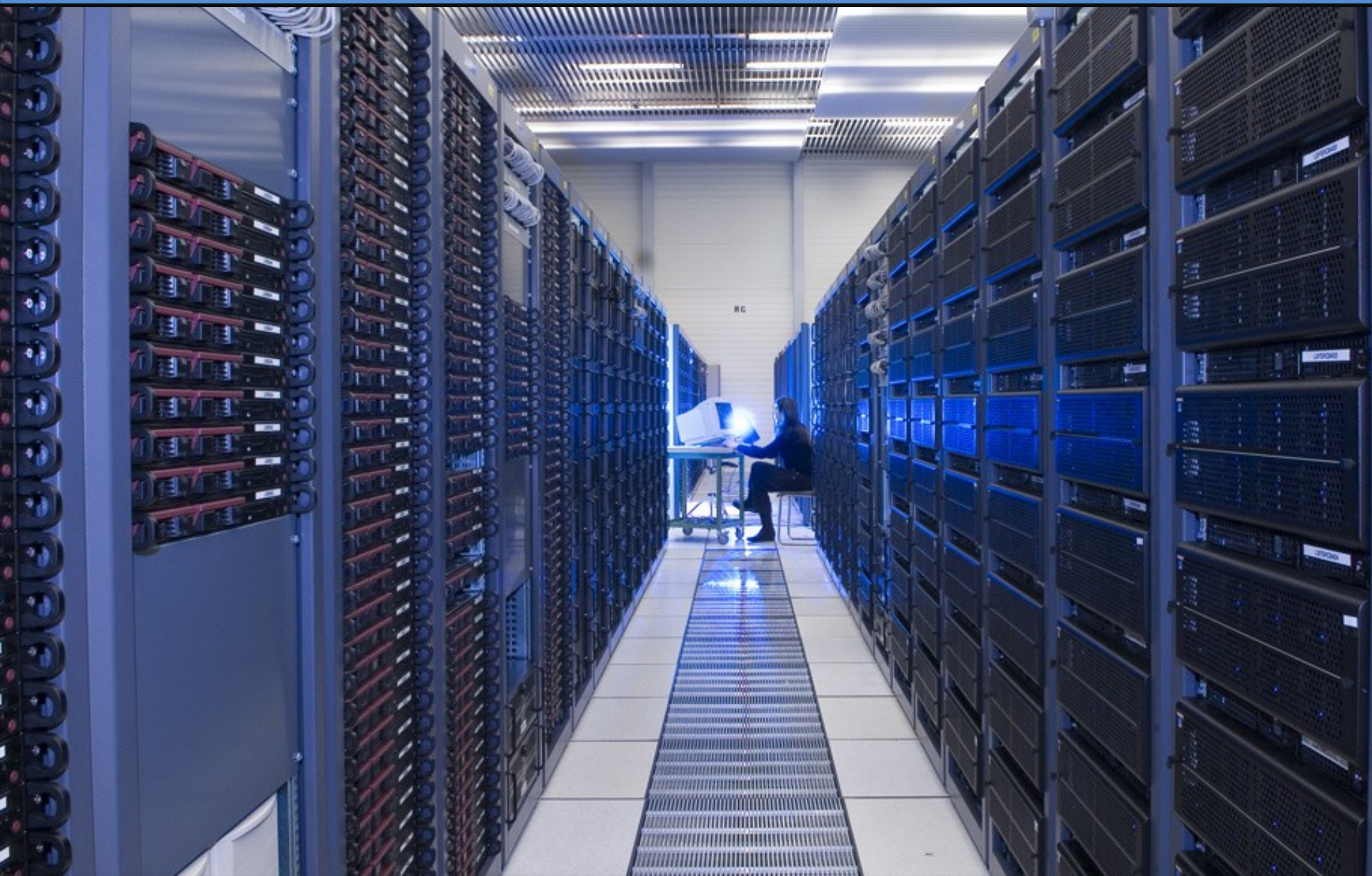
Cancer Sequencing



What is Cancer?

Definitions

- A class of diseases characterized by malignant growth of a group of cells
  - Growth is uncontrolled
  - Invasive and Damaging
  - Often able to metastasize
- An instance of such a disease (a malignant tumor)
- A disease of the genome

http://en.wikipedia.org/wiki/Cancer

http://faculty.ksu.edu.sa/tatiah/Pictures%20Library/normal%20male%20karyotyping.jpg

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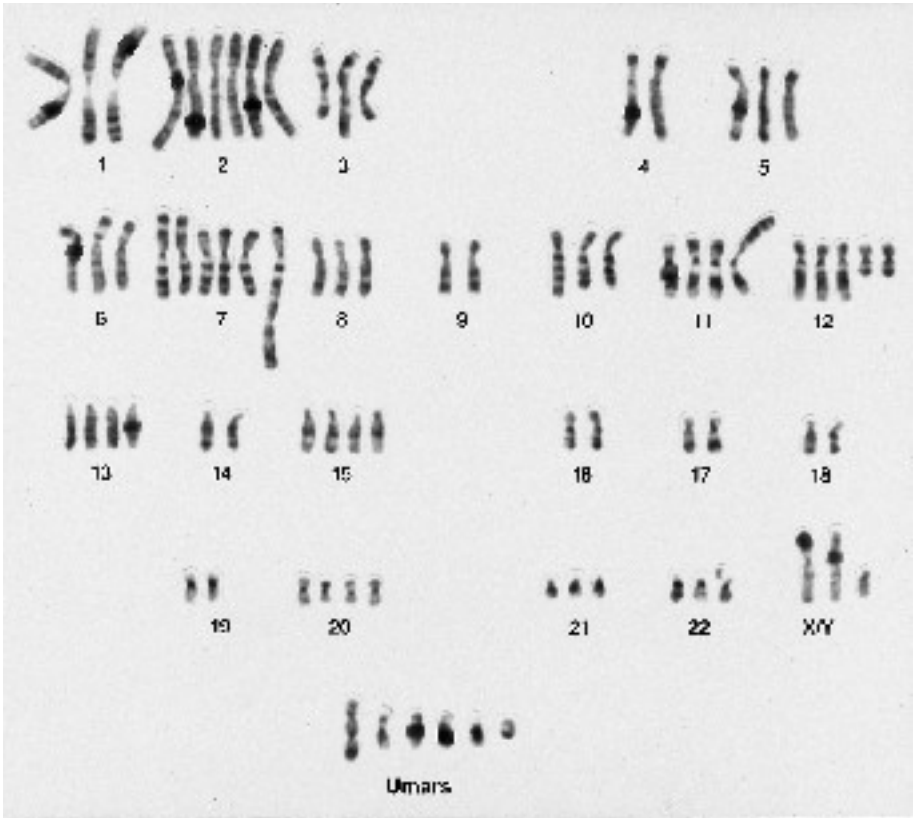
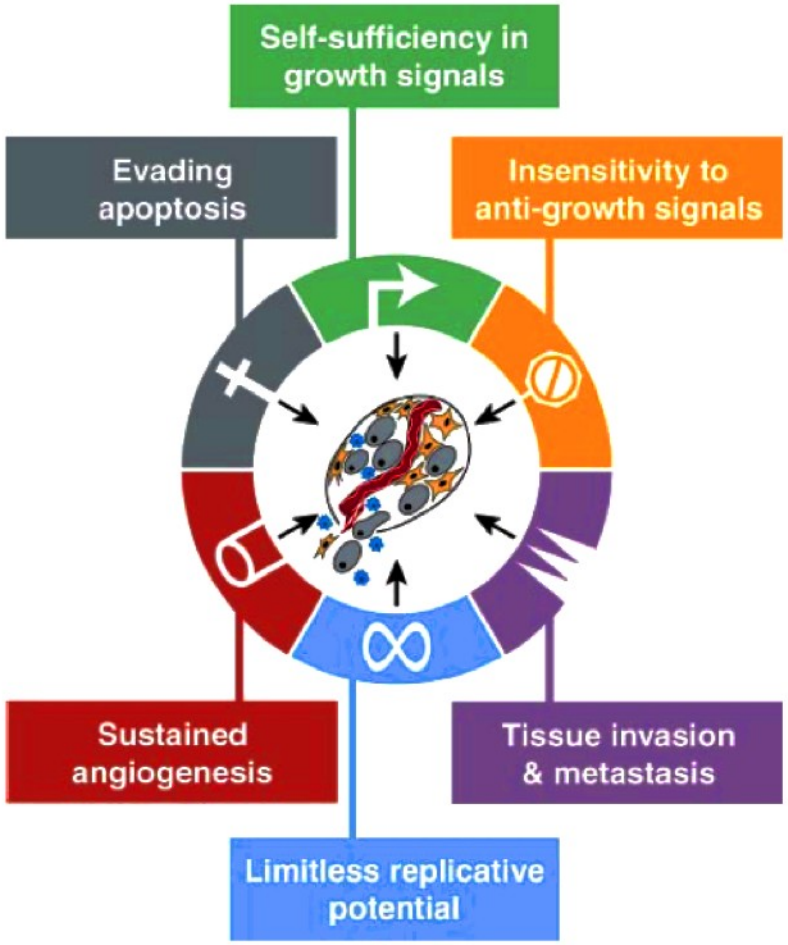


http://en.wikipedia.org/wiki/Cancer

http://www.moffitt.org/CCJRoot/v2n5/artcl2img4.gif

Fundamental Changes in Cancer Cell Physiology



Exploitation of natural pathways for cellular growth

- Growth Signals (e.g. TGF family)
- Angiogenesis
- Tissue Invasion & Metastasis

Evasion of anti-cancer control mechanisms

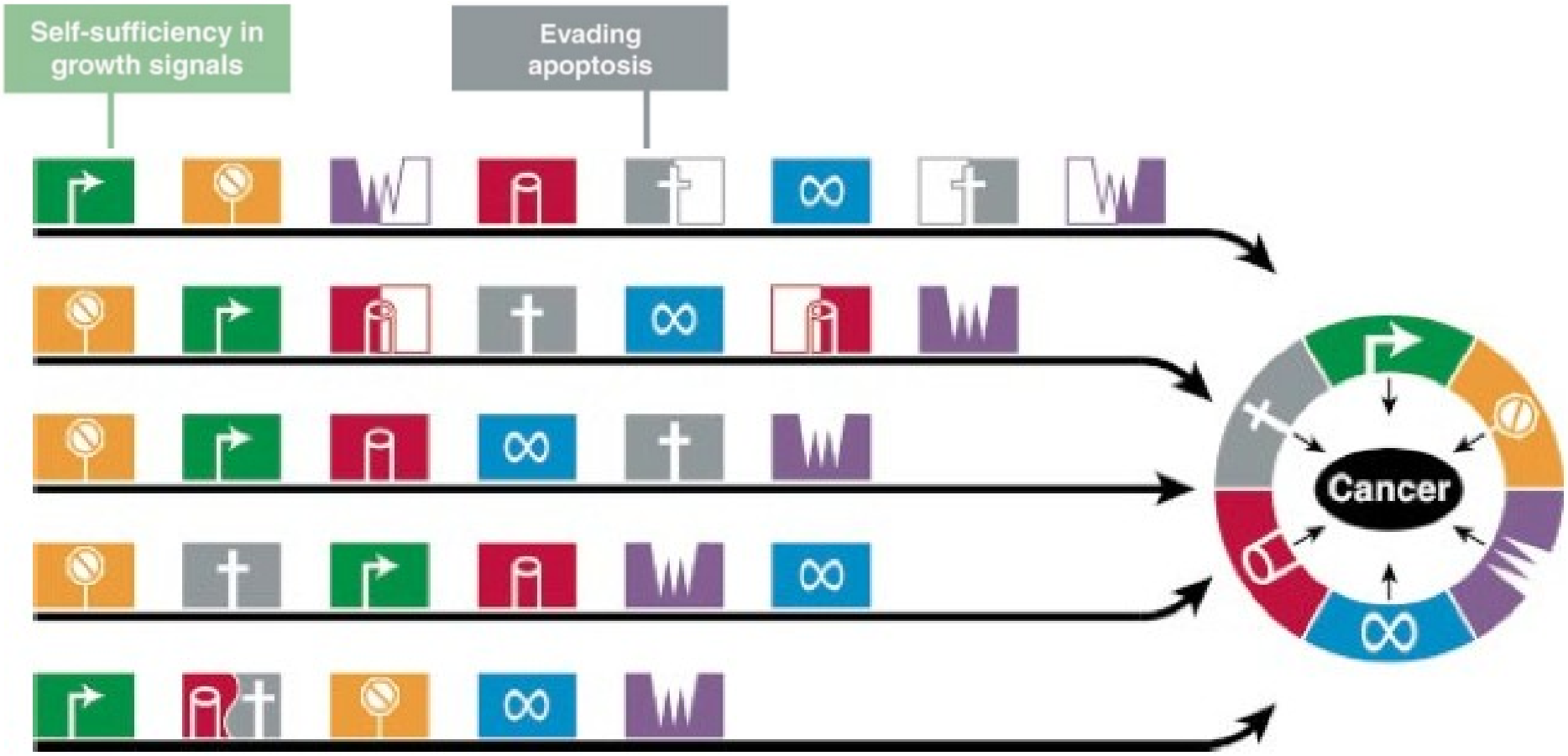
- Apoptosis (e.g. p53)
- Antigrowth signals (e.g. pRb)
- Cell Senescence

Acceleration of Cellular Evolution Via Genome Instability

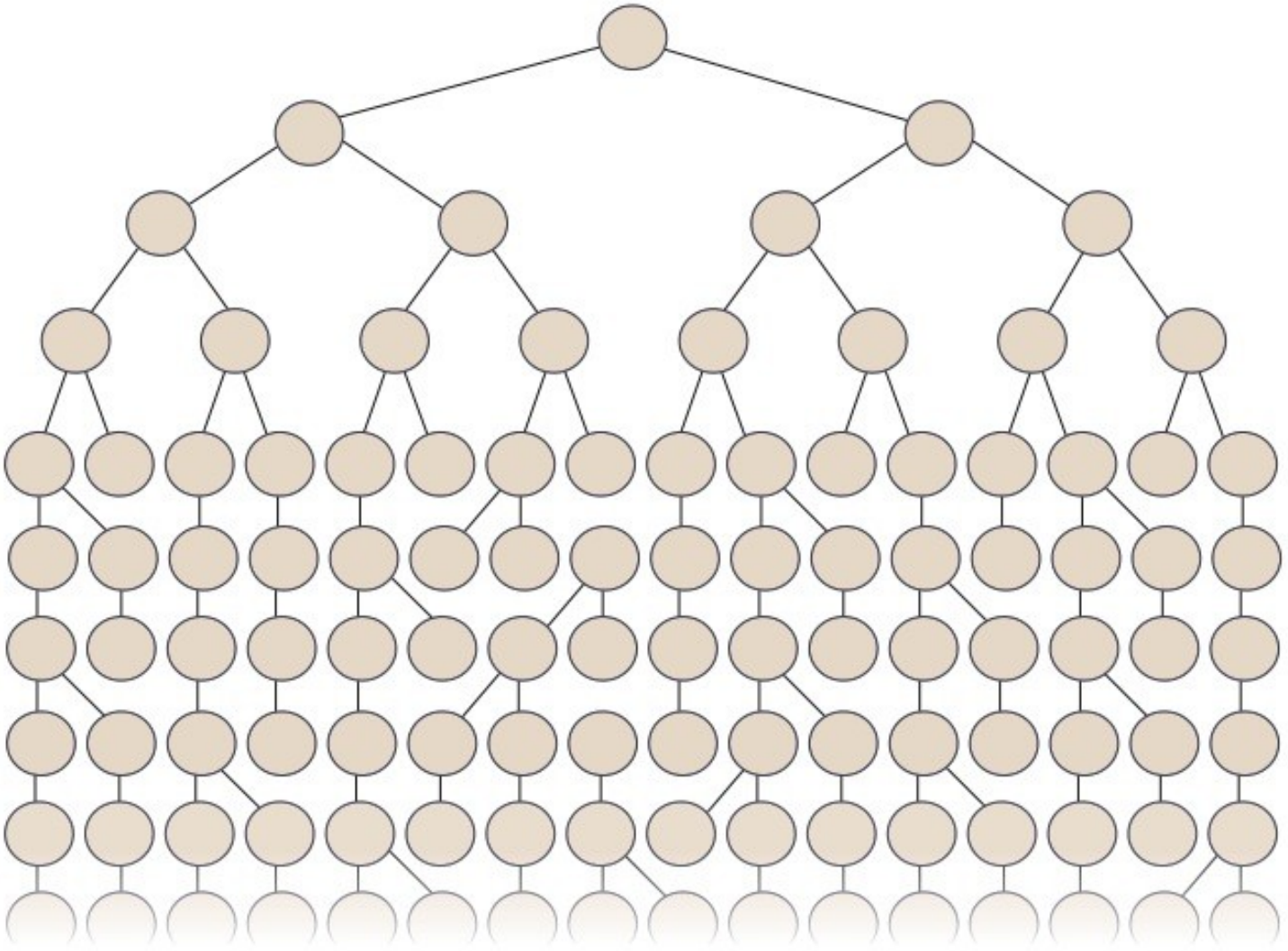
- DNA Repair
- DNA Polymerase

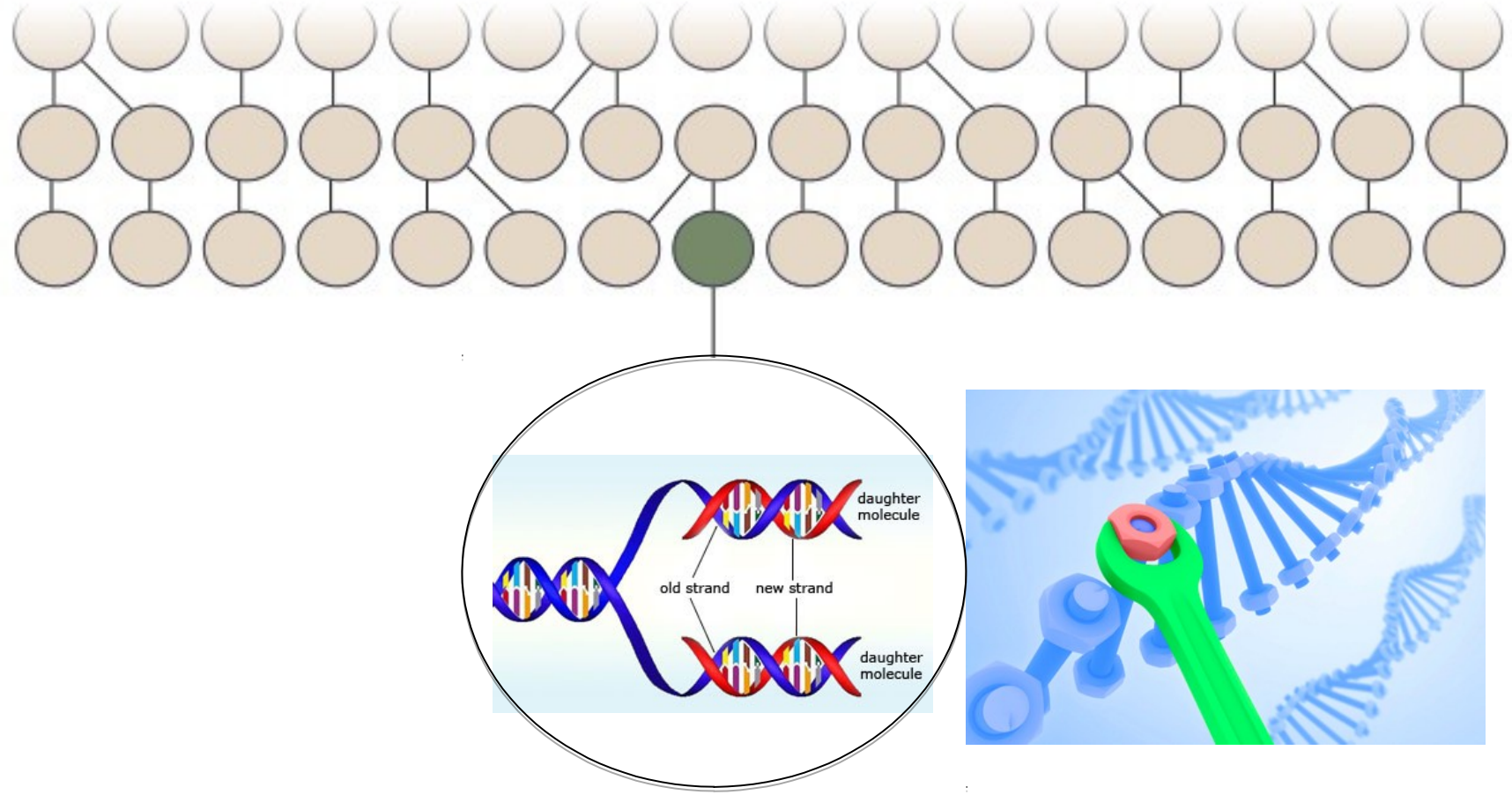
Hanahan and Weinberg. 2000. The hallmarks of cancer. Cell 100: 57-70.

