Canadian Centre for Computational Genomics

BIOINFORMATICS CANCER ANALYSIS

Mathieu Bourgey, *Ph.D*

MonBUG March meeting

2016-03-09

McGill

Outline

0. C3G presentation

1. Cancer genomics intro

2. Cancer interesting tools

3. GSoC 2016

What is C3G ?

- Genome Canada supported center lead by Dr Guillaume Bourque (McGill University) and Dr Michael Brudno (University of Toronto)
- C3G provides bioinformatics analysis and HPC services and solutions for the life science research community.
- C3G Montreal is divided in 2 sub-unit:
  - Services
  - R&D

C3GMTL - SERVICES

- Whole genome/exome analysis
- Whole transcriptome analysis
- Whole transcriptome assembly
- Genome assembly
- Cancer Analysis
- Small RNA analysis
- Whole genome bisulfite and SeqCap-Epi analysis
- Metagenomics analysis
- ChIP-seq analysis
- High coverage amplicon analysis
- Microarray analysis
- Gene and parthway analysis
- Customized Analysis

Webpage: http://computationalgenomics.ca/

C3GMTL – RESEARCH AND DEVELOPMENT

- Analysis pipelines:
  - https://bitbucket.org/mugqic/mugqic_pipelines
  - Python based framework
  - Reduce micro-management
  - WES/WGS; RNA; RNA assembly; ChipSeq; Pacbio small genome assembly; Illumina runProcessing
- Maintain an up-to-date set of bioinformatics tools (>80) and resources (18) to the community
  - Available through a MUGQIC - Compute Canada partnership
  - Based on the use of CernVM-FS
- Provide and maintain a semi-private local instance of Galaxy using Compute Canada resources
  - Light version of our DNA and RNA pipeline are available
- Integrate new technology and development
  - Single cell
  - Haloplex
  - PacBio assembly for Eukaryote
  - HLA typing
  - Bioinformatics tools: SCoNEs, popSV, BVAtools,…
  - Bioinformatics pipeline development: WGBS, Metagenomics, ***Cancer analysis***

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Cancer

- Cancer is one of the most common diseases in the developed world:
  - 3 out of 10 deaths are due to cancer in Canada
  - Lung cancer is the most common cancer in men
  - Breast cancer is the most common cancer in women
  - There are over 100 different forms of cancer

What causes cancer?

- Cancer arises from the mutation/alteration of a normal(s) gene(s).

- It is thought that several mutations need to occur to give rise to cancer

- Cells that are old or not functioning properly normally self destruct and are replaced by new cells.

- However, cancerous cells do not self destruct and continue to divide rapidly producing millions of new cancerous cells.

The genomic alterations model

- A wide range of genomic alterations can lead to the development of cancer:
  - Mutations
  - Copy number changes
  - Rearrangements

- Most of these alterations are somatic:
  - They are present in cancer cells but not in a patient's germ line

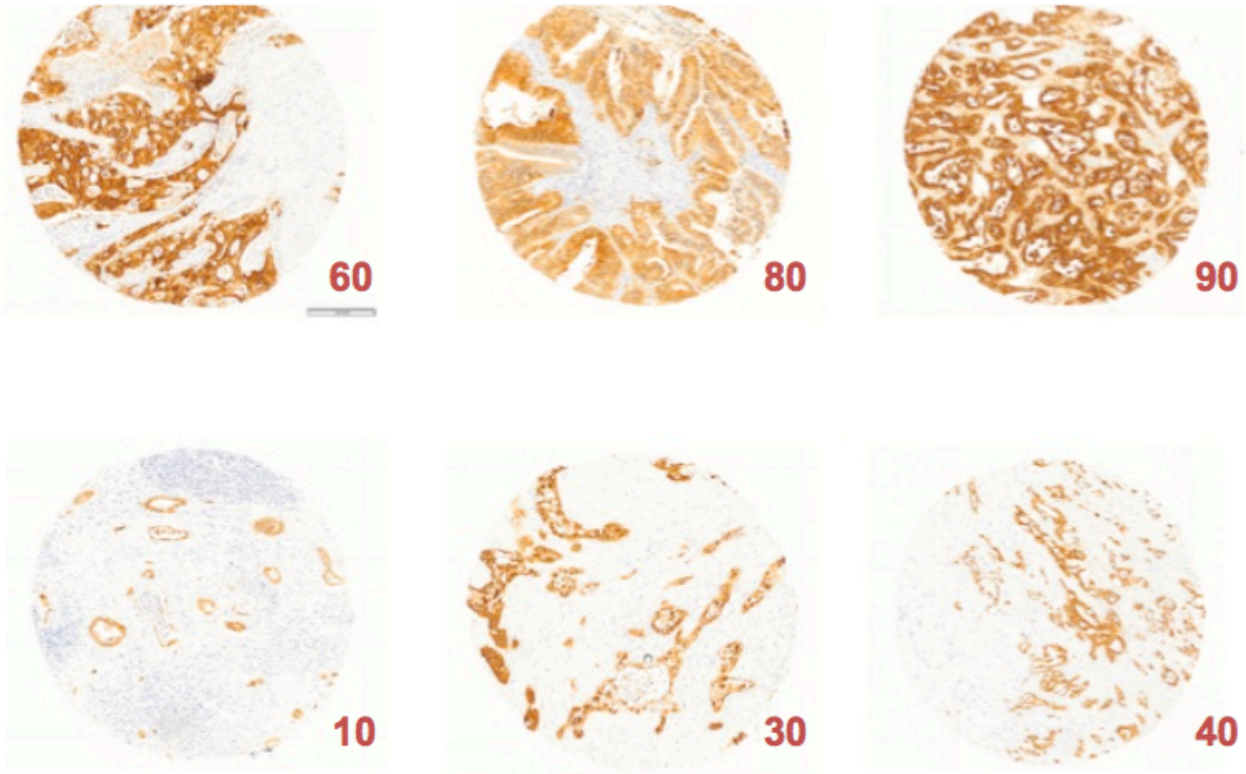
- Somatic genome alterations can provides potential therapies targeted:
  - Treatment with the inhibitors of the epidermal growth factor receptor kinase (EGFR) leads to a significant survival benefit in patients with lung cancer whose tumours carry *EGFR mutations, but no benefit in patients* whose tumours carry wild-type *EGFR*

Rosell, R. *et al. N. Engl. J. Med.*361, 958–967 (2009)

Characteristics of cancer samples

- Lower quantity
  - Quantity of material available is often smaller for cancer sample than for germ line sample
- Lower quality
  - Most cancer biopsy and resection specimens are formalin-fixed and paraffin-embedded (FFPE)
  - Cancer specimens often include substantial fractions of necrotic or apoptotic cells that reduce the average nucleic acid quality
- Lower purity
  - cancer specimen contains a mixture a mixture of cancer and normal genomes (**Cellularity**).
  - The cancers themselves may composed of different clones that have different genomes (heterogeneity)

