DISCLOSURE:

nothing to declare

Roberta Maestro
CRO Aviano

ESMO
GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

NEXT GENERATION SEQUENCING

a zoom on sarcoma genetics/genomics

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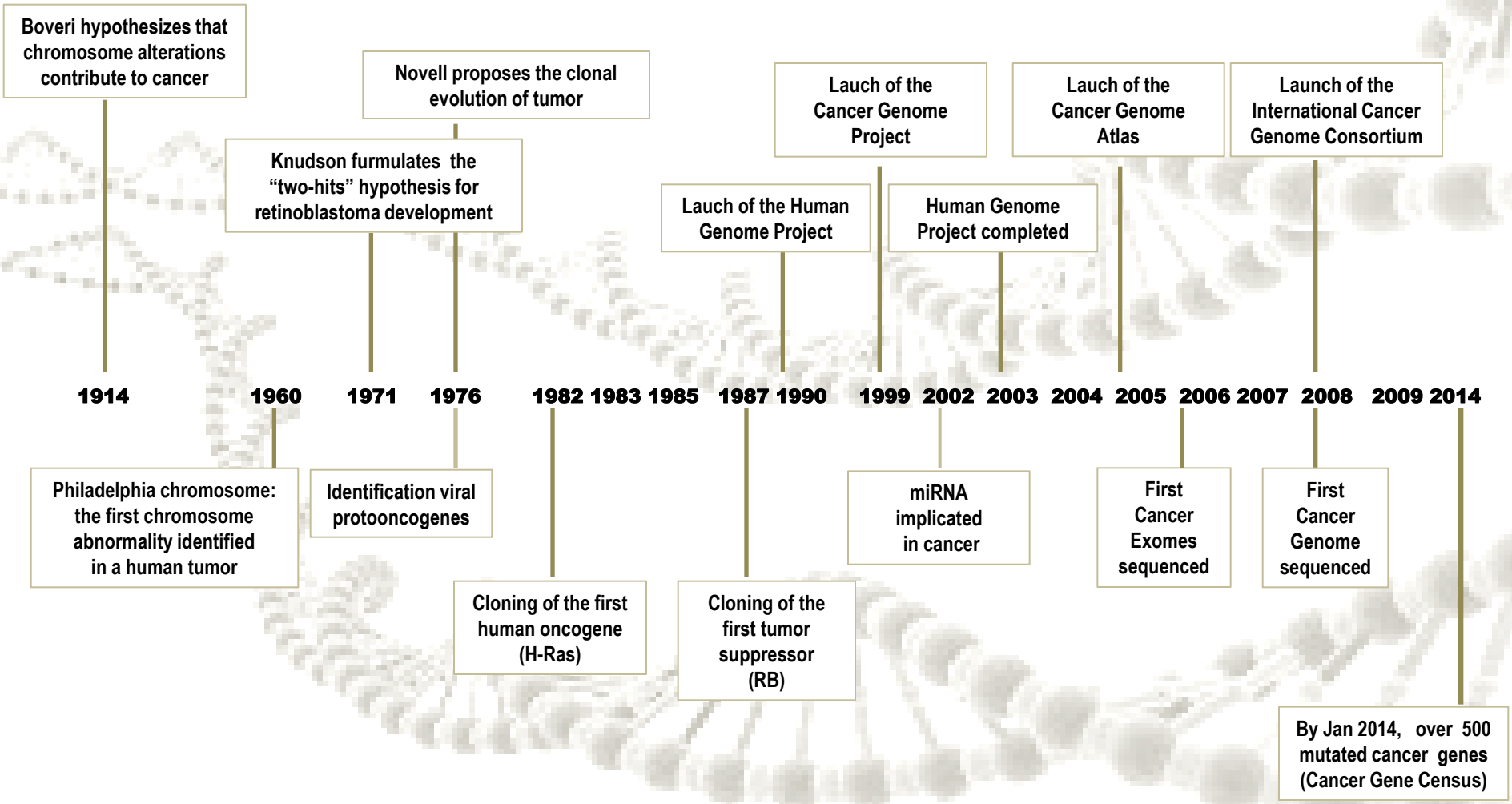
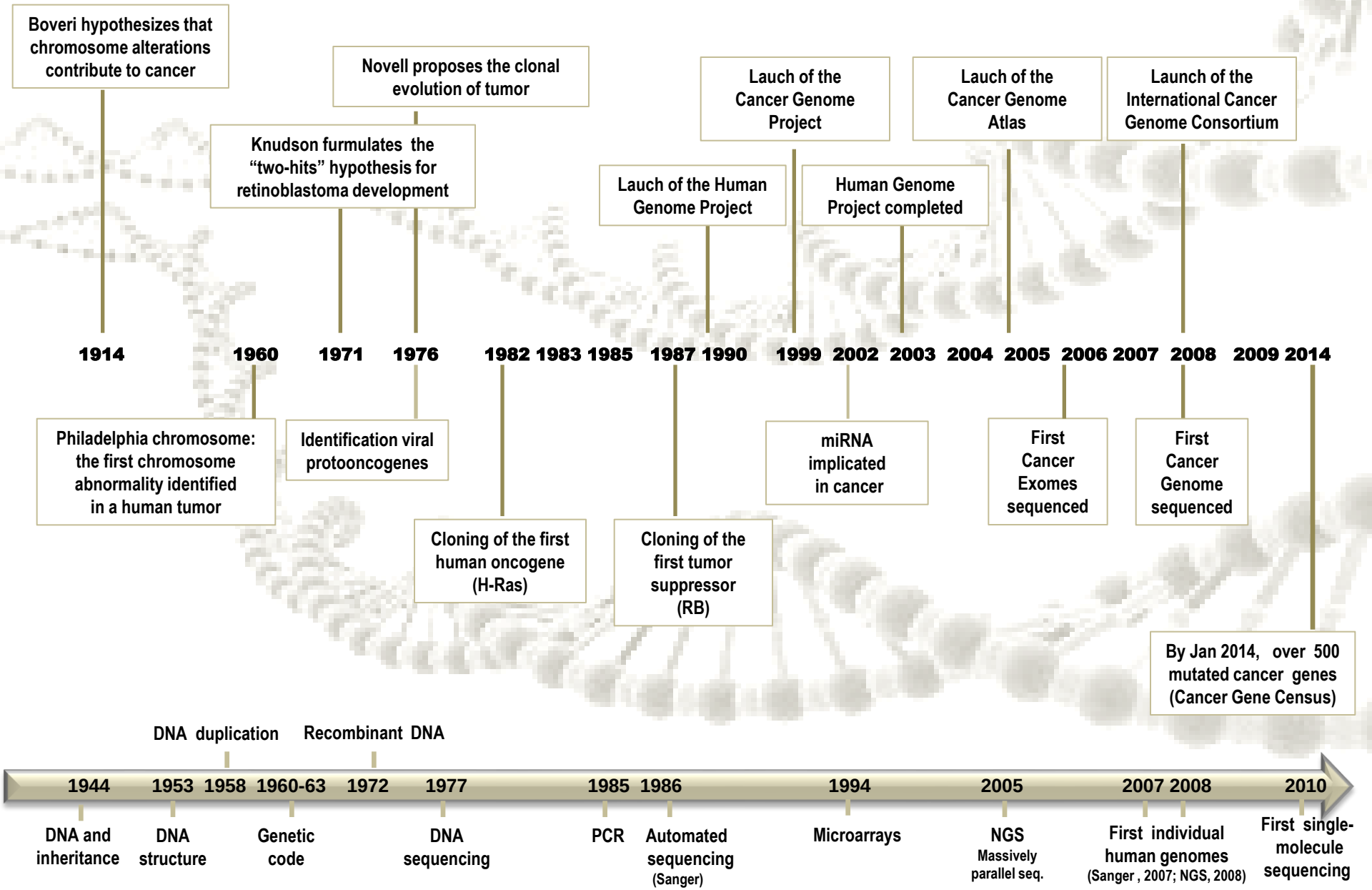
BEST PRACTICE

100 YEARS OF CANCER GENETICS



100 YEARS OF CANCER GENETICS



Cancer Genome Consortia

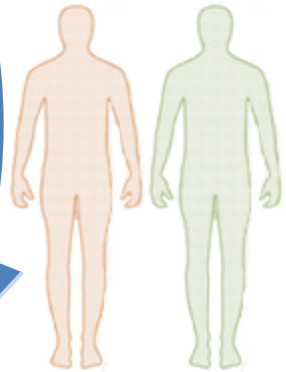
OVERALL GOALS

- characterization of the whole spectrum of genomic/genetic alterations
  within single tumors and across tumor types
- discrimination btw relevant and irrelevant mutations (drivers vs passengers)
- identification of prominent pathways involved in cancer
- identification of patterns that underpin specific cancer phenotypes
  (clonal evolution, histology, aggressiveness, resistance/sensitivity to therapies …)
- identification of potentially "actionable" molecules and mutation moieties
- pave the way to individualized treatments
  based on the genetic portrait of tumor and patient

Cycle of personalized cancer medicine

Genetic/genomic/epigenetic profile

Patient risk stratification
Personalized treatment



Further investigations

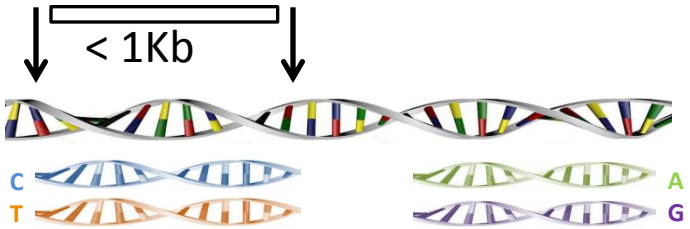
Improved diagnostic, prognostic therapeutic, monitoring approaches

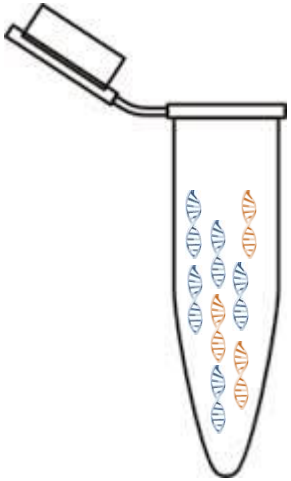
The NGS REVOLUTION

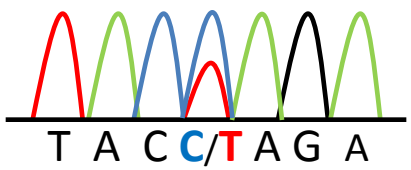
How did it start and where are we now?

1st generation sequencing

Sanger Sequencing

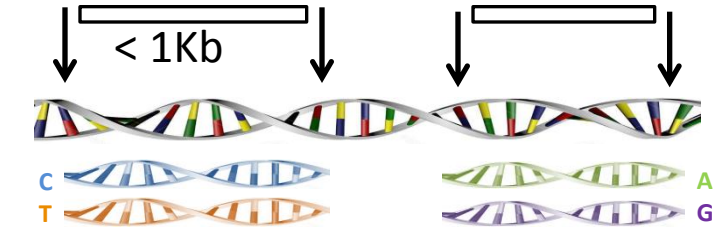


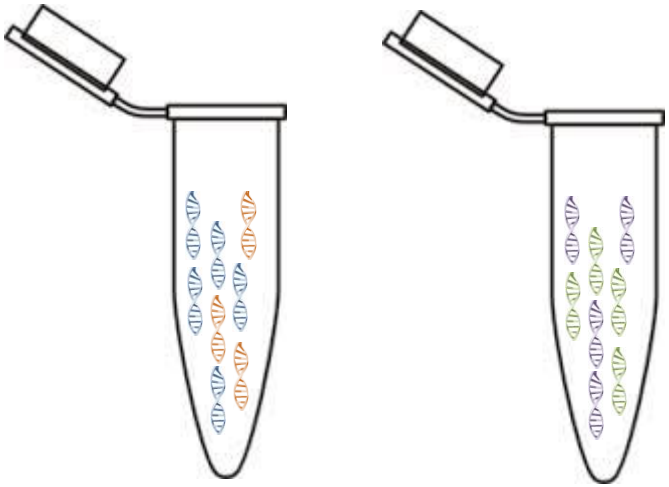


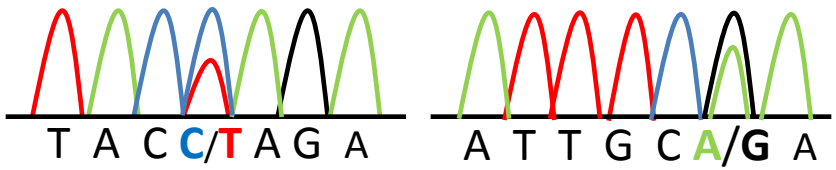


1st generation sequencing

Sanger Sequencing







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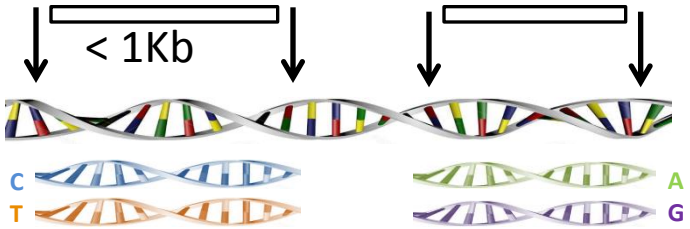
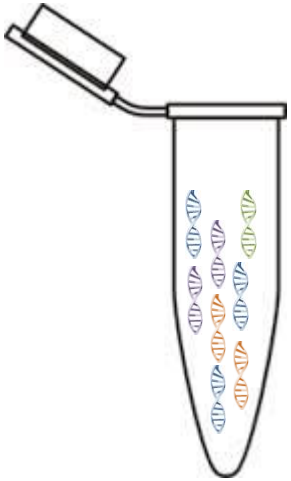
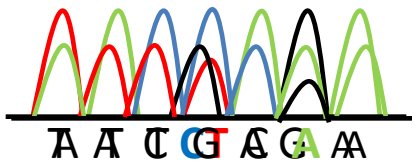
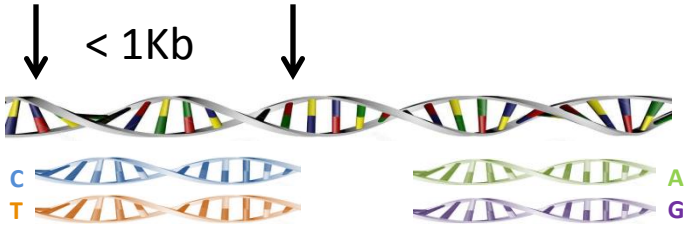
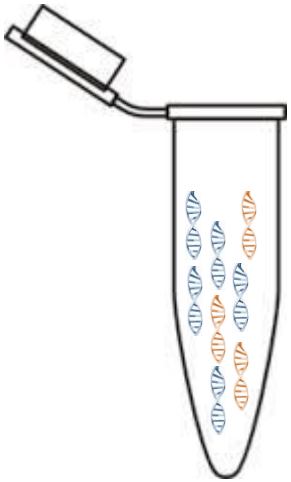


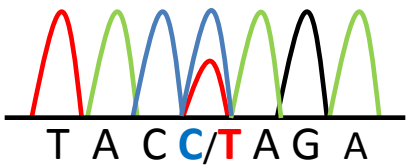




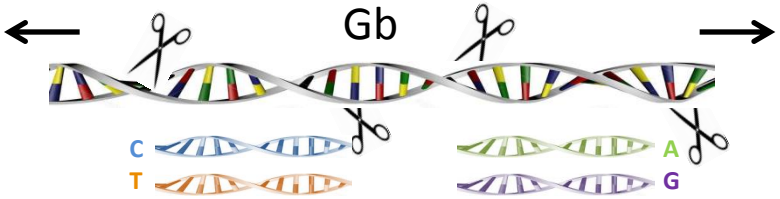
1st generation sequencing
Sanger Sequencing