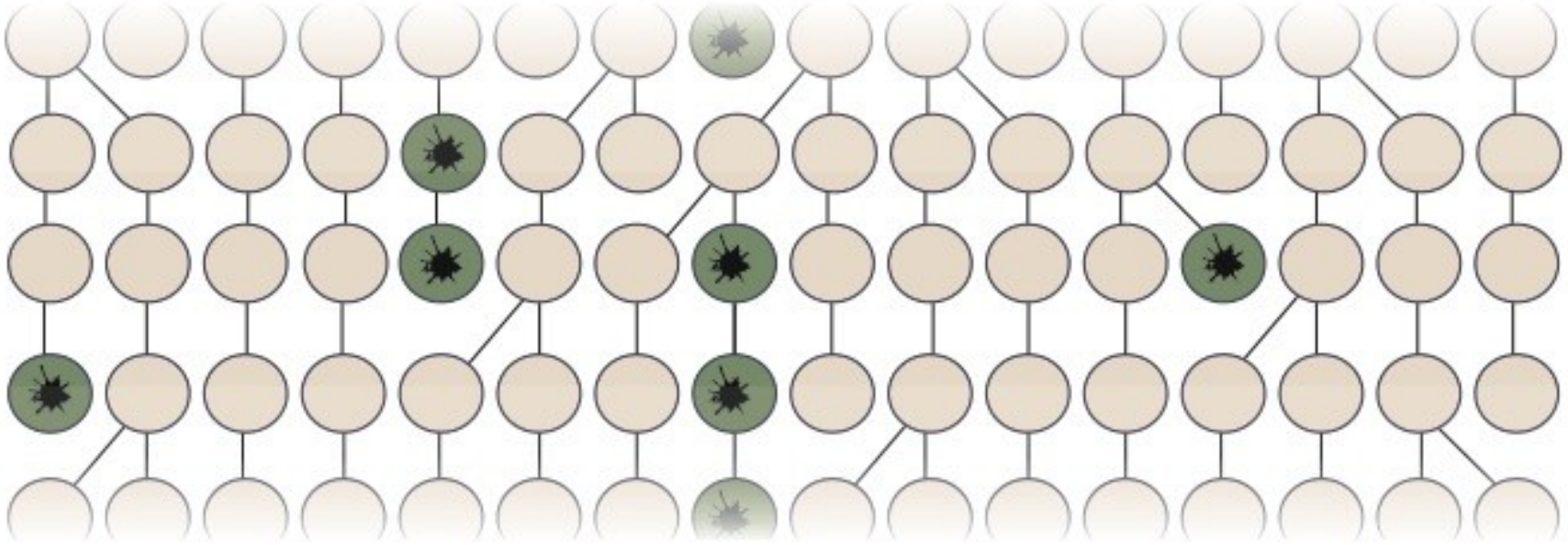
Many Paths Lead to Cancer Self-Sufficiency

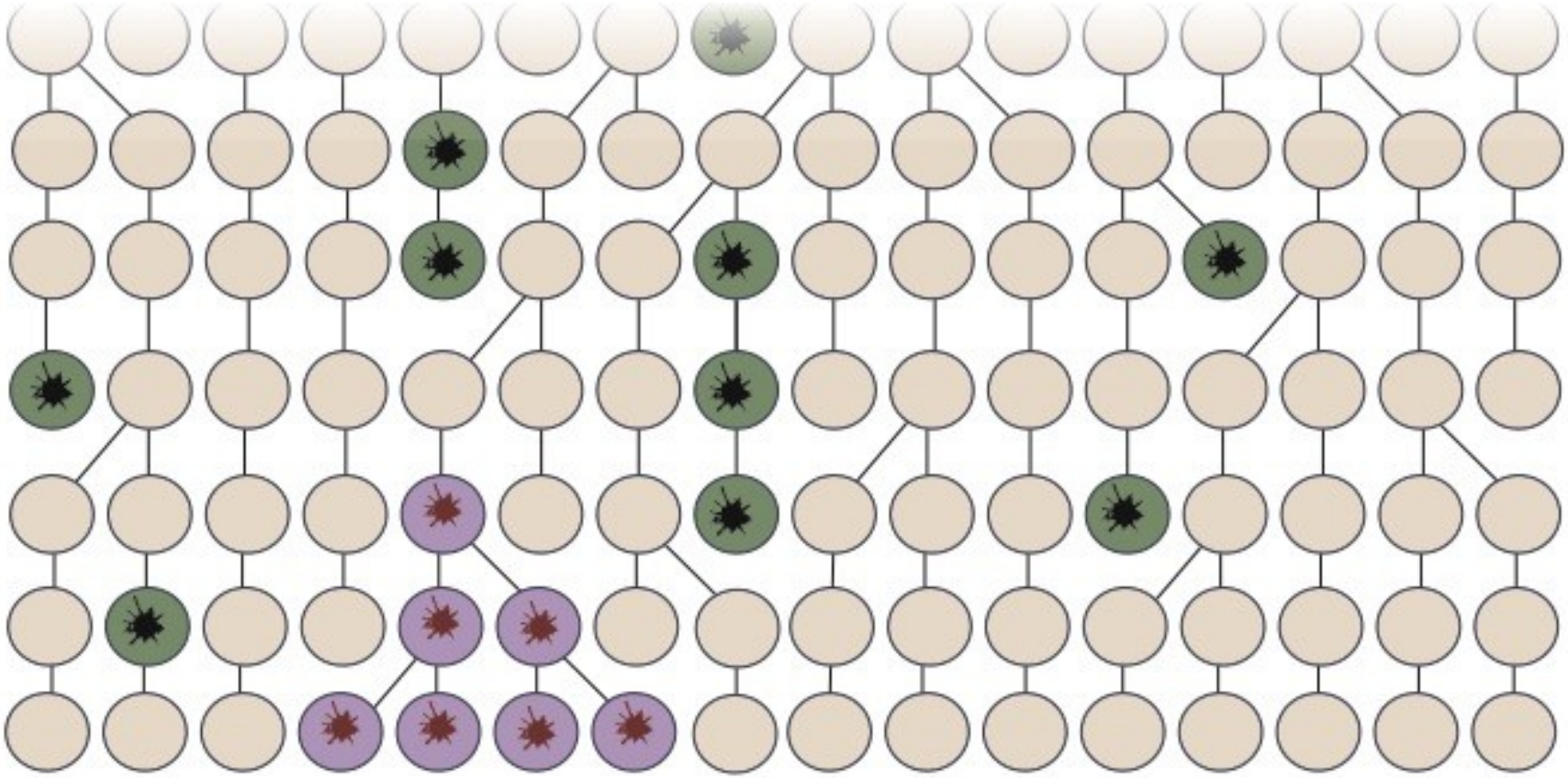


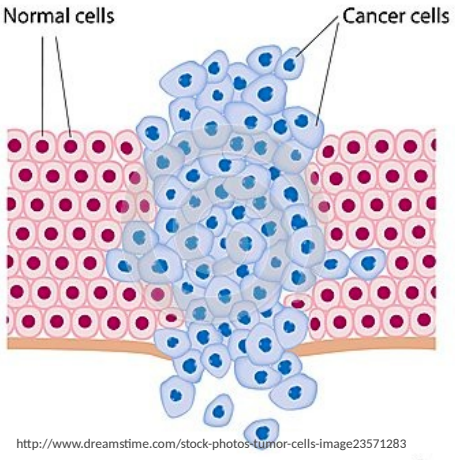
Hanahan, Douglas, and Ra Weinberg. 2000. The hallmarks of cancer. Cell 100: 57-70.



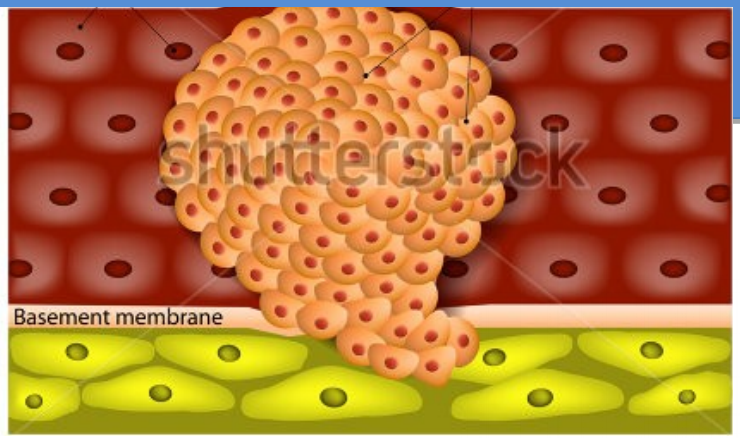

daughter molecule
old strand
new strand
daughter molecule

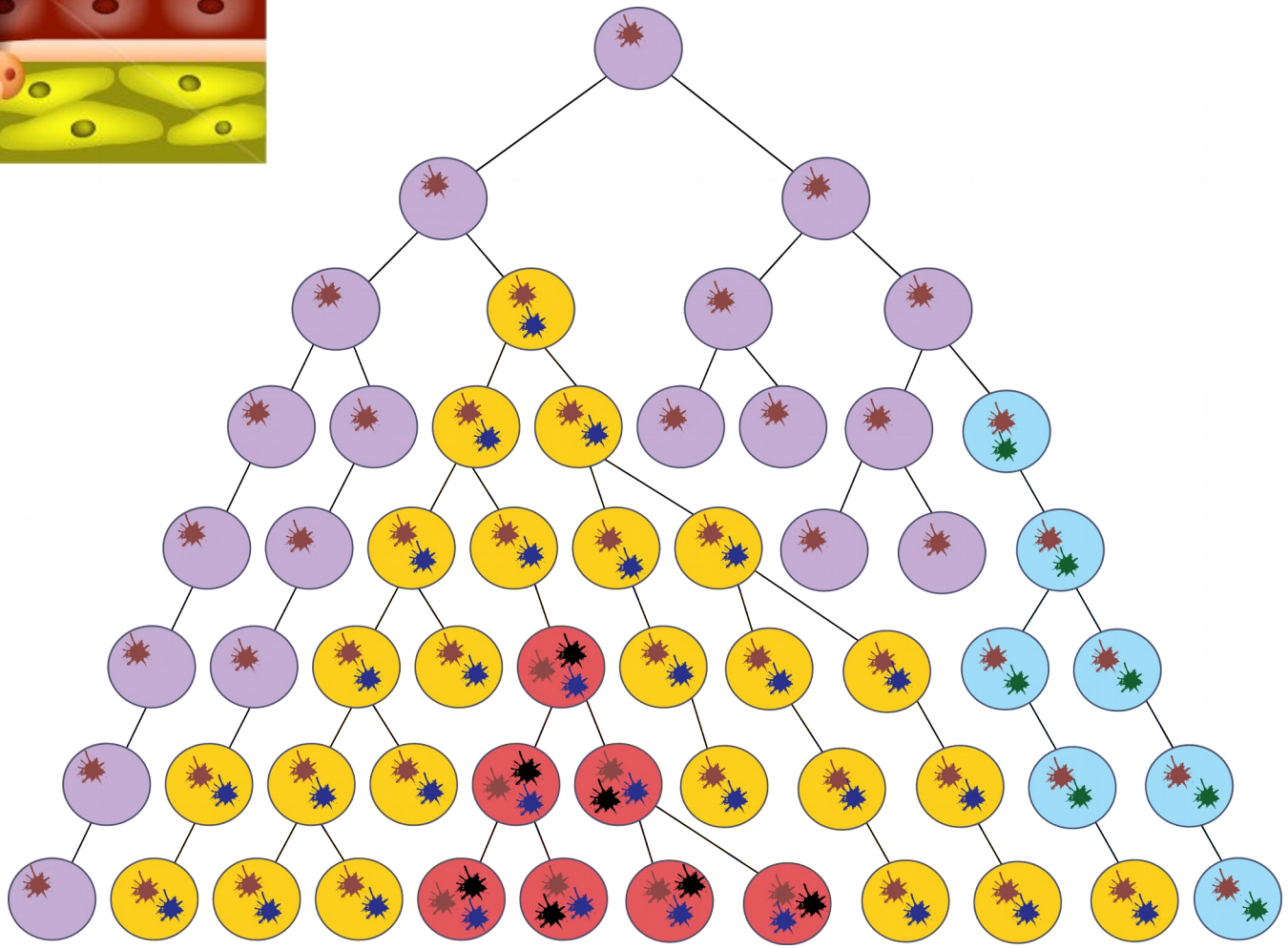


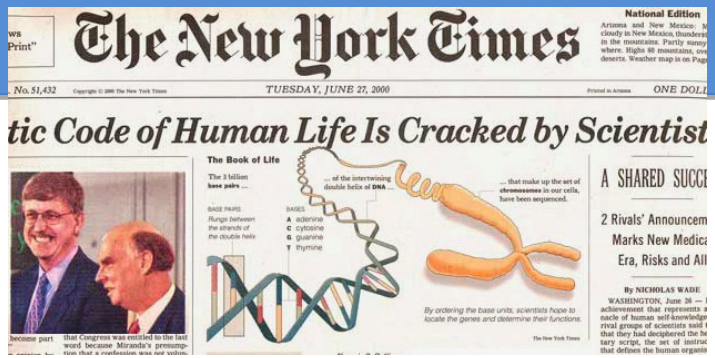


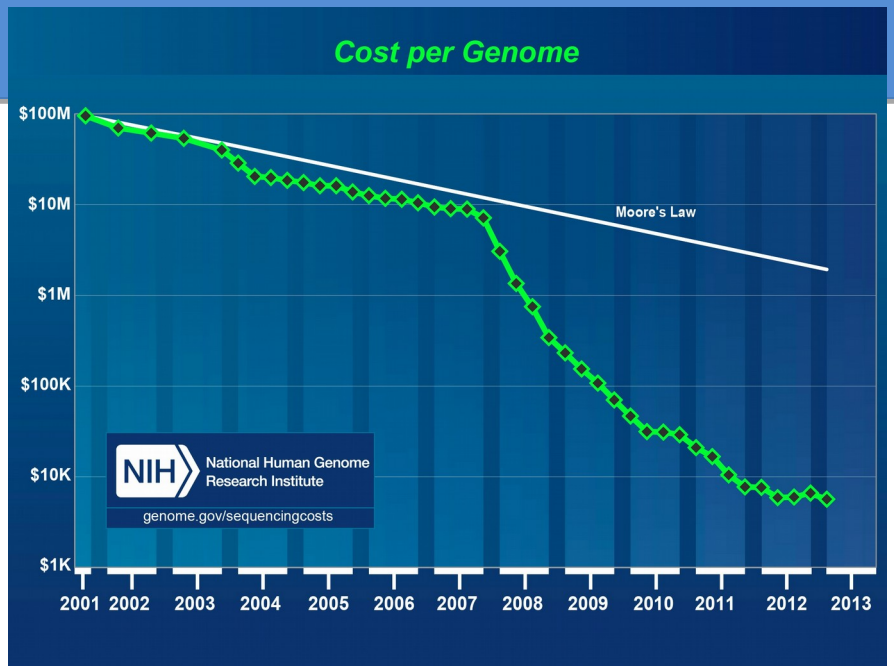


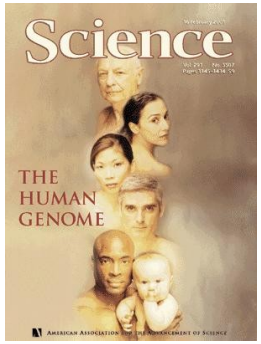
http://www.dreamstime.com/stock-photos-tumor-cells-image23571283


Basement membrane



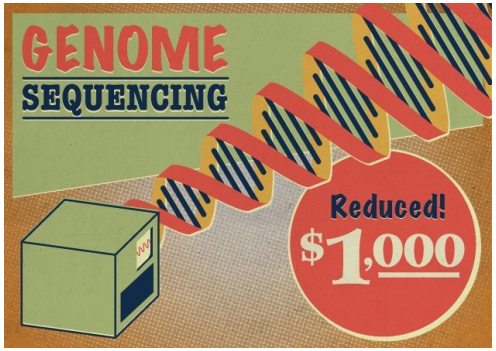






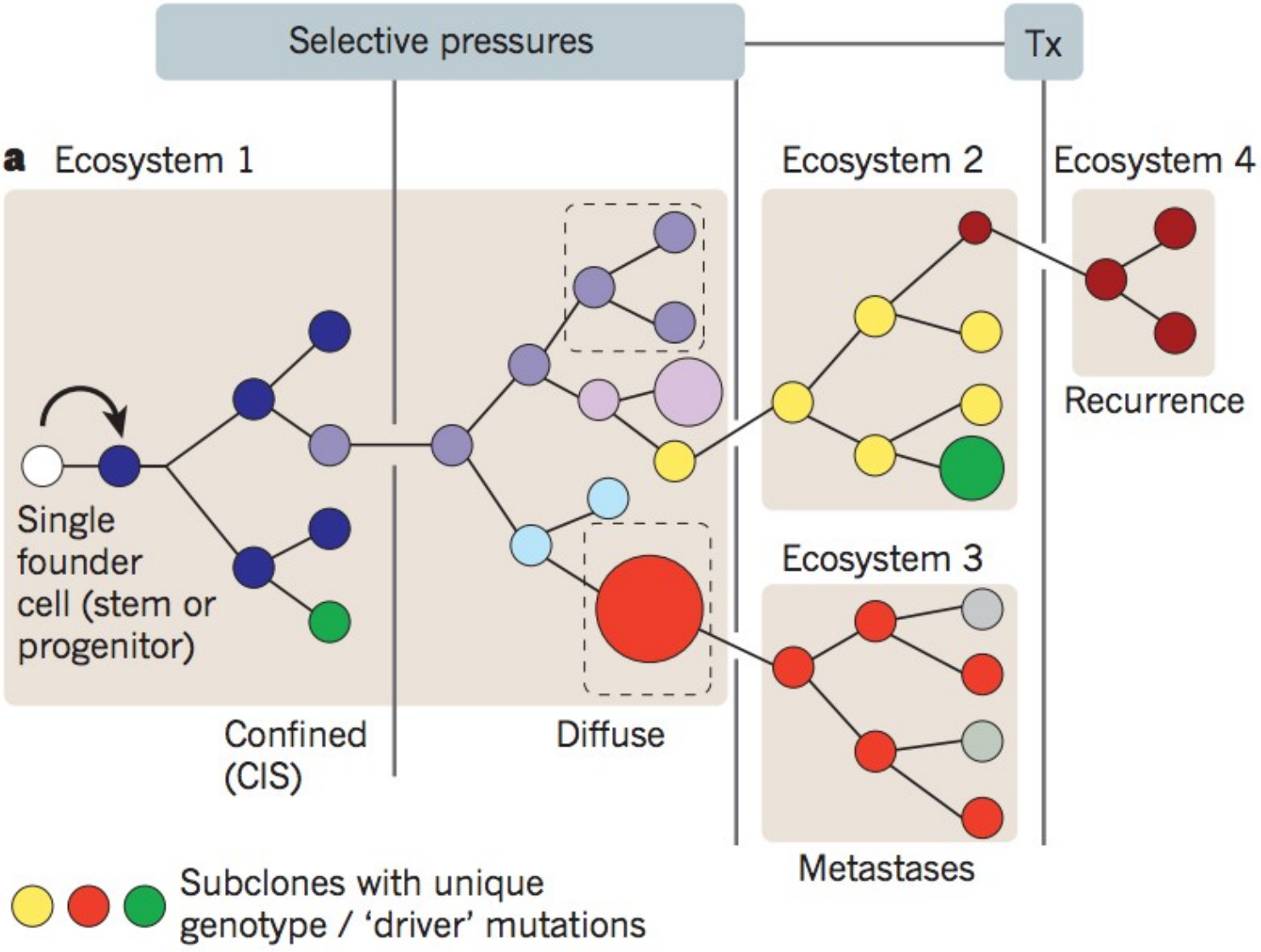
2000: initial draft

2003: Complete human reference genome ($3 billion)



2014

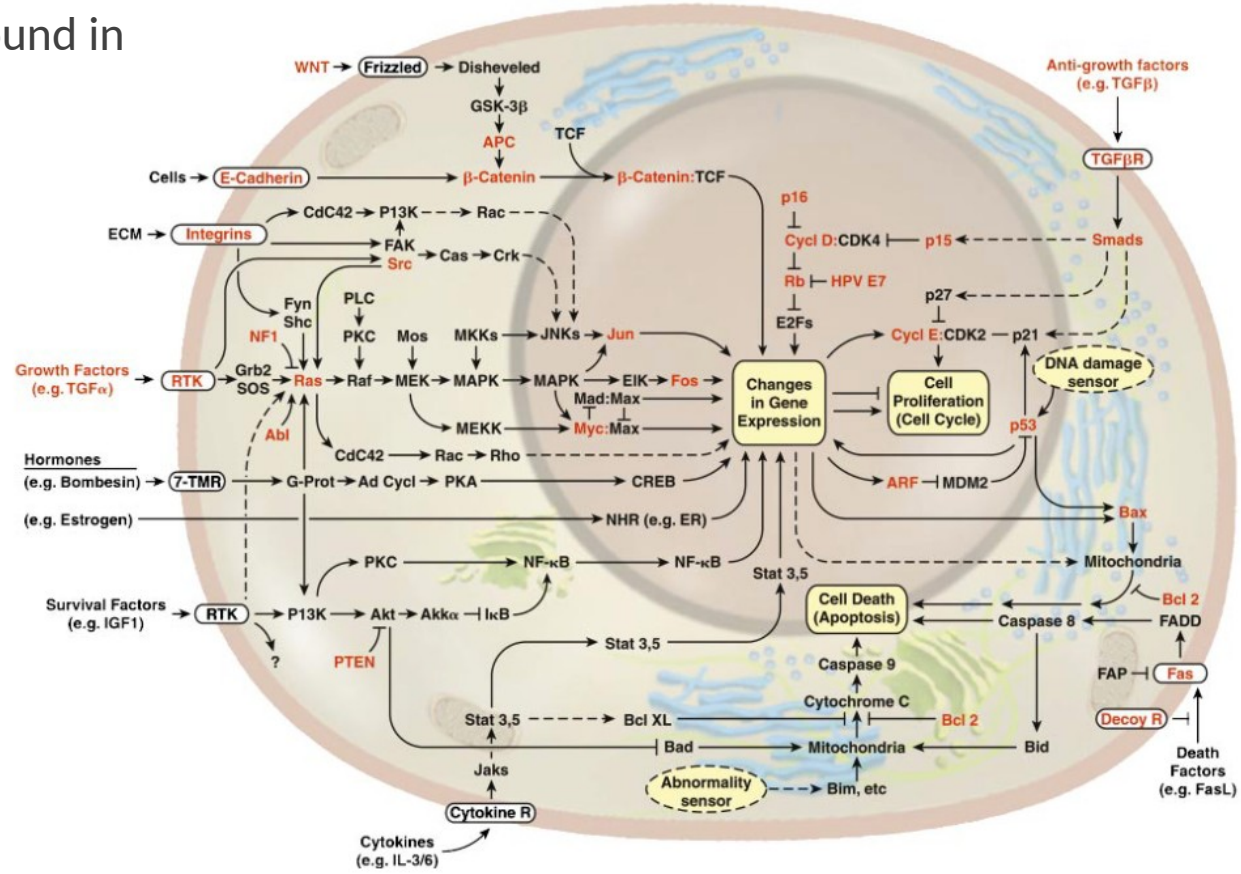
Cancer Heterogeneity



Greaves, M. & Maley, C. C. Clonal evolution in cancer. Nature 481, 306–13 (2012).

