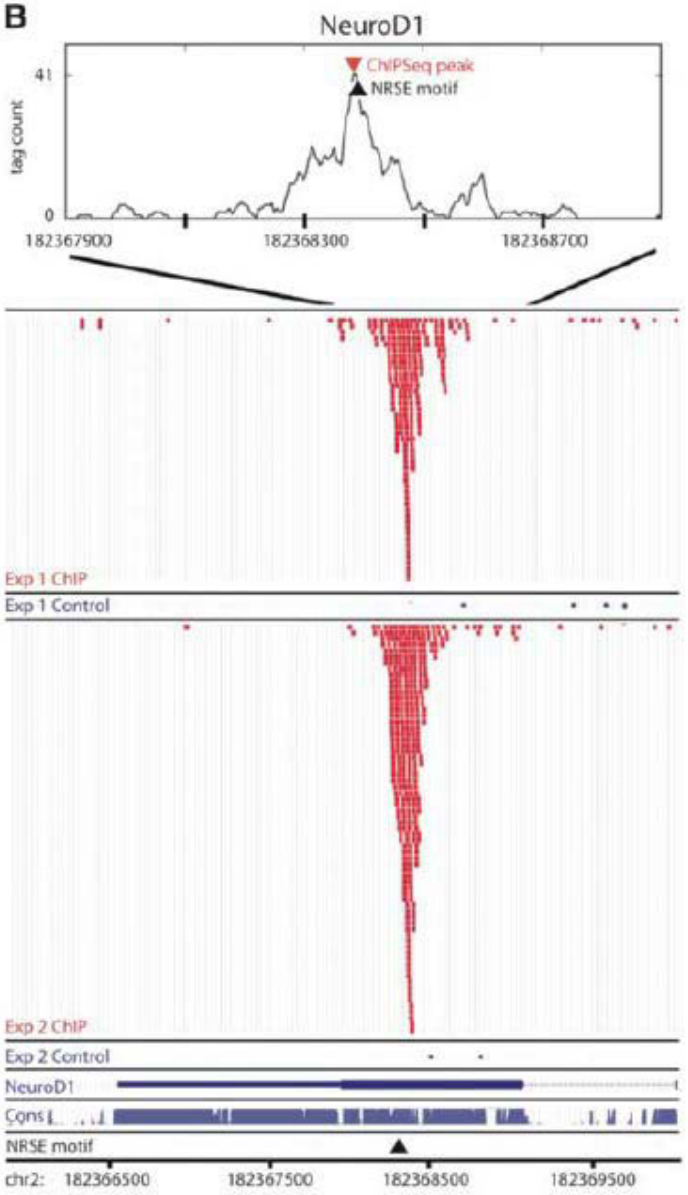
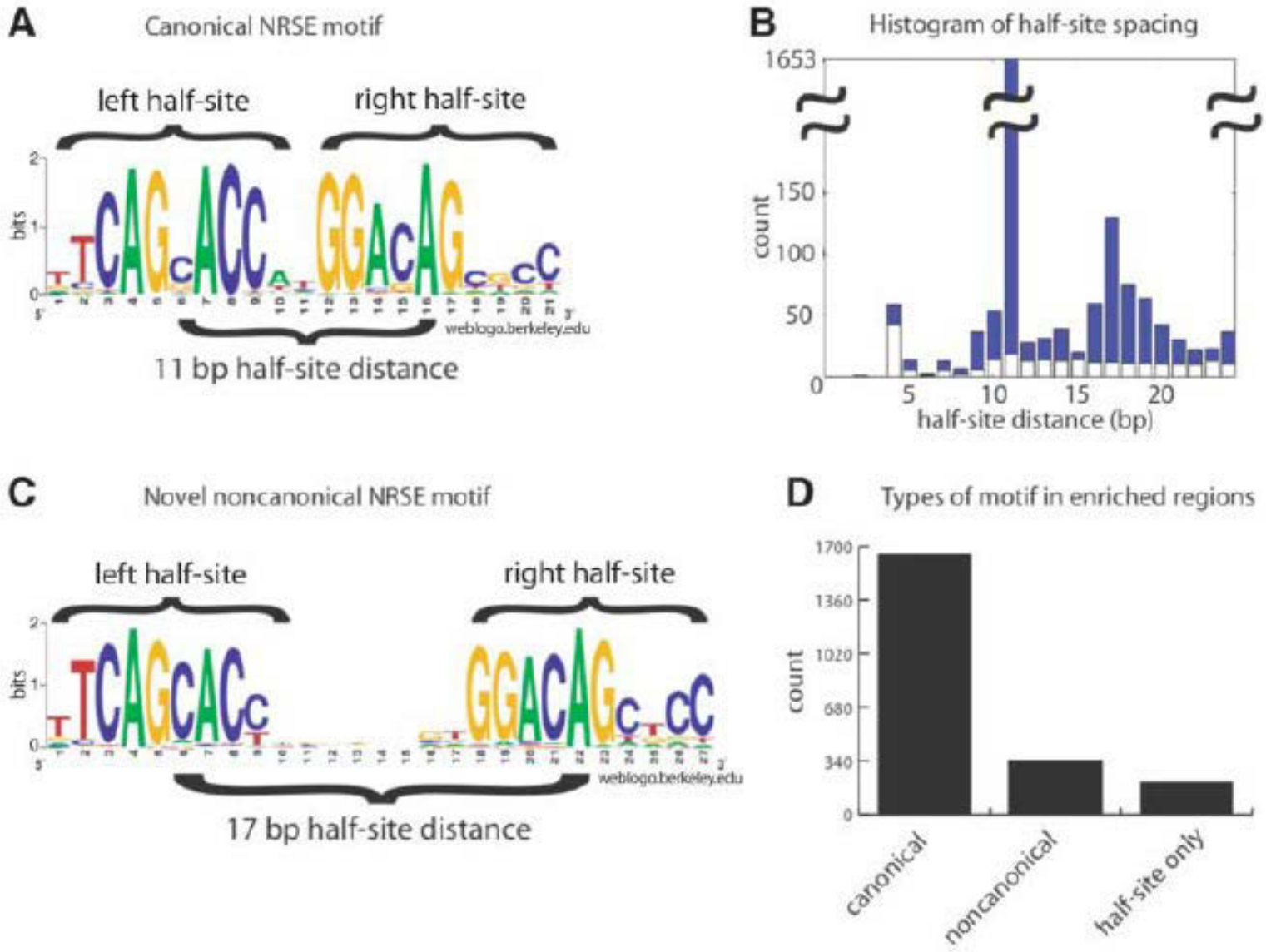


Figure 1 | Workflow of Chip-seq. DNA and proteins are cross-linked and purified; then bound DNA is analyzed by massively parallel short-read sequencing.

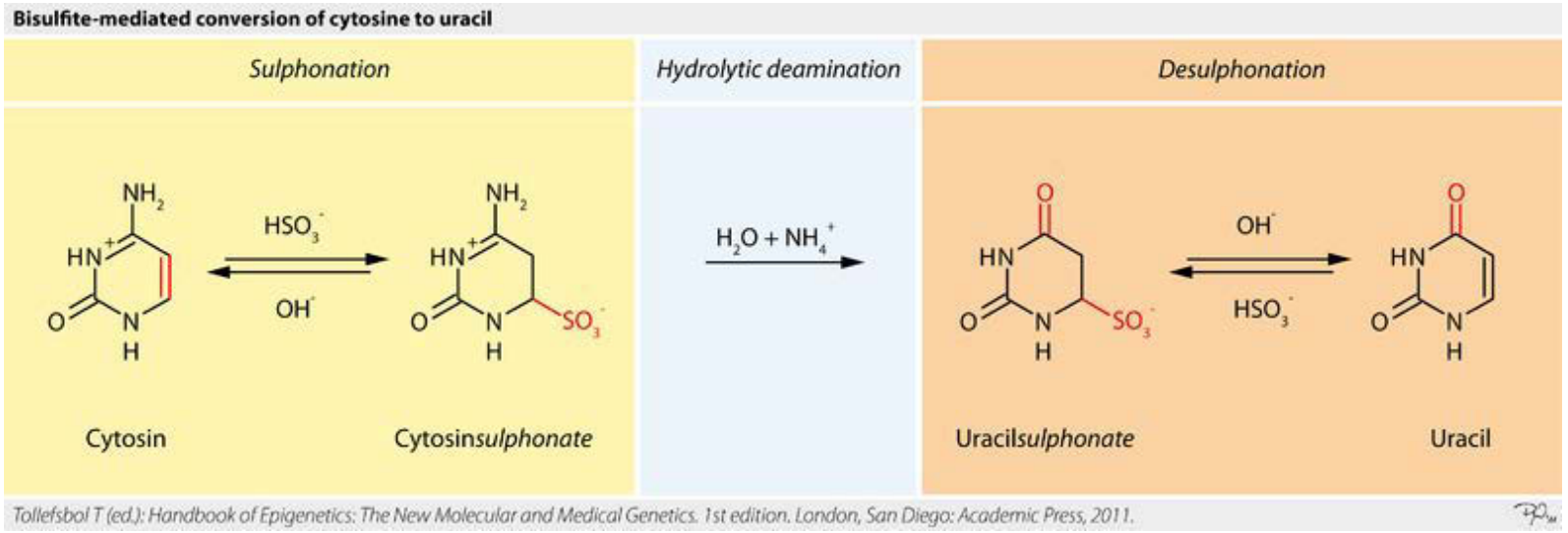
ChIP-Seq



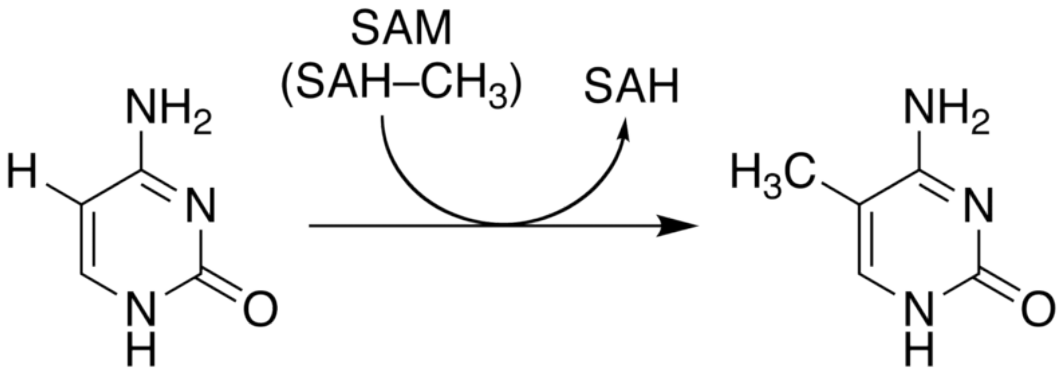
Johnson et al., Science 316:1497 (2007)