Example of cellularity

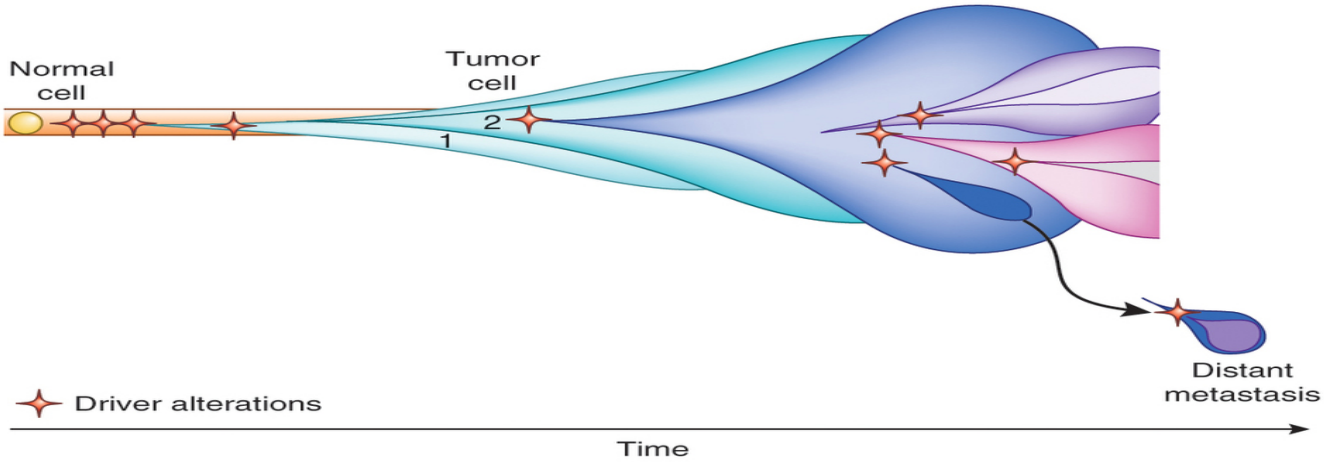


Modified from Dr Mark Cowley

Charateristics of cancer genomes

- Structural variability:
  - Mutation frequency (**Mutational signature**)
  - Global copy number or **Ploidy**
  - Genome structure

- Clonality



From Alizadeh et al. Nature Medicine 21, 846–853 (2015)

Cancer analysis challenge
Driver vs Passengers mutations

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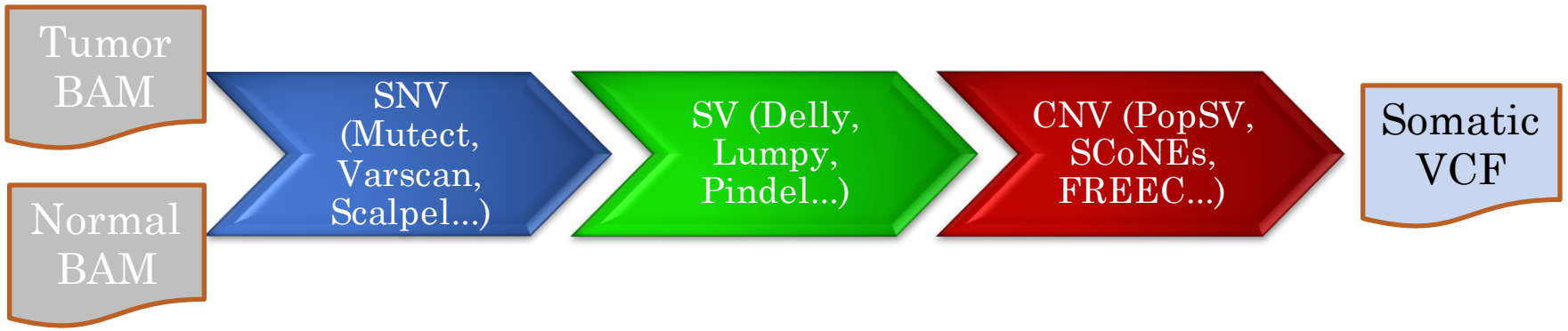
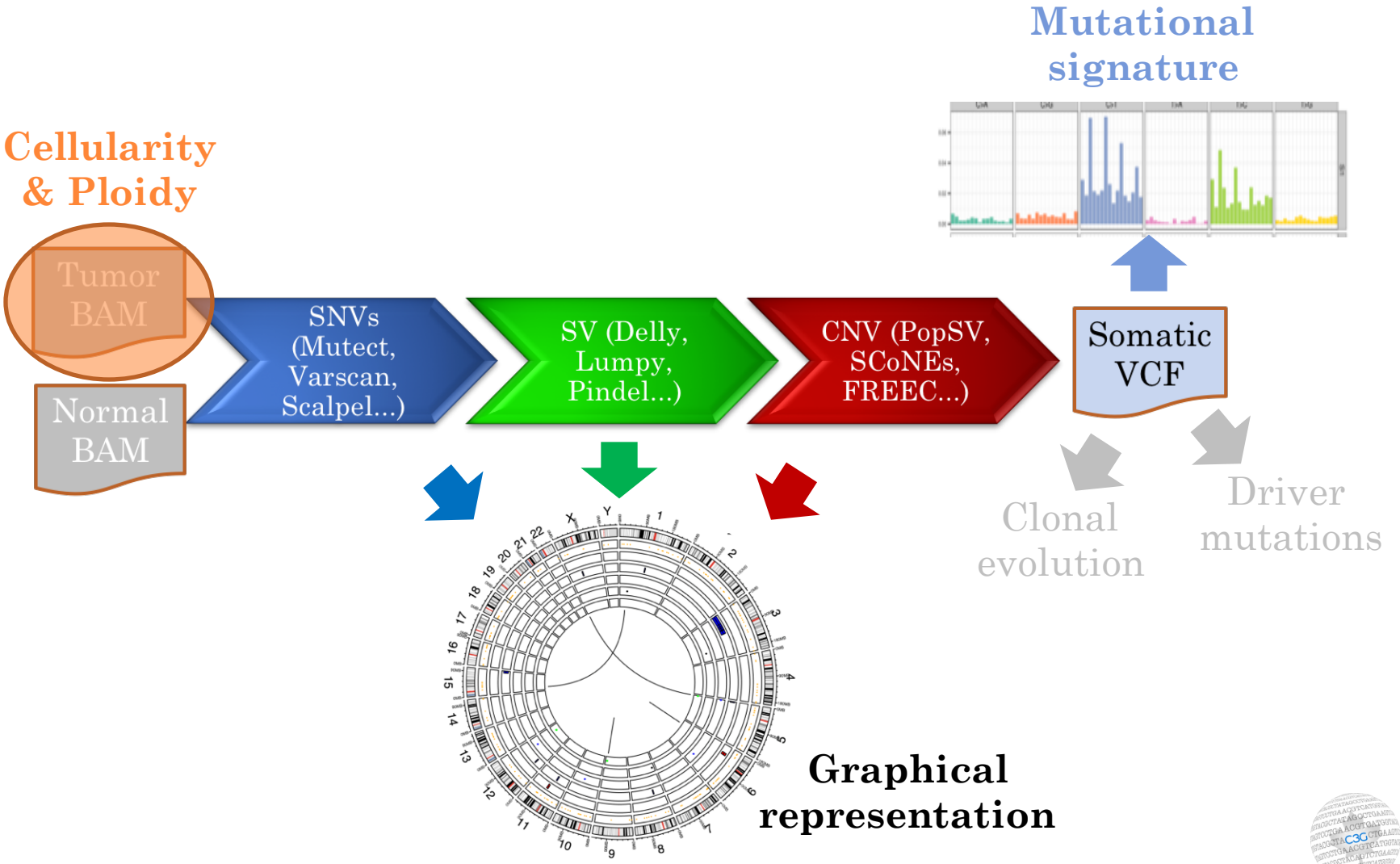
Cancer analysis summary



Lot of studies are conducted each year to define the set of best tools !