Why Sequence Cancer Genomes?

- Better understand cancer biology
  - Pathway information
  - Types of mutations found in different cancers



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- Cancer Diagnosis
  - Genetic signatures of cancer types will inform diagnosis
  - Non-invasive means of detecting or confirming presence of cancer
- Improve cancer therapies
  - Targeted treatment of cancer subtypes

Catalogue Of Somatic Mutations In Cancer	
Samples	544809
Mutations	141212
Papers	10383
Whole Genomes	29

COSMIC Database, v48, July 2010
http://www.sanger.ac.uk/genetics/CGP/cosmic/

Forbes et al. 2011. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Research 39: D945-D950

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Catalogue Of Somatic Mutations In Cancer	
Samples	1058292
Mutations	2710449
Papers	20247
Whole Genomes	15047

COSMIC Database, v71, Oct 2014
http://www.sanger.ac.uk/genetics/CGP/cosmic/

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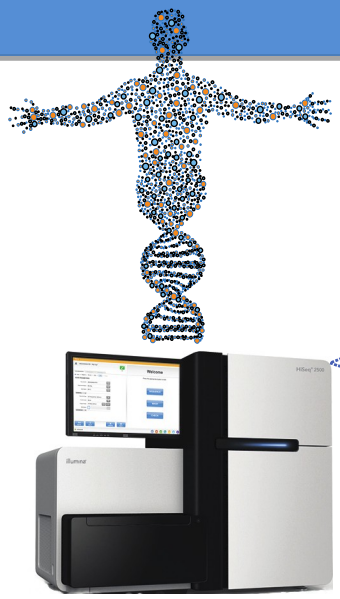
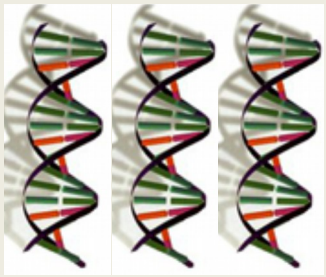
Catalogue Of Somatic Mutations In Cancer	
Samples	1,144,255
Mutations	3,480,051
Papers	22,276
Whole Genomes	22,690

COSMIC Database, v74, Sept 2015
http://www.sanger.ac.uk/genetics/CGP/cosmic/

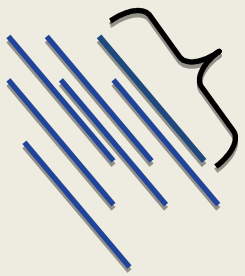
Genome Background





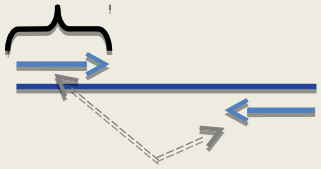
DNA is amplified

500 bp



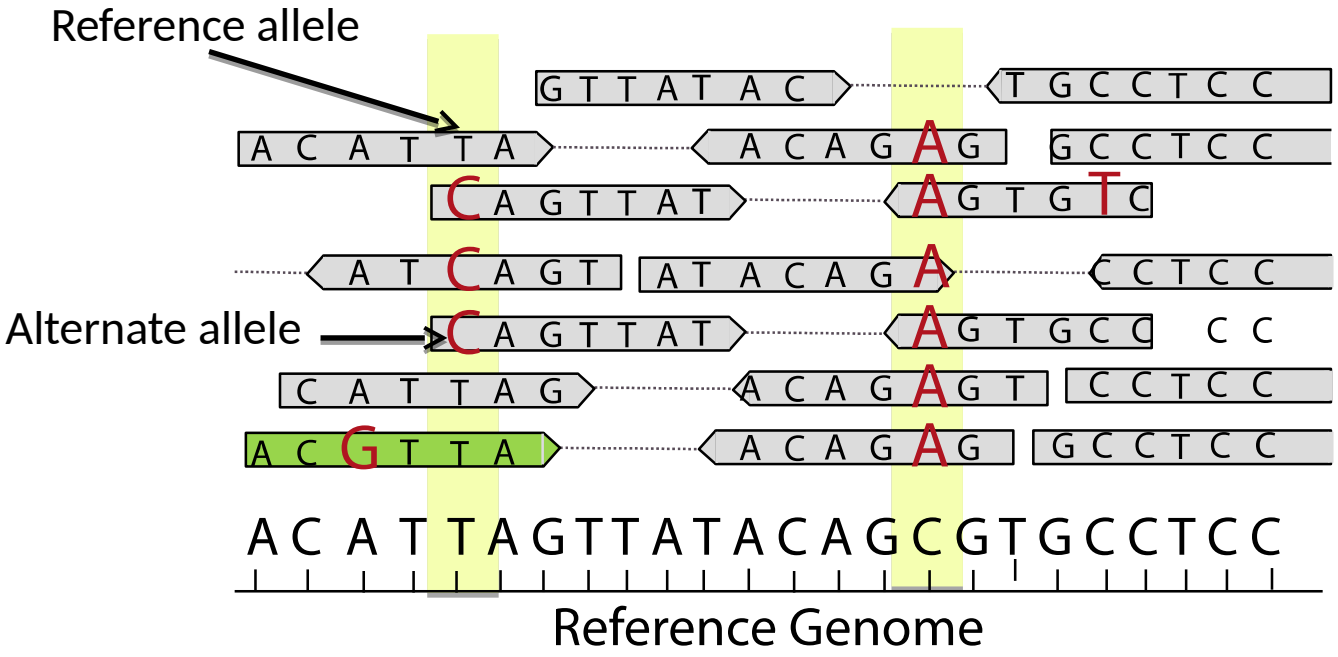
DNA is cut into small fragments

100 bp

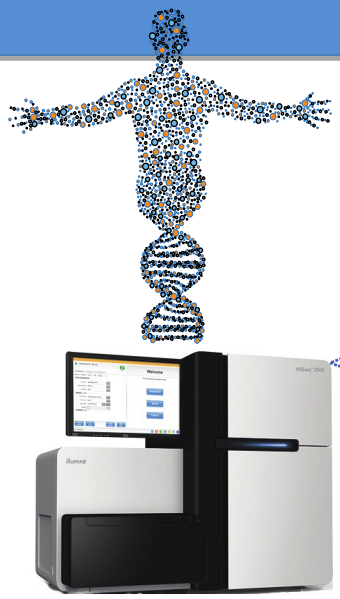


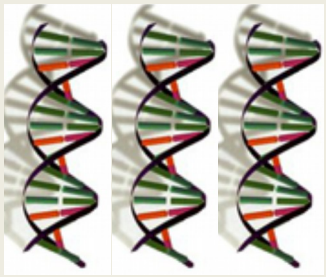
Sequenced reads

Two ends of the fragments are sequenced



Genome Background





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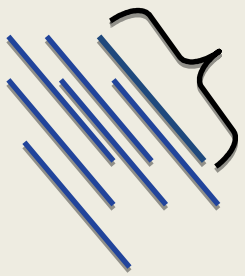

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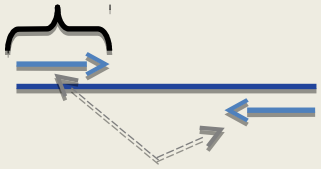
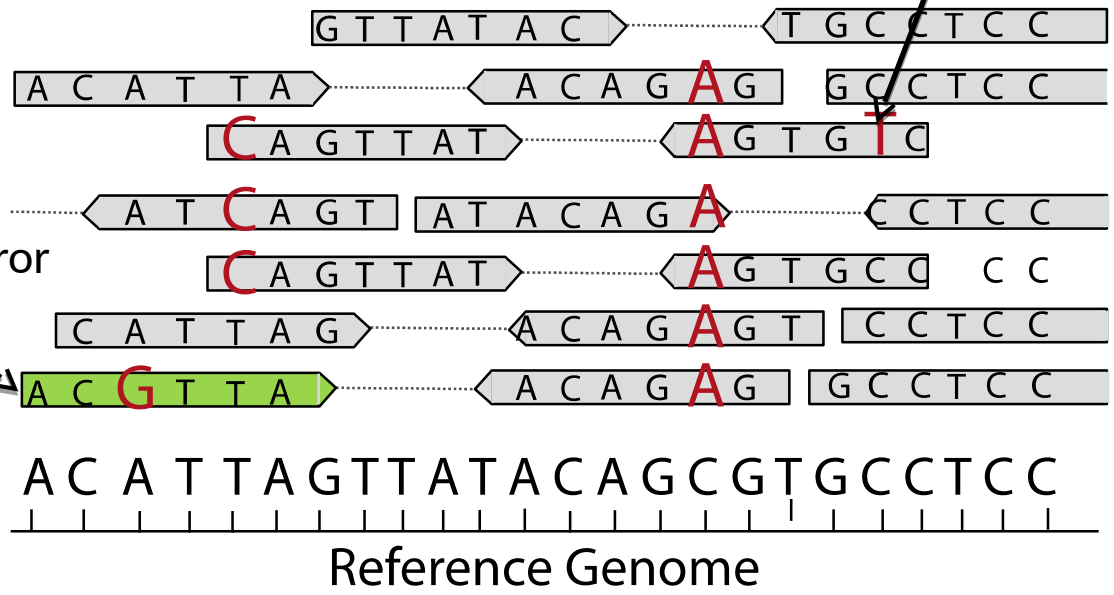


Sequenced reads

Two ends of the fragments are sequenced

Sequencing Error

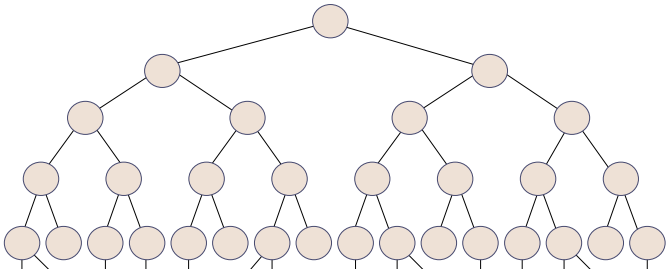
Alignment Error



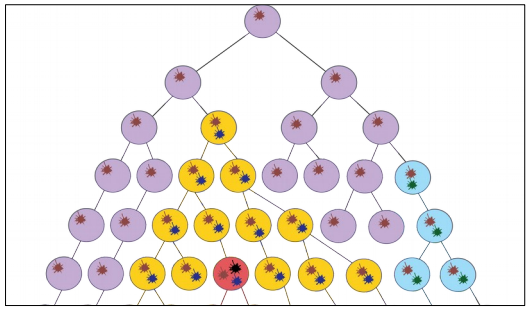
Genome Background

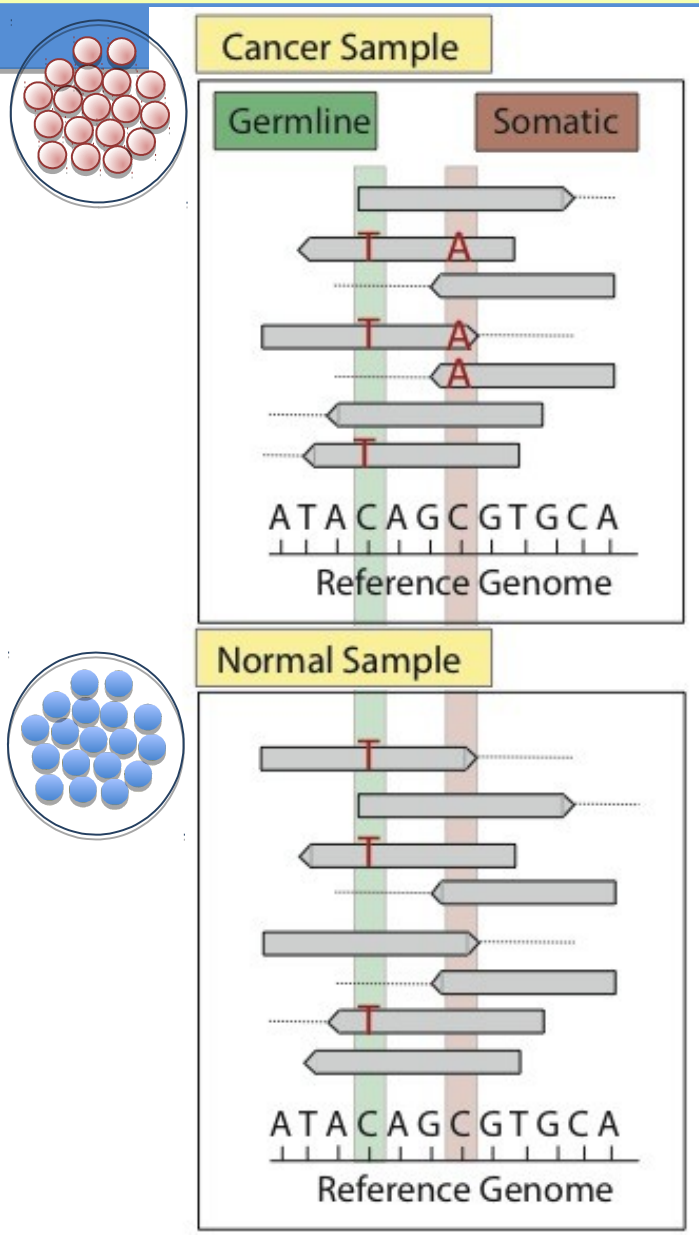
Types of SNVs in a cancer sample:

1. Germline (SNPs)
   - Inherited
   - All cells have it



2. Somatic
   - Acquired during cancer progression
   - Not present in normal cells





Considerations for Cancer Sequencing

- Factors that effect mutation signal
  - Limited genetic material (lower depth)
  - Mixture of tumor and normal tissue
  - Cancer Heterogeneity
- Factors that introduce noise
  - Formalin-fixed and Paraffin-embedded samples
  - Increased number of mutations and unusual genomic rearrangements
- General Consideration
  - Each individual has many unique mutations that could be confused with cancer causing mutations

Human Genome Variation

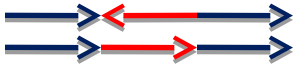
SNP

TGCTGAGA
TGCCGAGA

Novel Sequence

TGCTCGGAGA
TGC - - - GAGA

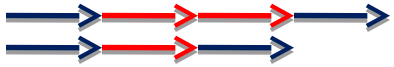
Inversion



Mobile Element or
Pseudogene Insertion

Translocation

Tandem Duplication



Microdeletion

TGC - - AGA
TGCCGAGA

Transposition

Large Deletion

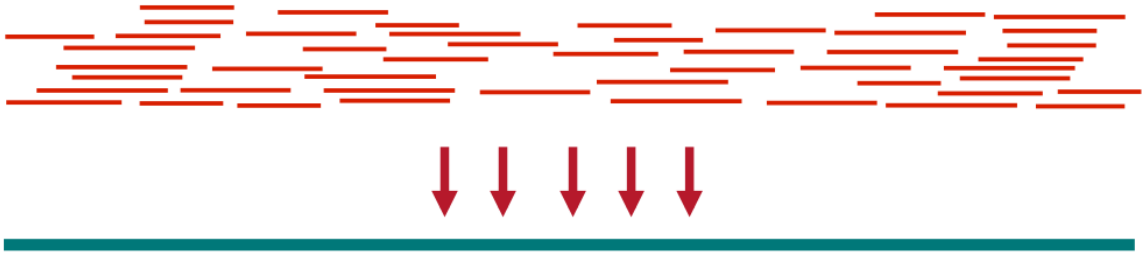
Novel Sequence
at Breakpoint

Variant Types

Variant Types
Single Nucleotide Variants(SNVs)
Small Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence

SNV Calling

Variant Types
Single Nucleotide Variants(SNVs)
Small Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence



```
                    CATCGACCGAGCGCGATGCTAGCTAGGTGATCGT......
                 TGCCGCATCGACCGAGCGCGATGCTAGCTAGGTGATCGT...
              GCATGCCGCATCGACCGAGCGCGATGCTAGCTAGGTGATCGT
           GTGCATGCCGCATCGACCGAGCGCGATGCTAGCTAGGTGATC
......AGGTGCATGCCGCATCGATCGAGCGCGATGCTAGCTAGCTGATCGT......
```

- A bayesian approach is the most general and common method of calling SNVs
  - MAQ, SOAPsnp, Genome Analyis ToolKit (GATK), SAMtools

SNV Calling

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Single Nucleotide Variants(SNVs)
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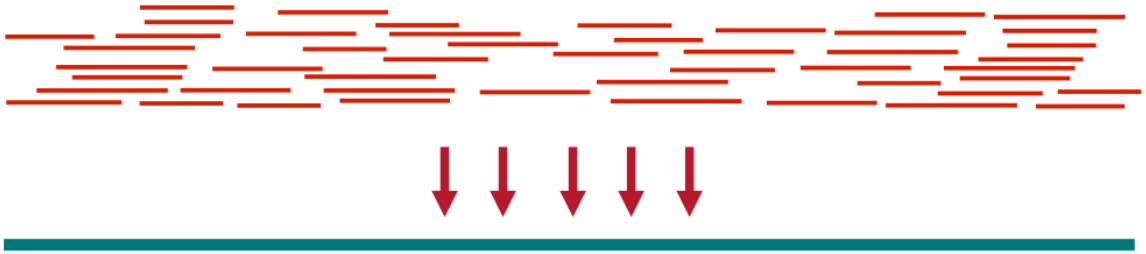
Novel Sequence



```
                CATCGACCGAGCGCGATGCTAGCTAGGTGATCGT.....
             TGCCGCATCGACCGAGCGCGATGCTAGCTAGGTGATCGT...
          GCATGCCGCATCGACCGAGCGCGATGCTAGCTAGGTGATCGT
       GTGCATGCCGCATCGACCGAGCGCGATGCTAGCTAGGTGATC
.....AGGTGCATGCCGCATCGATCGAGCGCGATGCTAGCTAGCTGATCGT.....
```