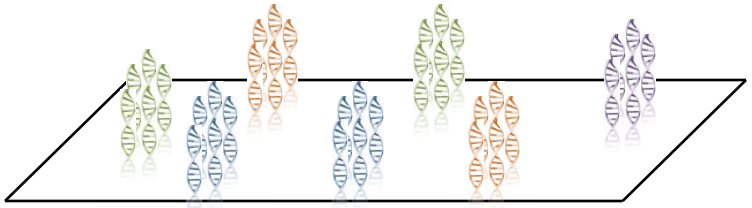




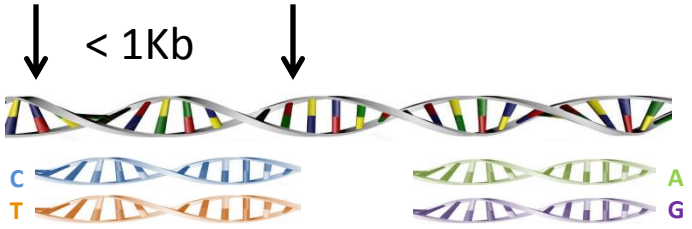


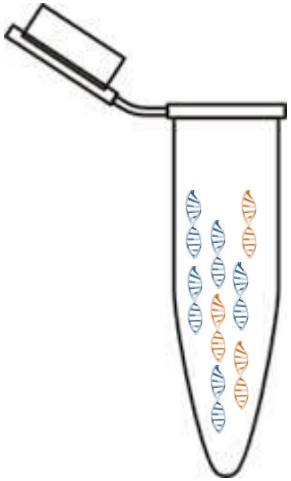
2nd generation sequencing
Massively Parallel Sequencing

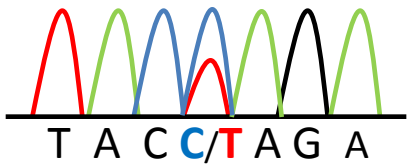




1st generation sequencing
Sanger Sequencing







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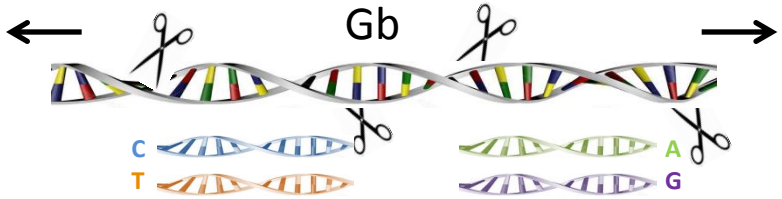

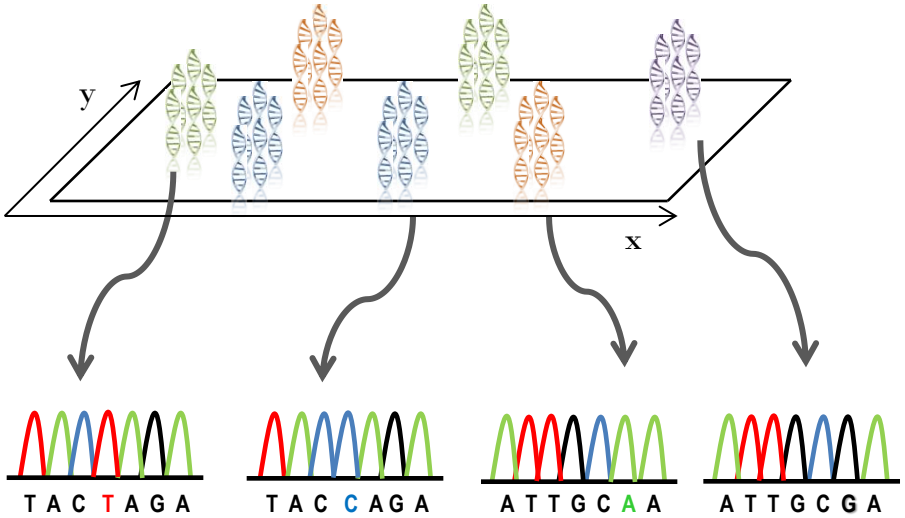

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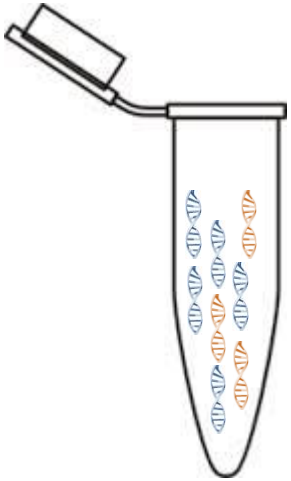

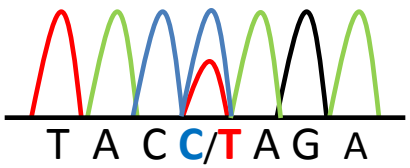

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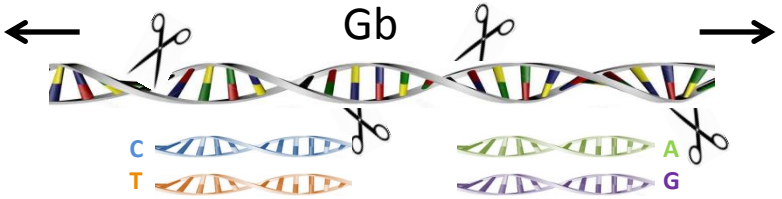
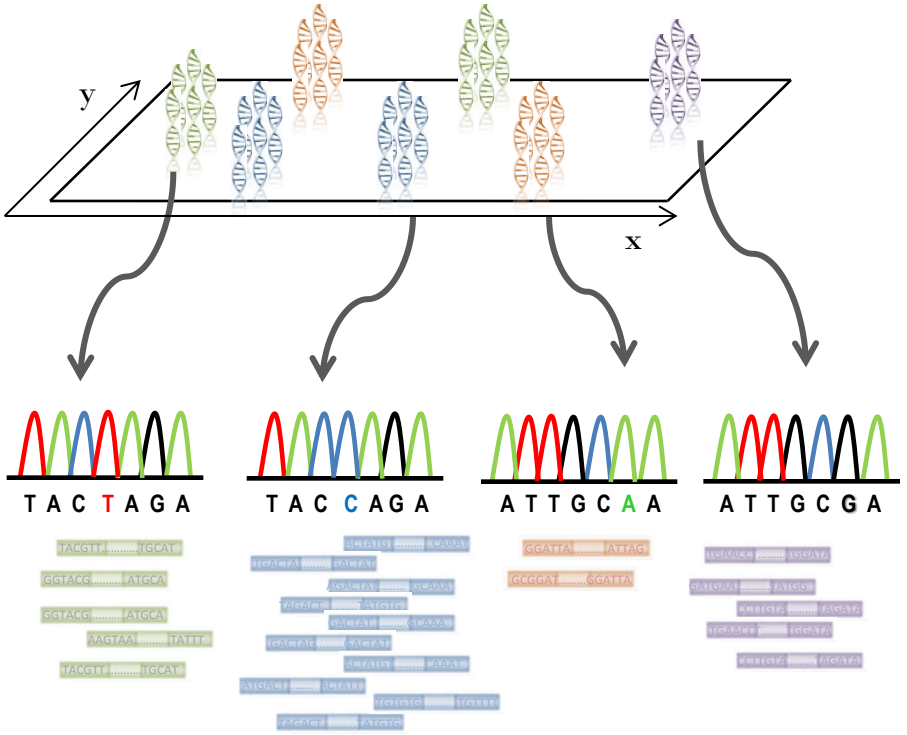
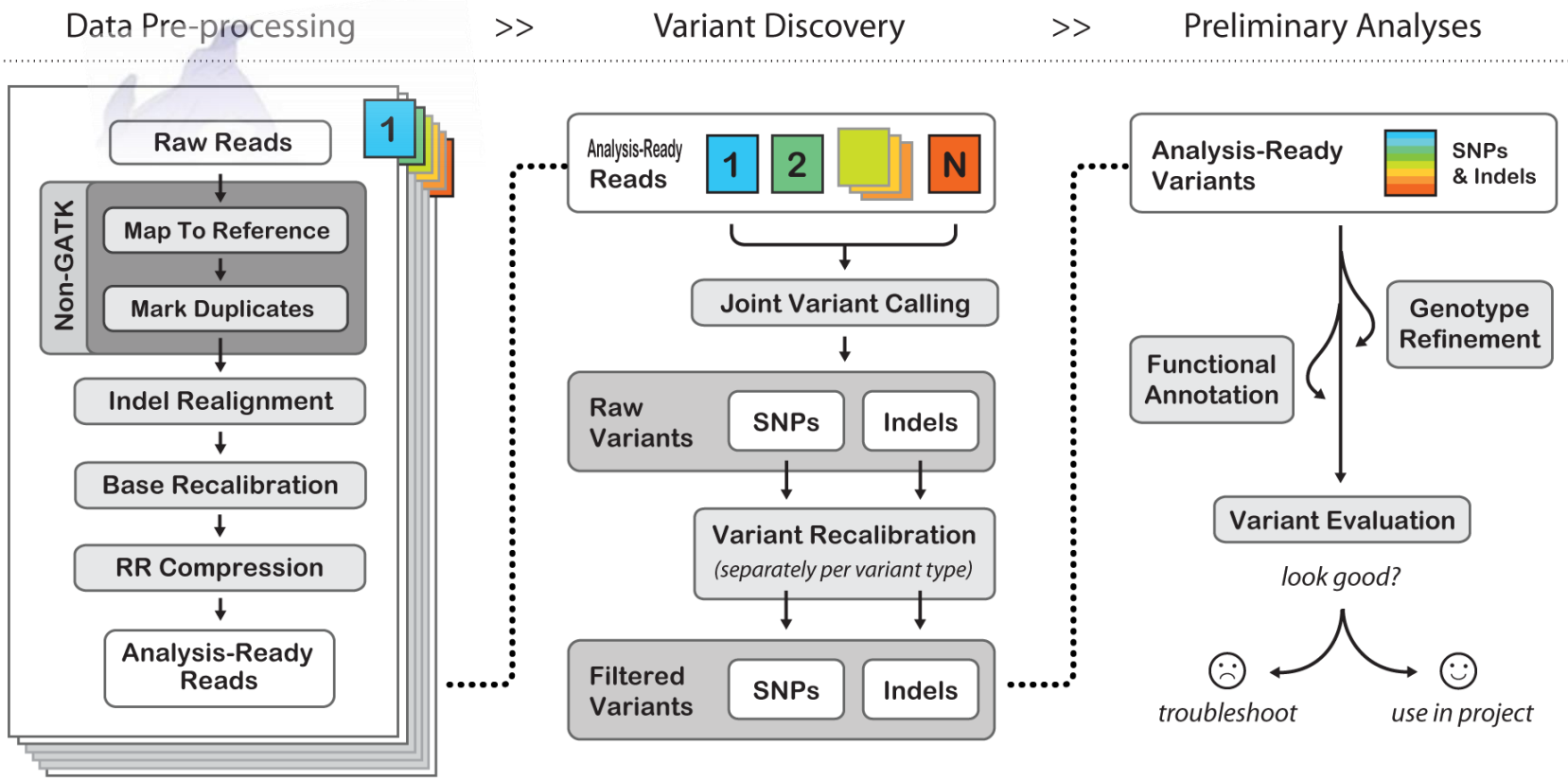


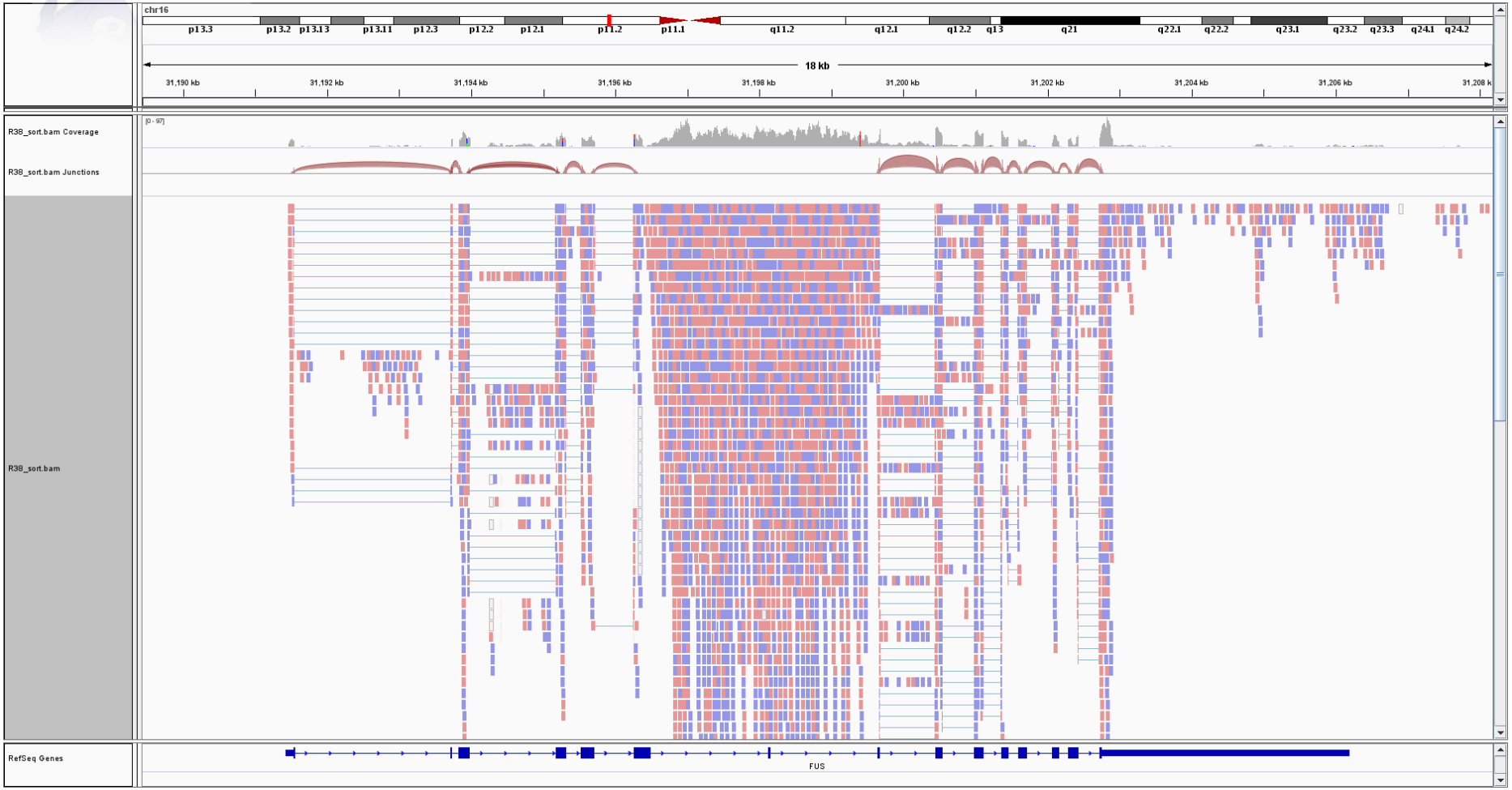


Bioinformagician...