BUT IS IT ENOUGH ?

Extended cancer analysis



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Celluarity and ploidy

Cellularity and ploidy

- As shown in introduction cancer analysis suffer from specificity of cancer sample (Cellularity) and of cancer genome (ploidy)
- Few cancer analysis tools take these parameters into account while it does variant calling. (SNV or SV)
- One method exists for CNV analysis using SNParray data:
  - ASCAT
- NGS methods:
  - ABSOLUTE
  - absCN-seq
  - *PurBayes*
  - *PurityEst*
  - *Titan*
- 2 methods have tried to extend ASCAT to NGS data:
  - Battenberg algorithm (from ASCAT co-author; alpha version)
  - ***Sequenza (published, good R vignettes)***

Annals of Oncology 26: 64–70, 2015
doi:10.1093/annonc/mdu479
Published online 15 October 2014

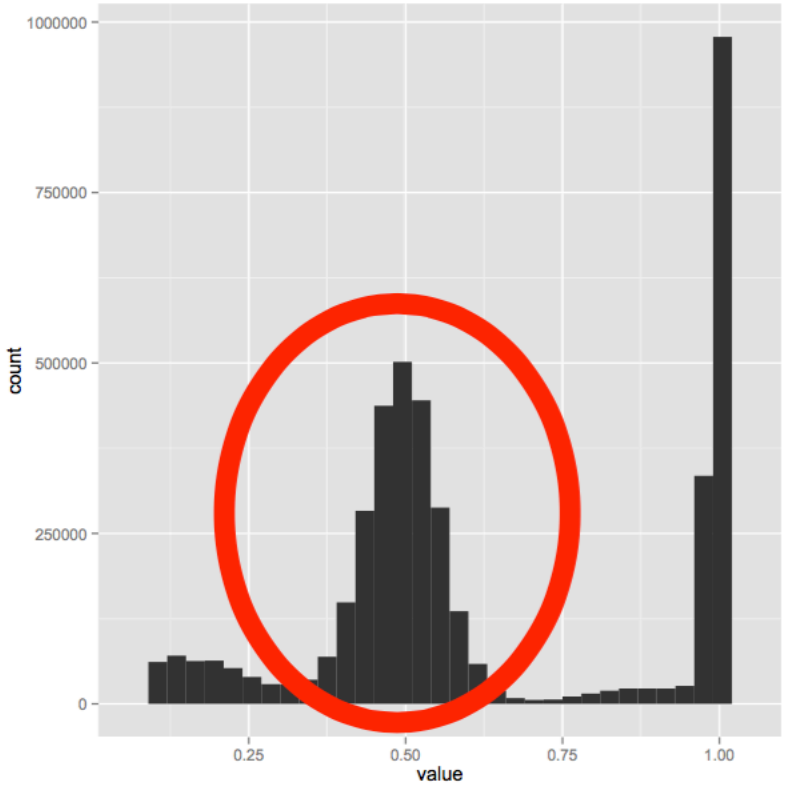
Sequenza: allele-specific copy number and mutation profiles from tumor sequencing data

F. Favero[1], T. Joshi[1], A. M. Marquard[1], N. J. Birkbak[1], M. Krzystanek[1], Q. Li[1,2], Z. Szallasi[1,3] & A. C. Eklund[1*]

[1]*Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark;* [2]*Medical School, Xiamen University, Xiamen, China;* [3]*Children's Hospital Informatics Program at the Harvard-MIT Division of Health Sciences and Technology (CHIP@HST), Harvard Medical School, Boston, USA*

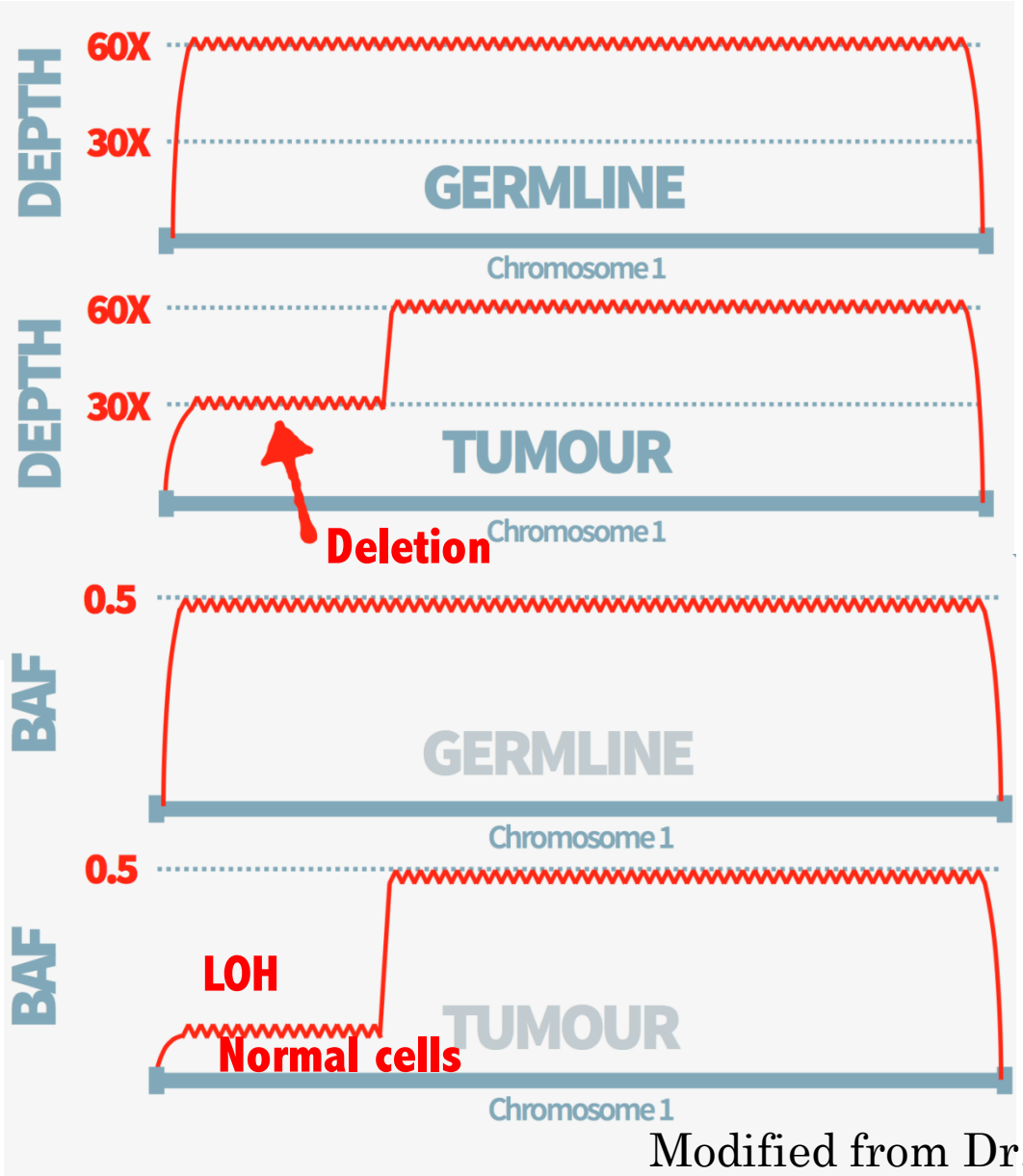
Received 7 April 2014; revised 8 October 2014; accepted 9 October 2014