Bayesian model

$$\Pr\{G|D\} = \frac{\overbrace{\Pr\{G\}}^{\text{Prior of the genotype}}\overbrace{\Pr\{D|G\}}^{\text{Likelihood of the genotype}}}{\Sigma_i \Pr\{G_i\}\Pr\{D|G_i\}}, \text{ [Bayes' rule]}$$

$$\Pr\{D|G\} = \prod_j \left(\frac{\Pr\{D_j|H_1\}}{2} + \frac{\Pr\{D_j|H_2\}}{2}\right) \text{ where } \overbrace{G = H_1 H_2}^{\text{Diploid assumption}}$$

$$\Pr\{D|H\} \text{ is the haploid likelihood function}$$

$$\Pr\{D_j|H\} = \Pr\{D_j|b\}, \text{ [single base pileup]}$$

$$\Pr\{D_j|b\} = \begin{cases} 1-\epsilon_j & D_j = b, \\ \epsilon_j & \text{otherwise.} \end{cases}$$

http://www.broadinstitute.org/gatk//events/2038/GATKwh0-BP-5-Variant_calling.p

SNV Calling

Variant Types
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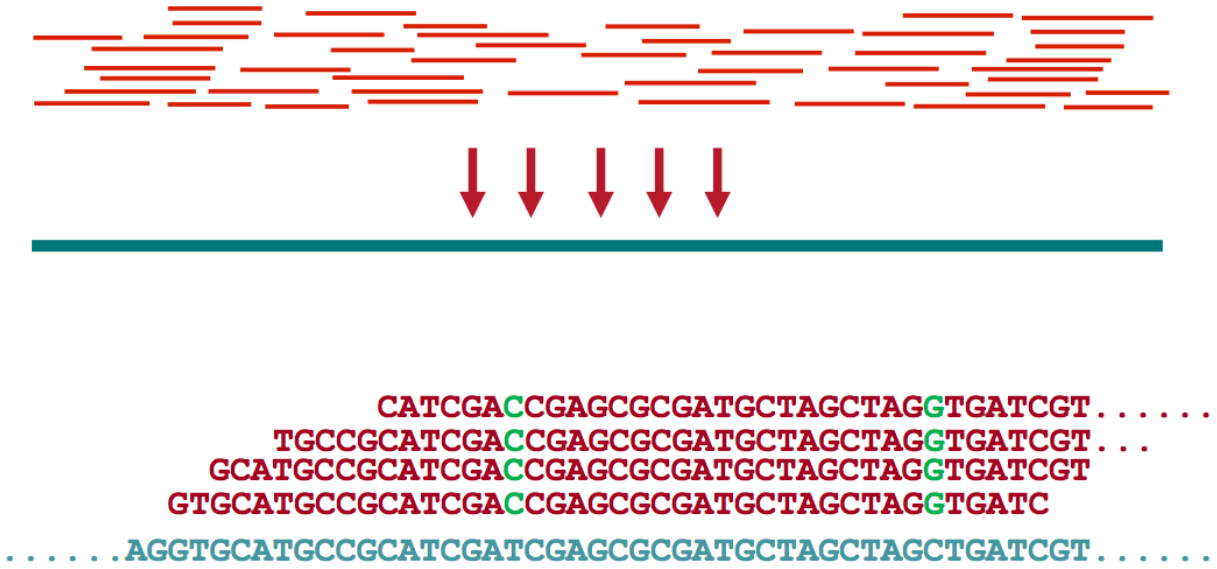
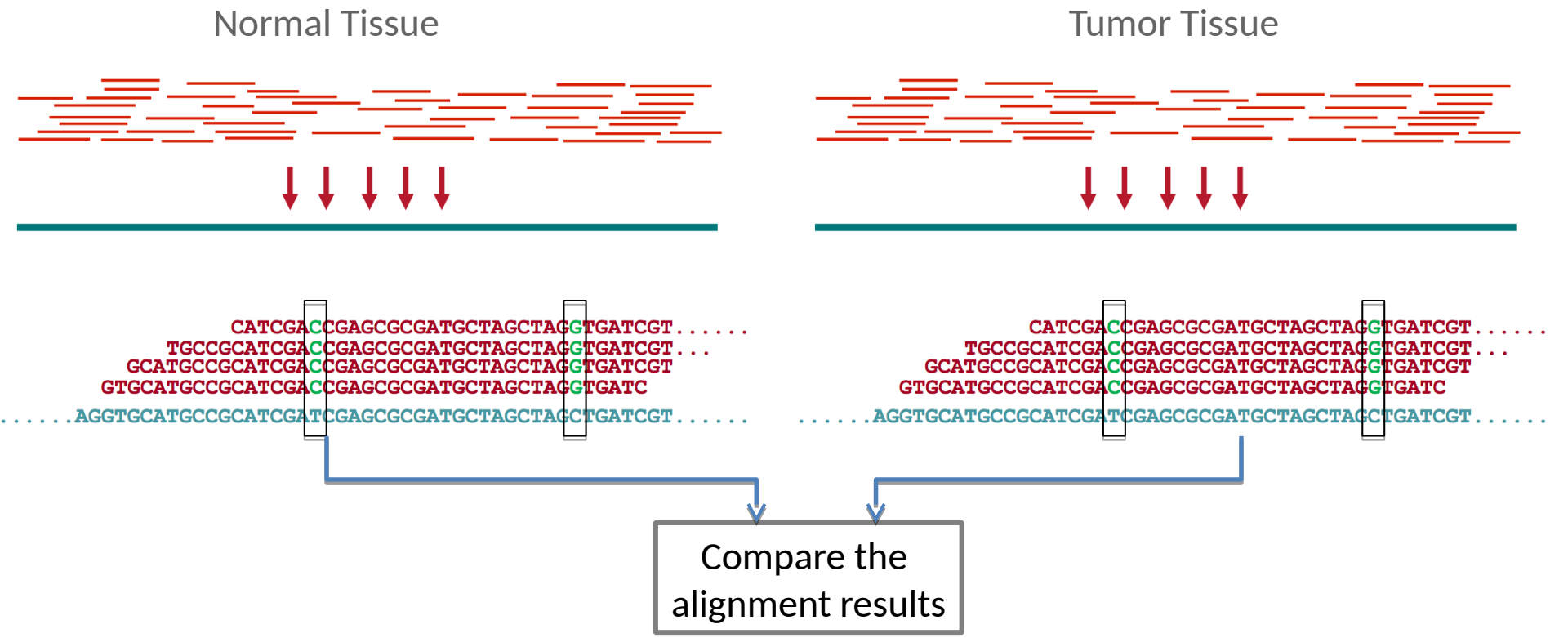


- A given human genome (germline) differs from the reference genome at millions of positions.
- A cancer genome differs from the healthy genome of its host by tens of thousands of positions at most, which is several orders of magnitude fewer differences than germline versus reference
- How do we distinguish germline mutations from somatic mutations?

Somatic SNV calling



- Most naïve: use a standard SNV caller on both datasets. If there is a mutation found in the tumor sample but not the normal, it is somatic!

Short Indel Calling

Variant Types
Single Nucleotide Variants(SNVs)
Short Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence

Short Indel Calling

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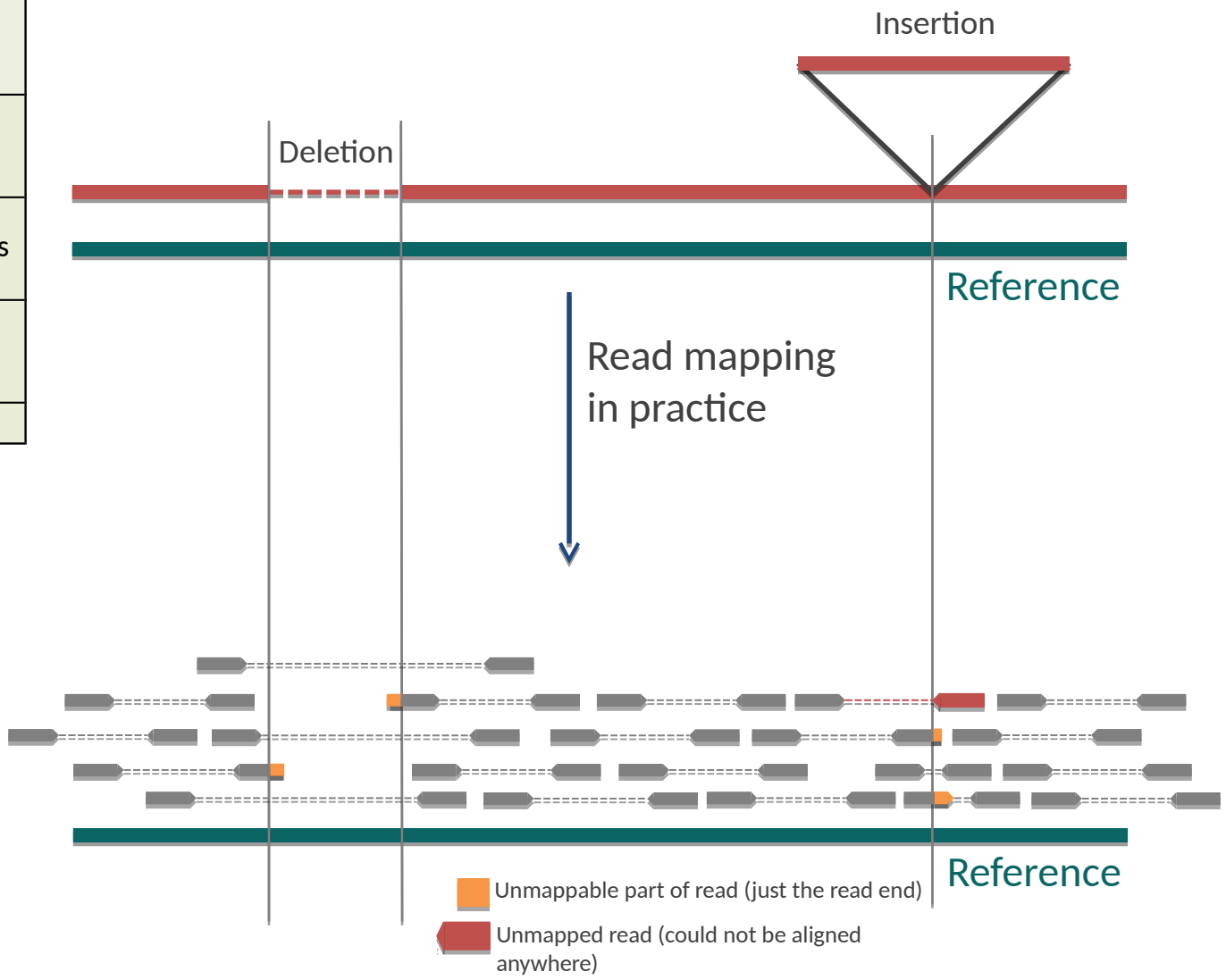

Short Indel Calling – Discordant Reads Pairs

Variant Types
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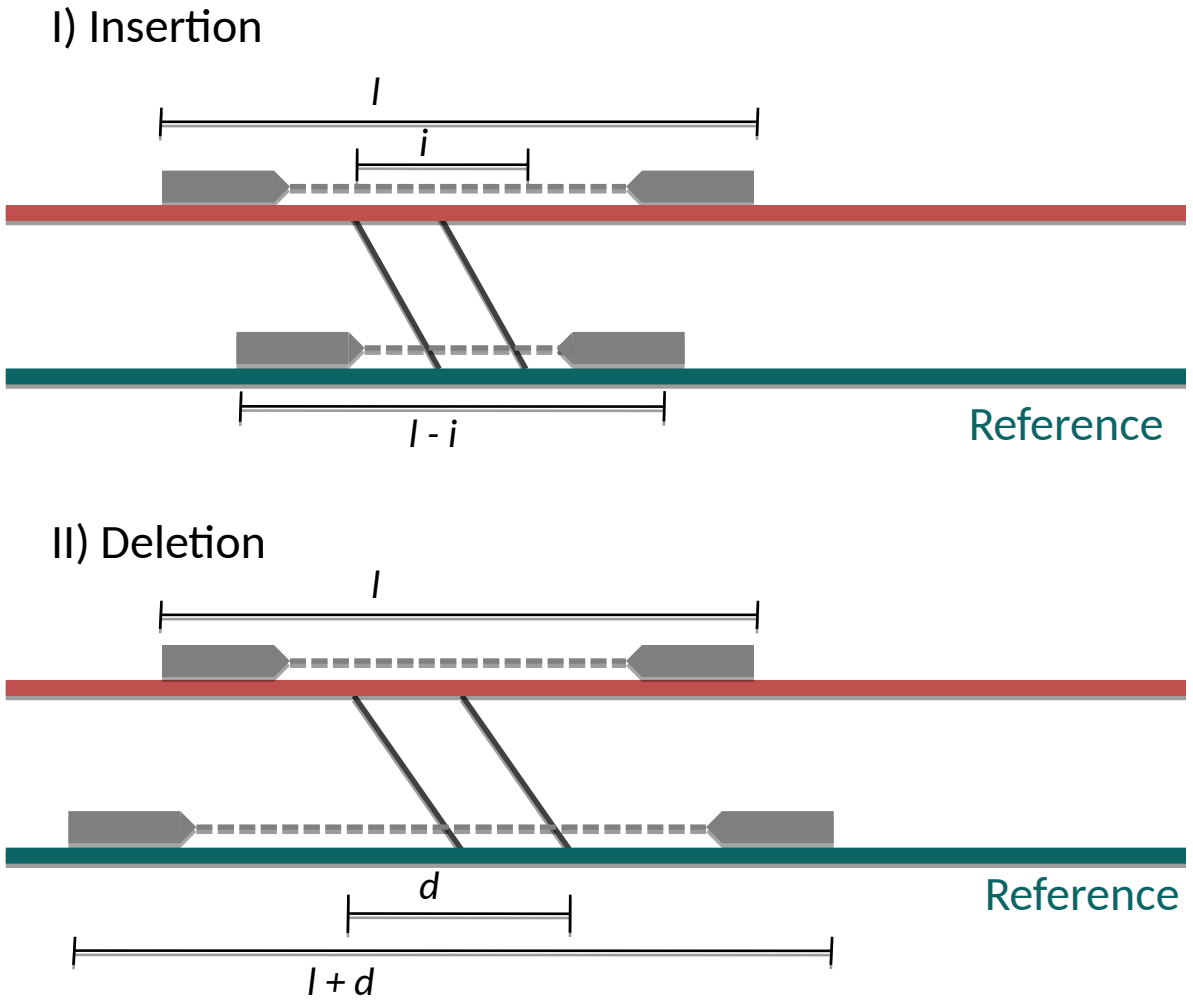
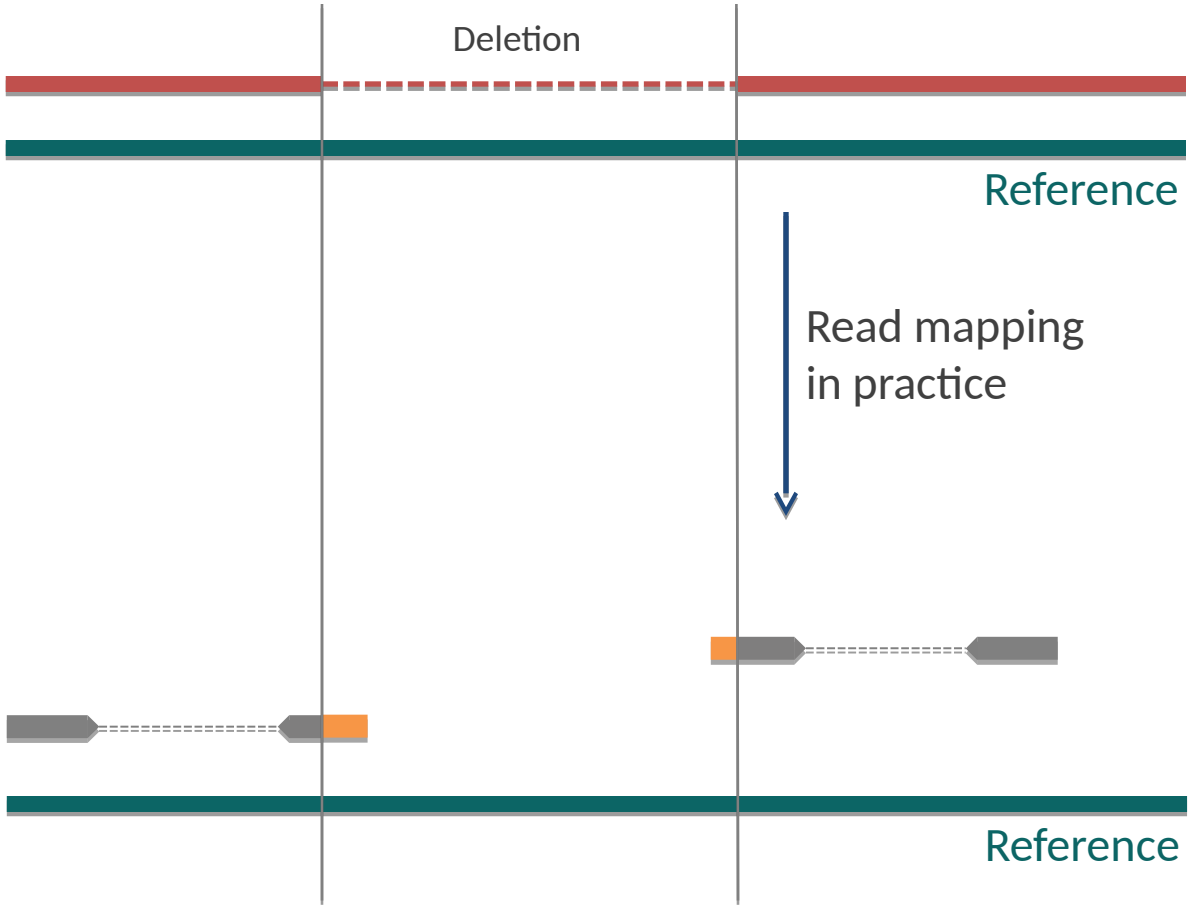
Short Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence



Short Indel Calling – Split Read Mapping

Variant Types
Single Nucleotide Variants(SNVs)
Short Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence



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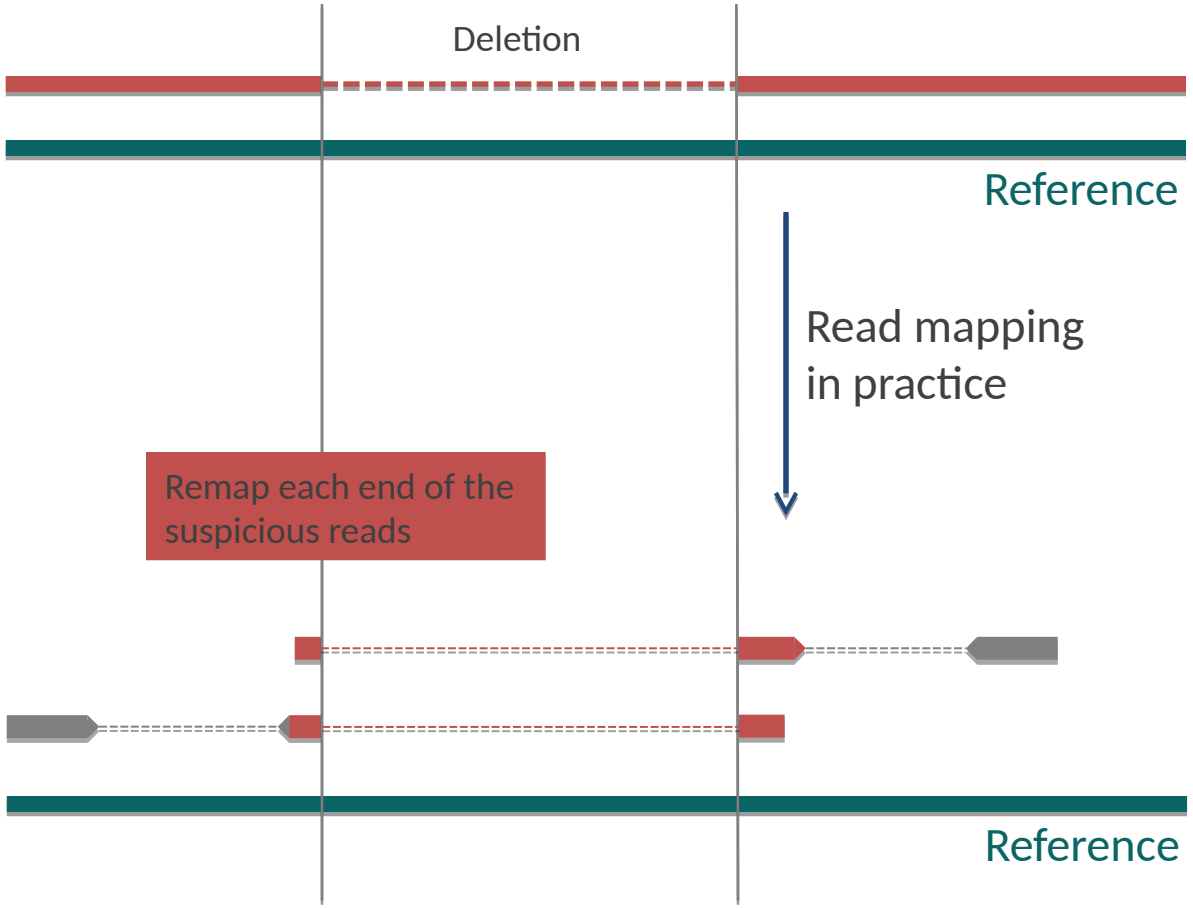

Copy Number Variants

Variant Types
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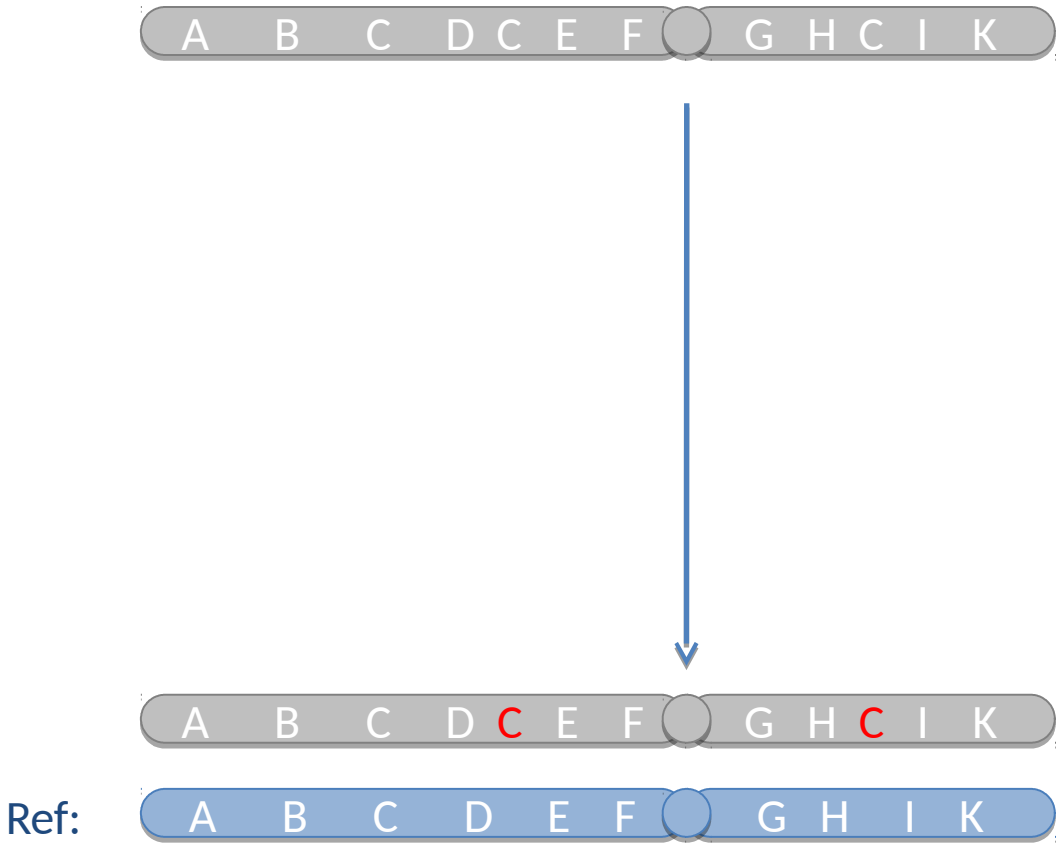
Novel Sequence