Methylation profiling

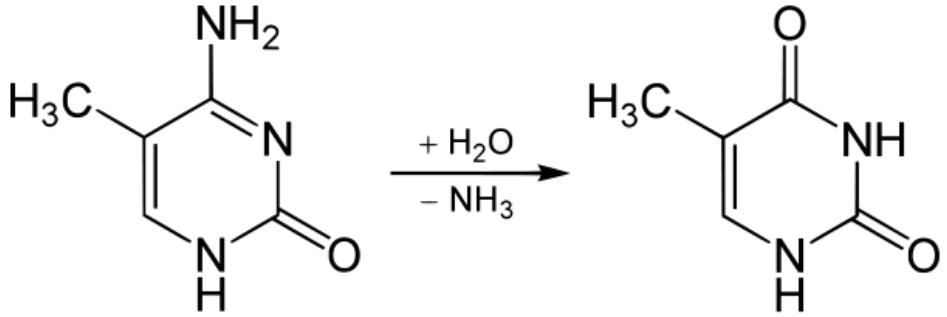
- Whole genome bisulfite sequencing
- MeDIP (Methylated DNA-IP)
- Reduced Representational Bisulfite Sequencing
- Specific Capture methods



Cytosine to 5-Methylcytosine to Thymine conversion



Cytosine 5-Methylcytosine



5-Methylcytosine Thymine

Johnson et al., Science 316:1497 (2007)

MeDIP-Seq

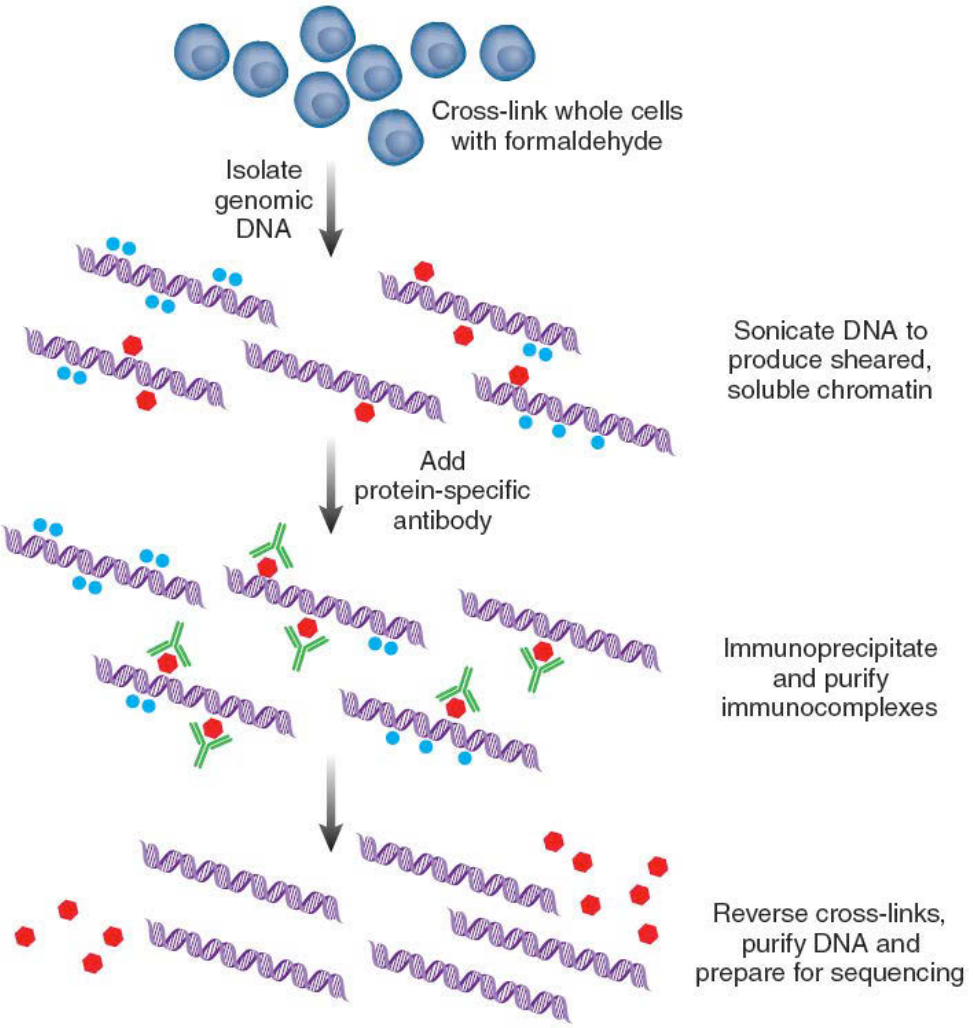
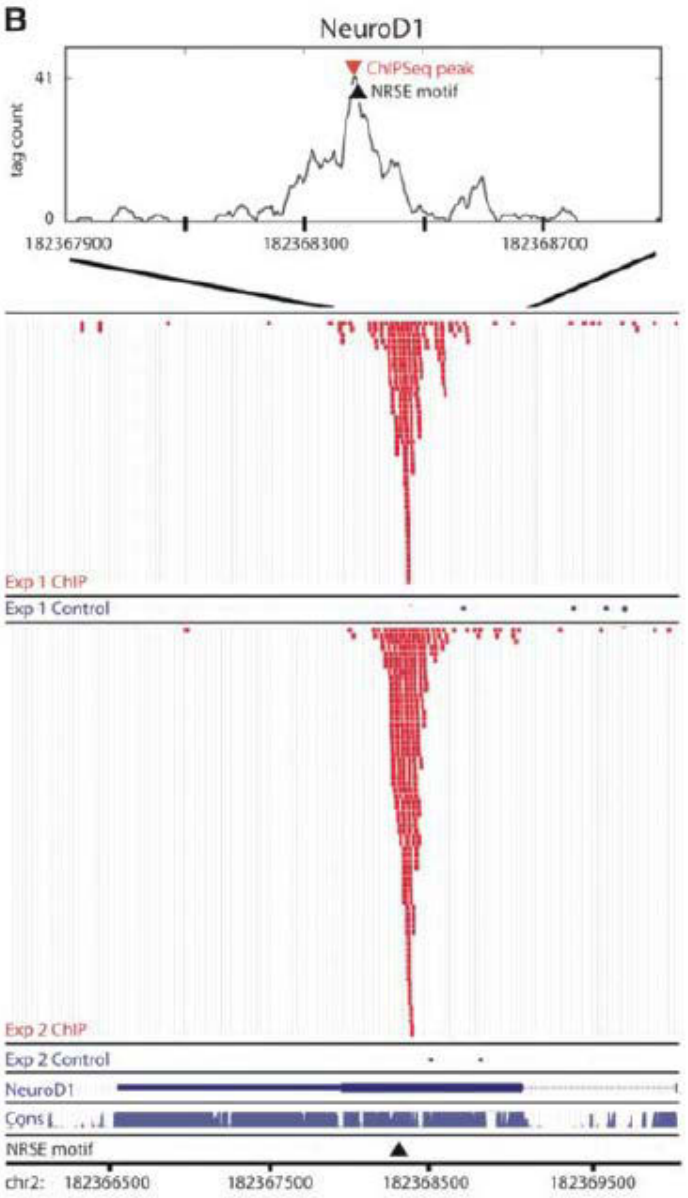
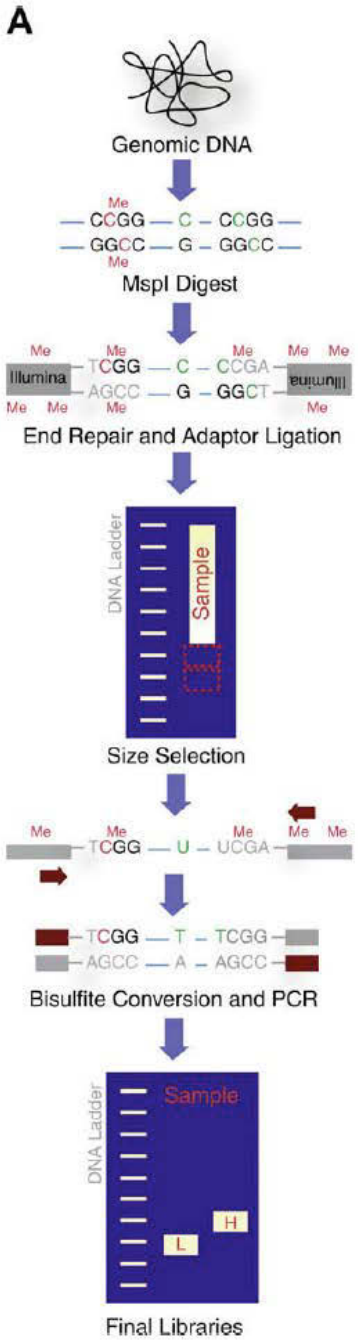
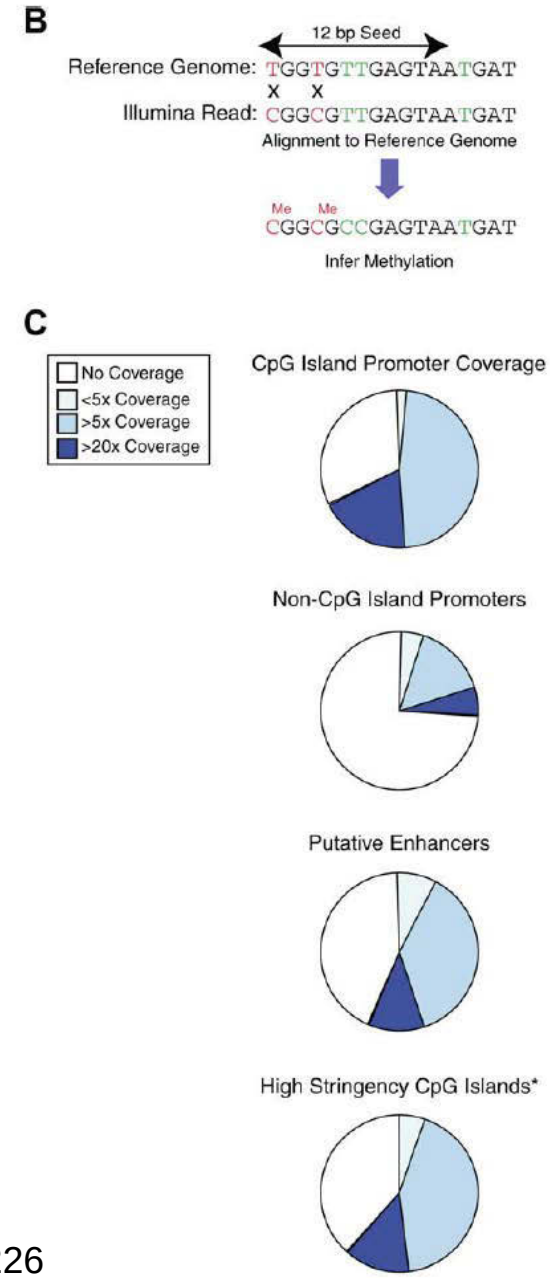