- Use read depth and BAF to estimate cellularity and ploidy.
- Use cellularity estimate to improve variants and LOH calls.



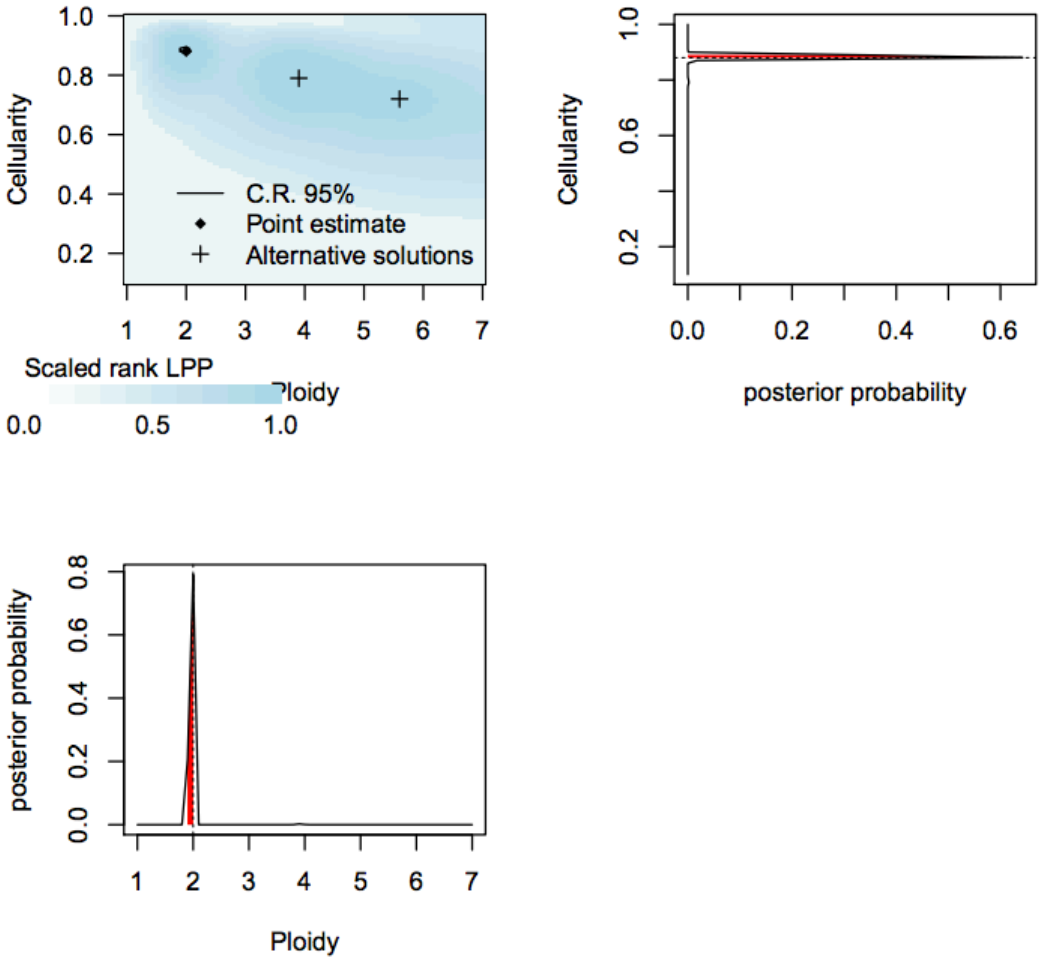
Modified from Dr. Velimir Gayevskiy

Cellularity estimation principle

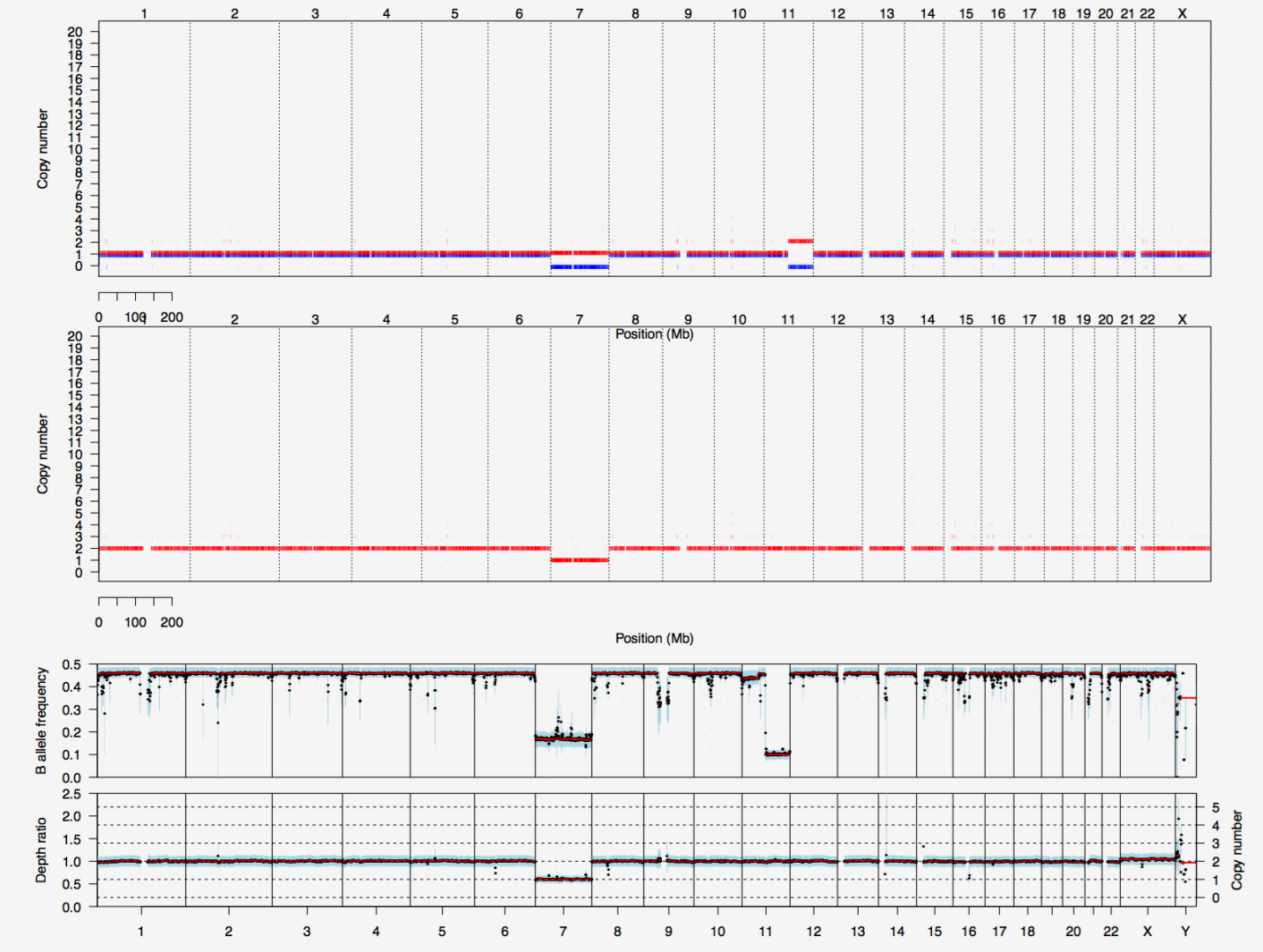


Modified from Dr. Velimir Gayevskiy

Outputs – parameters estimates

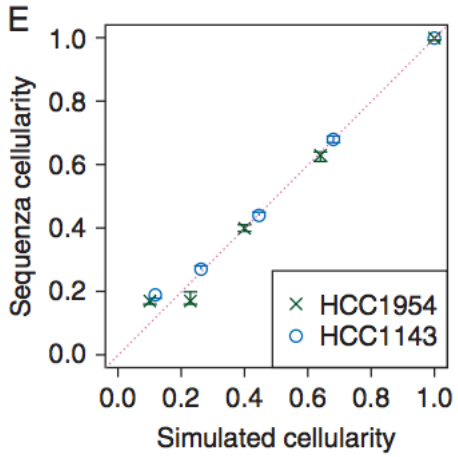


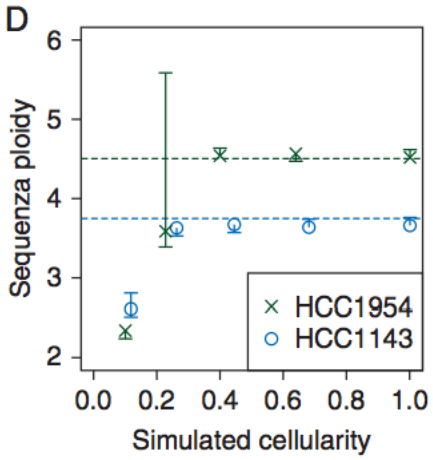
Outputs – Genome view



VALIDATION – SIMULATED DATA

- WGS, aligned to the hg19 genome at ×30 of coverage, of two cell lines HCC1143 and HCC1954, (matching normal blood)
- Simulated admixtures at tumor cellularity of 20%, 40%, 60%, and 80%





Cellularity estimates are goods and ploidy estimates seems accurate when cellularity values >= 30 %

VALIDATION – COMPARISON WITH OTHERS

- Run Sequenza, ABSOLUTE and absCN-seq on 30 WES data from TCGA and compare to ASCAT

Algorithm	r_ρ	r_ψ	$F_{\Delta CN=0}$
Sequenza	0.90 (0.91)	0.42 (0.94)	0.69
ABSOLUTE	0.19 (0.61)	0.13 (0.50)	0.08
absCN-seq	0.46 (0.65)	−0.26 (0.46)	0.02

Sequenza show an higher accuracy than its competitors

Application to real data

- By the authors:
  - Among the 29 renal cancer samples sequenza call 17 3p deletion (know to affect 70-80% of this cancer patients)
- By myself:
  - Applying the methods to 74 cagekid non-conventional paired WES sample.
  - Trying to understand:
    - Why some sample are hypermutated
    - Some interesting somatic mutations

chromoson	position	ref_allele	alt_alleles	gene_name	sequenza cellularity
12	133202816	C	T	POLE	
Patients with this POLE mutation					
	Depth	Allele Frequency	Variant normal	Variant tumor ratio	
K2150038	172 , 293	C:102 A:0 T:71 N:0 G:0,C:173 A:0 T:120 N:0 G:0	0,410404624	0,409556314	0.48
LR265	215 , 207	C:1 A:0 T:214 N:0 G:0,C:39 A:0 T:170 N:0 G:0	0,995348837	0,813397129	0.24
LR368	149 , 147	C:58 A:0 T:91 N:0 G:0,C:60 A:0 T:89 N:0 G:0	0,610738255	0,597315436	0.87
LR412	72 , 264	C:42 A:0 T:31 N:0 G:0,C:125 A:0 T:140 N:0 G:0	0,424657534	0,528301887	0.66
K2110054	299 , 205	C:147 A:0 T:152 N:0 G:0,C:110 A:0 T:95 N:0 G:0	0,508361204	0,463414634	0.49

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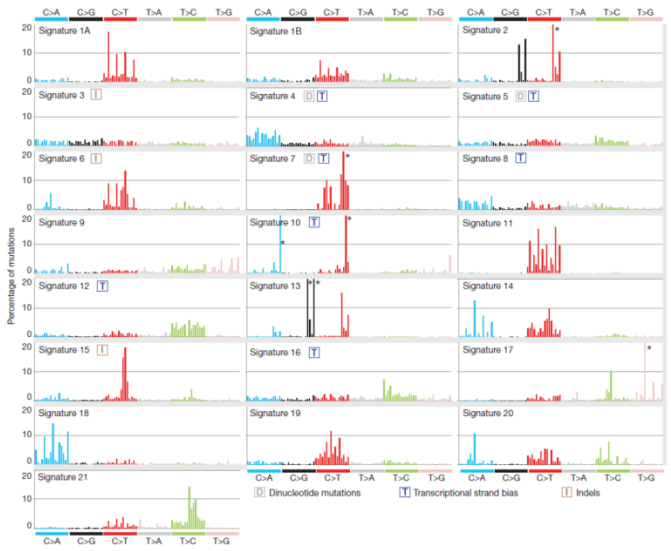
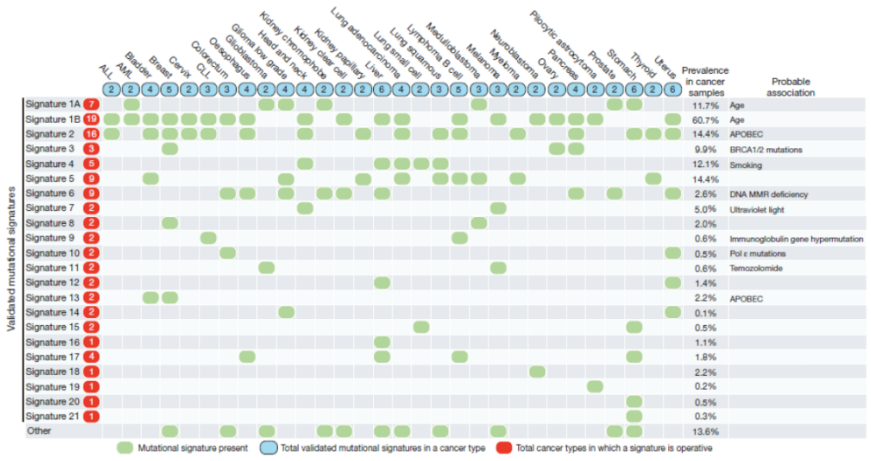
Mutational signature

What is somatic signature?