Example of NGS analysis pipeline for mutation detection, From GATK pipeline, Broad Inst

ALIGNMENT TO THE REFERENCE GENOME



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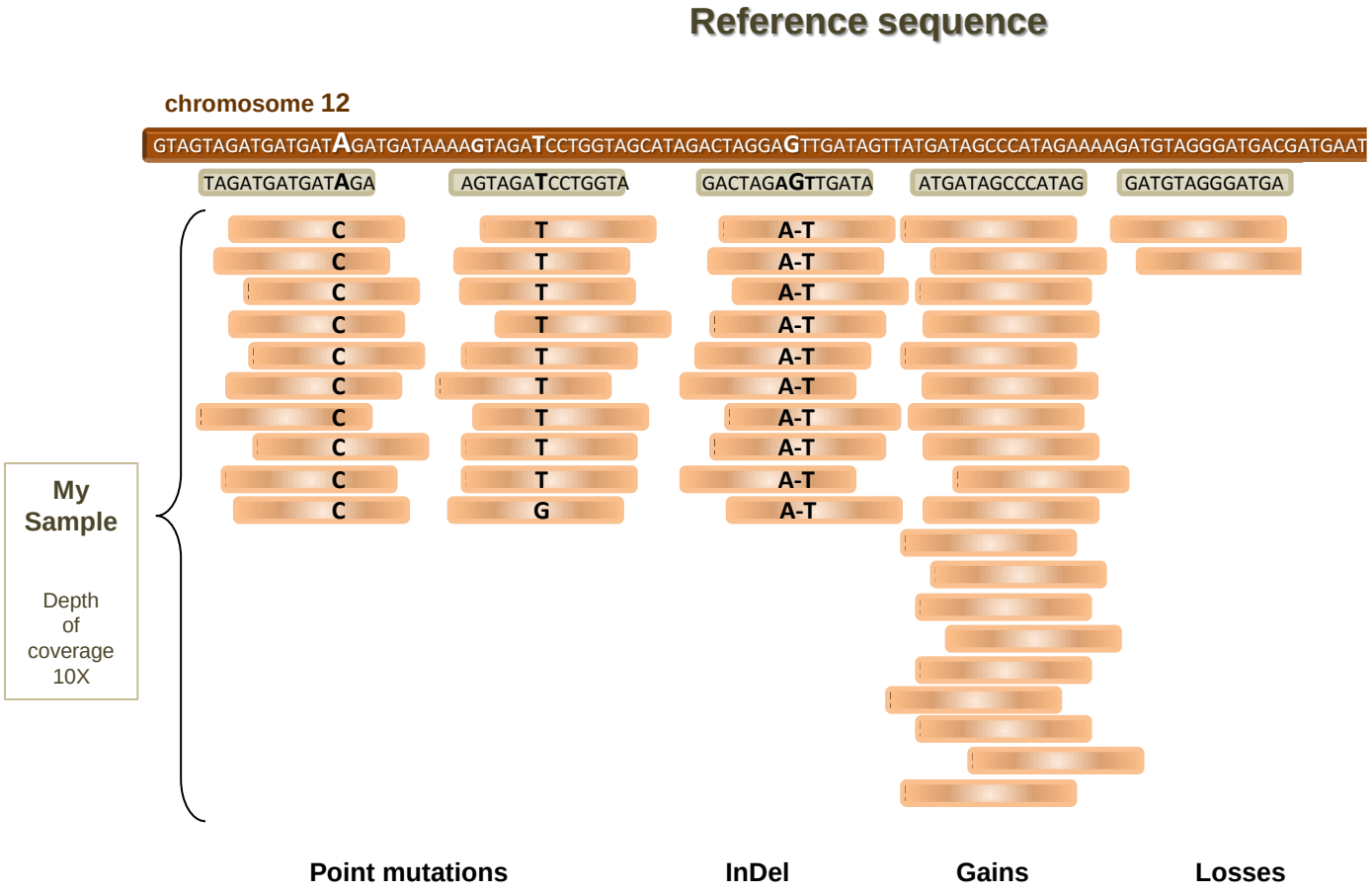

Depth of coverage The average number of times that a particular nucleotide is represented in a collection of random raw sequences.

Fold Coverage (Nr of reads* read length)/ target size

Instrinsic instrument error ~ 0.1%

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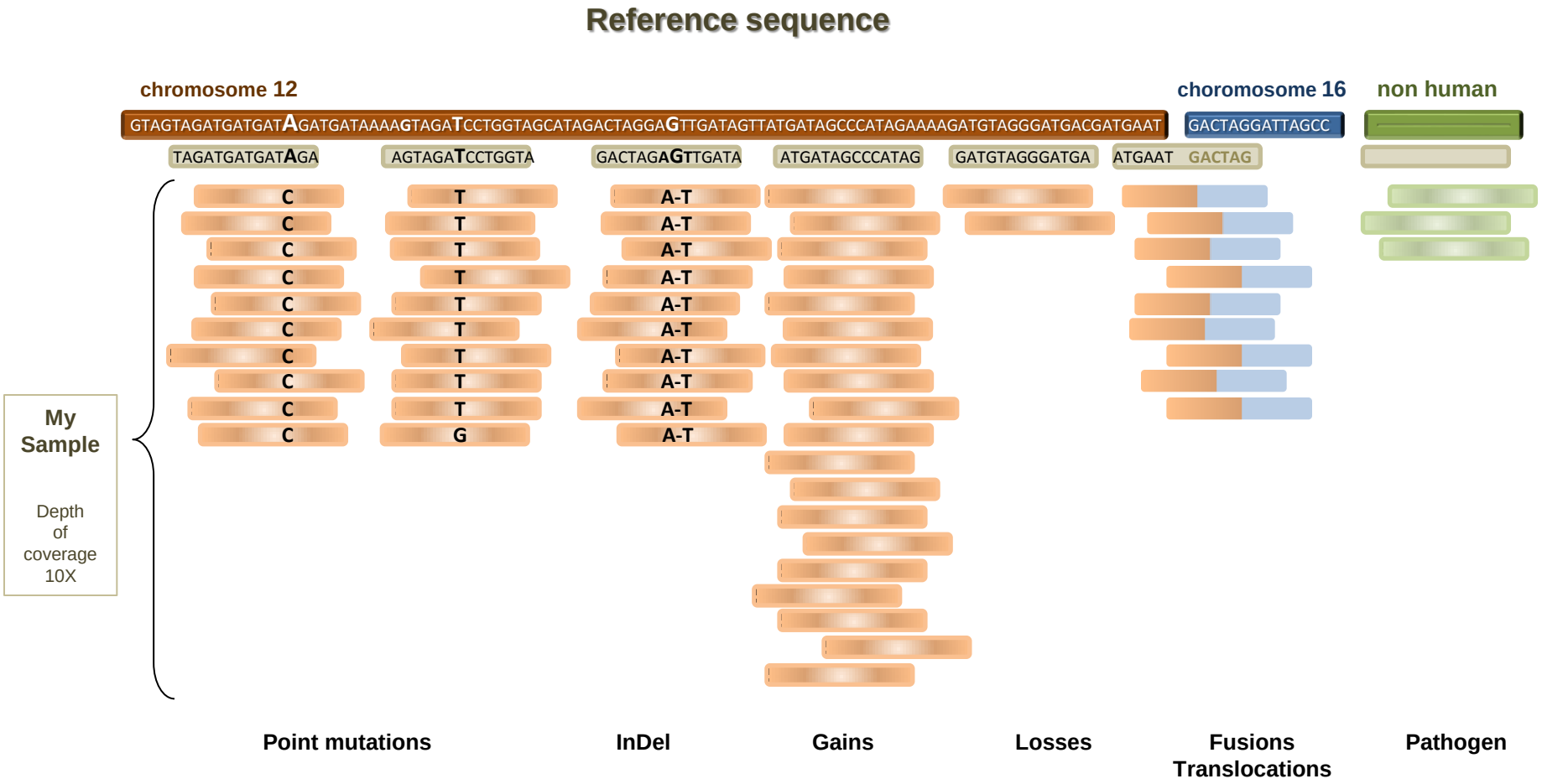

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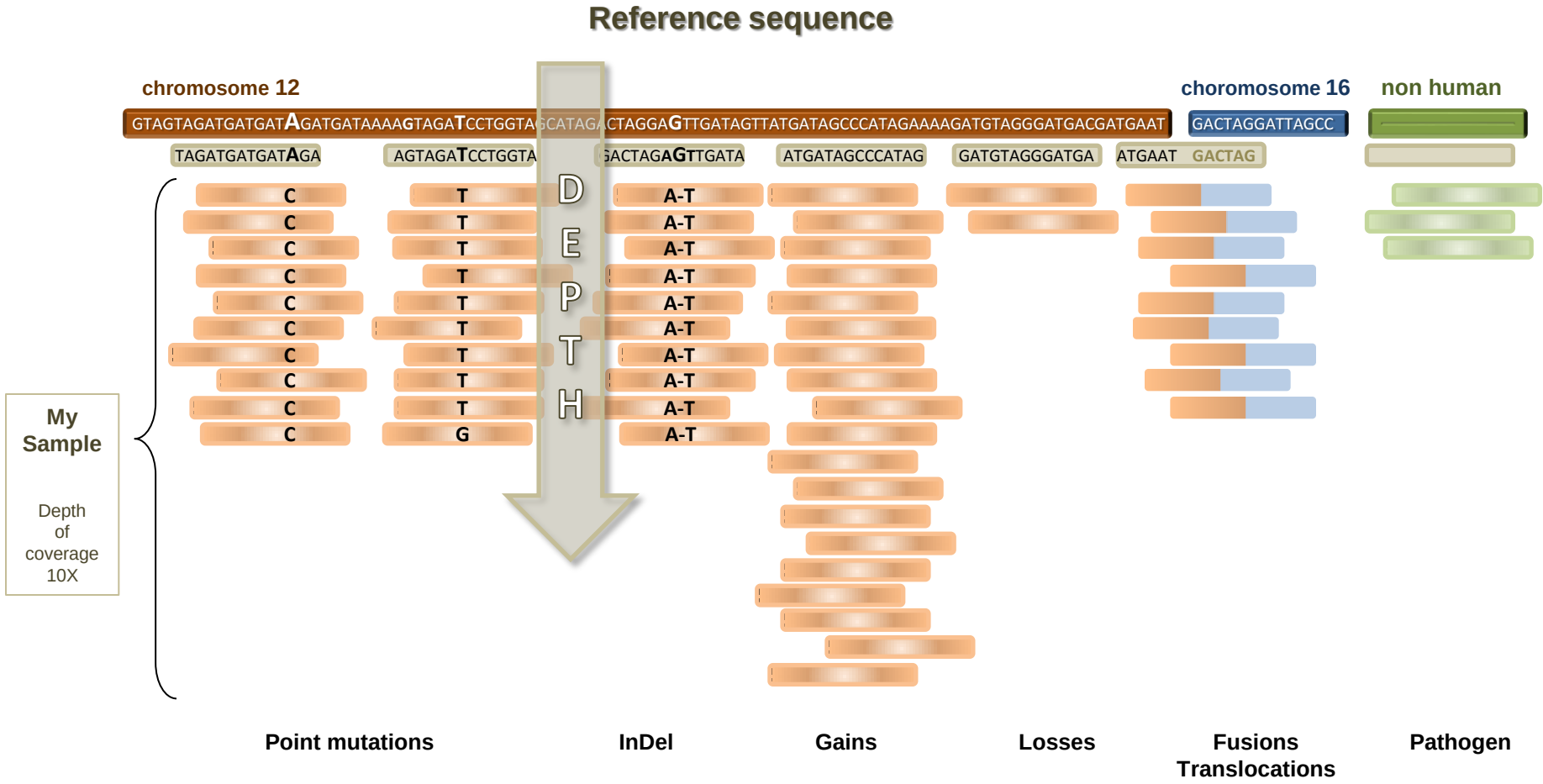

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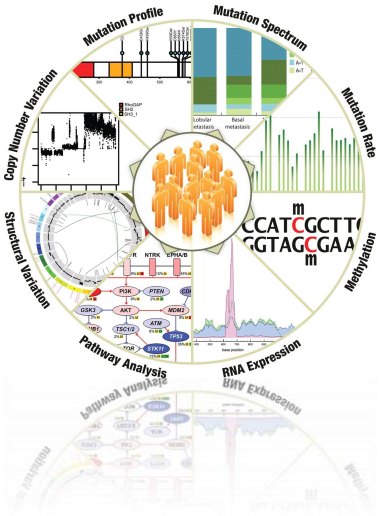
SEQUENCING

First generation	Second generation
Reaction occurs in solution	Reaction occurs on solid-phase
Each DNA template is sequenced individually	Thousands of DNA temples are sequenced in parallel
Long read lenght (~ 1kb)	Short read length (e.g. ~200 pb)
Low throughtput	High throughtput
Limited sensitivity *(max 20%)*	High sensitivity *(depends on coverage)*
Qualitative *Small-size abnormalities*	Quali/Quantitative *Multiple types of abnormalities* (SNV, InDels, CNV, Gene fusions, Translocations/Inversions, Transcriptome, Pathogen genomes)
High costs for large-scale projects Lower costs for small projects	"Low" costs for large-scale projects Higher costs for small projects

MPS Applications

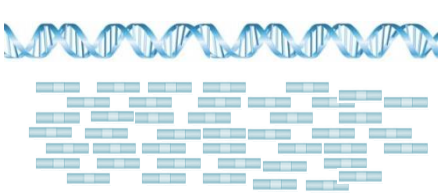
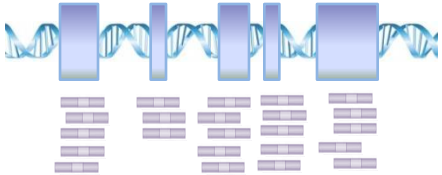
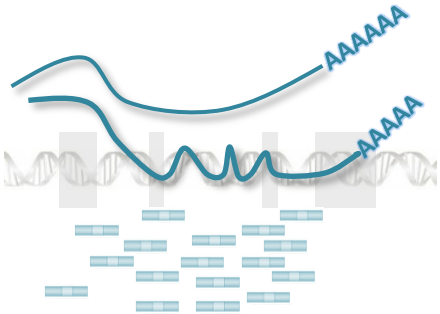
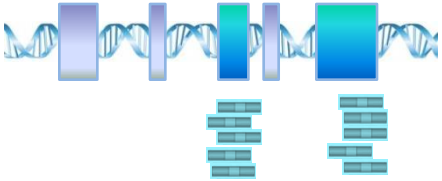
Method	Sequencing to determine:	Example reference	
DNA-Seq	A genome sequence	57	Comparison, 'anatomic' (isolation by anatomic site), flow cytometery, DNA extraction, mechanical shearing, adaptor ligation, PCR and sequencing
Targeted DNA-Seq	A subset of a genome (for example, an exome)	20	Comparison, cell culture, DNA extraction, mechanical shearing, adaptor ligation, PCR, hybridization capture, PCR and sequencing
Methyl-Seq	Sites of DNA methylation, genome-wide	34	Perturbation, genetic manipulation, cell culture, DNA extraction, mechanical shearing, adaptor ligation, bisulfite conversion, PCR and sequencing
Targeted methyl-Seq	DNA methylation in a subset of the genome	129	Comparison, cell culture, DNA extraction, bisulfite conversion, molecular inversion probe capture, circularization, PCR and sequencing
DNase-Seq, Sono-Seq and FAIRE-Seq	Active regulatory chromatin (that is, nucleosome-depleted)	113	Perturbation, cell culture, nucleus extraction, DNase I digestion, DNA extraction, adaptor ligation, PCR and sequencing
MAINE-Seq	Histone-bound DNA (nucleosome positioning)	130	Comparison, cell culture, MNase I digestion, DNA extraction, adaptor ligation, PCR and sequencing
ChIP-Seq	Protein-DNA interactions (using chromatin immunoprecipitation)	131	Comparison, 'anatomic', cell culture, cross-linking, mechanical shearing, immunoprecipitation, DNA extraction, adaptor ligation, PCR and sequencing
RIP-Seq, CLIP-Seq, HITS-CLIP	Protein-RNA interactions	46	Variation, cross-linking, 'anatomic', RNase digestion, immunoprecipitation, RNA extraction, adaptor ligation, reverse transcription, PCR and sequencing
RNA-Seq	RNA (that is, the transcriptome)	39	Comparison, 'anatomic', RNA extraction, poly(A) selection, chemical fragmentation, reverse transcription, second-strand synthesis, adaptor ligation, PCR and sequencing
FRT-Seq	Amplification-free, strand-specific transcriptome sequencing	119	Comparison, 'anatomic', RNA extraction, poly(A) selection, chemical fragmentation, adaptor ligation, reverse transcription and sequencing
NET-Seq	Nascent transcription	41	Perturbation, genetic manipulation, cell culture, immunoprecipitation, RNA extraction, adaptor ligation, reverse transcription, circularization, PCR and sequencing
Hi-C	Three-dimensional genome structure	71	Comparison, cell culture, cross-linking, proximity ligation, mechanical shearing, affinity purification, adaptor ligation, PCR and sequencing
Chia-PET	Long-range interactions mediated by a protein	73	Perturbation, cell culture, cross-linking, mechanical shearing, immunoprecipitation, proximity ligation, affinity purification, adaptor ligation, PCR and sequencing
Ribo-Seq	Ribosome-protected mRNA fragments (that is, active translation)	48	Comparison, cell culture, RNase digestion, ribosome purification, RNA extraction, adaptor ligation, reverse transcription, rRNA depletion, circularization, PCR and sequencing
TRAP	Genetically targeted purification of polysomal mRNAs	132	Comparison, genetic manipulation, 'anatomic', cross-linking, affinity purification, RNA extraction, poly(A) selection, reverse transcription, second-strand synthesis, adaptor ligation, PCR and sequencing
PARS	Parallel analysis of RNA structure	42	Comparison, cell culture, RNA extraction, poly(A) selection, RNase digestion, chemical fragmentation, adaptor ligation, reverse transcription, PCR and sequencing
Synthetic saturation mutagenesis	Functional consequences of genetic variation	93	Variation, genetic manipulation, barcoding, RNA extraction, reverse transcription, PCR and sequencing
Immuno-Seq	The B-cell and T-cell repertoires	86	Perturbation, 'anatomic', DNA extraction, PCR and sequencing
Deep protein mutagenesis	Protein binding activity of synthetic peptide libraries or variants	95	Variation, genetic manipulation, phage display, *in vitro* competitive binding, DNA extraction, PCR and sequencing
PhIT-Seq	Relative fitness of cells containing disruptive insertions in diverse genes	92	Variation, genetic manipulation, cell culture, competitive growth, linear amplification, adaptor ligation, PCR and sequencing

FAIRE-seq, formaldehyde-assisted isolation of regulatory elements–sequencing. MAINE-Seq, MNase-assisted isolation of nucleosomes-sequencing; RIP-Seq, RNA-binding protein immunoprecipitation-sequencing; CLIP-Seq, cross-linking immunoprecipitation-sequencing; HITS-CLIP, high-throughput sequencing of RNA isolated by cross-linking immunoprecipitation; FRT-Seq, on-flowcell reverse transcription–sequencing. NET-Seq, native elongating transcript sequencing. TRAP, translating ribosome affinity purification. PhIT-Seq, phenotypic interrogation via tag sequencing.