Figure 1 | Workflow of Chip-seq. DNA and proteins are cross-linked and purified; then bound DNA is analyzed by massively parallel short-read sequencing.



RRBS





Smith et al., 2009. Methods 48:226

ogenic microorganisms that literally share our body space" (Lederberg and McCray 2001). Initial efforts to determine the numbers of microbes in a community and their phylogenetic relationships comprised analyzing the relatively well conserved 16S rRNA genes in mixtures of organisms (Woese and Fox 1977; Stahl

[1]A complete list of authors and affiliations appears at the end of the paper, before the Acknowledgments section. See also, http://nihroadmap.nih.gov/hmp/members.asp.
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Article published online before print. Article and publication date are at http://www.genome.org/cgi/doi/10.1101/gr.096651.109. Freely available online through the *Genome Research* Open Access option

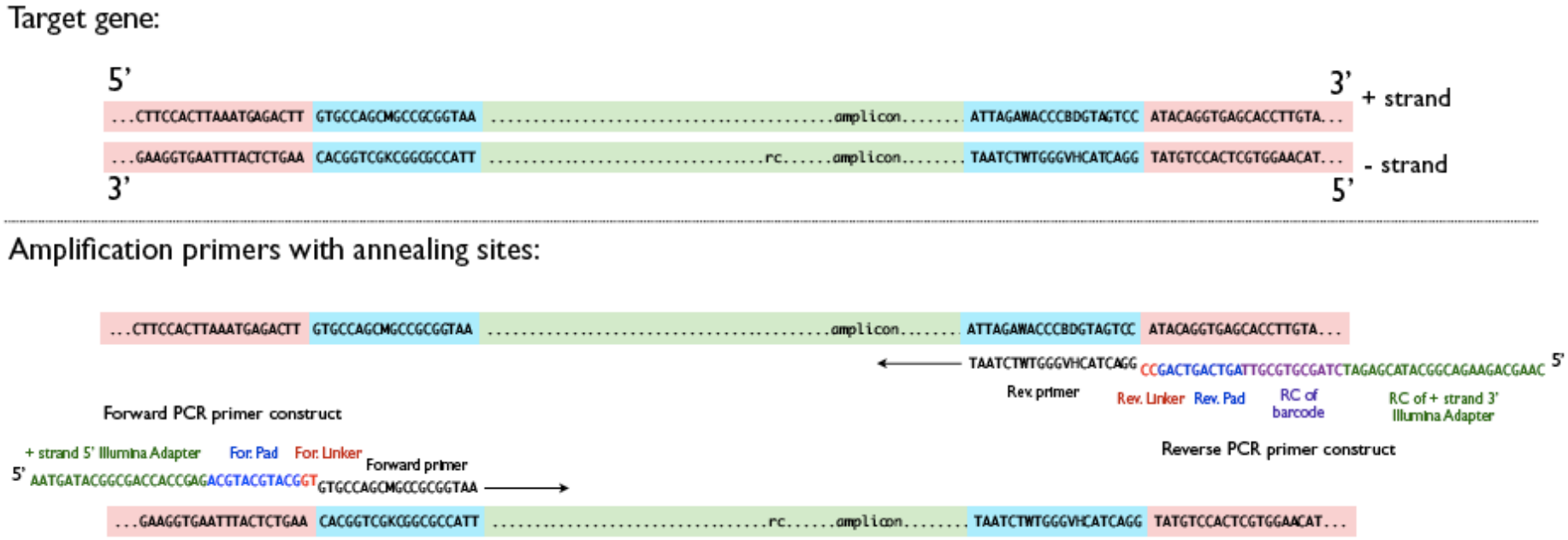
The early studies examining the microbiome stimulated i undertak ng a large s ale investigation of the human i microbiome. An international meeting was held in Par vember 2005 to disc ss su h an effort. This meeting, host French National Institute for Agricultural Research (IN chaired by Dusko Ehrlich, led to the recommendation th man Intestinal Metagenome Initiative (HIMI) be under define more completely the human intestinal microb health and disease. The meeting attendees also recommer an International Metagenome Consortium be formed together common efforts rom around the world to ac the goals of the HIMI (http //human microbiome.org).

would no longer define the biology at the site as
was done in order to reduce the number of exclu
make it, in the clinicians' opinion, possible to rec
There was concern that recruitment using a prot
volunteers who were "healthy" at each site (as
sample site experts) would have so many exclusi
recruitment would be very slow or impossible.

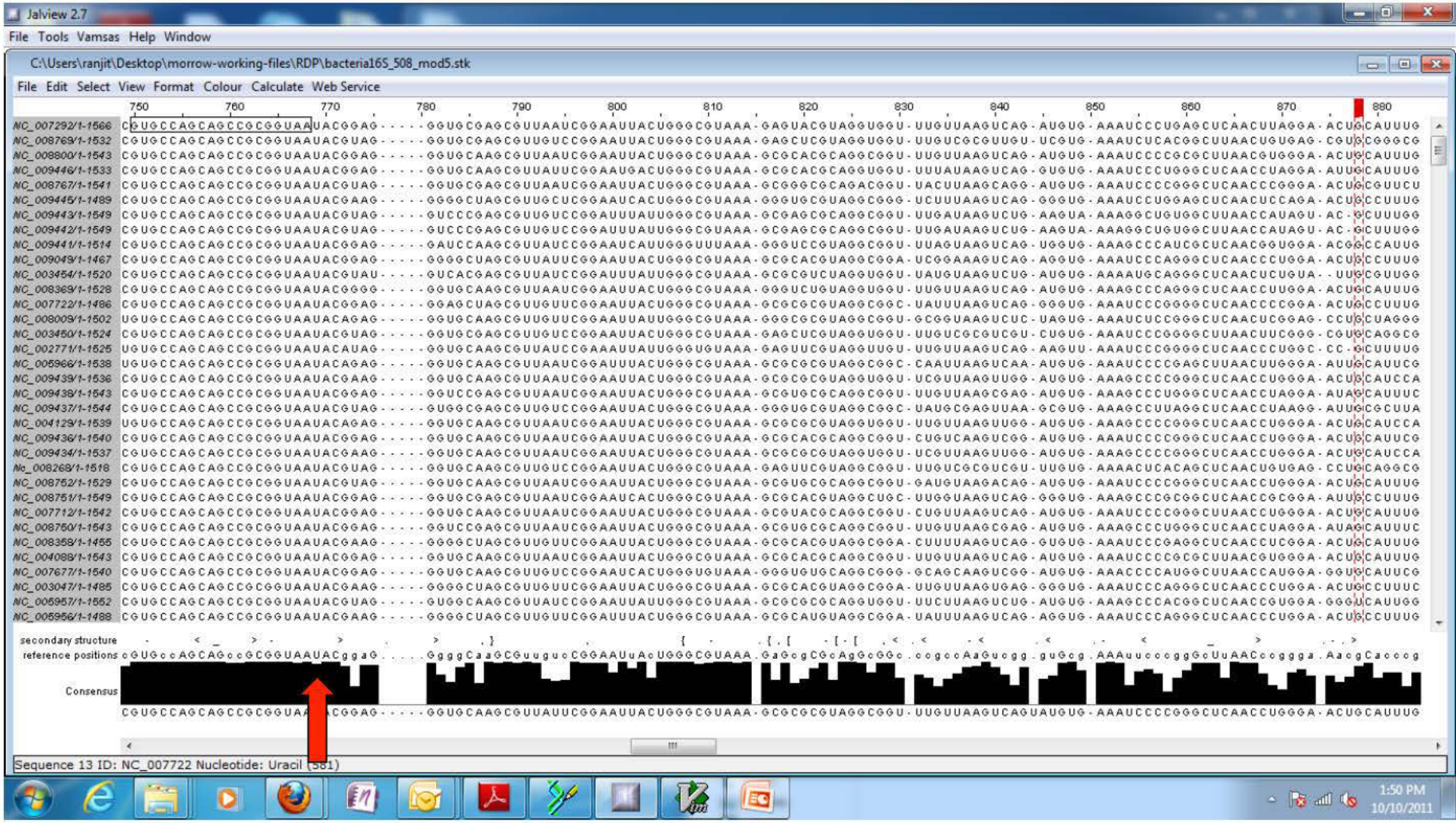
Special attention was paid to the informed cor
that potential sample donors were adequately infc
benefits and risks associated with participation in
resource" project. A template for an informed co
developed and then adapted for use at the twc
sampling took place (Baylor College of Medicine a
University; see http://hmpdacc.org/clinical.html for
Particular attention was given in the consent prc
ing donors about how their privacy would be pr
limitations of the available protections. Donors
that the microbiome data from the study of their sa

Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample

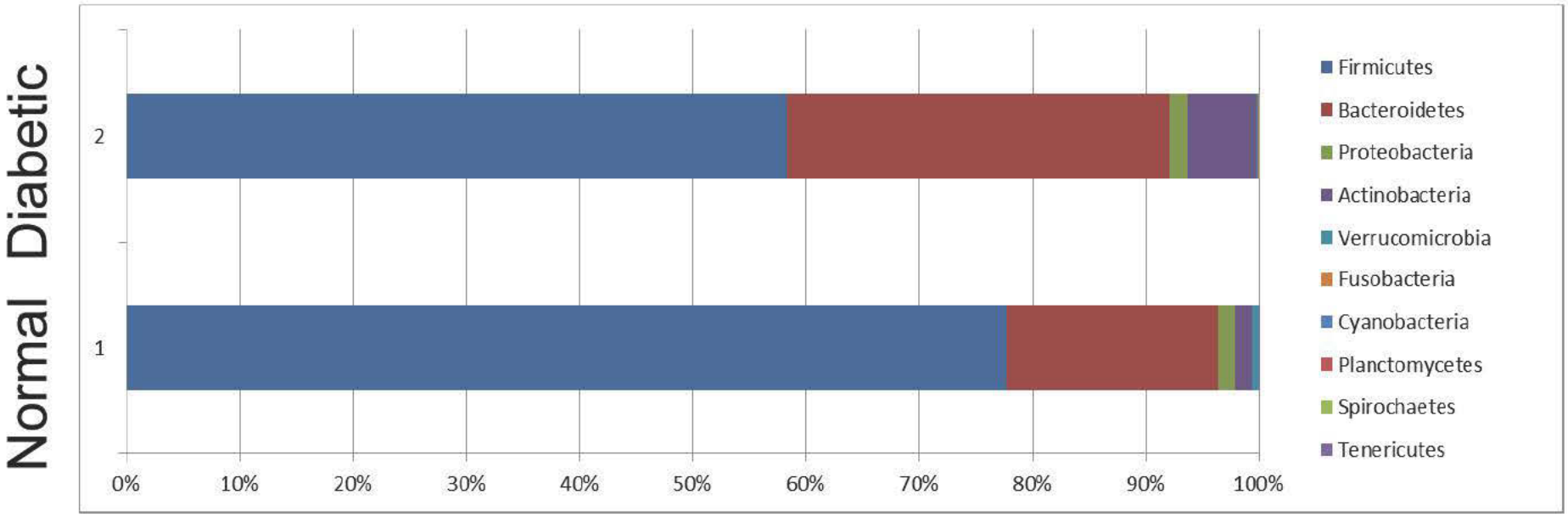
J. Gregory Caporaso[a], Christian L. Lauber[b], William A. Walters[c], Donna Berg-Lyons[b], Catherine A. Lozupone[a], Peter J. Turnbaugh[d], Noah Fierer[b,e], and Rob Knight[a,f,1]



MSA after forward primer



Microbiome at UAB



OPEN ACCESS Freely available online

PLoS one

Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults

Nadja Larsen[1]*, Finn K. Vogensen[1], Frans W. J. van den Berg[1], Dennis Sandris Nielsen[1], Anne Sofie Andreasen[2], Bente K. Pedersen[2], Waleed Abu Al-Soud[3], Søren J. Sørensen[3], Lars H. Hansen[3], Mogens Jakobsen[1]

1 Department of Food Science, University of Copenhagen, Frederiksberg, Denmark, 2 Department of Infectious Diseases and CMRC, University Hospital Rigshospitalet, Copenhagen, Denmark, 3 Department of Biology, University of Copenhagen, Copenhagen, Denmark

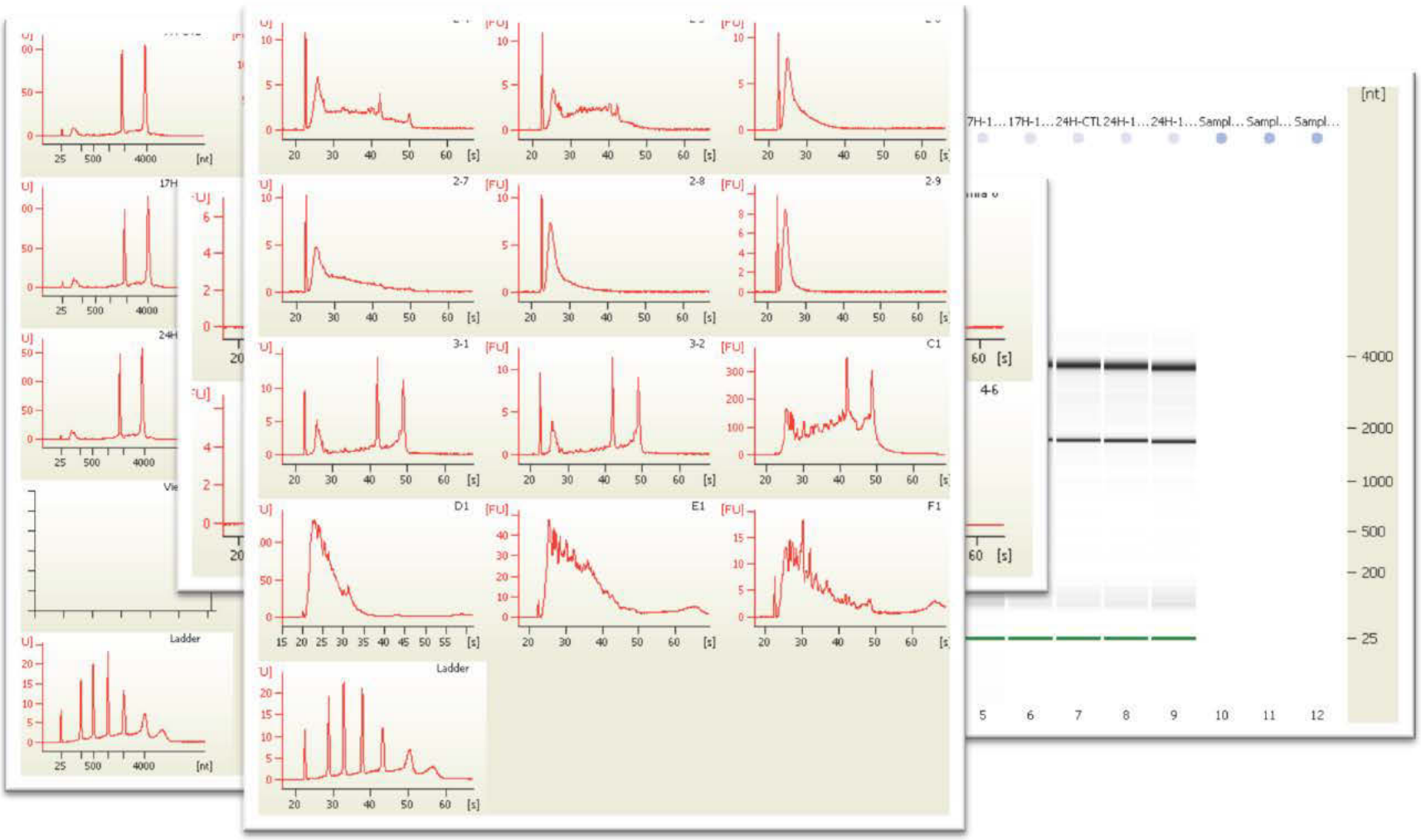
The proportions of phylum Firmicutes and class Clostridia were significantly reduced in the diabetic group compared to the control group ($P = 0.03$).