- The most common genetic model for cancer development is the accumulation of DNA mutations.
- Recently Alexandrov et al. (Nature 2013) developped a method to ***cluster cancer sample together by the type of the mutation and also what the neighbouring bases are.***
- Common mutational processes that are regularly identified in cancer sequencing have been linked to specific signature:





Analyzing somatic signature

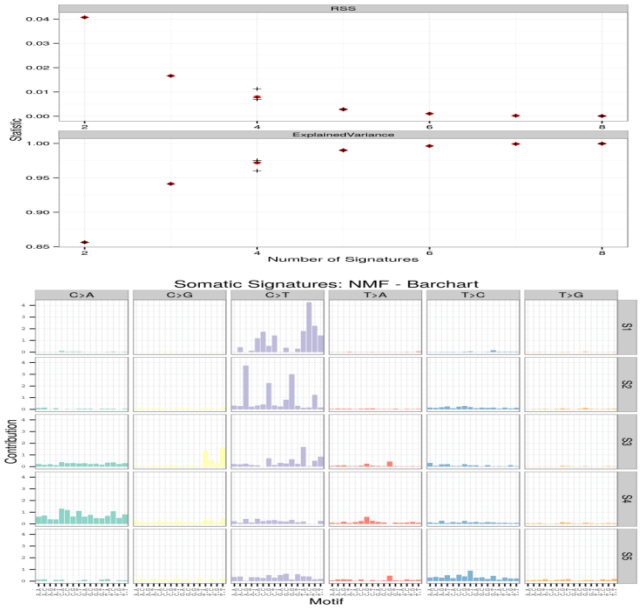
- Extraction of somatic signature
  - Alexandrov's method
    - WTSI Mutational Signature Framework
    - Matlab script
    - Not user friendly
    - Proprietary software
  - **J. Gehring's implementation *(Bioinformatics 2015)***
    - SomaticSignature R package
    - In Bioconductor
    - User friendly
    - With a good vignette

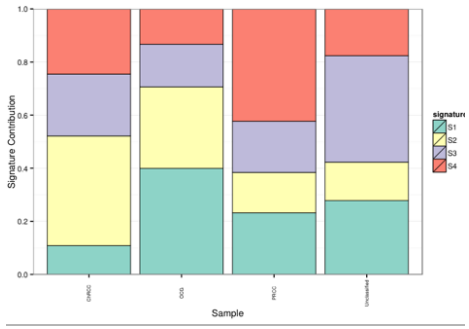
SOMATICSIGNATURE PRINCIPLE

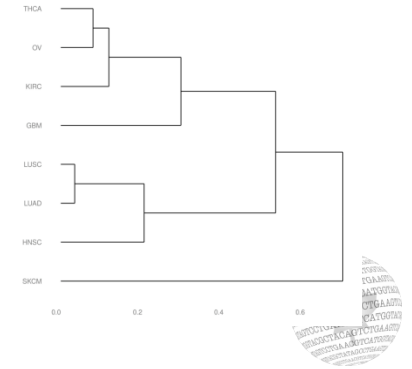
- The somatic motifs for each variant are retrieved from the reference sequence
  - mutationContext function and
- They are converted to a matrix representation
  - motifMatrix function.
- Somatic signatures are estimated with a method of choice
  - nmfDecomposition (alexandrov)
  - pcaDecomposition (large data set)
- The somatic signatures and their representation in the samples are assessed with a set of accessor and plotting functions.

How it works ?

- Once you generated the mutation context matrix
  - Find out how many signatures you have in the data
    - assessNumberSignatures
  - Identify the corresponding signature
    - identifySignatures
  - Determine signature contribution
    - plotSamples
  - Samples clustering
    - clusterSpectrum







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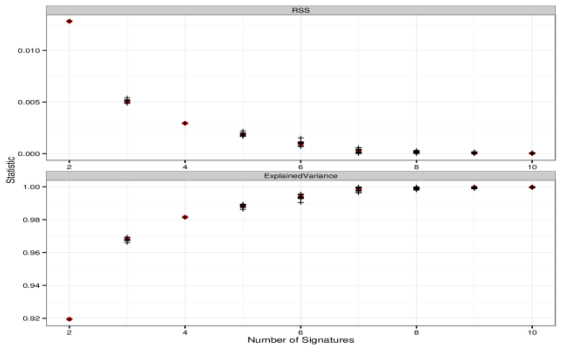
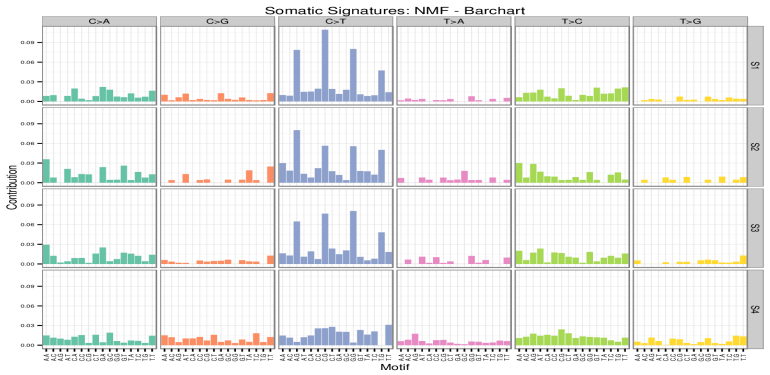
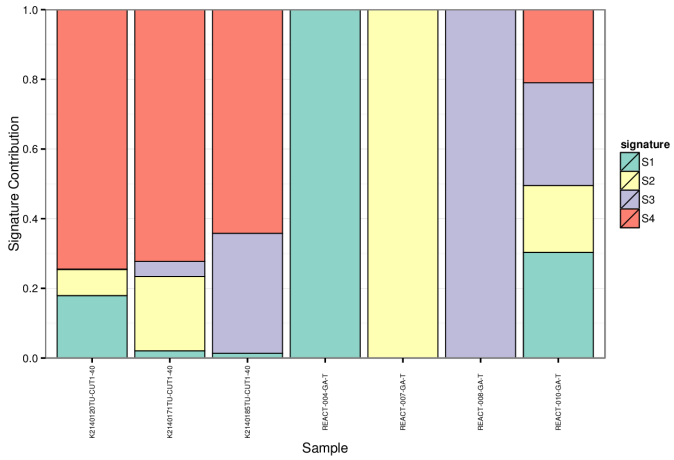
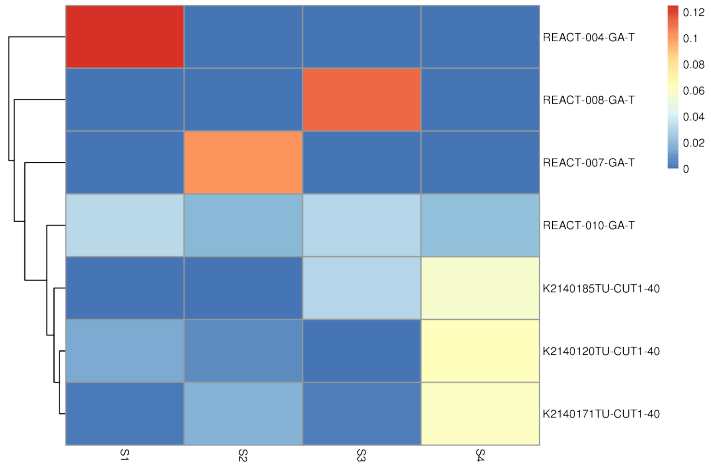
Real exemple