Copy Number Variants

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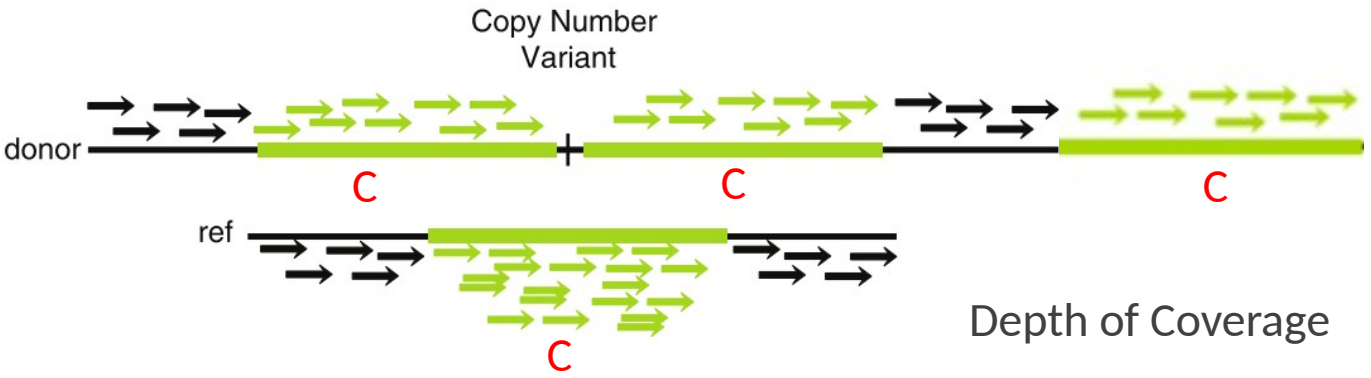
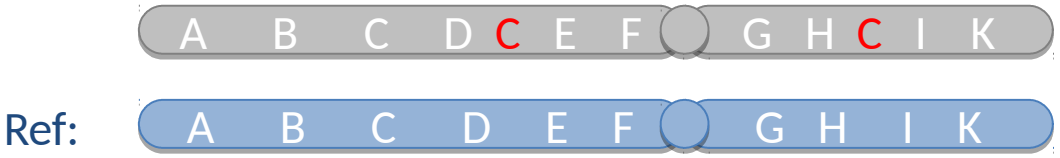
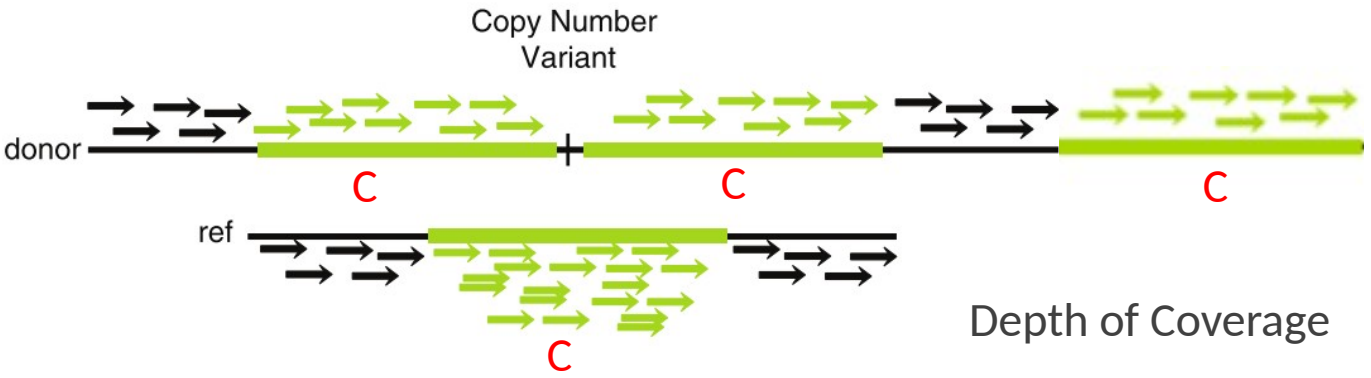


Modified from Dalca and Brudno. 2010. Genome variation discovery with high-throughput sequencing data. Briefings in bioinformatics 11, no. 1: 3-14

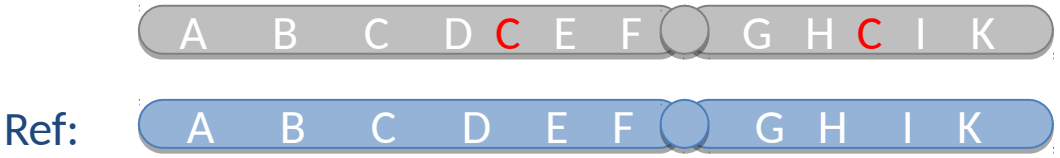


Copy Number Variants

Variant Types
Single Nucleotide Variants(SNVs)
Small Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence



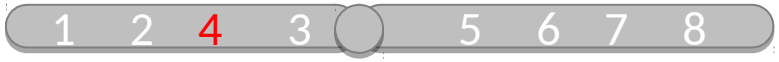
- Problems with DOC
  - Very sensitive to stochastic variance in coverage
  - Sensitive to bias coverage (e.g. GC content).
  - Impossible to determine non-reference locations of CNVs
- Graph methods using paired-end reads help overcome some of these problems



Variant Types

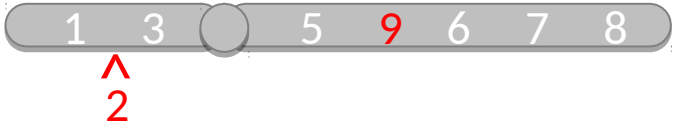
Variant Types
Single Nucleotide Variants(SNVs)
Short Insertion / Deletion (indels)
Copy Number Variants (CNVs)
Structural Variants (SVs)
Novel Sequence

Structural Rearrangement

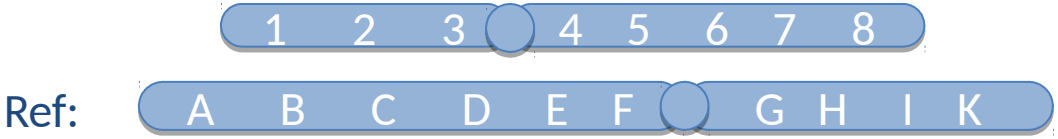


Translocation

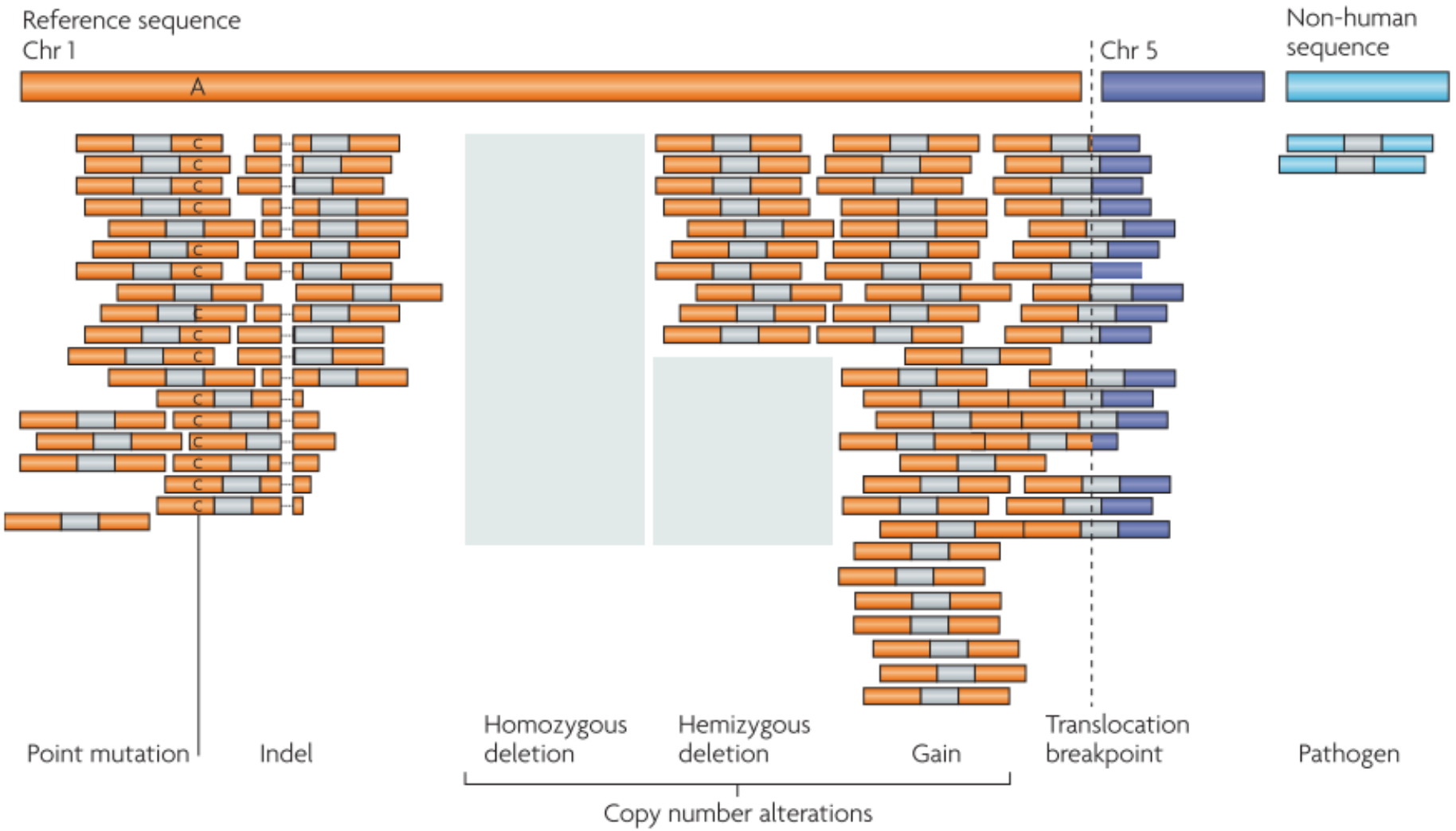
Inversion



Large Insertion / Deletion



Summary of Variant Types



Meyerson et al. . 2010. Advances in understanding cancer genomes through second-generation sequencing. Nature Reviews Genetics 11, no. 10 (October): 685-696

Tracking the Evolution of Cancer

Genome evolution during progression to breast cancer

Daniel E. Newburger, Dorna Kashef-Haghighi, Ziming Weng, et al.

Genome Res. 2013 23: 1097-1108 originally published online April 8, 2013
Access the most recent version at doi:10.1101/gr.151670.112

Cell

The Life History of 21 Breast Cancers

Serena Nik-Zainal,[1,19] Peter Van Loo,[1,2,3,19] David C. Wedge,[1,19] Ludmil B. Alexandrov,[1] Christopher D. Greenman,[1,4,5] King Wai Lau,[1] Keiran Raine,[1] David Jones,[1] John Marshall,[1] Manasa Ramakrishna,[1] Adam Shlien,[1] Susanna L. Cooke,[1] Jonathan Hinton,[1] Andrew Menzies,[1] Lucy A. Stebbings,[1] Catherine Leroy,[1] Mingming Jia,[1] Richard Rance,[1] Laura J. Mudie,[1] Stephen J. Gamble,[1] Philip J. Stephens,[1] Stuart McLaren,[1] Patrick S. Tarpey,[1] Elli Papaemmanuil,[1] Helen R. Davies,[1] Ignacio Varela,[1] David J. McBride,[1] Graham R. Bignell,[1] Kenric Leung,[1] Adam P. Butler,[1] Jon W. Teague,[1] Sancha Martin,[1] Goran Jönsson,[9] Odette Mariani,[7] Sandrine Boyault,[8] Penelope Miron,[9] Aquila Fatima,[9] Anita Langerød,[10] Samuel A.J.R. Aparicio,[11,12] Andrew Tutt,[6] Anieta M. Sieuwerts,[13] Åke Borg,[14] Gilles Thomas,[8] Anne Vincent Salomon,[7] Andrea L. Richardson,[9,15] Anne-Lise Børresen-Dale,[10,16] P. Andrew Futreal,[1] Michael R. Stratton,[1] Peter J. Campbell[1,17,18,*] and Breast Cancer Working Group of the International Cancer Genome Consortium

Cell

Mutational Processes Molding the Genomes of 21 Breast Cancers

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REVIEWS

Evolution of the cancer genome

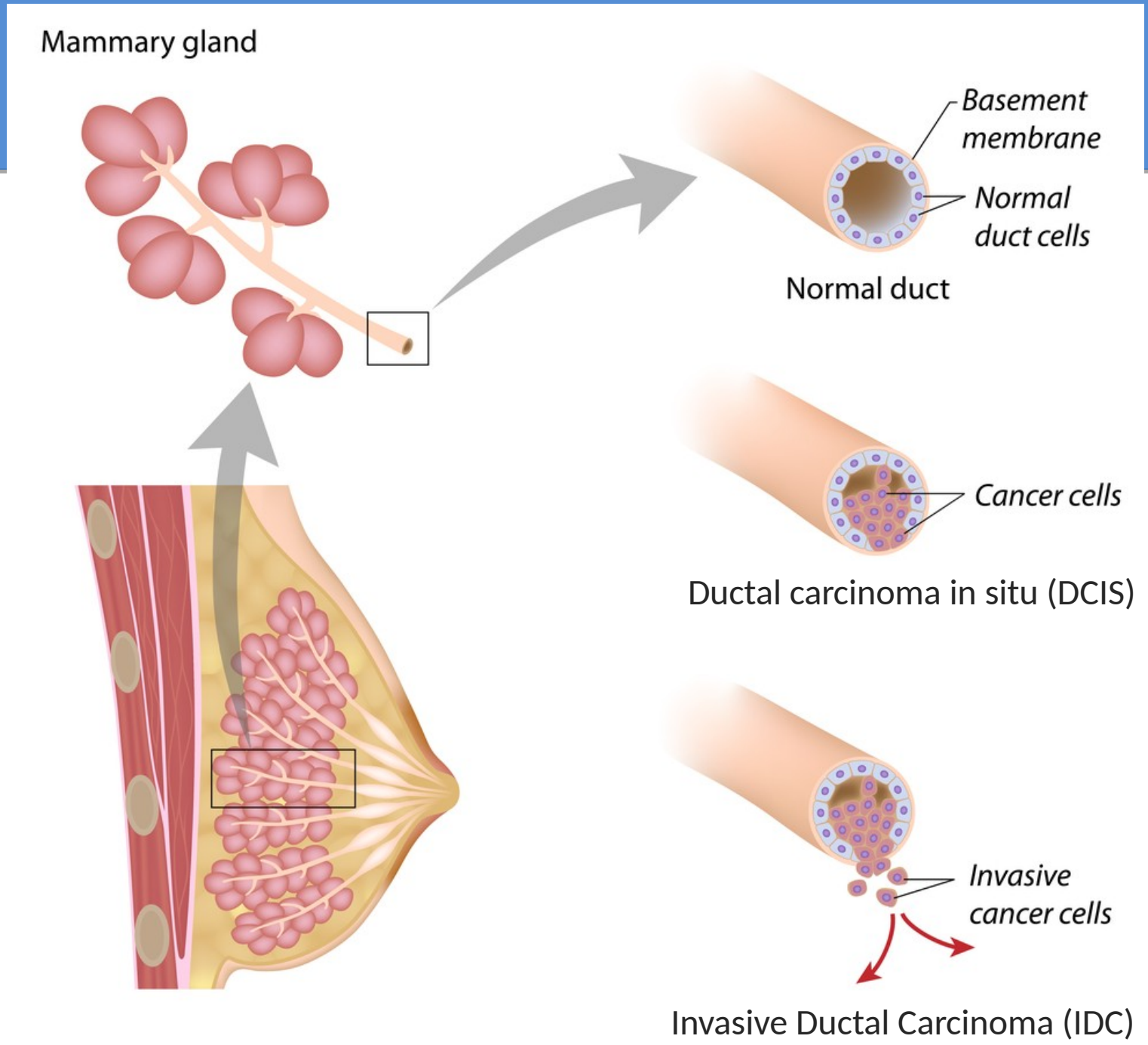
Lucy R. Yates[1] and Peter J. Campbell[1,2]

NATURE REVIEWS | GENETICS VOLUME 13 | NOVEMBER 2012

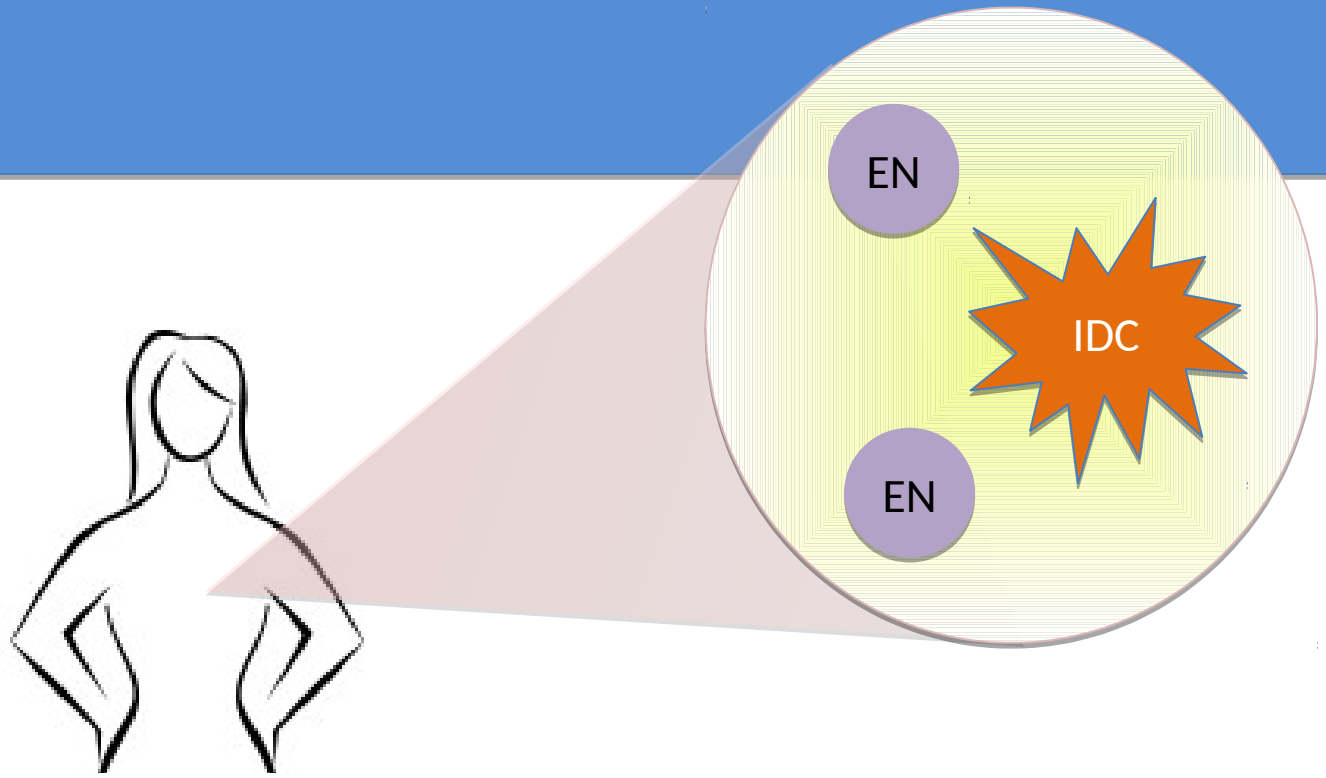
Clonal evolution in cancer

Mel Greaves[1] & Carlo C. Maley[2]

NATURE | VOL 481 | 19 JANUARY 2012



Source:http://marnieclark.com/category/types-of-breast-cancer/dcis/



Normal

Early Neoplasia

Ductal Carcinoma In situ

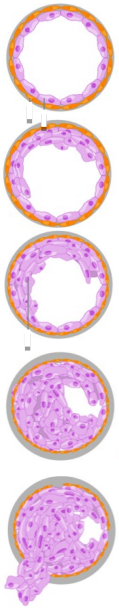
Invasive Ductal Carcinoma

Progression

Lesions

Patients

	P1	P2	P3	P4	P5	P6
Lymph	○			○	○	○
Normal	○	○	○			
EN	●	●	●	●	●	●
ENA	●				●	●
DCIS		●	●			●
IDC	●	●	●	●	● ●	●