Shendure J & Lieberman Aiden E, Nature Biotechnology (2012) 30:1084–1094
Figure form Ding L et al. , Hum. Mol. Genet. (2010) 9:R 188-196

Major MPS Applications

Whole genome-seq	Whole exome-seq	RNA-seq	Target-seq
			
Genome Structural variants Pont mutations/InDels CNV	**Exome** (protein-coding regions) Point mutations/InDels CNV	**Transcriptome** Gene expression Gene fusions Splice variants	**Selected list of genes/hot-spots** Point mutations/InDels CNV

Breath

NGS rule of thumb

Breath x Depth = Cost

'Complexity' x 'Accuracy' = Cost

Approach	Advantages	Disadvantages
Whole genome-Seq 1x diploid genome $6x10^9$ bp	Comprehensive landscape of whole genome alterations Any type of genomic alteration: - Qualitative (chromosome rearrangements, somatic mutations in coding and non-coding regions, active retrotrasposons, pathogen genomes) - Quantitative (gain /loss)	Expensive.. .. hence usually done at low/medium coverage to get a general picture (at the expence of accuracy) Huge amount of data to deal with, difficult to interpret Risk of incidental findings (ethical issues)
Whole exome-Seq 1x exome $60x10^6$ bp	Cost effective Good sensitivity (high coverage) Small datasets, easier to interpret Gene alterations (SNV, InsDel) within the coding regions	Covers only 1% of the genome Uneven capture efficiency across exons (may miss alterations) Off-target hybridizations Miss most fusion genes Risk of incidental findings (ethical issues)
RNA-Seq ncRNA-Seq Millions of reads	Cost effective - Qualitative (Fusion transcripts, Isoforms, RNA editing) and - Quantitative (mRNA and ncRNA expression levels) *Compared to Microarray: wider dinamic range; no dependent on known gene sequence; free of hybridization artifacts* Small datasets	Coverage dependends on expression levels Miss alterations in low-copy transcripts (low coverage) The inbalance in the representation of different mRNAs makes it hard the call of mutations
Targeted-seq A priori selected list of genes/mutations Variable length	Cost effective Mostly used to detect Point mutations/InDels/CNV Useful for diagnostics and NGS data validation Very small dataset, easy to interpret Very high sensitivity at high coverage Results are often actionable/Personalized medicine	Miss alterations outside the targeted regions A priori knowledge of the genes/mutations of interest

The power of NGS analyses

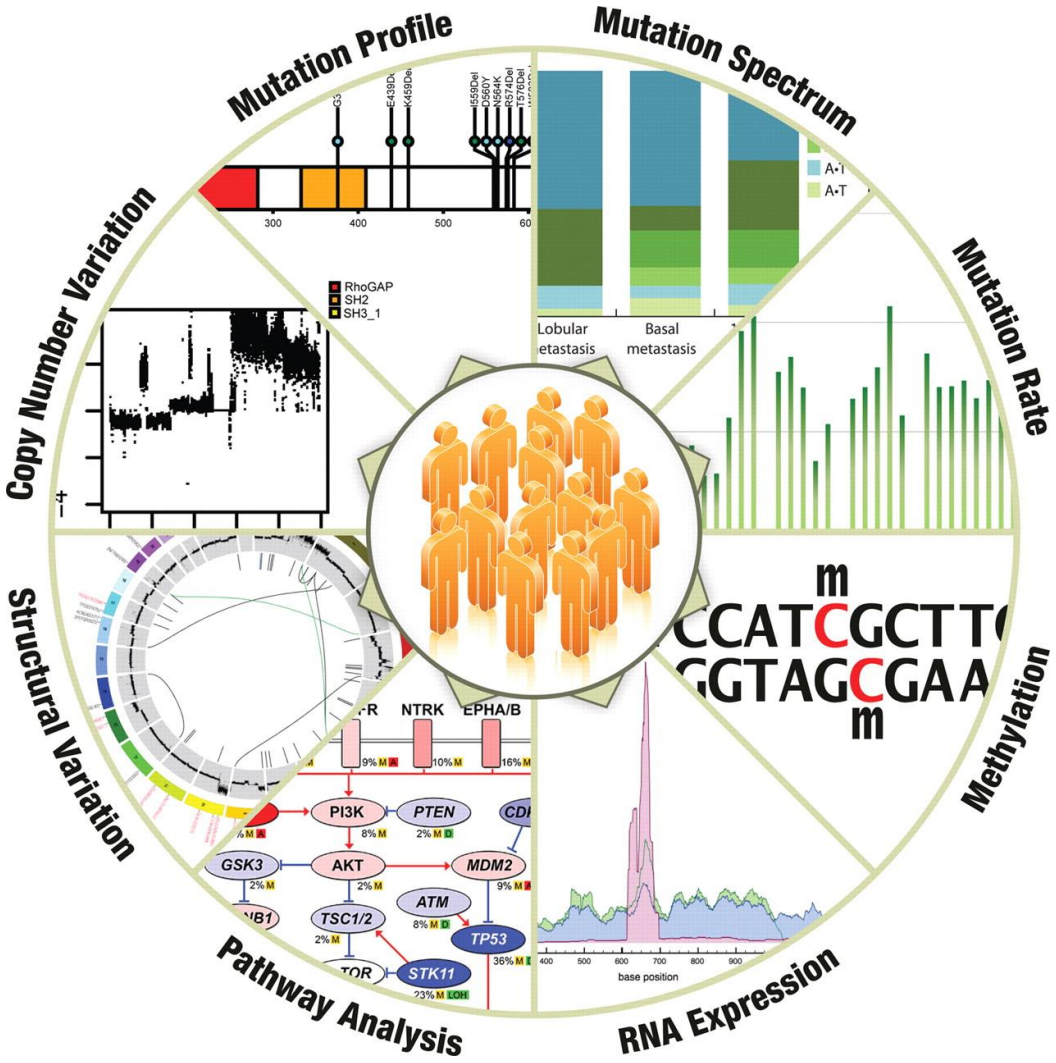


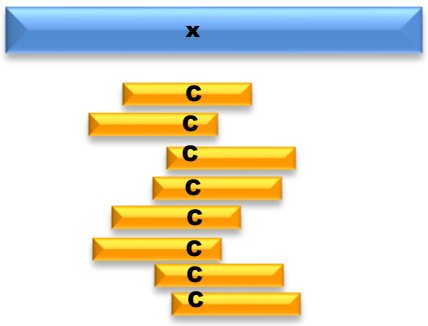
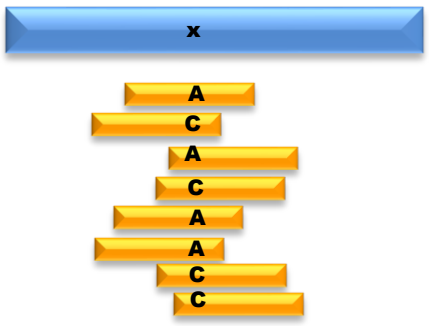
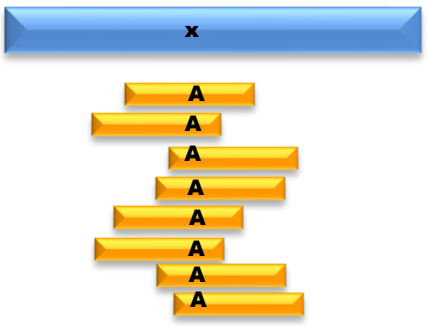
Image from Ding L et al. Hum. Mol. Genet. 2010;19:R188-R196

So far so good…

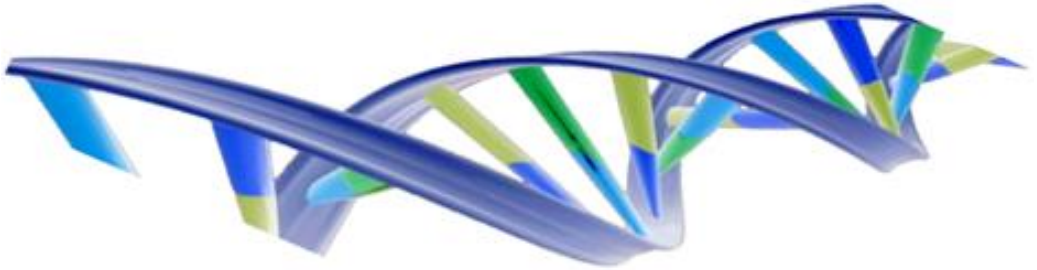
…but cancer is not a “simple” genetic disorder…

Genetics

Reference sequence
Chromosome 1

Homozygous C	Heterozygous A/C	Homozygous A
		
8/8 reads C (100% C)	4/8 reads C, 4/8 reads A (50% C, 50%A)	8/8 reads A (100% A)

8X coverage



ANALYSIS OF TUMOR SAMPLES: ISSUES

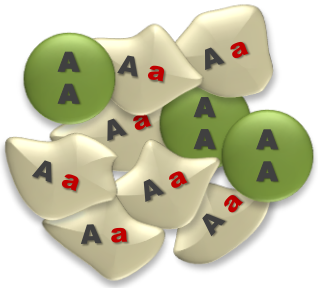
- Purity
- Clonality
- Aneuploidy & Rearrangments
- Sample quantity & quality

ANALYSIS OF TUMOR SAMPLES: ISSUES

Purity

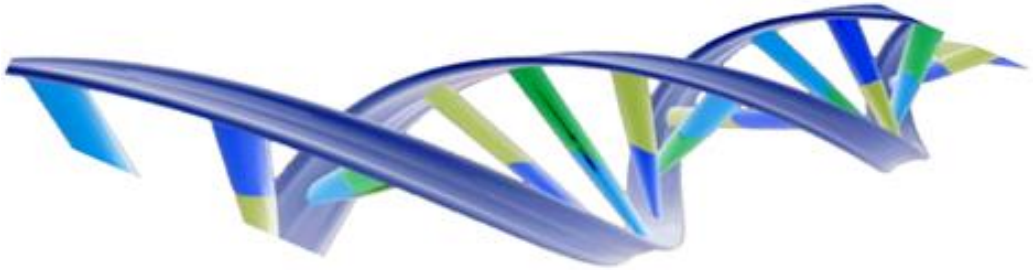
Contaminantion by non-tumoral cells affects the ability to detect mutations

Example: an heterozygous mutation in a tumor sample 70% pure (30% non-tumoral cells) will be detectable in 35% of the reads



10 cells, 2 alleles per cells (20 alleles total)
7 tumor cells (T) A**a**
3 normal cells (N) AA

A= 7 from T +(3+3) from N = 13/20 alleles are A
a = 7 from T + 0 from N = 7/20 alleles are **a**
The actual allelic frequency of **a** in the sample will be 7/20= 35%