- Mixing 3 samples from kidney cancer with 4 samples from Typhoid cancer







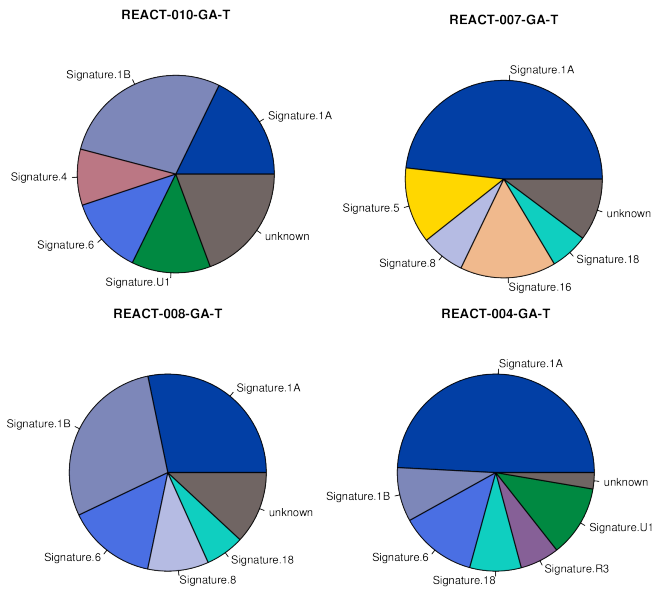


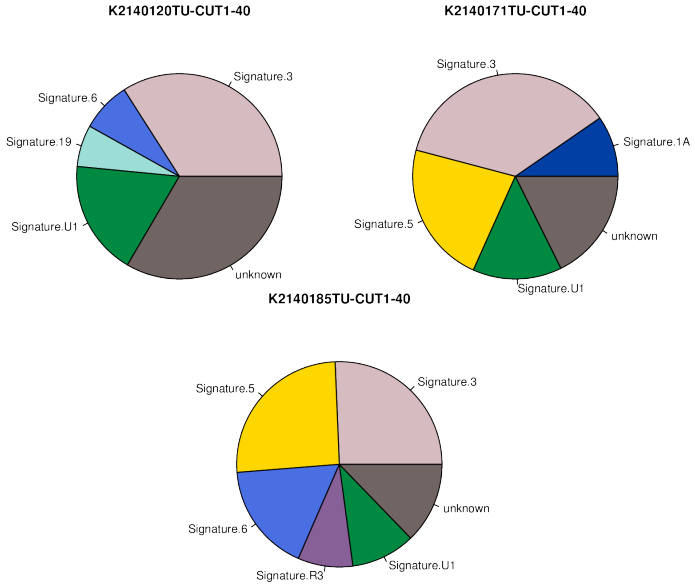
What is missing ?

- The limitation of this method is to not provide a way to link identified signature with a set of predefined signature
  - Is my signatures correspond to one of those detected by Alexendrov (or by a competitor) ?
- Riku et al. (Nat Gen 2015) proposed to use of the mean Kullback-Leibler divergence.
  - Available from R package entropy
  - It suffer from overparmetrization
  - Most of the time results are ambigous
    - All signature point to same one
- 2 weeks ago Rosenthal et al. (Genome Biology 2016) release deconstructSigs
  - Cran R package
  - I just start to test it
  - It is very promising

Testing DeconstructSig on real exemple

- Typhoid cancer
  - Mostly drive by signature 1.A and 1.B
    - Age
- Kidney
  - Mostly drive by signature 3 and Unknown
    - BRCA1/2 mutation





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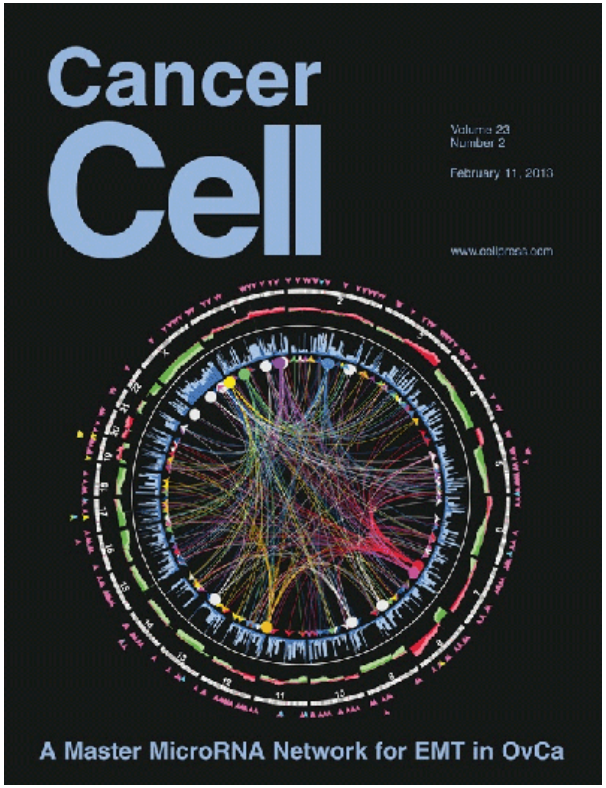
Visualization of cancer variants

Cancer data

- A major goal of cancer genomics analysis is to identify genetic changes which lead to the tumor evolution.
- In a more technical view, we ends-up with different types of genomics changes Point mutations Copy number variations Structural variants (insertion, inversion, deletion, duplication, translocation)
- A common visualization of these data is to give an overview of change for the 23 chromosome of the genome in circular view.