Sequencing RNA

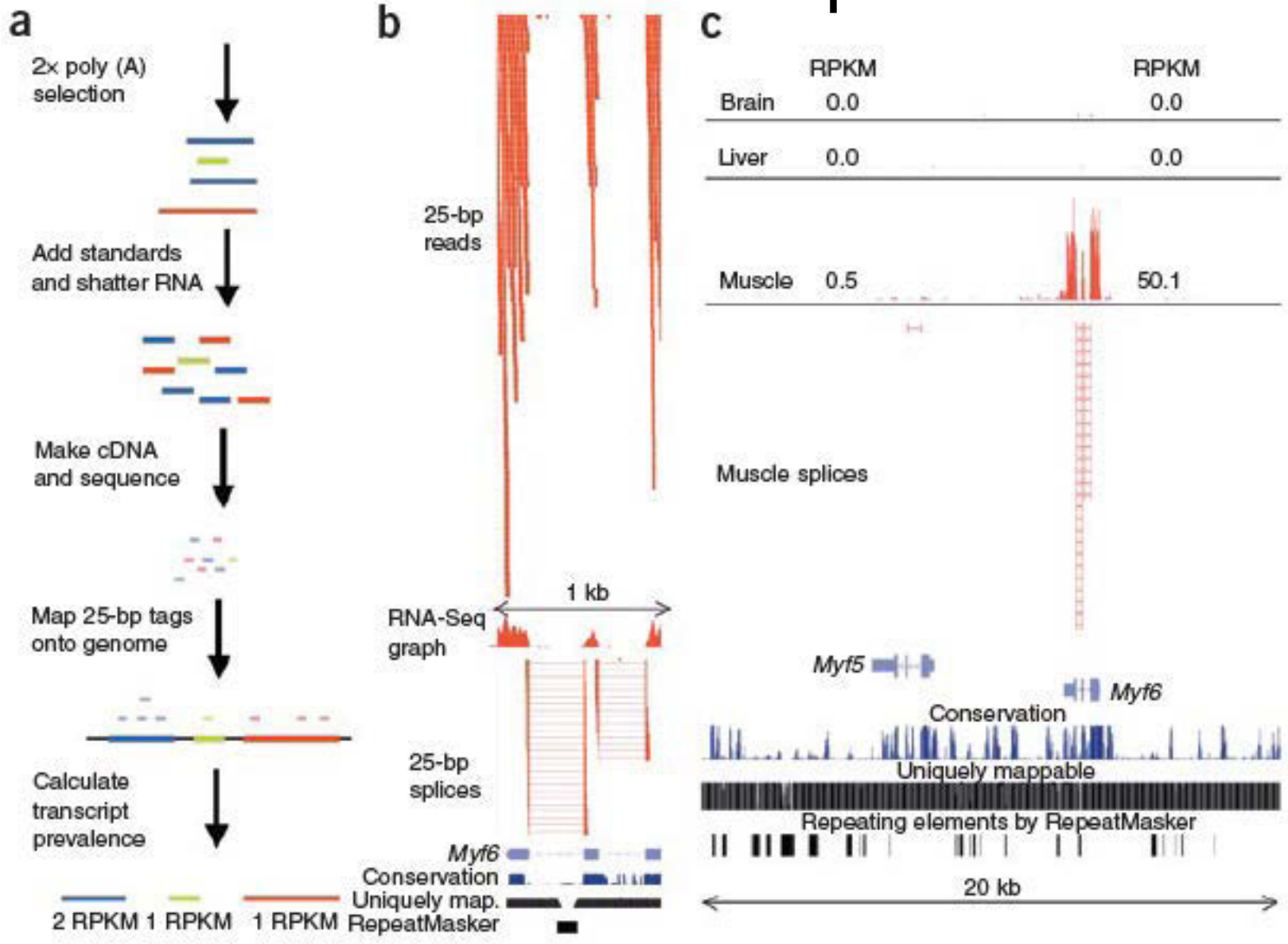
RNA Applications

- mRNA Sequencing (RefSeq, RNASeq)
- microRNA Sequencing
- RNA-IP-Sequencing

RNA Quality



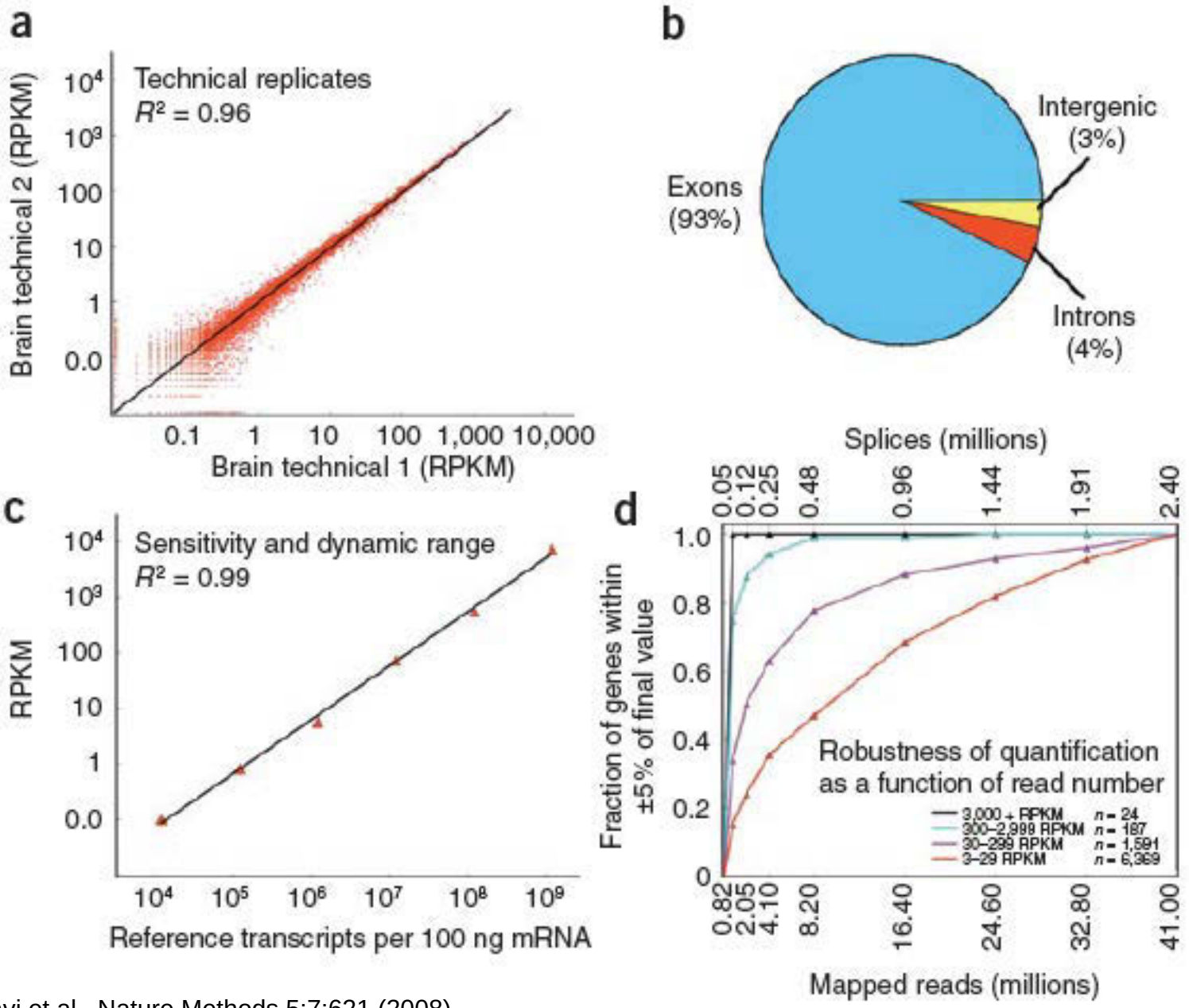
mRNA-Seq



Mortazavi et al., Nature Methods 5:7:621 (2008)

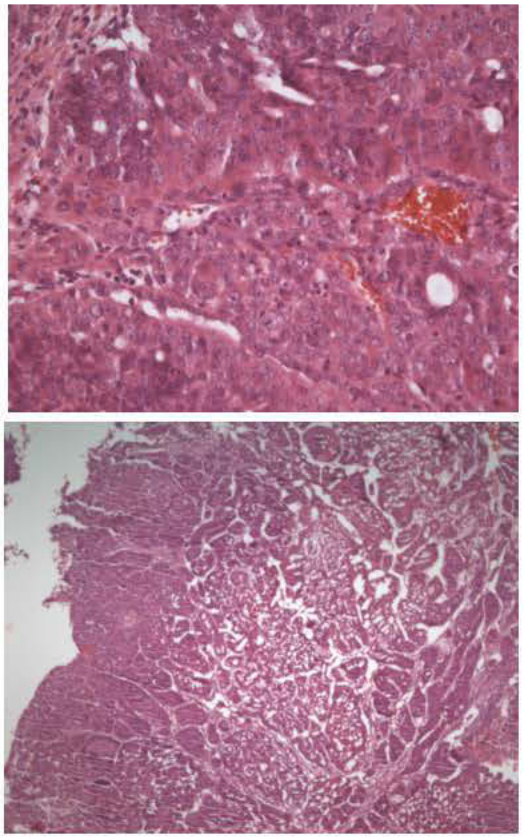
RPKM: reads per kilobase of exon model per million mapped reads