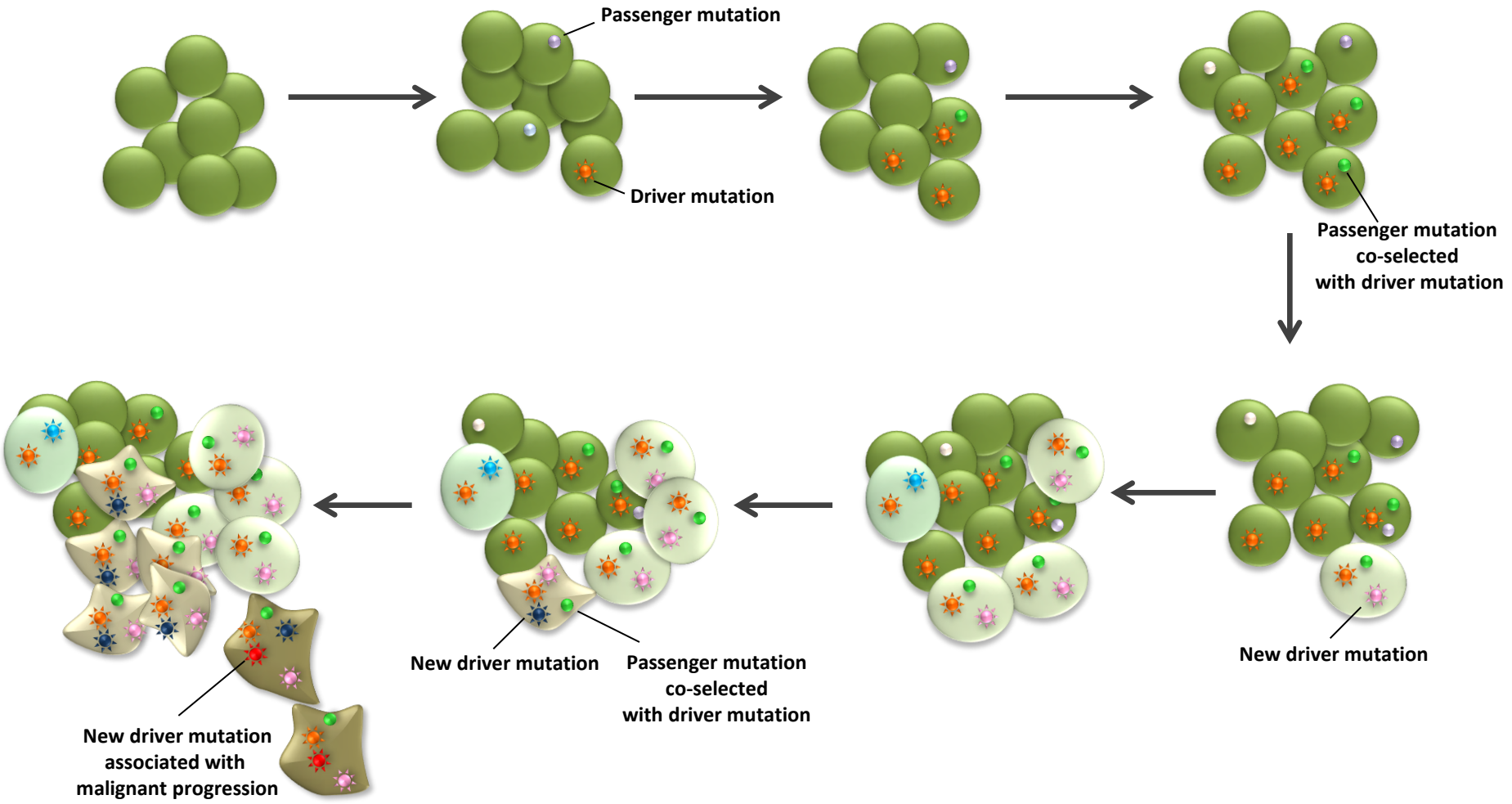


ANALYSIS OF TUMOR SAMPLES: ISSUES

Clonality

Tumors may be highly heterogeneous

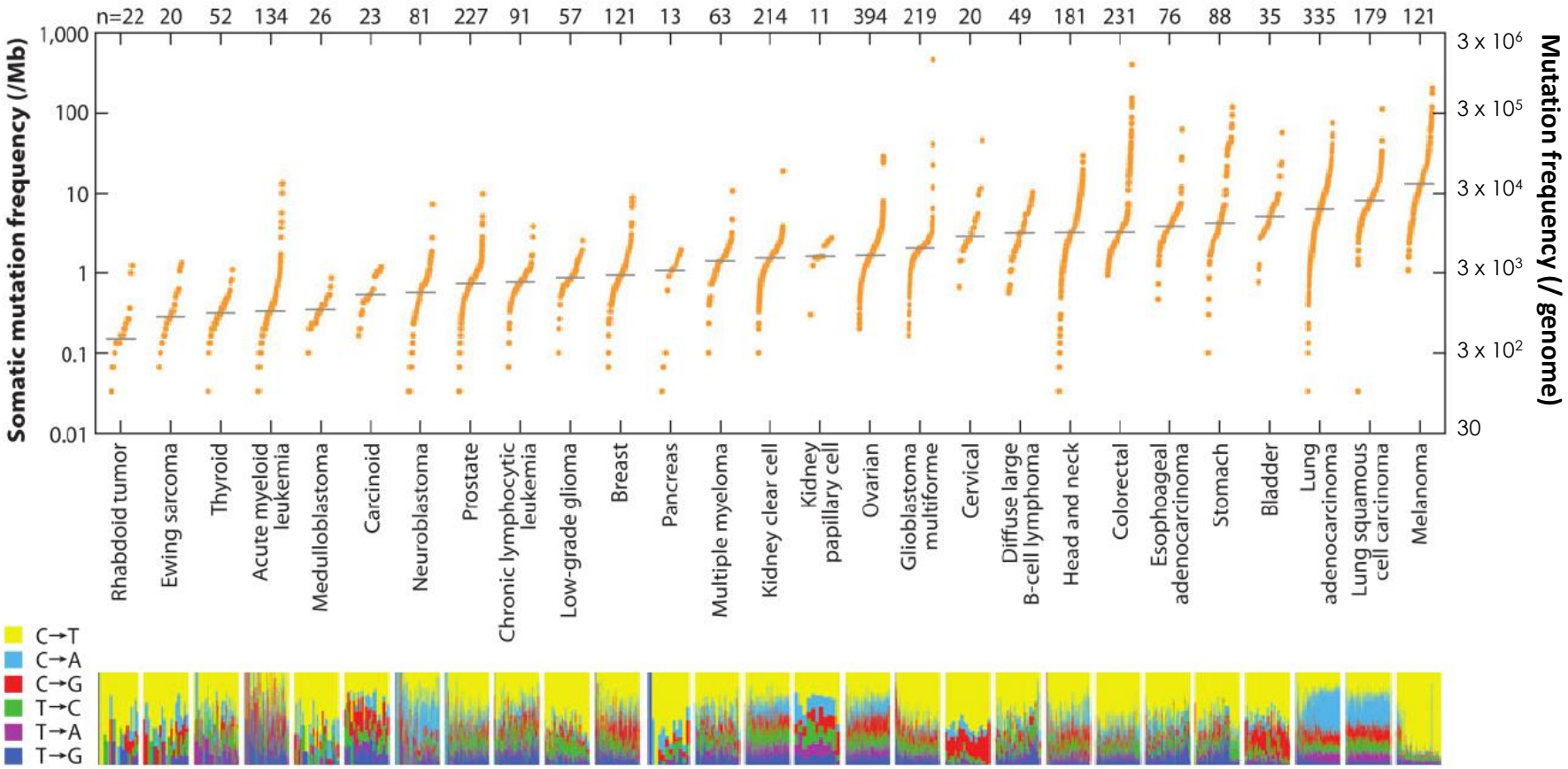
The “polyclonal” evolution of cancer



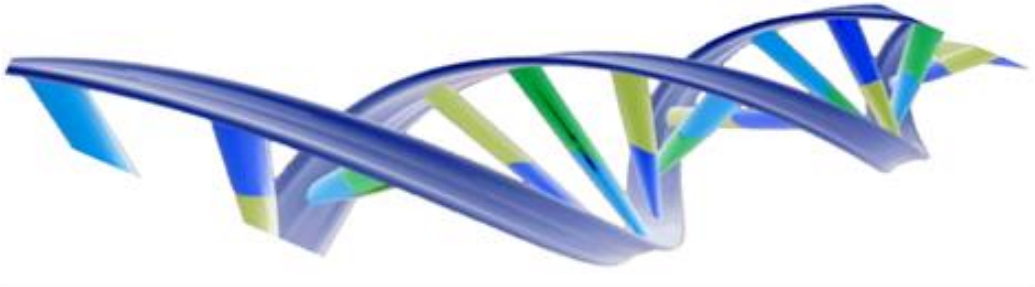
Driver mutation
Causally implicated in cancer.
It confers growth advantage to tumor cells, therefore undergoes positive selection

Passenger mutation
No role in cancer
No relevant impact on tumor cell growth or survival.
May be selected as a result of a bystander effect

Somatic mutation frequency in cancer



Modified from: Chmielecki J & Meyerson M. (2014)
DNA sequencing of cancer: what have we learned? Ann Rev Med. 65:63-79



ANALYSIS OF TUMOR SAMPLES: ISSUES

Somatic vs Germline (comparison with normal matched samples)

Drivers vs Passengers

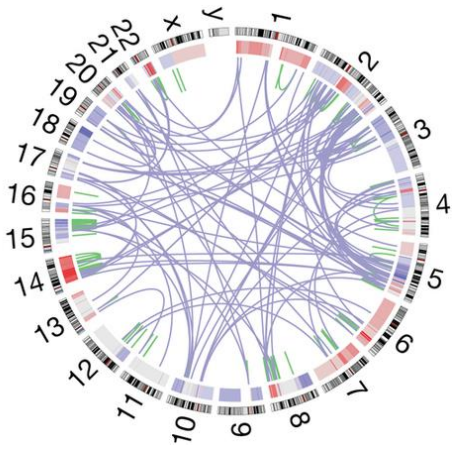
Functional validation of candidate driver mutations

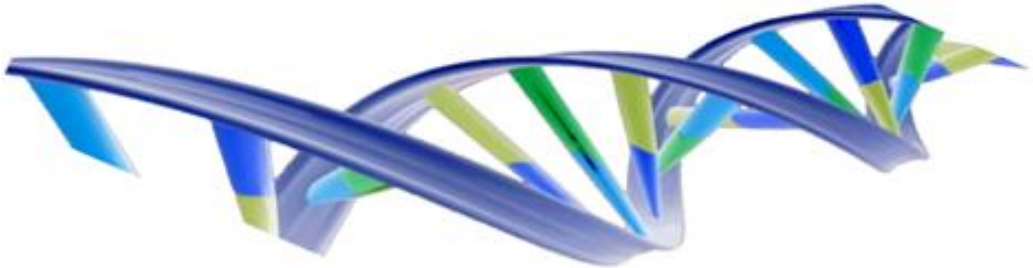
ANALYSIS OF TUMOR SAMPLES: ISSUES

Aneuploidy & Rearrangments

Tumors are genetically unbalanced and rearranged

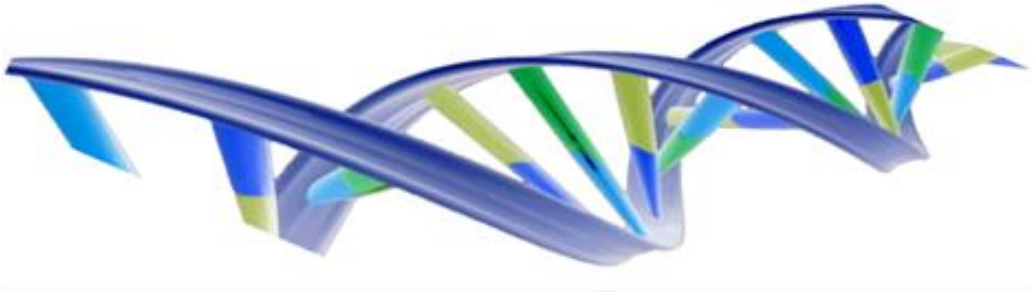
Alignment to the reference genome may be challening





ANALYSIS OF TUMOR SAMPLES: ISSUES

Sample quantity & quality (FFPE)



ANALYSIS OF TUMOR SAMPLES: ISSUES

Purity

Clonality

Aneuploidy & Rearrangments

Sample quantity & quality

Intrinsic error rate of the technology

Limits of bioinformatic tools

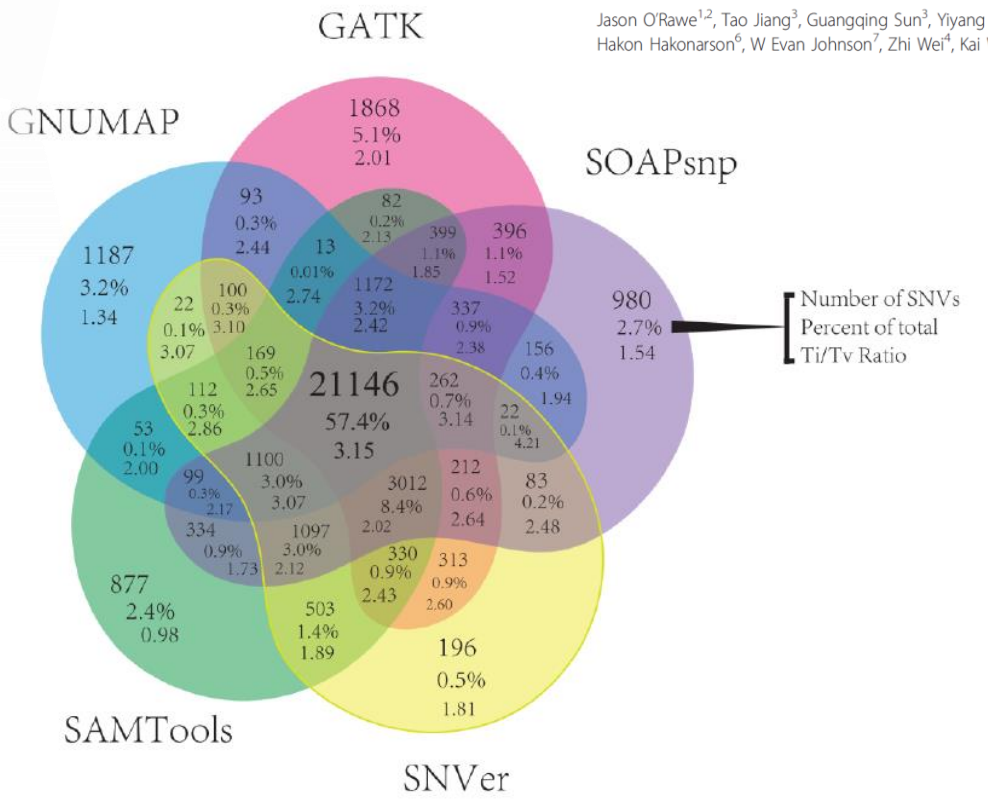
O'Rawe *et al. Genome Medicine* 2013, **5**:28
http://genomemedicine.com/content/5/3/28

Genome Medicine

RESEARCH Open Access

Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing

Jason O'Rawe[1,2], Tao Jiang[3], Guangqing Sun[3], Yiyang Wu[1,2], Wei Wang[4], Jingchu Hu[3], Paul Bodily[5], Lifeng Tian[6], Hakon Hakonarson[6], W Evan Johnson[7], Zhi Wei[4], Kai Wang[8,9*] and Gholson J Lyon[1,2,9*]



Conclusions: Our results suggest that more caution should be exercised in genomic medicine settings when analyzing individual genomes, including interpreting positive and negative findings with scrutiny,

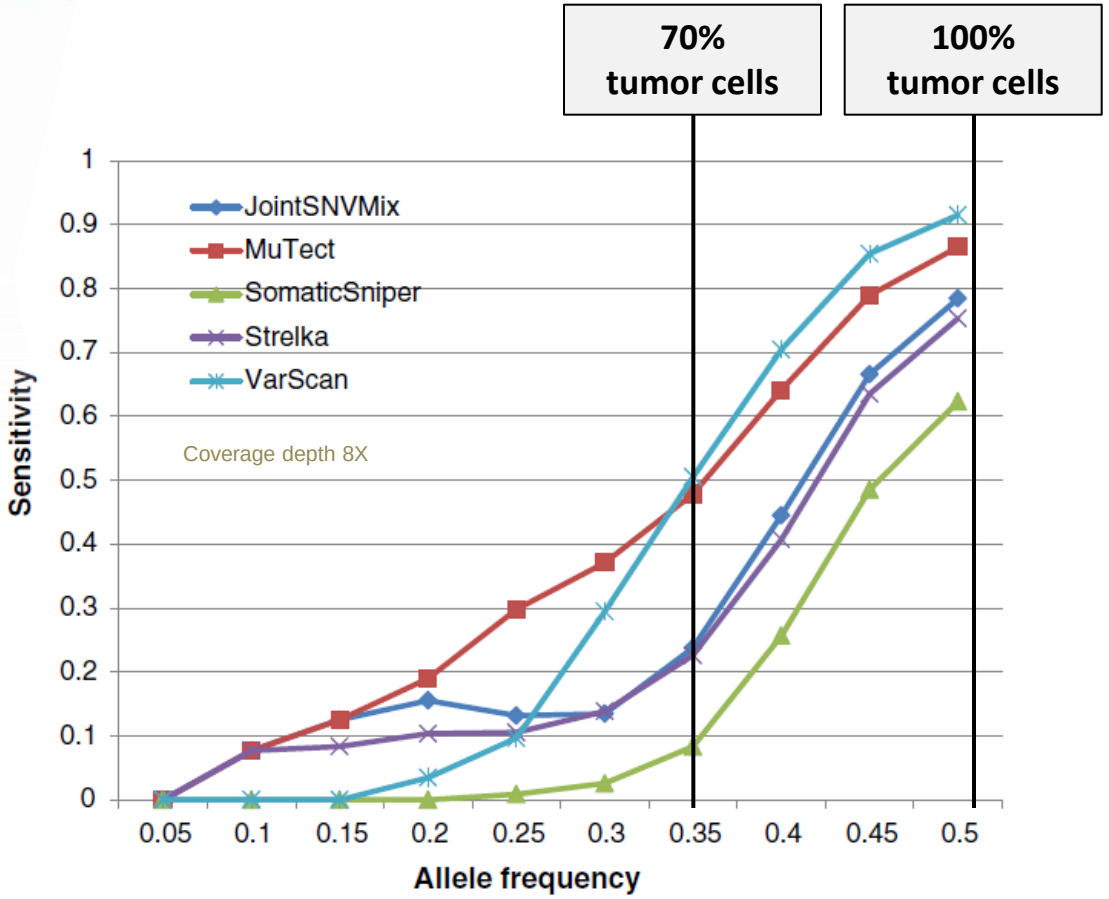
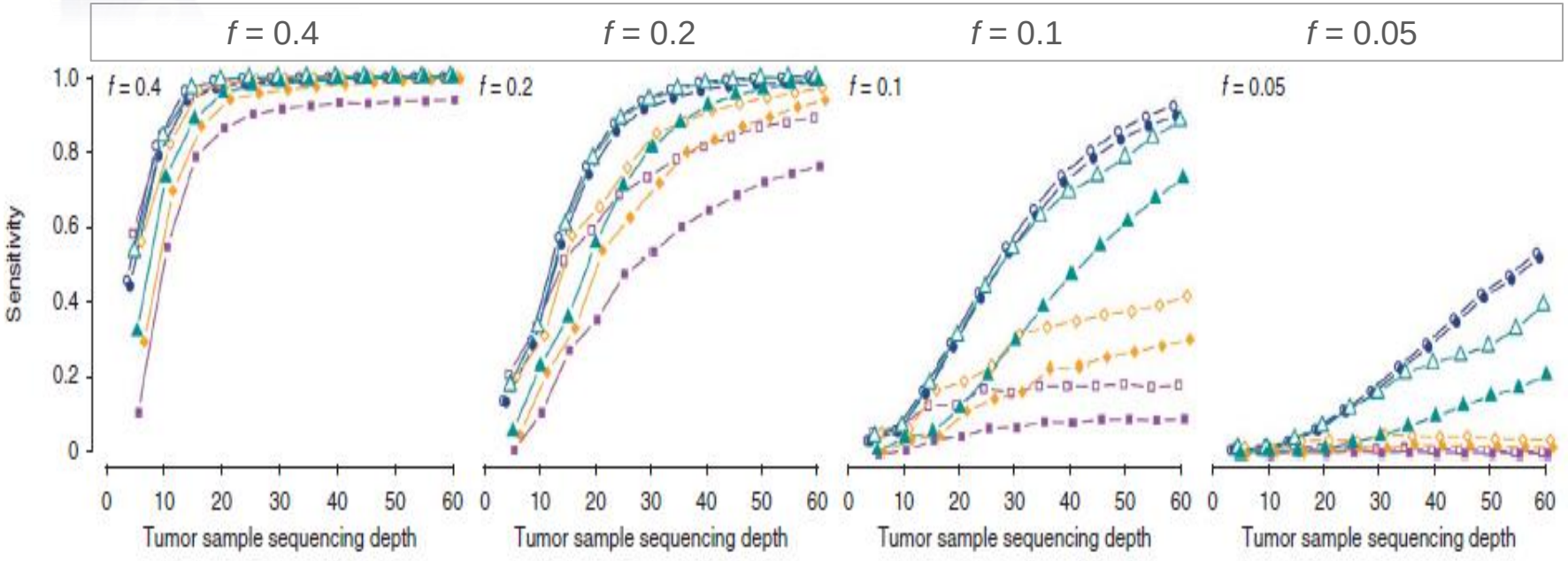