CIRCOS PLOT

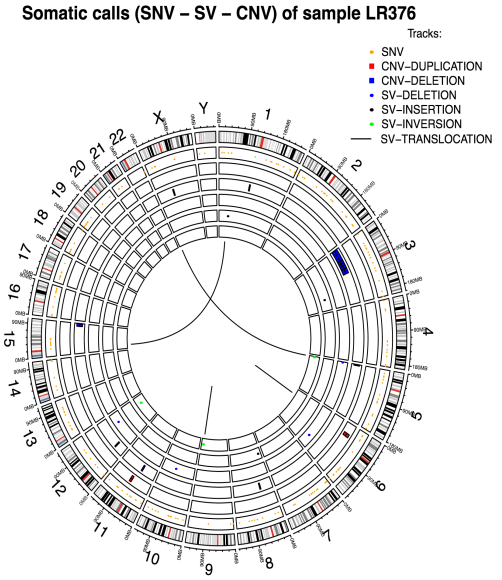
- What are the different options to draw it ?
  - Circos (www.circos.ca)
    - Not user friendly
    - Only recommended for very complex plot
  - Rcircos (R package, *Zhang H et al. Bioinformatics 2013*)
    - Limited vignettes
    - A little bit more complicated than circlize but a nicer rendering
  - circlize (R package, *Gu Z et al. Bioinformatics 2014*)
    - User friendly
    - Use bed format
    - Good vignettes
    - For genomic data: A set of methods and a specific vignette
    - Generic methods and vignettes

CIRCLIZE GENERIC WORKFLOW

- Set-up the generic graphical parameters
  - circos.par function
- Draw reference ideograms
  - circos.initializeWithIdeogram function
- draw 1 track for each type of variants
  - circos.genomicTrackPlotRegion function +
    - circos.genomicPoints function
    - circos.genomicRect function
    - circos.genomicLines function
- Draw positional links to represent transclocations
  - circos.link function
- A good graph needs title and legends
  - Use generic R methods

Real data example

- See details at: https://github.com/mbourgey/EBI_cancer_workshop_visualization/tree/Montreal_R_UserGroup
- Starting from 3 files
  - Breakdancer tsv for SVs
  - Mutect vcf for SNVs
  - SCoNEs tsv for CNVs



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Google Summer of Code (GSOC)

- Student gets $5000 for writing open source code for 3 months.
- Timeline:
  - Feb - Admins for open source organizations e.g. R, Bioconductor apply to Google.
  - Mar - Mentors suggest projects for each org. Students submit project proposals to Google. Google gives funding for n students to an org.
  - Apr - The top n students get $500 and begin coding.
  - Jul - Midterm evaluation, pass = $2250 (for student).
  - Aug - Final evaluation, pass = $2250 (for student).
  - Nov - Orgs get $500/student mentored

What makes a good GSOC project?

- Coding projects should:
  - Result in free/open-source software.
  - Be 3 months of full time work for a student.
  - Could include writing documentation and tests.
  - Should not include original research.

C3G IS AN ACCEPTED ORGANISATION FOR GSOC 2016

- https://summerofcode.withgoogle.com/organizations/5649665110310912/
- Details at: https://bitbucket.org/mugqic/gsoc2016
- List of proposed project so far :
  - Flowchart creator for MUGQIC Pipelines
  - Implement base modification analysis in the pacbio_assembly pipeline from MUGQIC Pipelines
  - Integrate structural variants calls in the tumor_pair pipeline from MUGQIC Pipeline
  - Improve SegAnnDB interactive genomic segmentation web app
  - Develop a noise reduction engine for SCoNEs
  - Develop add-ons for SCoNEs
  - EGA Data Submission database (mEGAdata)
  - Development of an HTML dynamic matrix to represent datasets with multiple dimensions
  - Population genetics simulation and modelling

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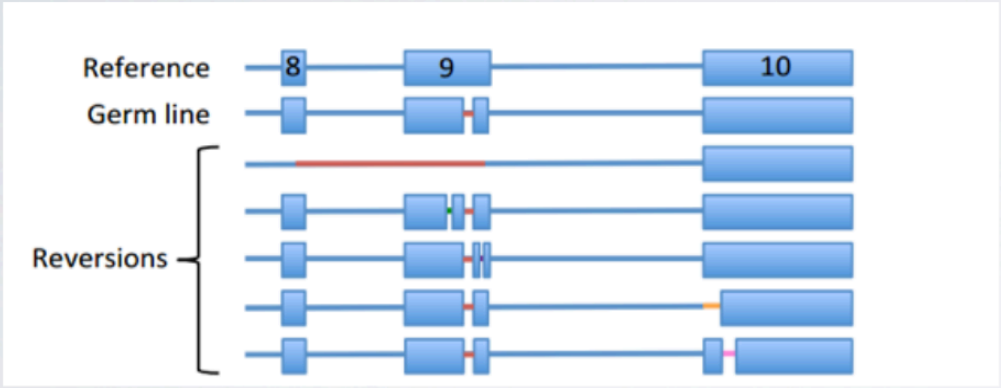
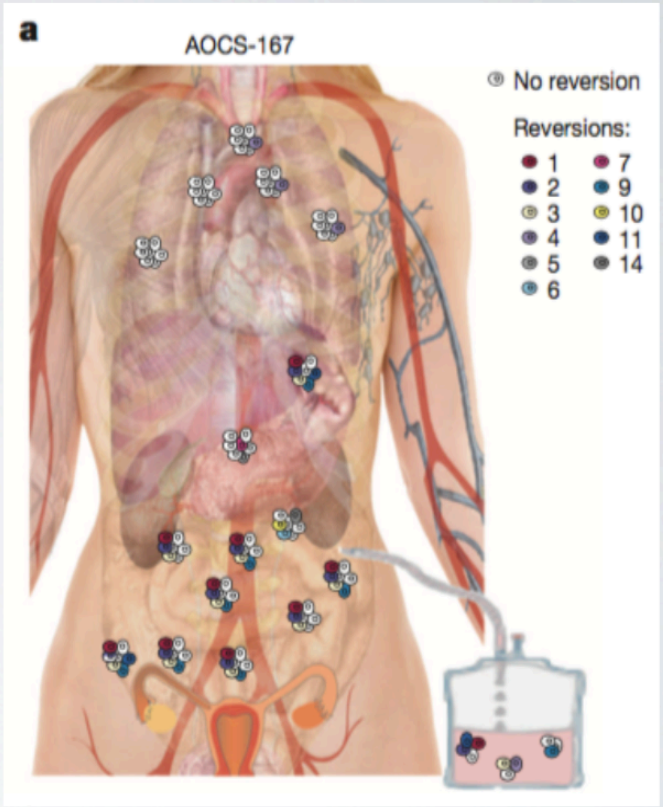
BONUS – SURPRIZING CANCER FEATURE

Ovarian Cancer - somatic reversions of inherited BRCA2 mutations





- germline 5bp deletion in BRCA2
- patient acquired resistance to PARP inhibitor olaparib and to carboplatin
- In 18 samples from same patient, 15/19 somatic mutations identified restored the reading frame

From Patch et al. Nature 2015

Acknowledgment

- C3G-Montreal
  - Guillaume Bourque
  - Robert Everleigh
  - Toby Hocking
  - François Lefebvre
  - Jean Monlong
  - Edouard Henrion
- Riazalhosseini's lab
  - Yasser Riazalhosseini
  - Madeleine Arseneault
- External:
  - Velimir Gayevskiy *(Garvan Insitut)*
  - Ann-Marie Patch *(Queensland Centre for Medical Genomics)*
  - Louis Letourneau *(sportlogIQ)*