Types of Variants

- Somatic SNVs
- Aneuploidies

Questions

>50x whole genome coverage per sample

1. What is the genetic relationship of lesions to one another?
2. What are the early genomic events?

Variant calling process

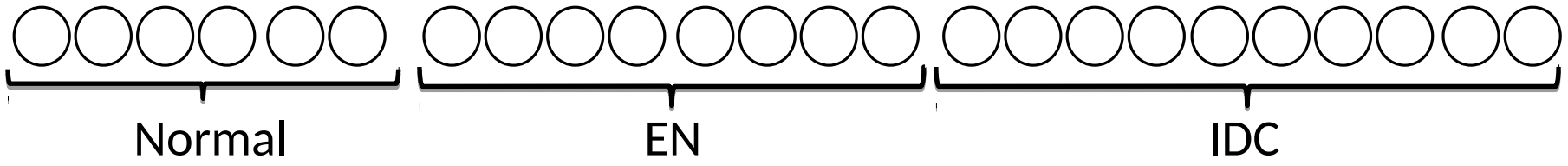
Challenges

- Sequencing Error
- Mapping Error
- Sequencing Coverage
- Normal Contamination

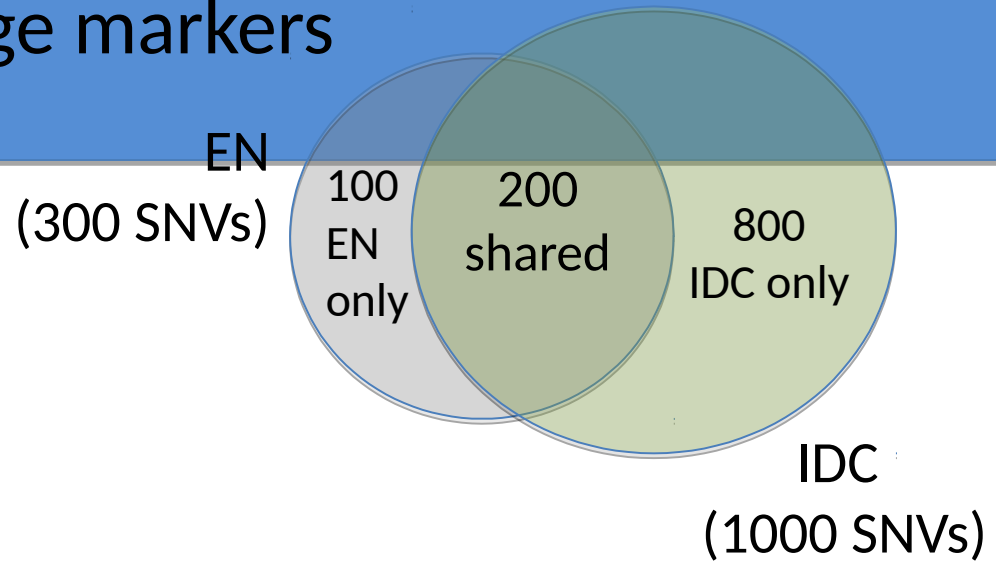
Pipeline

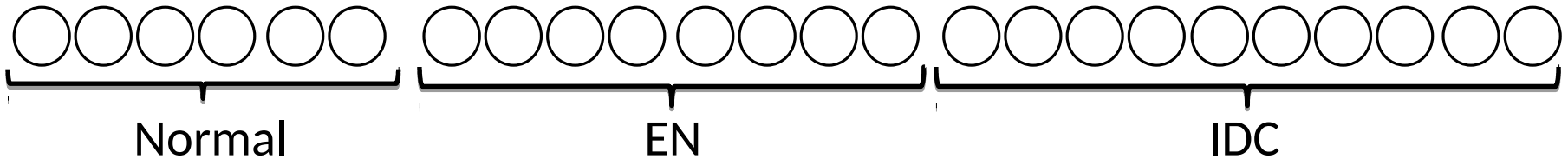
Alignment → PCR duplication removal → Base quality Recalibration → Local Realignment → GATK Unified-Genotyper → Filters → Rescue → Final Variants Set → Germline / Somatic