Figure 1 Sensitivity as a function of mutation allele frequency for five sSNV-detecting tools. Given an allele frequency value *f*, the sensitivity of a tool *T* (either JointSNVMix, MuTect, SomaticSniper, Strelka, or VarScan 2) is calculated as: $S_T = N_T/N_f$ where N_f is the total number of sSNVs with sequencing depth ≥8, the number of alternate allele-supporting reads ≥2 in the disease sample, and an allele frequency less than *f*, and N_T is the number of sSNVs that the tool *T* identified out of these N_f point mutations.

Cibulskis et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol. (2013) , 3:213-9.

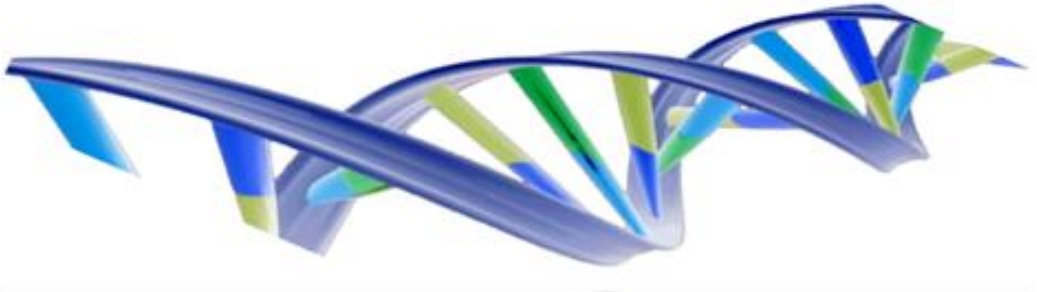
Sensitivity of mutation detection

as a function of sequencing depth and mutated allele frequency (f)



Cibulskis, K et al., Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples Nature Biotechnology (2013) 31, 213–219

Coverage of ~500X is required to detect mutations carried by a subclone representing ~ 1% of the tumor



ANALYSIS OF TUMOR SAMPLES: ISSUES

Higher coverage and

Dedicated Bioinformatic tools

Need of validation of mutation

Orthogonal approaches (Sanger, Pyroseq, ASO, others)

Interrogation of large cohorts

The power of NGS analyses

Modified from Ding L et al. Hum. Mol. Genet. 2010;19:R188-R196

NGS and Sarcomas

Human Cancer Biology

Clinical Cancer Research

Novel Clinically Relevant Genes in Gastrointestinal Stromal Tumors Identified by Exome Sequencing

Sebastian F. Schoppmann[1], Ursula Vinatzer[1], Niko Popitsch[2], Martina Mittlböck[2], Sai
Gerd Jomrich[1], Berthold Streubel[3], and Peter Birner[4]

Abstract

Purpose: Chromosomal gains and losses resulting in altered gene dosage a
gastrointestinal stromal tumors (GIST). The aim of our study was the identificat
in these candidate regions.
Material and Methods: A cohort of 174 GIST was investigated using DNA arr
sequencing ($n = 13$), and immunohistochemistry ($n = 145$).
Array analysis revealed recurrent copy number variations (CNVs) o
5q, 7p, 11q, 12p, 13q, 14q, 15q,
sociated with shorter disease-fre
egions showing recurrent CNVs
insertions, deletions) in each tu
served in 37 genes. After furth
HC. Immunohistochemical inve
ated with shorter DFS, expression

clusion: Using a novel approa
ntry, we were able to identify
relevance. Because the identifie
olved in MAP kinase signaling, o
ancer Res; 19(19); 5329–39. ©2

nature genetics

Frequent mutation of the major cartilage collagen gene *COL2A1* in chondrosarcoma

Patrick S Tarpey[1,8], Sam Behjati[1,2,8], Susanna L Cooke[1], Peter Van Loo[1,3], David C Wedge[1], Nischalan Pillay[4,5], John Marshall[1], Sarah O'Meara[1], Helen Davies[1], Serena Nik-Zainal[1], David Beare[1], Adam Butler[1], John Gamble[1], Claire Hardy[1], Jonathon Hinton[1], Ming Ming Jia[1], Alagu Jayakumar[1], David Jones[1], Calli Latimer[1], Mark Maddison[1], Sancha Martin[1], Stuart McLaren[1], Andrew Menzies[1], Laura Mudie[1], Keiran Raine[1], Jon W Teague[1], Jose M C Tubio[1], Dina Halai[4], Roberto Tirabosco[4], Fernanda Amary[4], Peter J Campbell[1,6,7], ew Futreal[1]

in children and adolescents, bei
features of sarcomeric differentiatio
as been defined as an entity, separat
outcome. So far, no recurrent gene
udied a case of adult spindle cell RI
approach, we identified 31 tumou
nal relevance to muscle differentiati
or mutations using Sanger sequenci
mples. The highly conserved sequer
d us to screen the basic DNA–bindi
ted 17 samples, seven (41%) show
btype of adult spindle cell RMS, wh
differentiation. The p.L122R mutati
nsactivation and MYC-like functio

SDHA Loss-of-Function Mutations in *KIT–PDGFRA* Wild-Type Gastrointestinal Stromal Tumors Identified by Massively Parallel Sequencing

Maria A. Pantaleo, Annalisa Astolfi, Valentina Indio, Richard Moore,
Nina Thiessen, Michael C. Heinrich, Chiara Gnocchi, Donatella Santi
Fausto Catena, Serena Formica, Pier Luigi Martelli, Rita Casadio,
Andrea Pession, Guido Biasco

Manuscript received December 7, 2010; revised March 10, 2011; accepted
2011.

Correspondence to: Maria A. Pantaleo, Department of Hematology and Oncolog
"L.A.Seragnoli," Sant'Orsola-Malpighi Hospital, University of Bologna, Via Massar
Bologna, Italy (e-mail: maria.pantaleo@unibo.it).

**Approximately 10%–15% of gastrointestinal stromal tumors (GISTs) i
not harbor any mutation in the *KIT* or *PDGFRA* genes (ie, KIT/PDGFR
GISTs). Recently, mutations in *SDHB* and *SDHC* (which encode succina
genase subunits B and C, respectively) but not in *SDHA* and *SDHD* (wh
subunits A and D, respectively) were identified in KIT/PDGFRA wild-typ
search for novel pathogenic mutations, we sequenced the tumor trans
two young adult patients who developed sporadic KIT/PDGFRA wild-ty
using a massively parallel sequencing approach. The only variants i
disease related by computational analysis were in *SDHA*. One patient**

nature genetics

Distinct *H3F3A* and *H3F3B* driver mutations define chondroblastoma and giant cell tumor of bone

Sam Behjati[1,2,12], Patrick S Tarpey[1,12], Nadège Presneau[3,4], Susanne Scheipl[3,5], Nischalan Pillay[3,6], Peter Van Loo[1,7], David C Wedge[1], Susanna L Cooke[1], Gunes Gundem[1], Helen Davies[1], Serena Nik-Zainal[1], Sancha Martin[1], Stuart McLaren[1], Victoria Goodie[1], Ben Robinson[1], Adam Bu
Ola Myklebost[8], Daniel Baumhoer[9], Gernot Jundt[9], Rifat Ha
P Andrew Futreal[1], Michael R Stratton[1], Peter J Campbell[1,10]

It is recognized that some mutated cancer genes contribute to the development of many cancer types, whereas others are cancer type specific. For genes that are mutated in multiple cancer classes, mutations are usually similar in the different affected cancer types. Here, however, we report exquisite tumor type specificity for different histone H3.3 driver alterations. In 73 of 77 cases of chondroblastoma (95%), we found p.Lys36Met alterations predominantly encoded
genes for histone H3.3,
giant cell tumors of bone,
ions exclusively in *H3F3A*,
e case, p.Gly34Leu alterations.
to the stromal cell population
oclasts or their precursors.
ported *H3F3A* mutations
ly34Arg or p.Gly34Val alterations
emarkable picture of tumor
3 driver alterations emerges,
sidues, mutations and genes

nature genetics

A common single-nucleotide variant in *T* is strongly associated with chordoma

Nischalan Pillay[1,2], Vincent Plagnol[3], Patrick S Tarpey[4], Samira B Lobo[5], Nadège Presneau[5], Karoly Szuhai[5], Dina Halai[6], Fitnat Berisha[7], Stephen R Cannon[8], Simon Mead[7], Dalia Kasperaviciute[9], Jutta Palmen[9], Philippa J Talmud[9], Lars-Gunnar Kindblom[10], M Fernanda Amary[6], Roberto Tirabosco[6] & Adrienne M Flanagan[1,2]

Chordoma is a rare malignant bone tumor that expresses the transcription factor T. We conducted an association study of 40 individuals with chordoma and 158 ancestry-matched controls, with replication in an independent cohort. Whole-exome and Sanger sequencing of *T* exons showed strong association of the common nonsynonymous SNP rs2305089 with chordoma risk (allelic odds ratio (OR) = 6.1, 95% confidence interval (CI) = 3.1–12.1; $P = 4.4 \times 10^{-9}$), a finding that is exceptional in cancers with a non-Mendelian mode of inheritance.

IJC
International Journal of Cancer

Identification of a novel, recurrent *MBTD1-CXorf67* fusion in low-grade endometrial stromal sarcoma

Barbara Dewaele[1], Joanna Przybyl[1,2,3], Anna Quattrone[1], Julio Finalet Ferreiro[1], Vanessa Vanspauwen[1], Ellen Geerdens[1], Valentina Gianfelici[1], Zeynep Kalender[4], Agnieszka Wozniak[1], Philippe Moerman[5], Raf Sciot[4], Sabrina Croce[7], Frederic Amant[8], Peter Vandenberghe[1], Jan Cools[1]* and Maria Debiec-Rychter[1]*

[1]Department of Human Genetics, KU Leuven and University Hospitals Leuven, Leuven, Belgium
[2] Department of Molecular and Translational Oncology, The Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland
[3]Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland
[4] Department of Human Genetics, KU Leuven and Flanders Interuniversity Institute for Biotechnology (VIB), Leuven, Belgium
[5]Laboratory of Experimental Oncology, Department of Oncology, KU Leuven and Department of General Medical Oncology, University Hospitals Leuven, Leuven, Belgium
[6] Department of Pathology, KU Leuven and University Hospitals Leuven, Leuven, Belgium
[7] Department of Pathology, Institute Bergonié, Bordeaux, France
[8] Department of Oncology, KU Leuven and Leuven Cancer Institute, University Hospitals Leuven, Leuven, Belgium

Endometrial stromal sarcomas (ESSs) are a genetically heterogeneous group of rare uterine neoplasms that are commonly driven by recurrent gene rearrangements. In conventional low-grade ESS, *JAZF1-SUZ12*, *PHF1-JAZF1*, *EPC1-PHF1* and *MEAF6-PHF1*, and recently described *ZC3H7-BCOR* chim
t(3;0;17)(q22;p13) translocation yields YWHAE-FA
and clinically more aggressive ESS. Integrating
hybridization (FISH) and banding cytogenetics,
(chromosome X open reading frame 67) as the
two independent low-grade ESS of classical hist
both cases using reverse-transcription polymera
developed to detect the novel t(0;17) translocat
of an additional low-grade ESS case positive for
undifferentiated endometrial sarcomas (UESs)
files of seven ESS (including three with *YWHAE*
somal aberrations indicated clustering of tumor
The chimeric *MBTD1-CXorf67* fusion identifies y
opportunity to shed light on the functions of tw

RESEARCH BRI

Frequent Alterations and Epigenetic Silencing of Differentiation Pathway Genes in Structurally Rearranged Liposarcomas

V. Angeles[2], onescu[4], Joseph M. Scandura[2,3], d Samuel Singer[2]

erations contributing to liposarcomagenesis b
xome, transcriptome, and cytosine methylome
osarcoma (DLPS) from distinct chemotherapy/r
enomes had complex structural rearrangements
ts on the structure and expression of affected g
ntegrative analyses and additional screening id
of DLPS. Liposarcoma methylomes revealed al
uding CEBPA methylation in 24% of DLPS. Treati
f CEBPA expression in DLPS cells, was antiprolif
tumor growth *in vivo*. Both genetic and epigeneti
NAs in liposarcomagenesis, typified by methyla
PS but not its well-differentiated counterpart. T
pigenetic abnormalities in DLPS tumors and sug
utics.