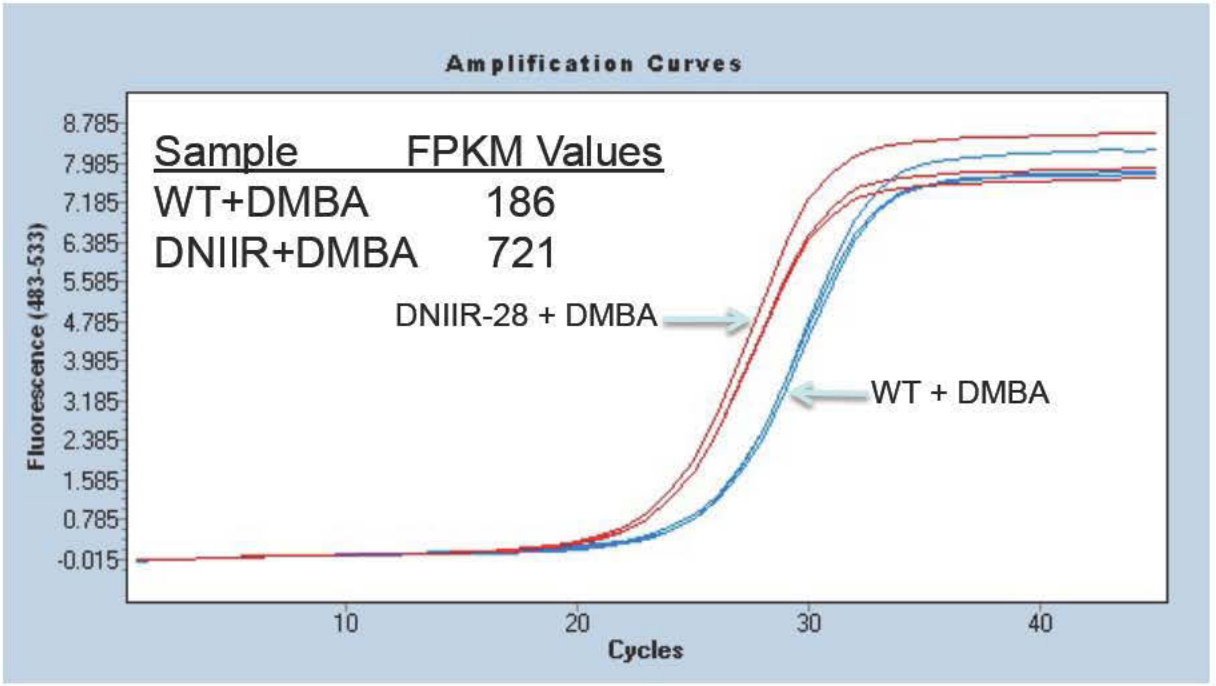
FPKM: fragment of reads per kilobase of exon model per million mapped reads (usually 25bp fragments).



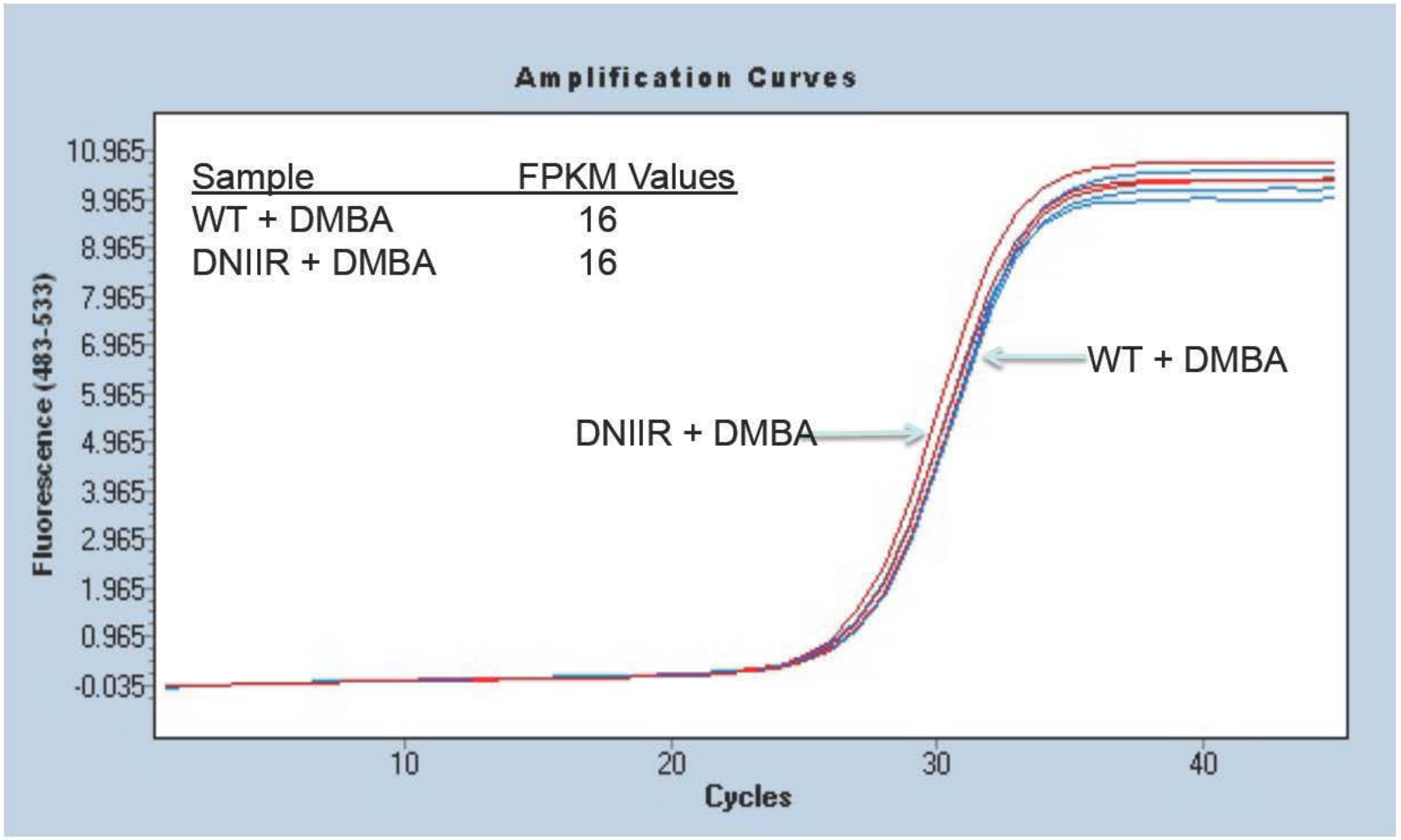
Mortazavi et al., Nature Methods 5:7:621 (2008)

Keratin 8

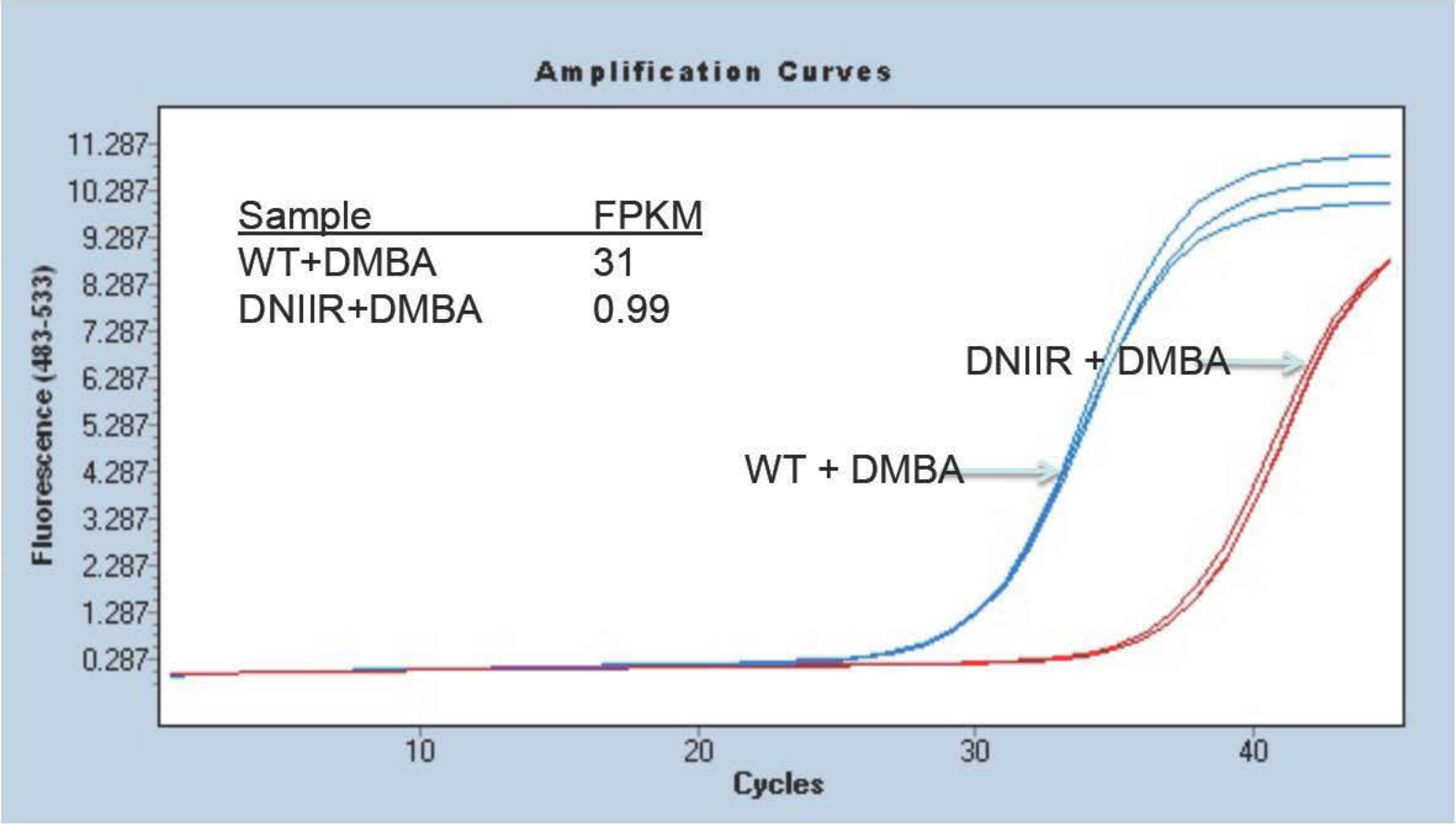



Amplification Curves
Sample FPKM Values
WT+DMBA 186
DNIIR+DMBA 721
DNIIR-28 + DMBA
WT + DMBA
Fluorescence (483-533)
8.785
7.985
7.185
6.385
5.585
4.785
3.985
3.185
2.385
1.585
0.785
-0.015
10
20
30
40
Cycles