Somatic SNVs as lineage markers

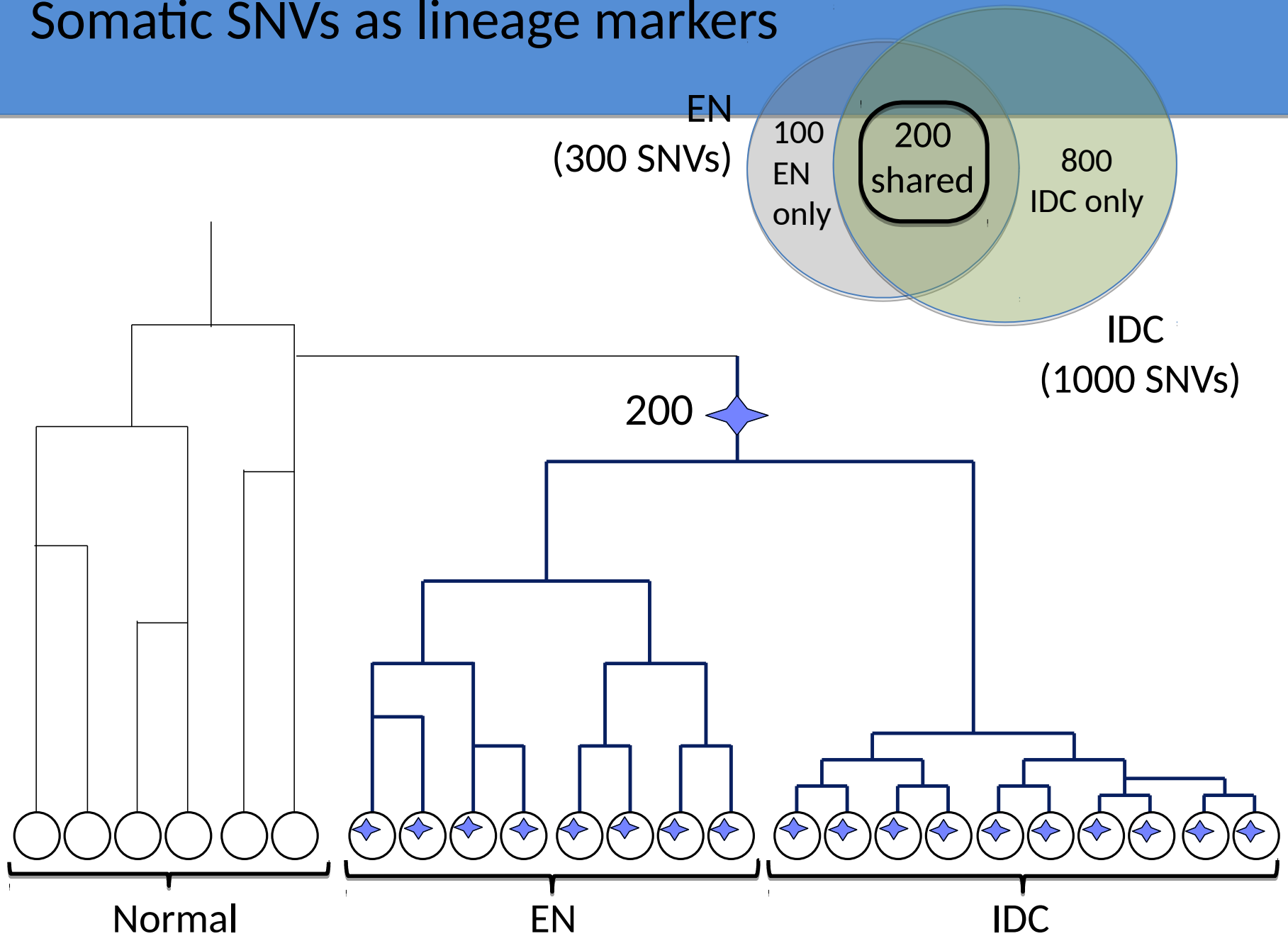


Somatic SNVs as lineage markers

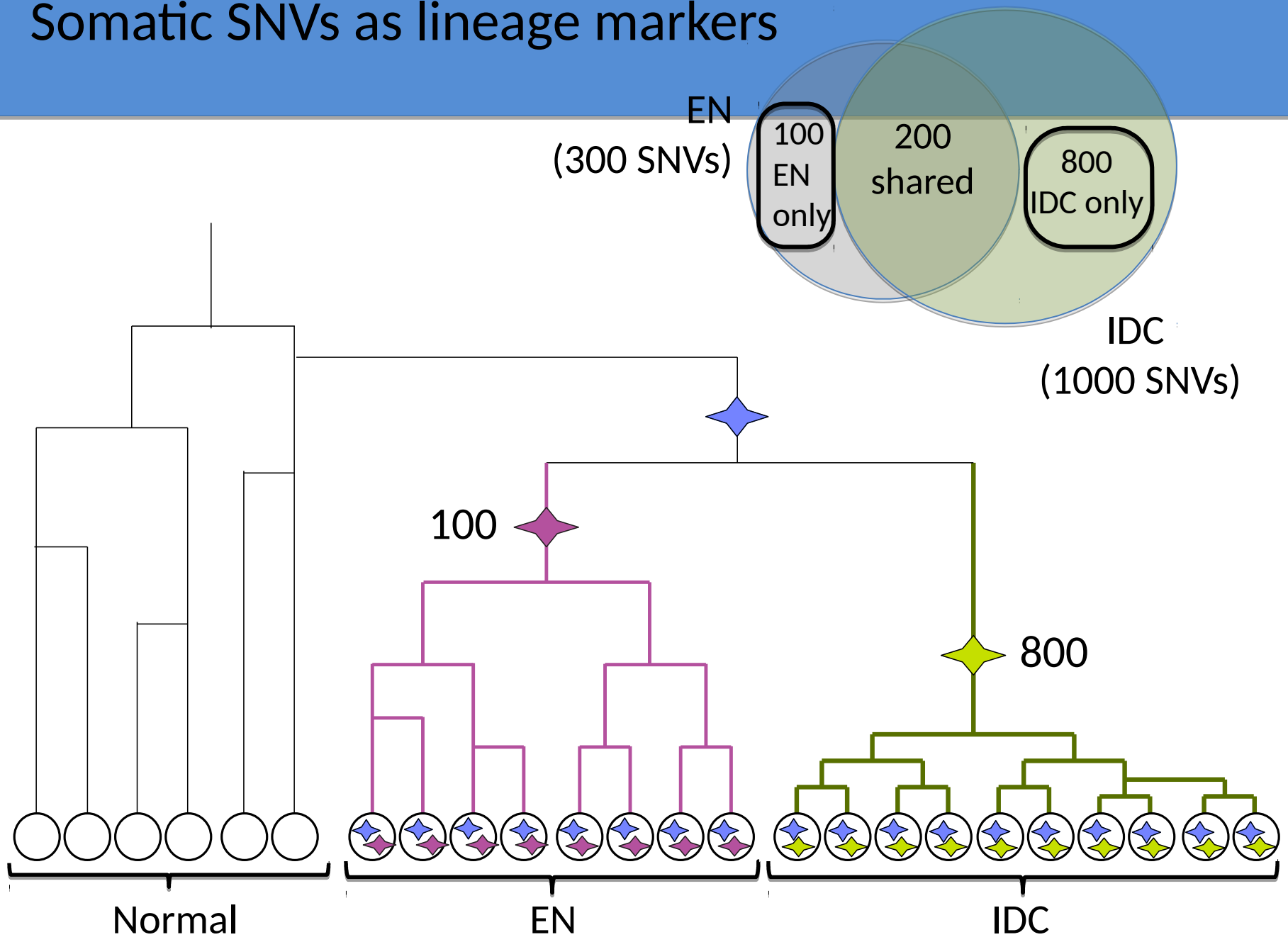




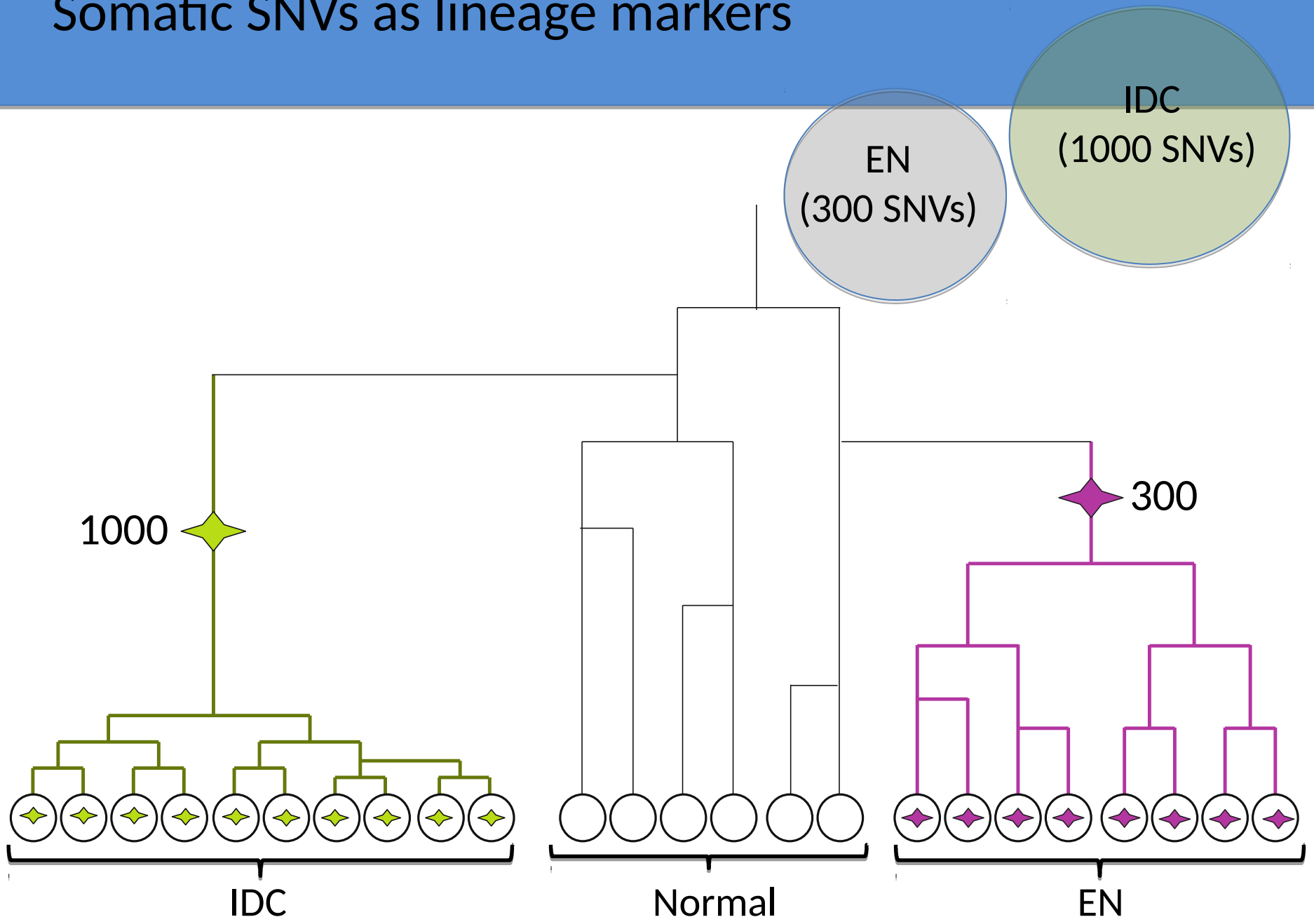
Somatic SNVs as lineage markers



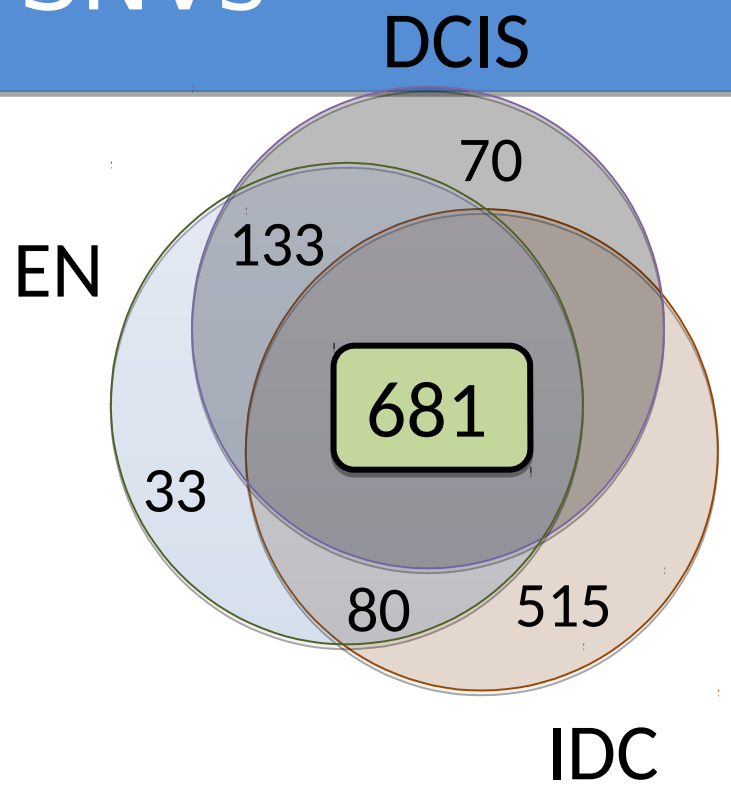
Somatic SNVs as lineage markers



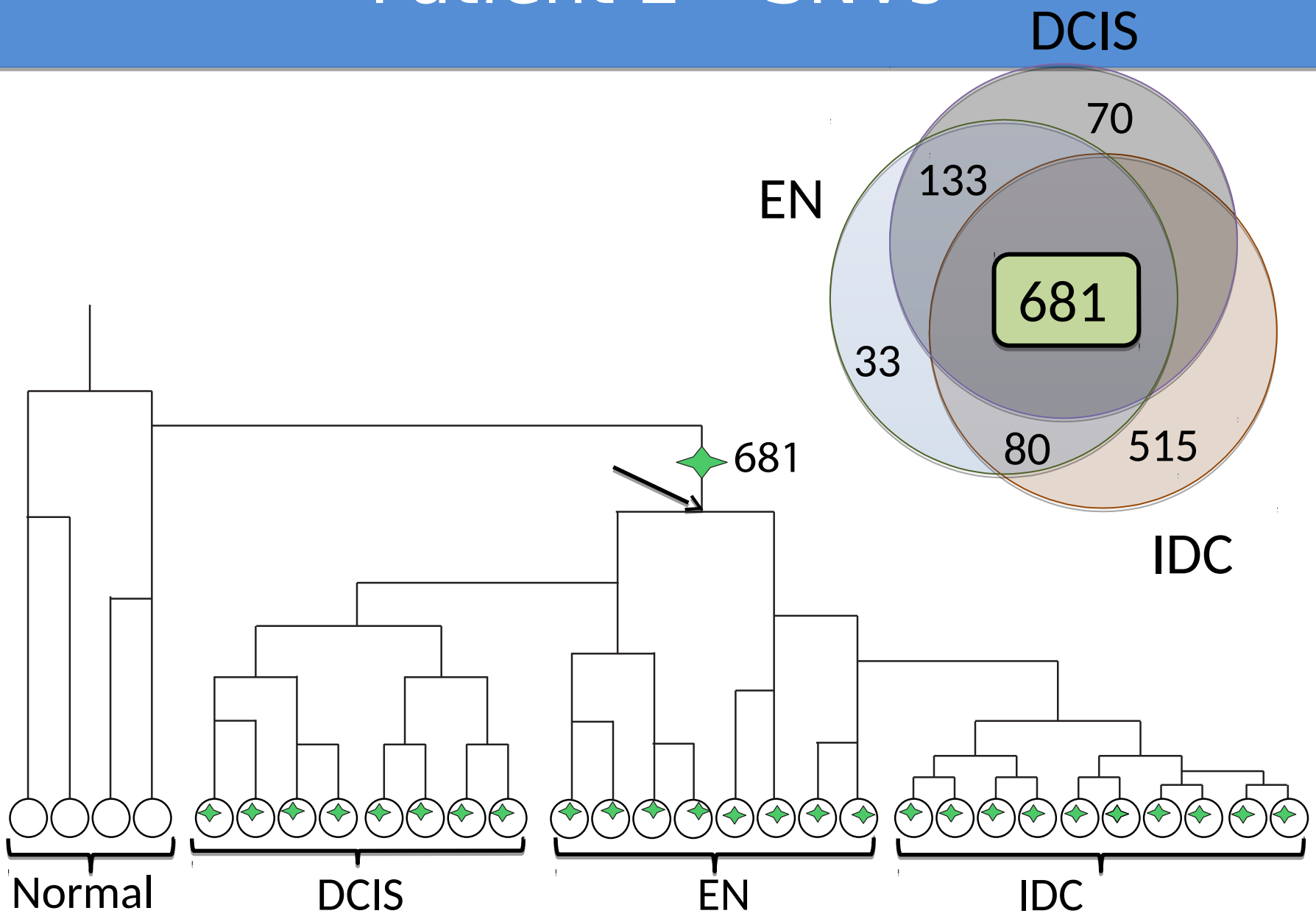
Somatic SNVs as lineage markers



Patient 2 – SNVs

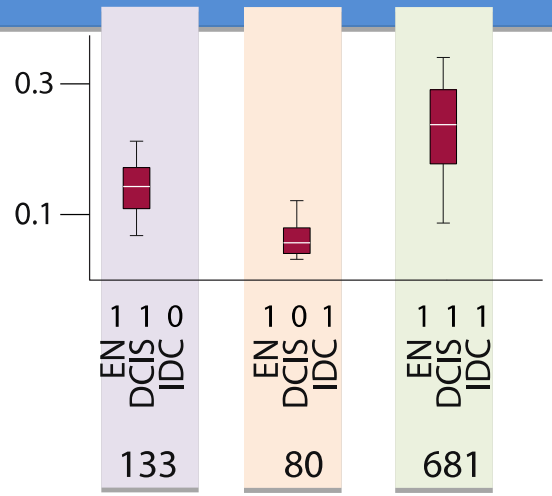


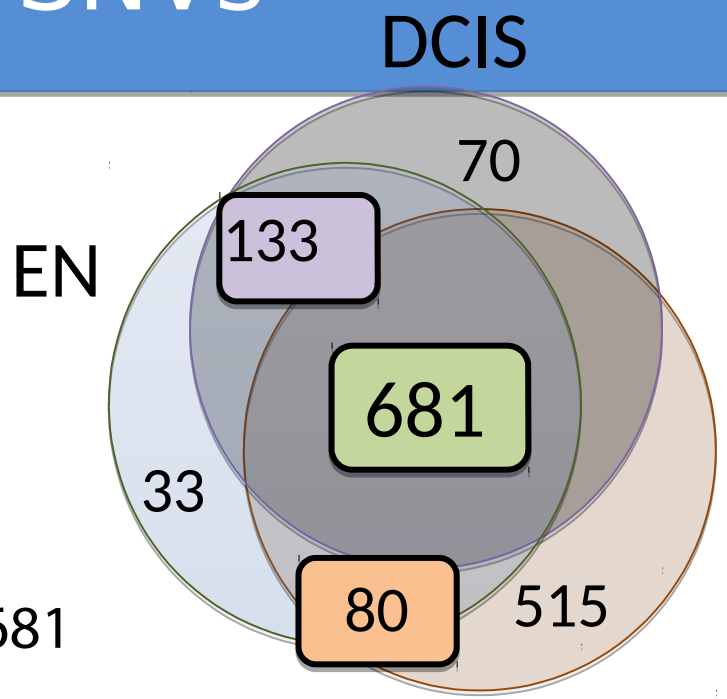
Patient 2 – SNVs

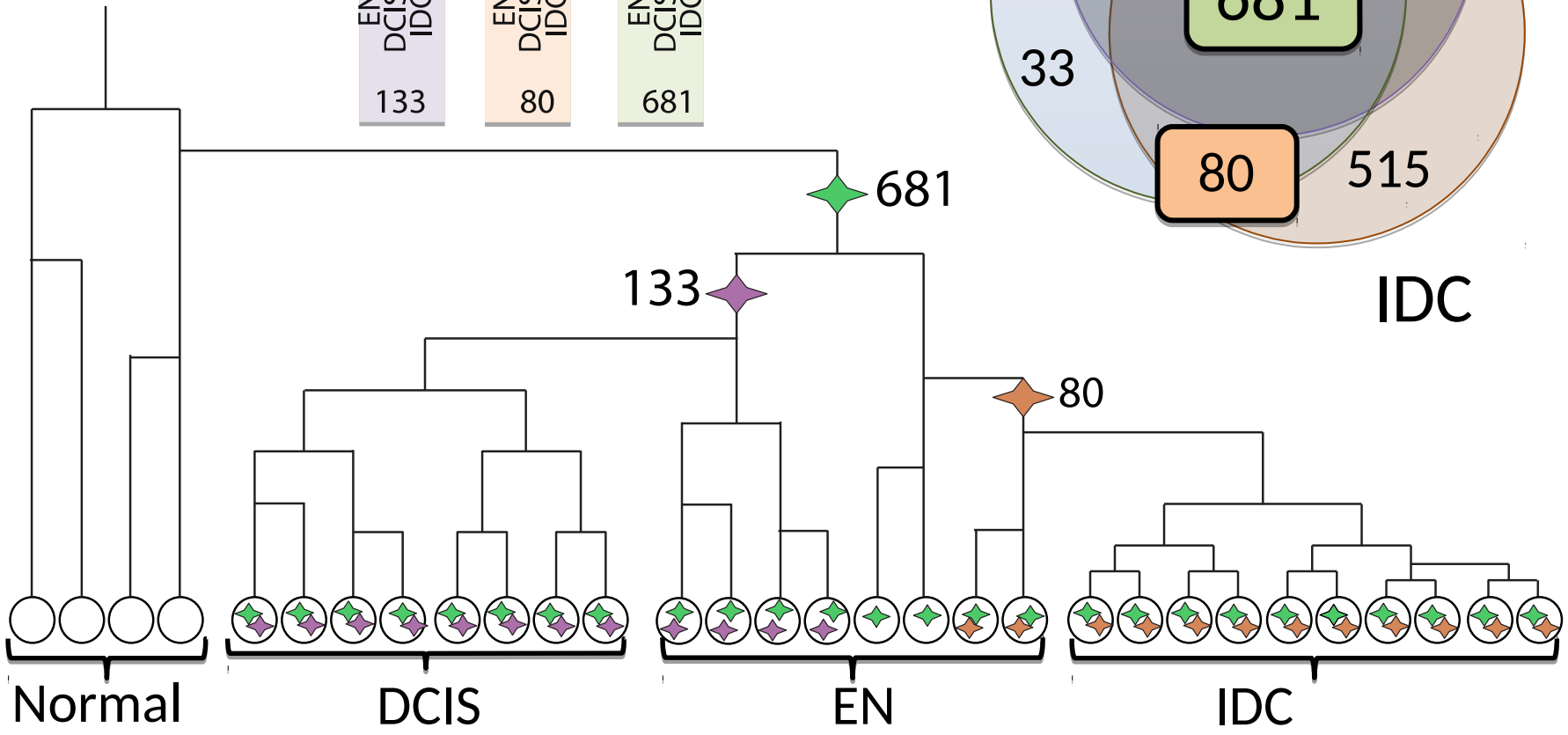


Patient 2 – SNVs

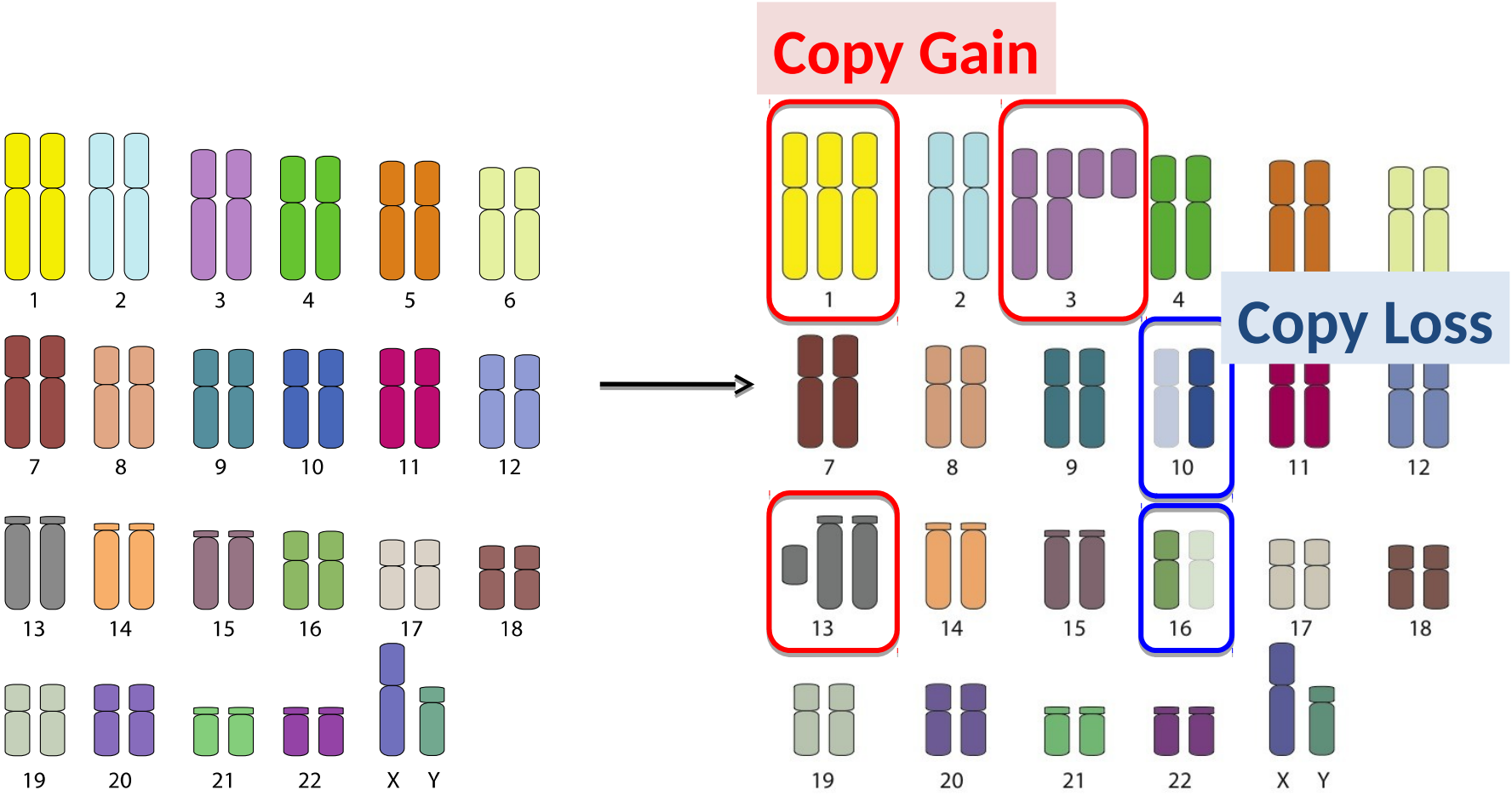
Alternate Allele Fraction in EN



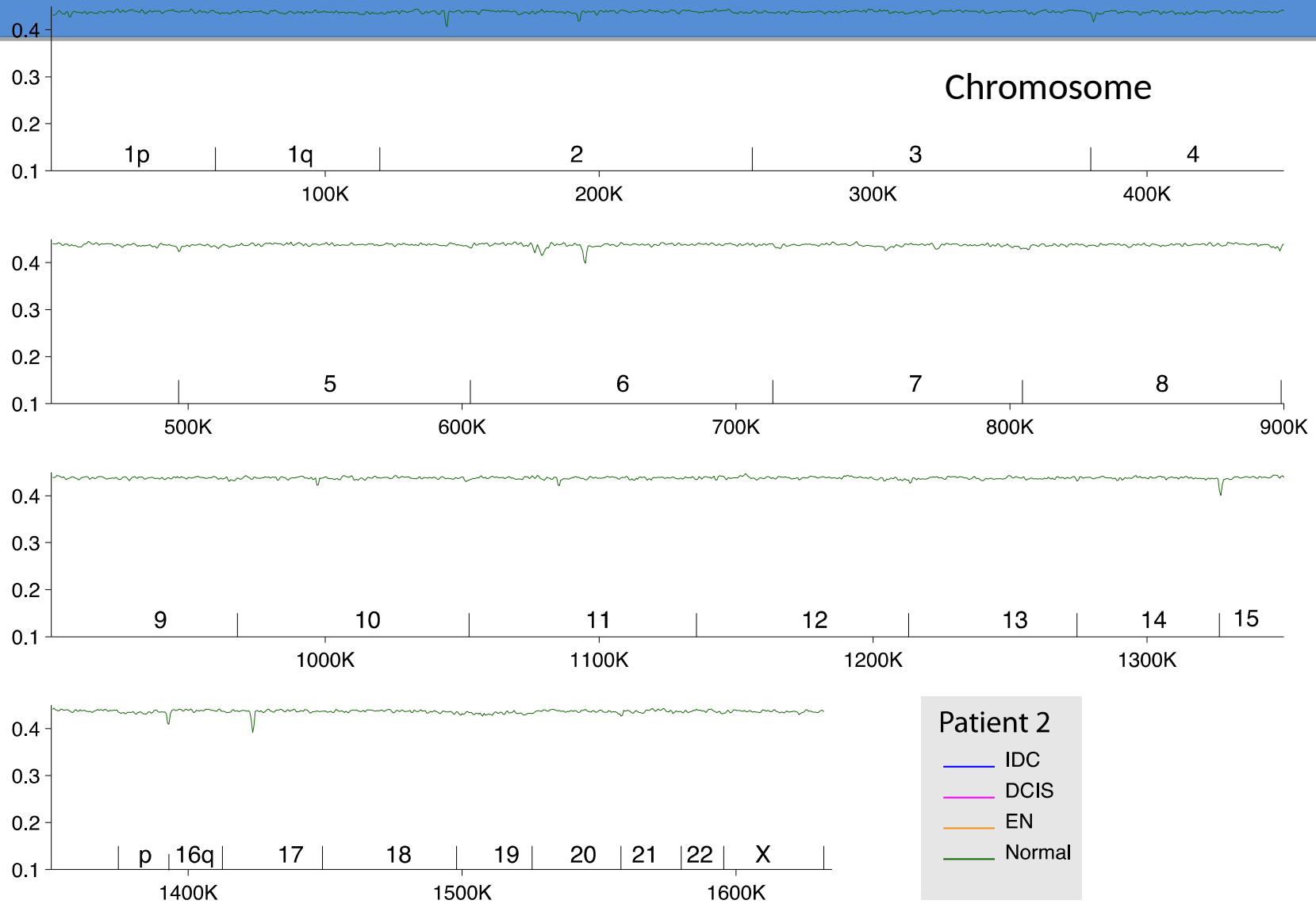




Aneuploidies

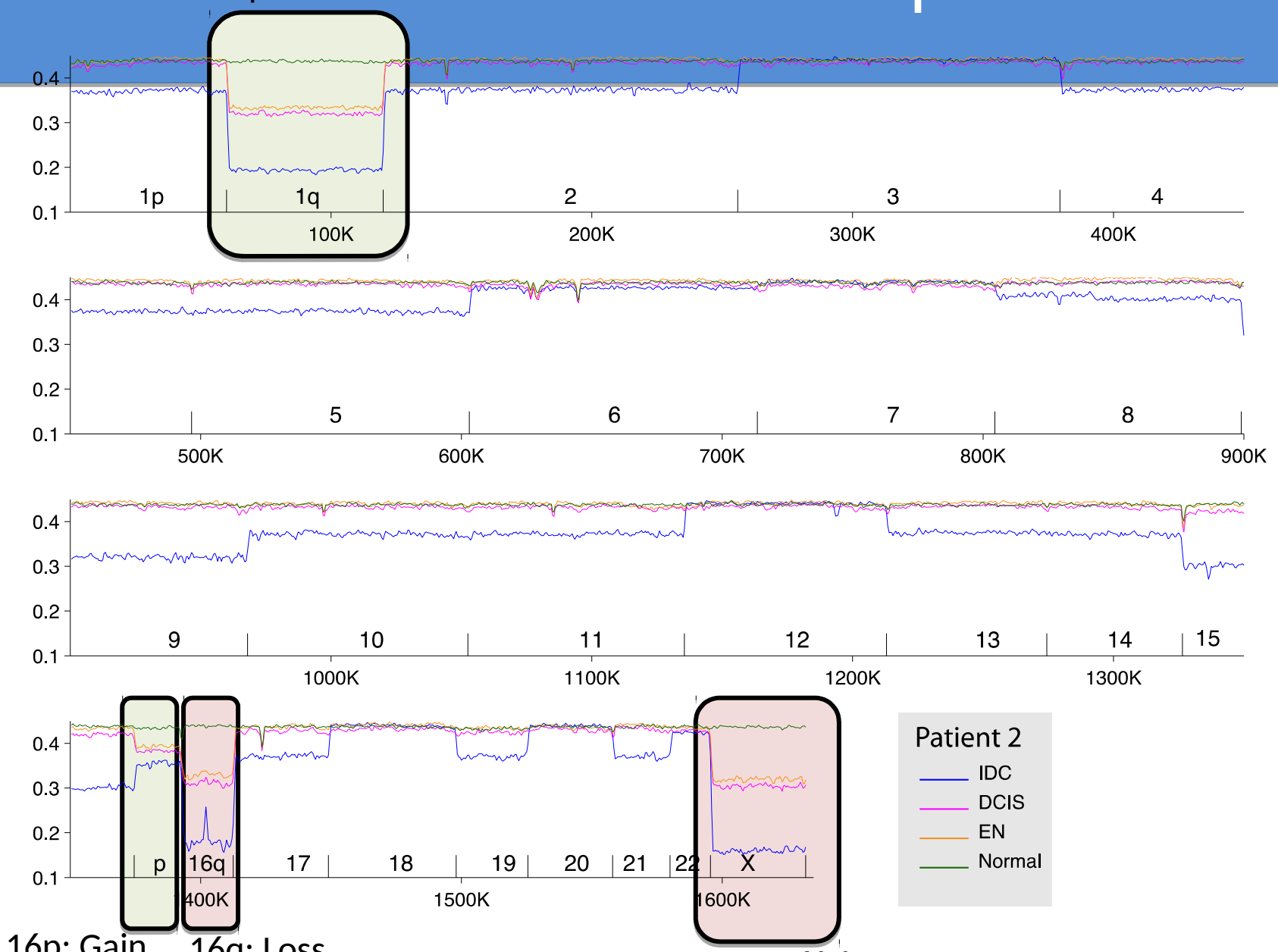


Patient 2 - Aneuploidies

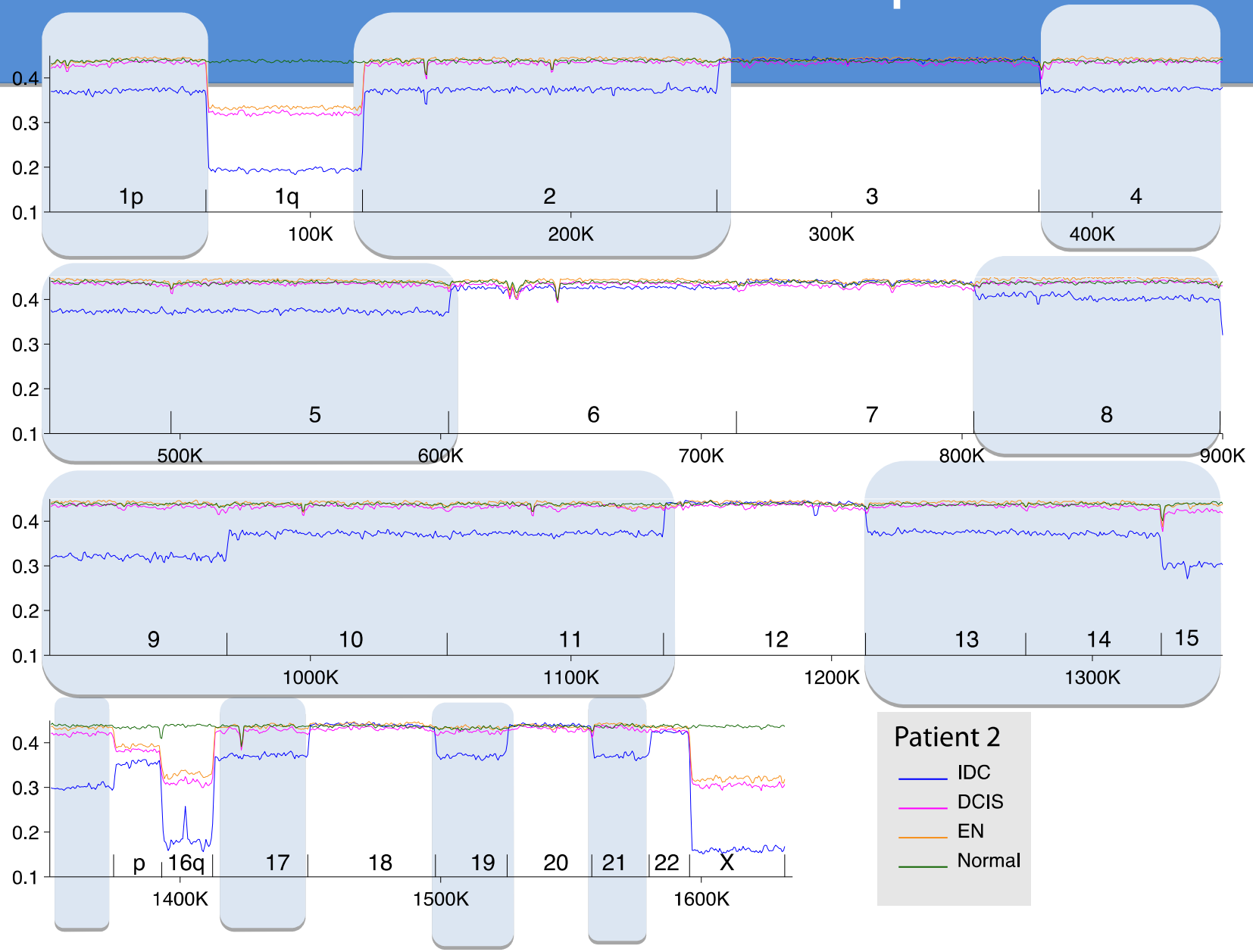


Plots are windows of 1000 SNPs overlapping by 500

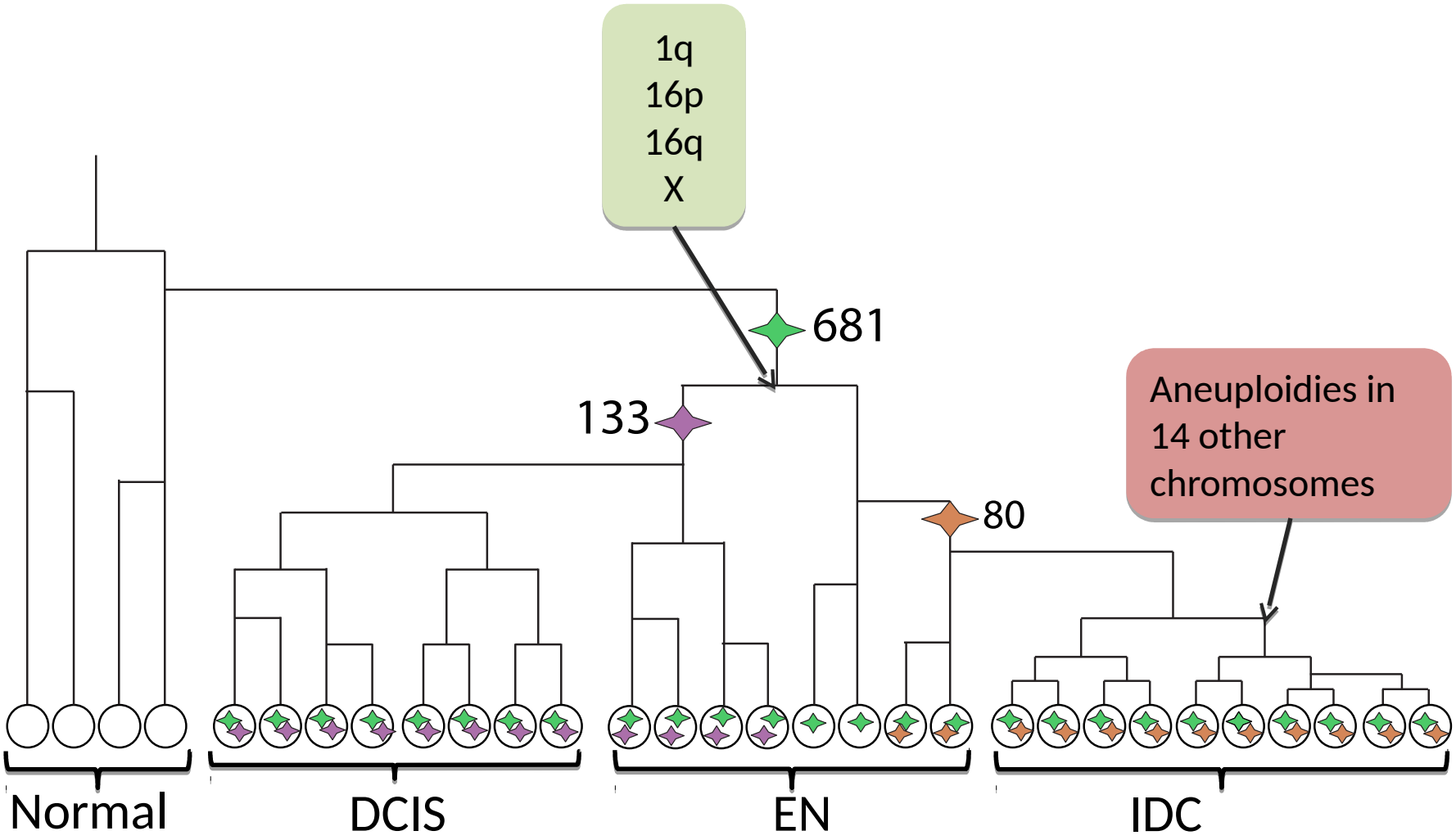
Patient 2 - Aneuploidies



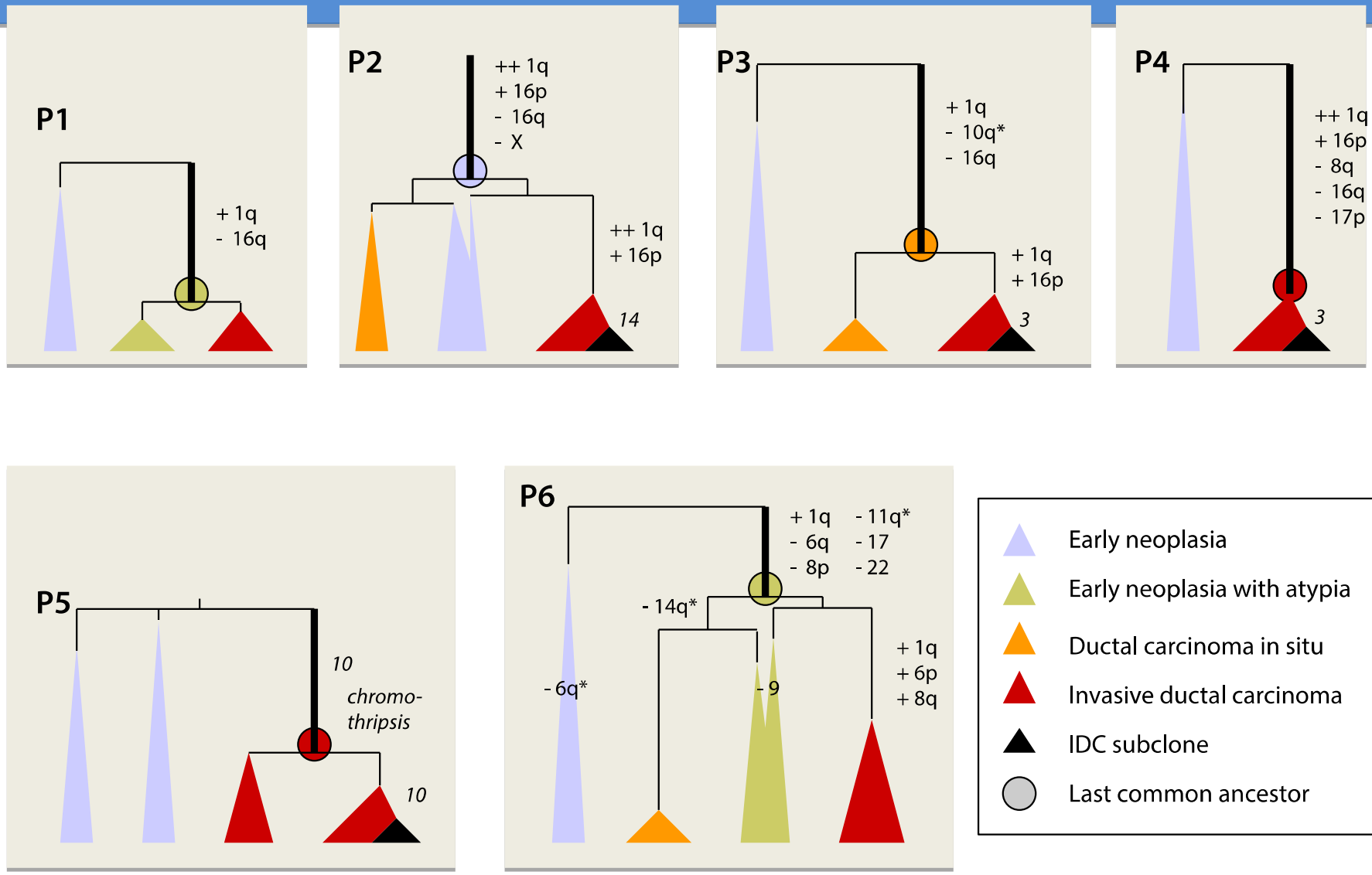