OPEN ACCESS Freely available online

PLOS ONE

Whole-Transcriptome Sequencing Identifies Novel *IRF2BP2-CDX1* Fusion Gene Brought about by Translocation t(1;5)(q42;q32) in Mesenchymal Chondrosarcoma

Kaja B. Nyquist[1,2,3]*, Ioannis Panagopoulos[1,2], Jim Thorsen[1,2], Lisbeth Haugom[1,2], Ludmila Gorunova[1,2], Bodil Bjerkehagen[4], Alexander Fossà[5], Marianne Guriby[2,6], Torfinn Nome[2,6], Ragnhild A. Lothe[2,6], Rolf I. Skotheim[2,6], Sverre Heim[1,2,3], Francesca Micci[1,2]

1 Section for Cancer Cytogenetics, Institute for Medical Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, 2 Centre for Cancer Biomedicine, University of Oslo, Oslo, Norway, 3 Faculty of Medicine, University of Oslo, Oslo, Norway, 4 Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, 5 Department of Oncology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, 6 Department of Cancer Prevention, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Abstract

Mesenchymal chondrosarcomas (MCs) account for 3–10% of primary chondrosarcomas. The cytogenetic literature includes only ten such tumours with karyotypic information and no specific aberrations have been identified. Using a purely molecular genetic approach a *HEY1-NCOA2* fusion gene was recently detected in 10 of 15 investigated MCs. The fusion probably arises through intrachromosomal rearrangement of chromosome arm 8 q. We report a new case of MC showing a t(1;5)(q42;q32) as the sole karyotypic aberration. Through FISH and whole transcriptome sequencing analysis we found a novel fusion between the *IRF2BP2* gene and the transcription factor *CDX1* gene arising from the translocation. The *IRF2BP2-CDX1* has not formerly been described in human neoplasia. In our hospital's archives three more cases of MC were found, and we examined them looking for the supposedly more common *HEY1-NCOA2* fusion, finding it in all three tumours but not in the case showing t(1;5) and *IRF2BP2-CDX1* gene fusion. This demonstrates that genetic heterogeneity exists in mesenchymal chondrosarcoma.

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PLOS ONE

Genetic Aberrations in Imatinib-Resistant Dermatofibrosarcoma Protuberans Revealed by Whole Genome Sequencing

Jung Yong Hong[1]*, Xiao Liu[2,4]*, Mao Mao[3], Miao Li[2], Dong Il Choi[5], Shin Woo Kang[6], Jeeyun Lee[1], Yoon La Choi[7]*

1 Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, 2 BGI-Shenzhen, Shenzhen, China, 3 Pfizer Oncology, San Diego, California, United States of America, 4 Department of Biology, Copenhagen University, Copenhagen, Denmark, 5 Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, 6 Department of Mathematics, Korea University, Seoul, Korea, 7 Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract

Dermatofibrosarcoma protuberans (DFSP) is a very rare soft tissue sarcoma. DFSP often reveals a specific chromosome translocation, t(17;22)(q22;q13), which results in the fusion of collagen 1 alpha 1 (*COL1A1*) gene and platelet-derived growth factor-B (*PDGFB*) gene. The COL1A1-PDGFB fusion protein activates the PDGFB receptor and resultant constitutive activation of PDGFR receptor is essential in the pathogenesis of DFSP. Thus, blocking PDGFR receptor activation with imatinib has shown promising activity in the treatment of advanced and metastatic DFSP. Despite the success with targeted agents in cancers, acquired drug resistance eventually occurs. Here, we tried to identify potential drug resistance mechanisms against

Cell PRESS

Cancer Cell
Article

Targeting Oxidative Stress in Embryonal Rhabdomyosarcoma

Xiang Chen[1,2,3] Elizabeth Stewart[1,2,3] Anang A. Shelat,[1] Chunxu Qu,[1] Armita Bahrami,[2] Mark Hatley,[2] Gang Wu,[1] Cori Bradley,[2] Justina McEvoy,[2] Alberto Pappo,[2] Shari Spunt,[4] Marcus B. Valentine,[5] Virginia Valentine,[5] Fred Krafcik,[5] Walter H. Lang,[6] Monika Wendt,[6] Lyudmila Tsurkan,[6] Viktor Tollenaar,[7] Sara M. Federico,[7] Chris Morton,[7] Charles Lu,[1] Li Ding,[3,8,10,11] John Easton,[1] Michael Rusch,[1] Panduka Nagahawatte,[1] Jianmin Wang,[1] Matthew Parker,[1] Lei Wei,[1] Erin Hedlund,[1] David Finkelstein,[1] Michael Edmonson,[1] Sheila Shurtleff,[1] Kristy Boggs,[1] Heather Mulder,[1] Donald Yergeau,[1] Steve Skapek,[12] Douglas S. Hawkins,[13] Nilsa Ramirez,[13] Philip M. Potter,[2] John A. Sandoval,[2] Andrew M. Davidoff,[2] Elaine R. Mardis,[3,8,11] Richard K. Wilson,[3,8,11] Jinghui Zhang,[1] James R. Downing,[2] and Michael A. Dyer,[1,14]* on behalf of the St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project

[1]Department of Computational Biology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
[2]Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
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[5]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
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[8]The Genome Institute, Washington University School of Medicine in St. Louis, St. Louis, MO 63108, USA
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[12]Nationwide Children's Hospital, Columbus, OH 43205, USA
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[14]Division of Hematology-Oncology, Seattle Children's Hospital, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA 98105, USA
[15]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
[15]These authors contributed equally to this work.
*Correspondence: michael.dyer@stjude.org

http://dx.doi.org/10.1016/j.ccr.2013.11.002

RESEARCH ARTICLE

Comprehensive Genomic Analysis of Rhabdomyosarcoma Reveals a Landscape of Alterations Affecting a Common Genetic Axis in Fusion-Positive and Fusion-Negative Tumors

Jack F. Shern[1], Li Chen[1], Juliann Chmielecki[3,4], Jun S. Wei[1], Rajesh Patidar[1], Mara Rosenberg[2], Lauren Ambrogio[3], Daniel Auclair[3], Jianjun Wang[1], Young K. Song[1], Catharine Tolman[1], Laura Hurd[1], Hongling Liao[1], Shile Zhang[1], Dominik Bogen[1], Andrew S. Brohl[1], Sivasish Sindiri[2], Daniel Catchpoole[5], Thomas Badgett[1], Gad Getz[3], Jaume Mora[10], James R. Anderson[6], Stephen X. Skapek[11], Frederic G. Barr[12], Matthew Meyerson[3,4,5], Douglas S. Hawkins[6], and Javed Khan[1]

ABSTRACT Despite gains in survival, outcomes for patients with metastatic or recurrent rhabdomyosarcoma remain dismal. In a collaboration between the National Cancer Institute, Children's Oncology Group, and Broad Institute, we performed whole-genome, whole-exome, and transcriptome sequencing to characterize the landscape of somatic alterations in 147 tumor/normal pairs. Two genotypes are evident in rhabdomyosarcoma tumors: those characterized by the PAX3 or PAX7 fusion and those that lack these fusions but harbor mutations in key signaling pathways. The overall burden of somatic mutations in rhabdomyosarcoma is relatively low, especially in tumors that harbor a PAX3/7 gene fusion. In addition to previously reported mutations in *NRAS*, *KRAS*, *HRAS*, *FGFR4*, *PIK3CA*, and *CTNNB1*, we found novel recurrent mutations in *FBXW7* and *BCOR*, providing potential new avenues for therapeutic intervention. Furthermore, alteration of the receptor tyrosine kinase/RAS/PIK3CA axis affects 93% of cases, providing a framework for genomics-directed therapies that might improve outcomes for patients with rhabdomyosarcoma.

SIGNIFICANCE: This is the most comprehensive genomic analysis of rhabdomyosarcoma to date. Despite a relatively low mutation rate, multiple genes were recurrently altered, including *NRAS*, *KRAS*, *HRAS*, *FGFR4*, *PIK3CA*, *CTNNB1*, *FBXW7*, and *BCOR*. In addition, a majority of rhabdomyosarcoma tumors alter the receptor tyrosine kinase/RAS/PIK3CA axis, providing an opportunity for genomics-guided intervention. *Cancer Discov.* 4(2); 216–31. ©2014 AACR.

NGS and Sarcomas

Screening

Diagnosis

Prognosis

Personalized therapy

Disease monitoring

Human Cancer Biology

Clinical Cancer Research

Novel Clinically Relevant Genes in Gastrointestinal Stromal Tumors Identified by Exome Sequencing

Sebastian F. Schoppmann[1], Ursula Vinatzer[1], Niko Popitsch[5], Martina Mittlböck[2], Sai
Gerd Jomrich[1], Berthold Streubel[3], and Peter Birner[4]

Abstract

Purpose: Chromosomal gains and losses resulting in altered gene dosage a
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Material and Methods: A cohort of 174 GIST was investigated using DNA arr
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Array analysis revealed recurrent copy number variations (CNVs) o
5q, 7p, 11q, 12p, 13q, 14q, 15q,
sociated with shorter disease-fre
gions showing recurrent CNVs
insertions, deletions) in each tu
served in 37 genes. After furth
NA. Immunohistochemical inve
with shorter DFS, expression

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(wileyonlinelibrary.com) DOI: 10.1002/path.4307

ORIGINAL PAPER

Transactivating mutation of the *MYOD1* gene is a frequent event in adult spindle cell rhabdomyosarcoma

Karoly Szuhai,[1]* Daniëlle de Jong,[1] Wai Yi Leung,[2] Christopher DM Fletcher[3] and Pancras CW Hogen

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nature genetics

Frequent mutation of the major cartilage collagen gene *COL2A1* in chondrosarcoma

Patrick S Tarpey[1,8], Sam Behjati[1,2,8], Susanna L Cooke[1], Peter Van Loo[1,3], David C Wedge[1], Nischalan Pillay[4,5], John Marshall[1], Sarah O'Meara[1], Helen Davies[1], Serena Nik-Zainal[1], David Beare[1], Adam Butler[1], John Gamble[1], Claire Hardy[1], Jonathon Hinton[1], Ming Ming Jia[1], Alagu Jayakumar[1], David Jones[1], Calli Latimer[1], Mark Maddison[1], Sancha Martin[1], Stuart McLaren[1], Andrew Menzies[1], Laura Mudie[1], Keiran Raine[1], Jon W Teague[1], Jose M C Tubio[1], Dina Halai[4], Roberto Tirabosco[4], Fernanda Amary[4], Peter J Campbell[1,6,7], ew Futreal[1]

in children and adolescents, bei
features of sarcomeric differentiatio
as been defined as an entity, separat
outcome. So far, no recurrent gene
udied a case of adult spindle cell rh
approach, we identified 31 tumou
nal relevance to muscle differentiati
or mutations using Sanger sequenci
mples. The highly conserved sequen
d us to screen the basic DNA–bindi
ted 17 samples, seven (41%) show
btype of adult spindle cell RMS, wh
differentiation. The p.L122R mutati
nsactivation and MYC-like functio

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PLOS ONE

Genetic Aberrations in Imatinib-Resistant Dermatofibrosarcoma Protuberans Revealed by Whole Genome Sequencing

Jung Yong Hong[1]*, Xiao Liu[2,4]*, Mao Mao[3], Miao Li[2], Dong Il Choi[5], Shin Woo Kang[6], Jeeyun Lee[1], Yoon La Choi[7]*

1 Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, 2 BGI-Shenzhen, Shenzhen, China, 3 Pfizer Oncology, San Diego, California, United States of America, 4 Department of Biology, Copenhagen University, Copenhagen, Denmark, 5 Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, 6 Department of Mathematics, Korea University, Seoul, Korea, 7 Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract

Dermatofibrosarcoma protuberans (DFSP) is a very rare soft tissue sarcoma. DFSP often reveals a specific chromosome translocation, t(17;22)(q22;q13), which results in the fusion of collagen 1 alpha 1 (*COL1A1*) gene and platelet-derived growth factor-B (*PDGFB*) gene. The COL1A1-PDGFB fusion protein activates the PDGFB receptor and resultant constitutive activation of PDGFR receptor is essential in the pathogenesis of DFSP. Thus, blocking PDGFR receptor activation with imatinib has shown promising activity in the treatment of advanced and metastatic DFSP. Despite the success with targeted agents in cancers, acquired drug resistance eventually occurs. Here, we tried to identify potential drug resistance mechanisms against

Cell PRESS

Cancer Cell
Article

SDHA Loss-of-Function Mutations in *KIT–PDGFRA* Wild-Type Gastrointestinal Stromal Tumors Identified by Massively Parallel Sequencing

Maria A. Pantaleo, Annalisa Astolfi, Valentina Indio, Richard Moore,
Nina Thiessen, Michael C. Heinrich, Chiara Gnocchi, Donatella Santi
Fausto Catena, Serena Formica, Pier Luigi Martelli, Rita Casadio,
Andrea Pession, Guido Biasco

Manuscript received December 7, 2010; revised March 10, 2011; accepted
2011.

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"L.A.Seragnoli," Sant'Orsola-Malpighi Hospital, University of Bologna, Via Massar
Bologna, Italy (e-mail: maria.pantaleo@unibo.it).

Approximately 10%–15% of gastrointestinal stromal tumors (GISTs) i
not harbor any mutation in the *KIT* or *PDGFRA* genes (ie, KIT/PDGFR
GISTs). Recently, mutations in *SDHB* and *SDHC* (which encode succina
genase subunits B and C, respectively) but not in *SDHA* and *SDHD* (wh
subunits A and D, respectively) were identified in KIT/PDGFRA wild-typ
search for novel pathogenic mutations, we sequenced the tumor trans
two young adult patients who developed sporadic KIT/PDGFRA wild-ty
using a massively parallel sequencing approach. The only variants i
disease related by computational analysis were in *SDHA*. One patient

nature genetics

Distinct *H3F3A* and *H3F3B* driver mutations define chondroblastoma and giant cell tumor of bone

Sam Behjati[1,2,12], Patrick S Tarpey[1,12], Nadège Presneau[3,4], Susanne Scheipl[3,5], Nischalan Pillay[3,6], Peter Van Loo[1,7], David C Wedge[1], Susanna L Cooke[1], Gunes Gundem[1], Helen Davies[1], Serena Nik-Zainal[1], Sancha Martin[1], Stuart McLaren[1], Victoria Goodie[1], Ben Robinson[1], Adam Bu
Ola Myklebost[8], Daniel Baumhoer[9], Gernot Jundt[9], Rifat Ha
P Andrew Futreal[1], Michael R Stratton[1], Peter J Campbell[1,10]

It is recognized that some mutated cancer genes contribute to the development of many cancer types, whereas others are cancer type specific. For genes that are mutated in multiple cancer classes, mutations are usually similar in the different affected cancer types. Here, however, we report exquisite tumor type specificity for different histone H3.3 driver alterations. In 73 of 77 cases of chondroblastoma (95%), we found p.Lys36Met alterations predominantly encoded genes for histone H3.3, giant cell tumors of bone, ions exclusively in *H3F3A*, e case, p.Gly34Leu alterations. to the stromal cell population oclasts or their precursors. ported *H3F3A* mutations ly34Arg or p.Gly34Val alterations emarkable picture of tumor 3 driver alterations emerges, sidues, mutations and genes

nature genetics

A common single-nucleotide variant in *T* is strongly associated with chordoma

Nischalan Pillay[1,2], Vincent Plagnol[3], Patrick S Tarpey[4], Samira B Lobo[5], Nadège Presneau[5], Karoly Szuhai[5], Dina Halai[6], Fitnat Berisha[7], Stephen R Cannon[8], Simon Mead[7], Dalia Kasperaviciute[9], Iutta Palmen[9], Philippa J Talmud[9], Lars-Gunnar Kindblom[10], M Fernanda Amary[6], Roberto Tirabosco[6] & Adrienne M Flanagan[1,2]

Chordoma is a rare malignant bone tumor that expresses the transcription factor T. We conducted an association study of 40 individuals with chordoma and 358 ancestry-matched controls, with replication in an independent cohort. Whole-exome and Sanger sequencing of *T* exons showed strong association of the common nonsynonymous SNP rs2305089 with chordoma risk (allelic odds ratio (OR) = 6.1, 95% confidence interval (CI) = 3.1–12.1; $P = 4.4 \times 10^{-9}$), a finding that is exceptional in cancers with a non-Mendelian mode of inheritance.