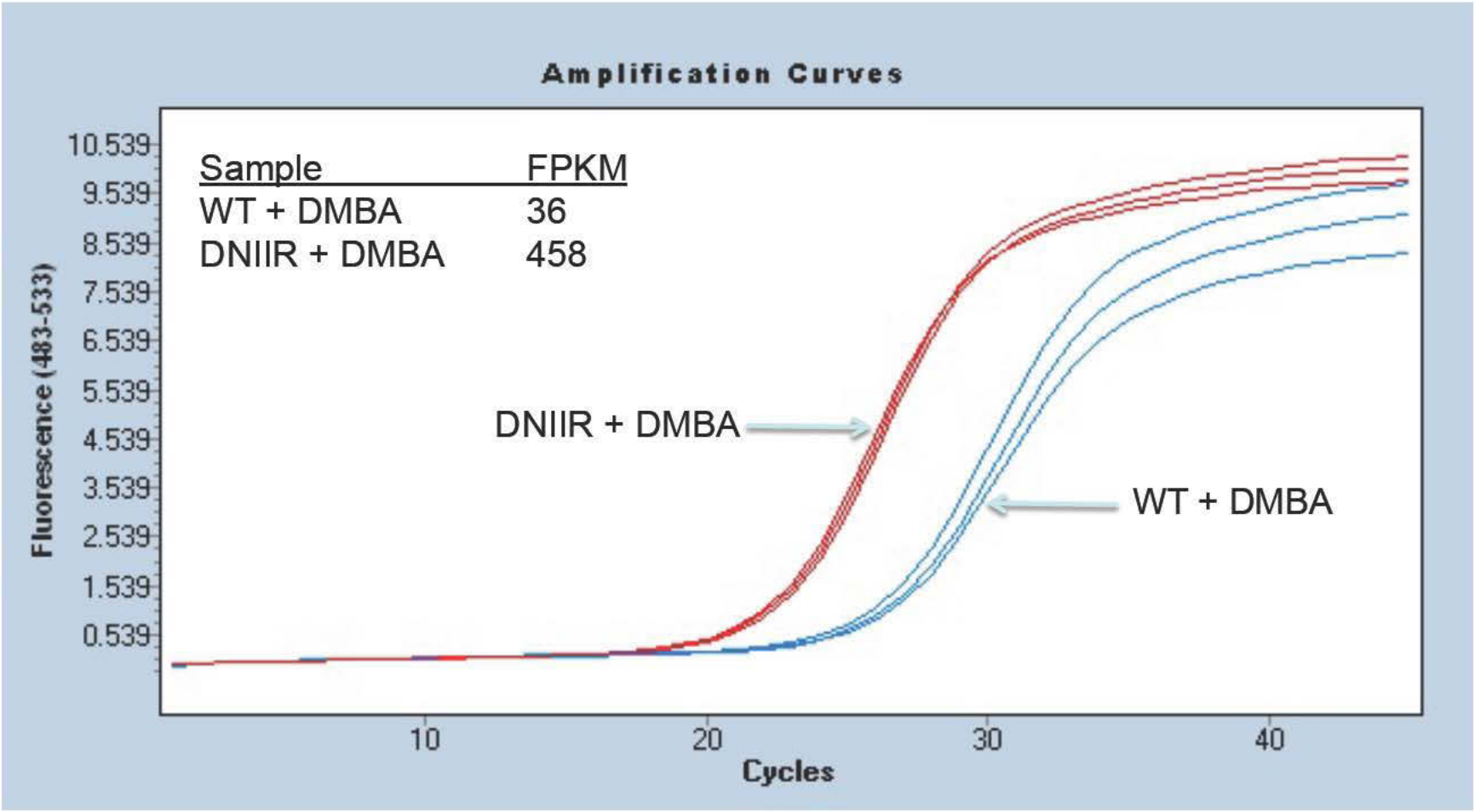
Lipase Maturation Factor 1 (Lmf1)



Lysophosphatidic acid receptor 3

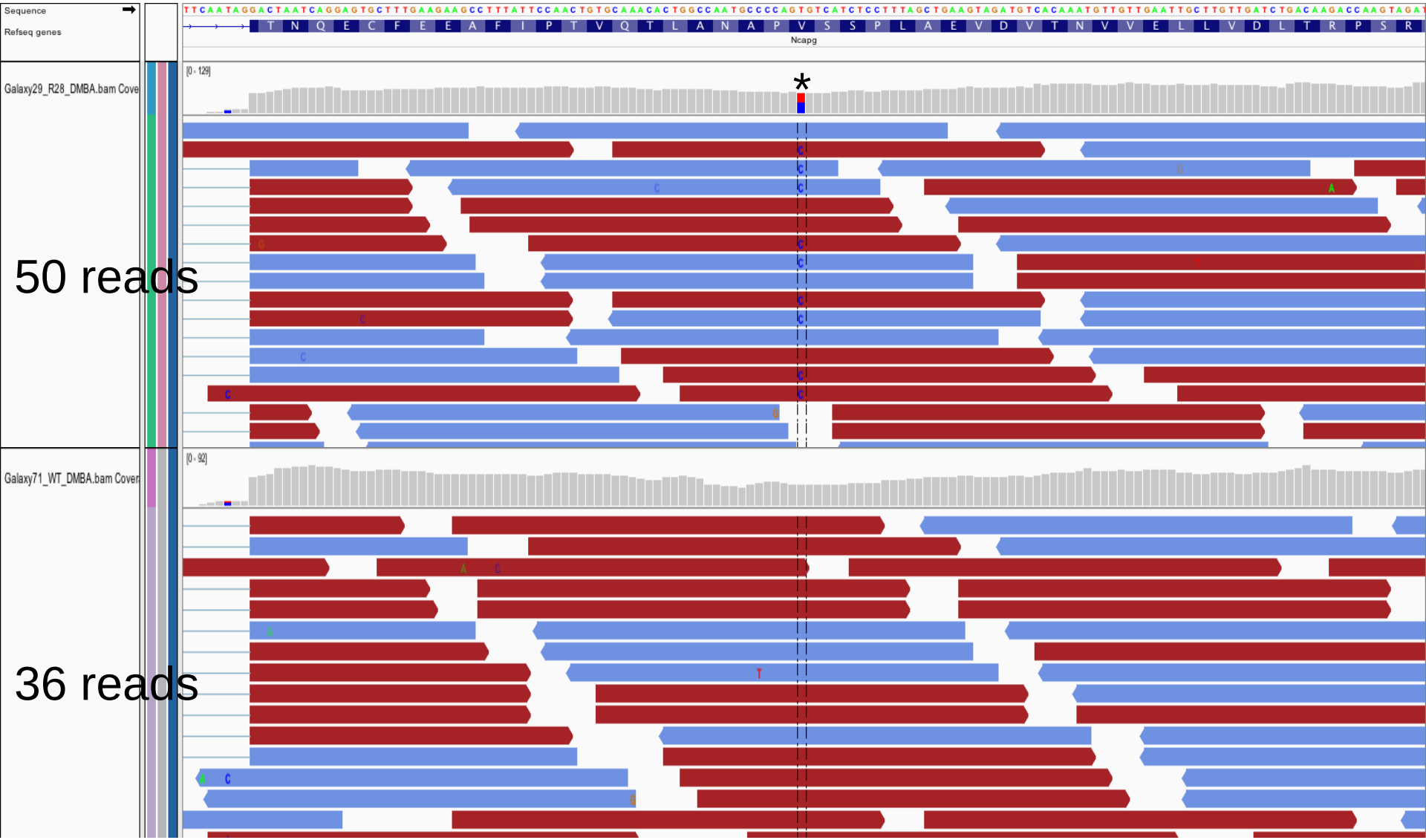


Insulin like growth factor binding protein 3 (Igfbp3)