IJC
International Journal of Cancer

Identification of a novel, recurrent *MBTD1-CXorf67* fusion in low-grade endometrial stromal sarcoma

Barbara Dewaele[1], Joanna Przybyl[2,3], Anna Quattrone[1], Julio Finalet Ferreiro[1], Vanessa Vanspauwen[1], Ellen Geerdens[1], Valentina Gianfelici[4], Zeynep Kalender[4], Agnieszka Wozniak[1], Philippe Moerman[4], Raf Sciot[4], Sabrina Croce[5], Frederic Amant[6], Peter Vandenberghe[1], Jan Cools[1]* and Maria Debiec-Rychter[1]*

[1]Department of Human Genetics, KU Leuven and University Hospitals Leuven, Leuven, Belgium
[2]Department of Molecular and Translational Oncology, The Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland
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[4]Department of Human Genetics, KU Leuven and Flanders Interuniversity Institute for Biotechnology (VIB), Leuven, Belgium
[5]Laboratory of Experimental Oncology, Department of Oncology, KU Leuven and Department of General Medical Oncology, University Hospitals Leuven, Leuven, Belgium
[6]Department of Pathology, KU Leuven and University Hospitals Leuven, Leuven, Belgium
[7]Department of Pathology, Institute Bergonié, Bordeaux, France
[8]Department of Oncology, KU Leuven and Leuven Cancer Institute, University Hospitals Leuven, Leuven, Belgium

Endometrial stromal sarcomas (ESSs) are a genetically heterogeneous group of rare uterine neoplasms that are commonly low-grade ESS, JAZF1-SUZ12, PHF1-JAZF1, EPC1-PHF1 and MEAF6-

RESEARCH BRI

Frequent Alterations and Epigenetic Silencing of Differentiation Pathway Genes in Structurally Rearranged Liposarcomas

V. Angeles[2], onescu[2], Joseph M. Scandura[1,3], d Samuel Singer[3]

PLOS ONE

erations contributing to liposarcomagenesis b
xome, transcriptome, and cytosine methylome
osarcoma (DLPS) from distinct chemotherapy/r
enomes had complex structural rearrangements
ts on the structure and expression of affected g
ntegrative analyses and additional screening id
of DLPS. Liposarcoma methylomes revealed al
uding CEBPA methylation in 24% of DLPS. Treat
f CEBPA expression in DLPS cells, was antiprol
tumor growth in vivo. Both genetic and epigeneti
NAs in liposarcomagenesis, typified by methyla
PS but not its well-differentiated counterpart. T
pigenetic abnormalities in DLPS tumors and sug
utics.

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Whole-Transcriptome Sequencing Identifies Novel *IRF2BP2-CDX1* Fusion Gene Brought about by Translocation t(1;5)(q42;q32) in Mesenchymal Chondrosarcoma

Kaja B. Nyquist[1,3]*, Ioannis Panagopoulos[1,2], Jim Thorsen[1,2], Lisbeth Haugom[1,2], Ludmila Gorunova[1,2], Bodil Bjerkehagen[4], Alexander Fossä[5], Marianne Guriby[2,6], Torfinn Nome[2,6], Ragnhild A. Lothe[2,6], Rolf I. Skotheim[2,6], Sverre Heim[1,2,3], Francesca Micci[1,2]

1 Section for Cancer Cytogenetics, Institute for Medical Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, 2 Centre for Cancer Biomedicine, University of Oslo, Oslo, Norway, 3 Faculty of Medicine, University of Oslo, Oslo, Norway, 4 Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, 5 Department of Oncology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, 6 Department of Cancer Prevention, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Abstract

Mesenchymal chondrosarcomas (MCs) account for 3–10% of primary chondrosarcomas. The cytogenetic literature includes only ten such tumours with karyotypic information and no specific aberrations have been identified. Using a purely molecular genetic approach a *HEY1-NCOA2* fusion gene was recently detected in 10 of 15 investigated MCs. The fusion probably arises through intrachromosomal rearrangement of chromosome arm 8 q. We report a new case of MC showing a t(1;5)(q42;q32) as the sole karyotypic aberration. Through FISH and whole transcriptome sequencing analysis we found a novel fusion between the *IRF2BP2* gene and the transcription factor *CDX1* gene arising from the translocation. The *IRF2BP2-CDX1* has not formerly been described in human neoplasia. In our hospital's archives three more cases of MC were found, and we examined them looking for the supposedly more common *HEY1-NCOA2* fusion, finding it in all three tumours but not in the case showing t(1;5) and *IRF2BP2-CDX1* gene fusion. This demonstrates that genetic heterogeneity exists in mesenchymal chondrosarcoma.

RESEARCH ARTICLE

Comprehensive Genomic Analysis of Rhabdomyosarcoma Reveals a Landscape of Alterations Affecting a Common Genetic Axis in Fusion-Positive and Fusion-Negative Tumors

Jack F. Shern[1], Li Chen[1], Juliann Chmielecki[3,4], Jun S. Wei[1], Rajesh Patidar[1], Mara Rosenberg[2], Lauren Ambrogio[3], Daniel Auclair[3], Jianjun Wang[1], Young K. Song[1], Catharine Tolman[1], Laura Hurd[1], Hongling Liao[1], Shile Zhang[1], Dominik Bogen[3], Andrew S. Brohl[1], Sivasish Sindiri[2], Daniel Catchpoole[5], Thomas Badgett[1], Gad Getz[3], Jaume Mora[10], James R. Anderson[6], Stephen X. Skapek[11], Frederic G. Barr[2], Matthew Meyerson[3,4,5], Douglas S. Hawkins[8], and Javed Khan[1]

ABSTRACT

Despite gains in survival, outcomes for patients with metastatic or recurrent rhabdomyosarcoma remain dismal. In a collaboration between the National Cancer Institute, Children's Oncology Group, and Broad Institute, we performed whole-genome, whole-exome, and transcriptome sequencing to characterize the landscape of somatic alterations in 147 tumor/normal pairs. Two genotypes are evident in rhabdomyosarcoma tumors: those characterized by the PAX3 or PAX7 fusion and those that lack these fusions but harbor mutations in key signaling pathways. The overall burden of somatic mutations in rhabdomyosarcoma is relatively low, especially in tumors that harbor a PAX3/7 gene fusion. In addition to previously reported mutations in *NRAS*, *KRAS*, *HRAS*, *FGFR4*, *PIK3CA*, and *CTNNB1*, we found novel recurrent mutations in *FBXW7* and *BCOR*, providing potential new avenues for therapeutic intervention. Furthermore, alteration of the receptor tyrosine kinase/RAS/PIK3CA axis affects 93% of cases, providing a framework for genomics-directed therapies that might improve outcomes for patients with rhabdomyosarcoma.

SIGNIFICANCE: This is the most comprehensive genomic analysis of rhabdomyosarcoma to date. Despite a relatively low mutation rate, multiple genes were recurrently altered, including *NRAS*, *KRAS*, *HRAS*, *FGFR4*, *PIK3CA*, *CTNNB1*, *FBXW7*, and *BCOR*. In addition, a majority of rhabdomyosarcoma tumors alter the receptor tyrosine kinase/RAS/PIK3CA axis, providing an opportunity for genomics-guided intervention. *Cancer Discov* 4(2); 216–31. ©2014 AACR.

Targeting Oxidative Stress in Embryonal Rhabdomyosarcoma

Xiang Chen[1,16], Elizabeth Stewart[1,16], Anang A. Shelat[1], Chunxu Qu[1], Armita Bahrami[2], Mark Hatley[2], Gang Wu[1], Cori Bradley[2], Justina McEvoy[2], Alberto Pappo[2], Shari Spunt[2], Marcus B. Valentine[2], Virginia Valentine[2], Fred Krafcik[2], Walter H. Lang[2], Monika Wendt[2], Lyudmila Tsurkan[3], Viktor Tollenaar[3], Sara M. Federico[3], Chris Morton[1], Charles Lu[1], Li Ding[3,15,17], John Easton[1], Michael Rusch[1], Panduka Nagahawatte[1], Jianmin Wang[1], Matthew Parker[1], Lei Wei[1], Erin Hedlund[1], David Finkelstein[1], Michael Edmonson[1], Sheila Shurtleff[1], Kristy Boggs[1], Heather Mulder[1], Donald Yergeau[1], Steve Skapek[11], Douglas S. Hawkins[14], Nitza Ramirez[12], Philip M. Potter[1], John A. Sandoval[1], Andrew M. Davidoff[1], Elaine R. Mardis[3,4,5], Richard K. Wilson[3,4,5], Jinghui Zhang[1], James R. Downing[13], and Michael A. Dyer[1,16] on behalf of the St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project

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[10]Department of Medicine, Washington University School of Medicine in St. Louis, St. Louis, MO 63108, USA
[11]Sherman Cancer Center, Washington University School of Medicine in St. Louis, St. Louis, MO 63108, USA
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[16]These authors contributed equally to this work.
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http://dx.doi.org/10.1016/j.ccr.2013.11.002

SUMMARY

Rhabdomyosarcoma is a soft-tissue sarcoma with molecular and cellular features of developing skeletal muscle. Rhabdomyosarcoma has two major histologic subtypes, embryonal and alveolar, each with distinct clinical, molecular, and genetic features. Genomic analysis shows that embryonal tumors have more struc-

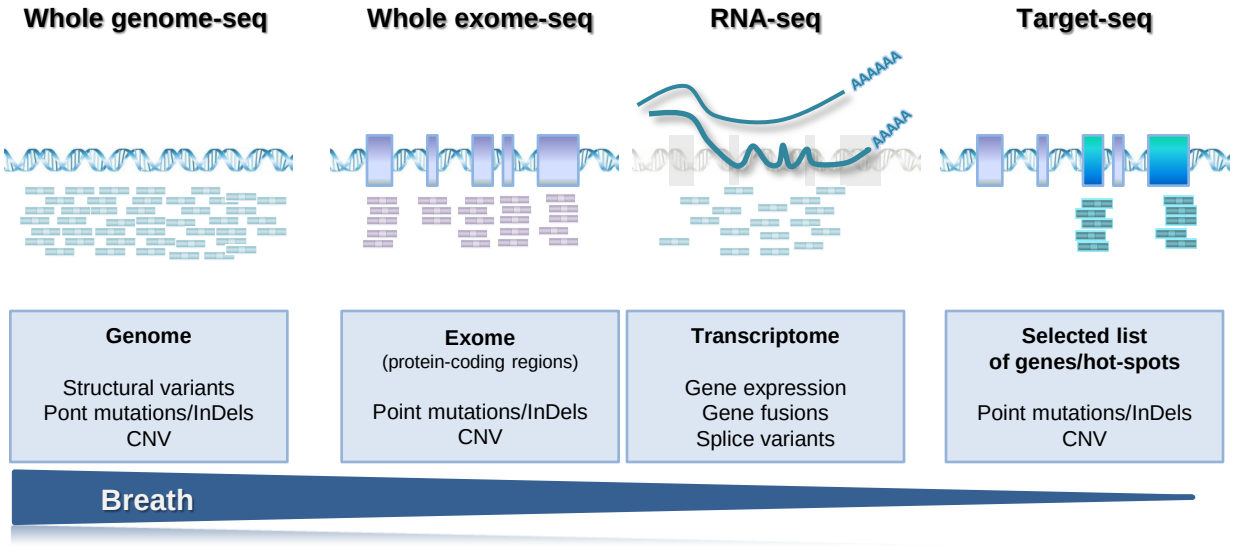
Screening

Diagnosis

Prognosis

Personalized therapy

Disease monitoring

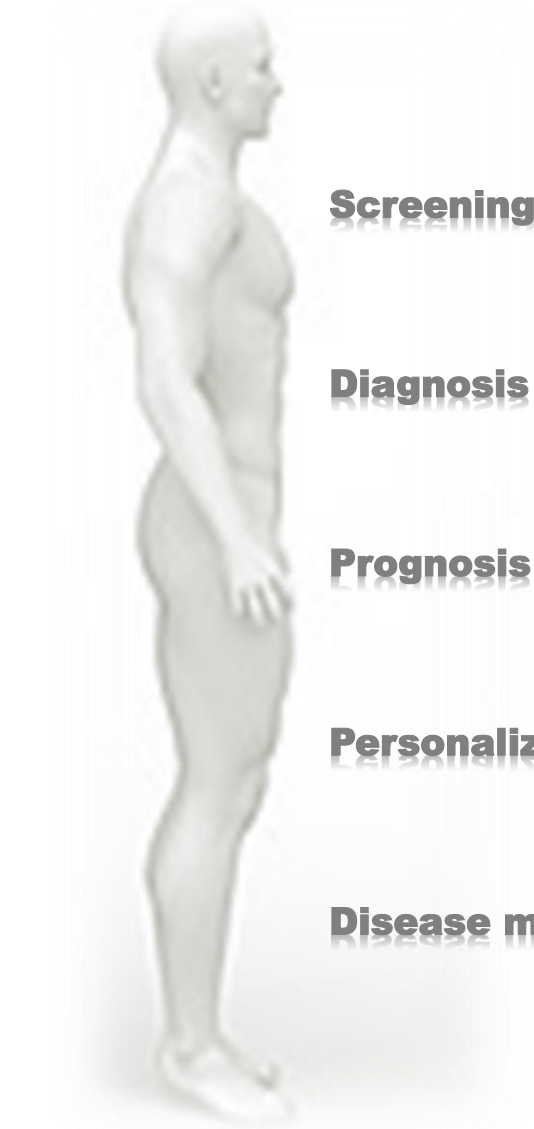


NGS rule of thumb

Breath x Depth = Cost

'complexity' x 'accuracy' = cost

TARGETED SEQUENCING



Screening

Diagnosis

Prognosis

Personalized therapy

Disease monitoring

Accurate, fast and cost-effective analysis of a selected set of clinically actionable mutations

TARGETED SEQUENCING

Screening

Diagnosis

Prognosis

Personalized therapy

Disease monitoring

The NEW ENGLAND JOURNAL of MEDICINE

Perspective

DECEMBER 19, 2013

FDA Approval of Illumina Miseq for genetic/genomic testing

First FDA Authorization for Next-Generation Sequencer

Francis S. Collins, M.D., Ph.D., and Margaret A. Hamburg, M.D.

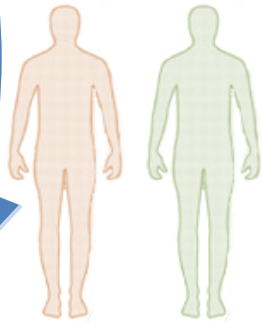
Accurate, fast and cost-effective analysis of a selected set of clinically actionable mutations

Cycle of personalized cancer medicine

Genetic/genomic/epigenetic investigations

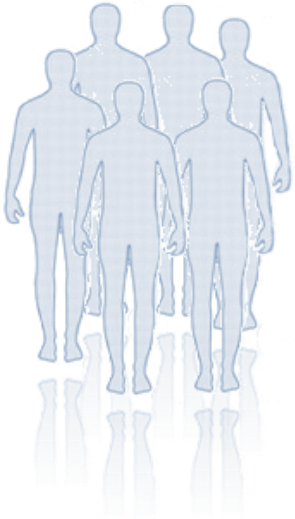
Increased knowledge

Hypotheses for Patient risk stratification



Validation/Exploitation

Personalized medicine



Thank you

Roberta Maestro
CRO Aviano

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New technologies may be upsetting….

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J Pathol 2010; **220**: 307–315
Published online 17 November 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/path.2636

THE JOURNAL OF Pathology

Invited Review

Does massively parallel DNA resequencing signify the end of histopathology as we know it?

Samuel AJR Aparicio[1,2,3]* and David G Huntsman[2,3]

[1]*Molecular Oncology, BC Cancer Agency, 675 W10th Avenue, Vancouver V5Z 1L3, Canada*
[2]*Centre for Translational and Applied Genomics, BC Cancer Agency, Vancouver V5Z 1L3, Canada*
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Journal of Pathology
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THE JOURNAL OF Pathology

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Samuel AJR Aparicio[1,2,3]* and David G Huntsman[2,3]
[1]*Molecular Oncology, BC Cancer Agency, 675 W10th Avenue, Vancouver V5Z 1L3, Canada*
[2]*Centre for Translational and Applied Genomics, BC Cancer Agency, Vancouver V5Z 1L3, Canada*
[3]*Department of Pathology, University of British Columbia, G227-2211 Wesbrook Mall, Vancouver, BC V6T 2B5 Canada*

These technologies **will become part of histopathology practice** and will augment microscopy, either directly or indirectly, through the **incorporation of novel sequencing-based diagnostics into routine pathology practice**