Ncapg: Non-SMC condensin I complex, subunit G

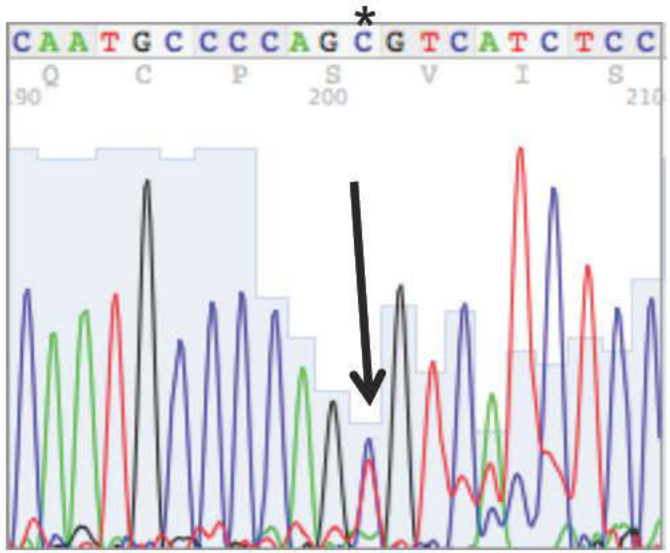
Exon 16 of Ncapg



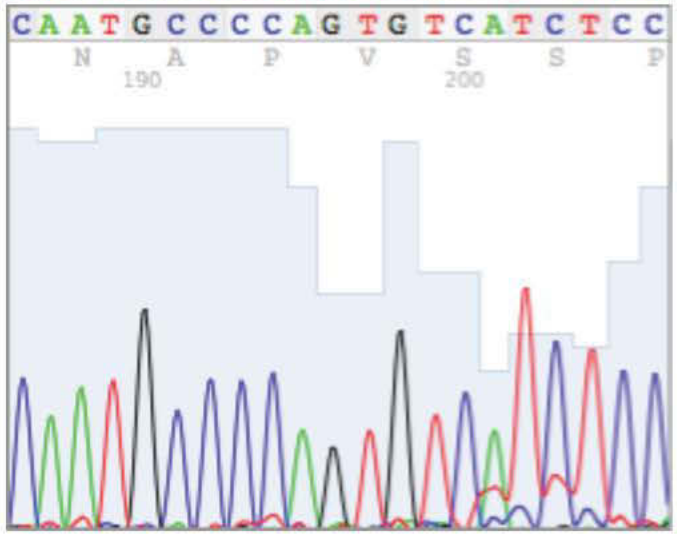
T-C mutation resulting in a Val-Ala change in the protein

Sequence Confirmation of Ncapg mutation

DNIIR

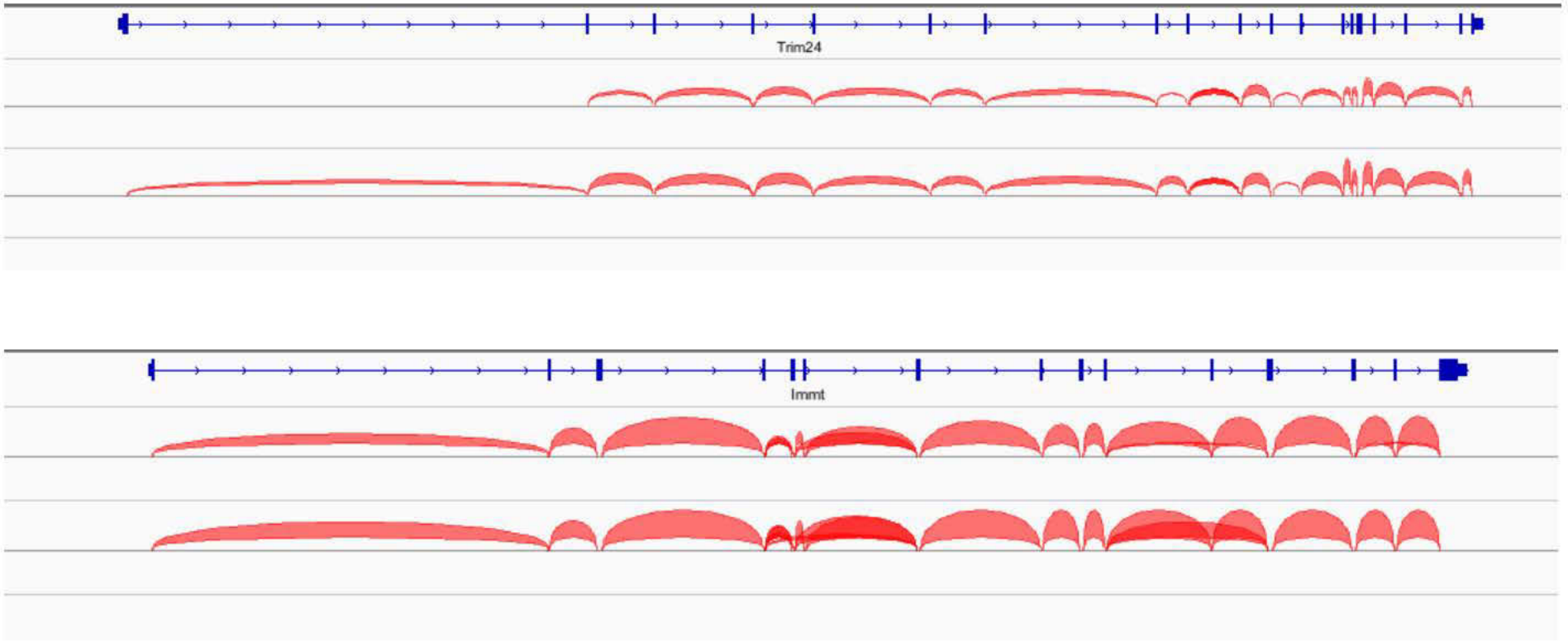


WT

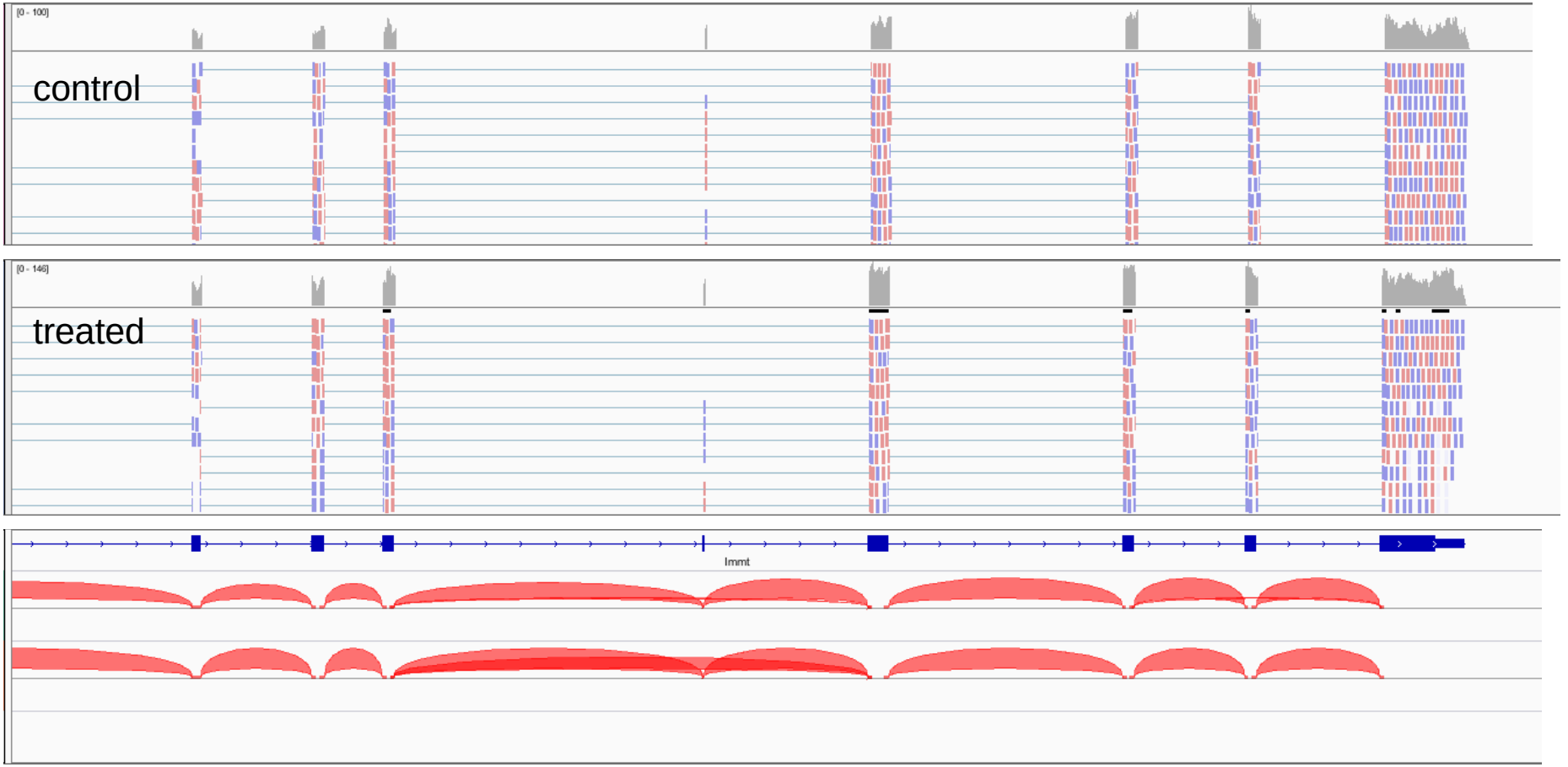


T>C mutation resulting in an Ala>Val change at position aa784 in the protein. The other mutations were a polymorphic T>C change at aa242 and an A>G change at aa347 resulting in a non-synonymous change from Arg>Lys.

Alternative Exon Usage



Alternative splicing



Summary

- Several different platforms exist utilizing different technologies.
- Generate between 500 million to 600 Billion bases of sequence information per run.
- Several applications including Whole genome sequencing, Targeted genomic seq., ChIP-Seq and mRNA-Seq, among others.
- Data files are very large ≥1Tb of information.
- Personalized medicine via genome sequencing is not far away.