Patient 2 - Aneuploidies



Patient 2 – Final Tree

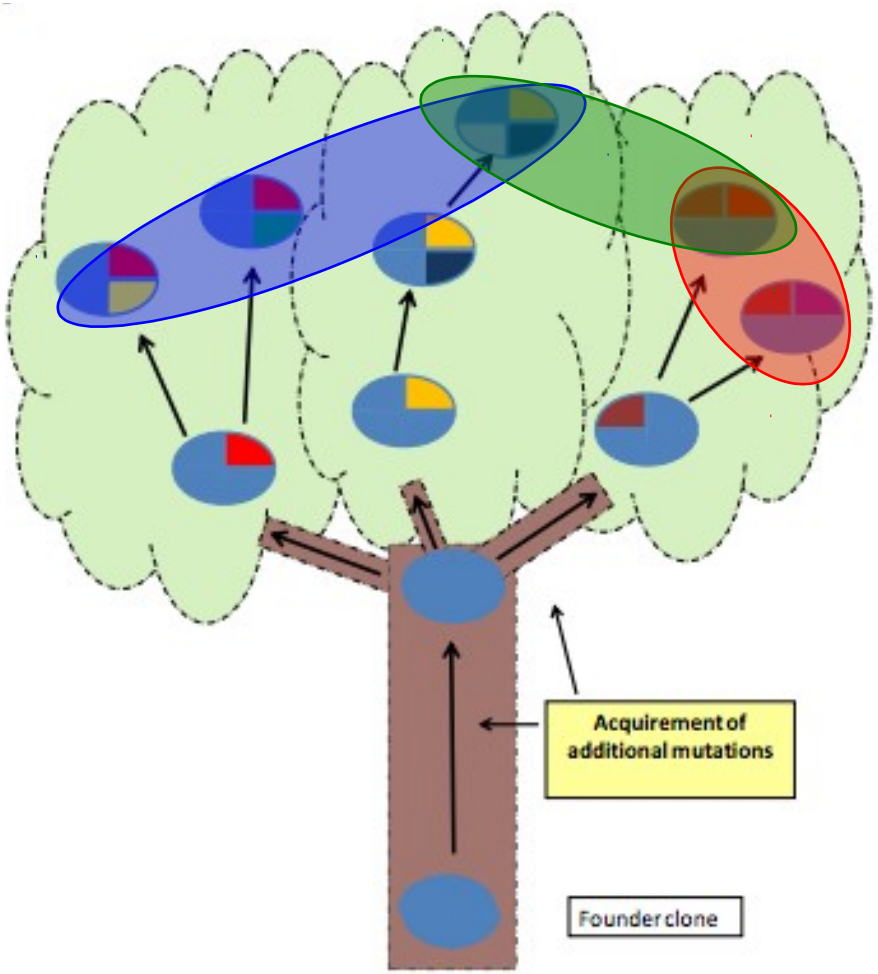


Results

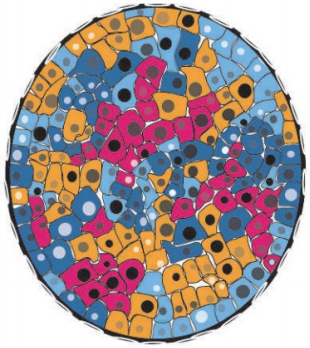

P1
+ 1q
- 16q
P2
++ 1q
+ 16p
- 16q
- X
++ 1q
+ 16p
14
P3
+ 1q
- 10q*
- 16q
+ 1q
+ 16p
3
P4
++ 1q
+ 16p
- 8q
- 16q
- 17p
3
P5
10
chromo-
thripsis
10
P6
+ 1q
- 6q
- 8p
- 11q*
- 17
- 22
- 14q*
- 6q*
- 9
+ 1q
+ 6p
+ 8q
Early neoplasia
Early neoplasia with atypia
Ductal carcinoma in situ
Invasive ductal carcinoma
IDC subclone
Last common ancestor

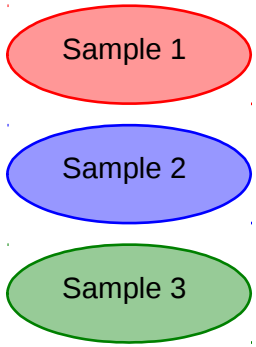
Automated Inference of Multi-Sample Cancer Phylogenies

Victoria Popic
Raheleh Salari



Branched tree model



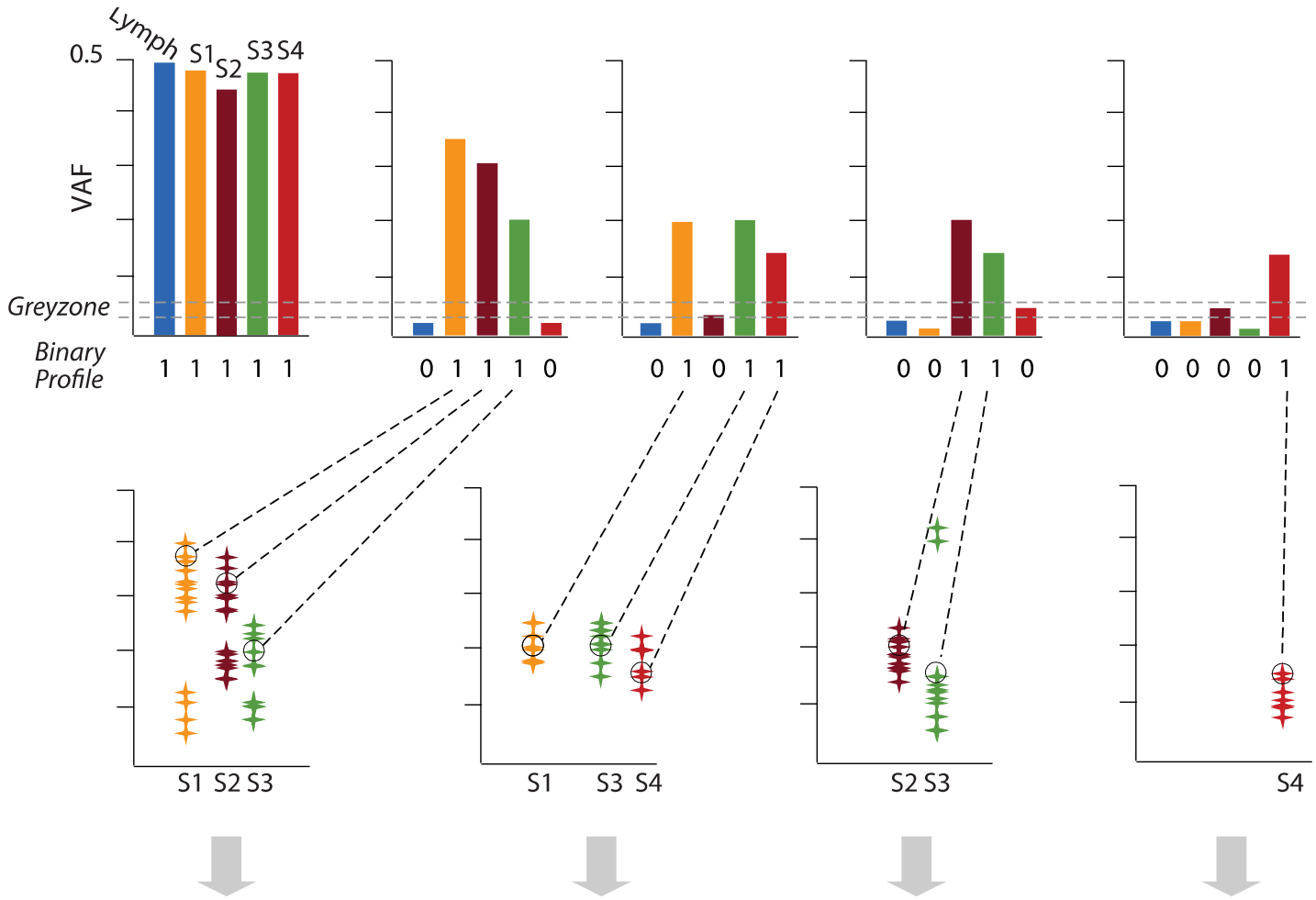


SMutH: Somatic Mutation Hierarchies

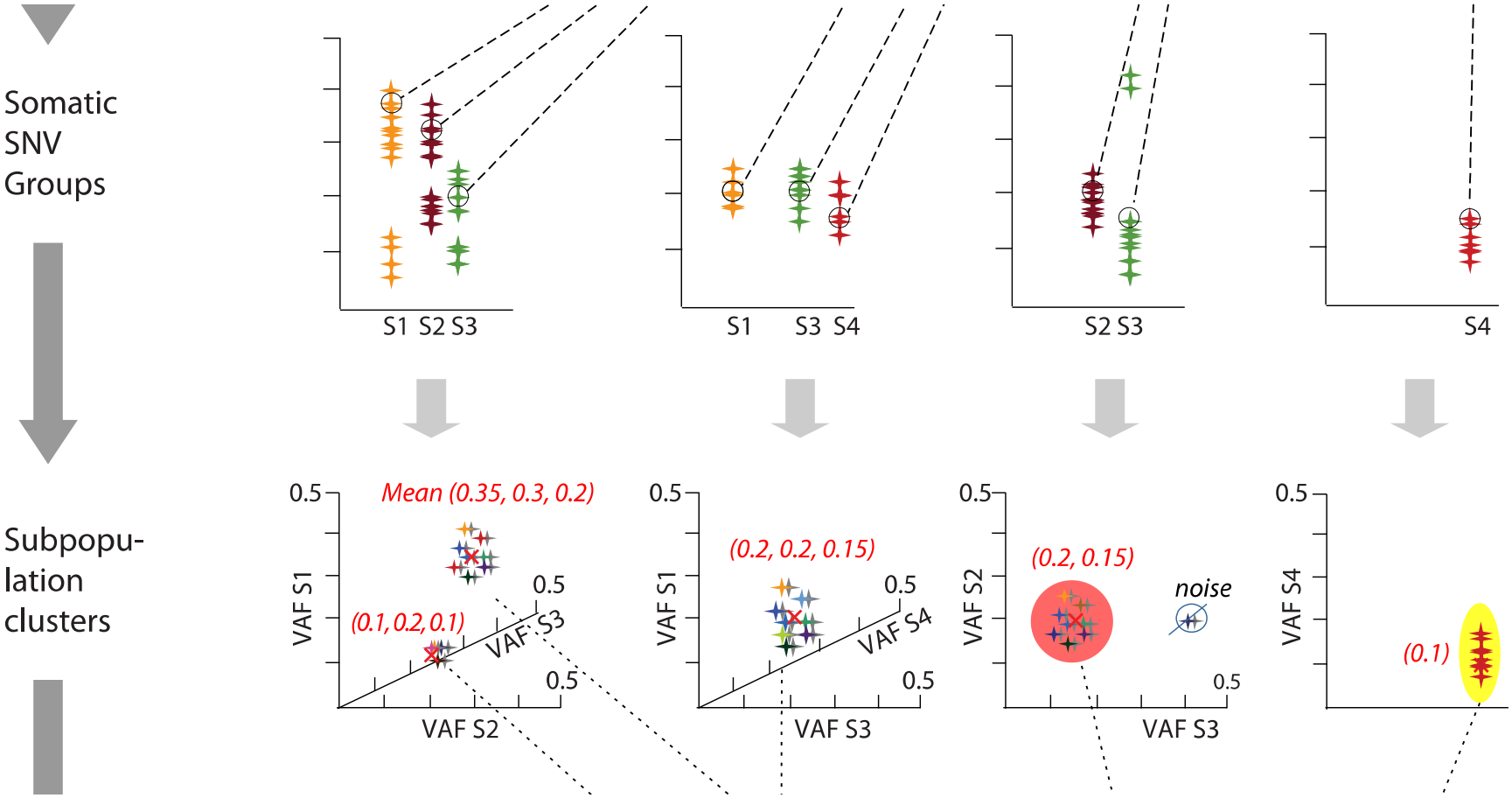
VAF profiles of SNVs across samples

SNV VAF profiles across samples S0 ... S4

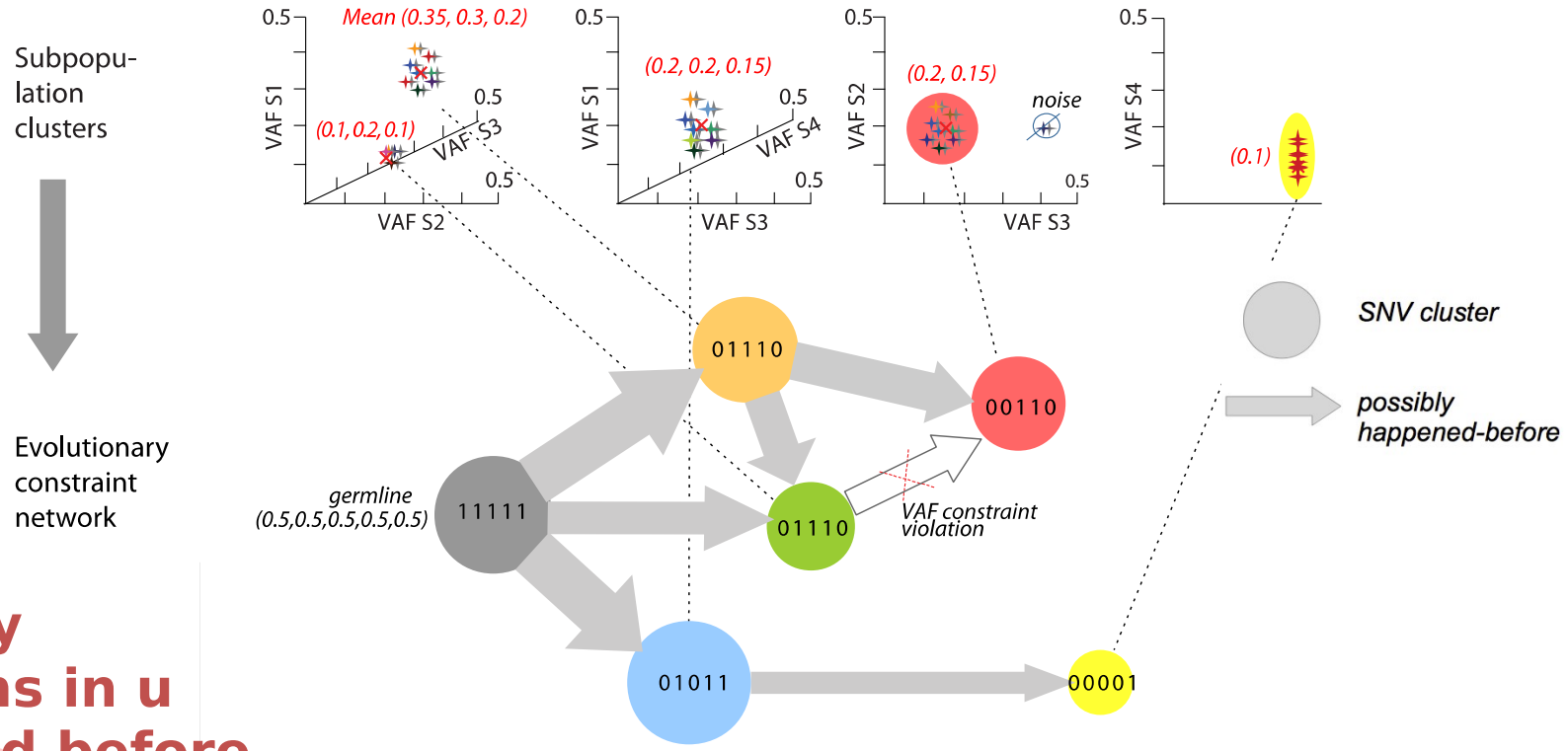
Somatic SNV Groups



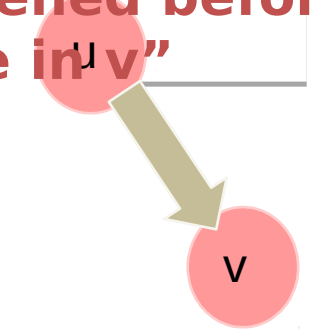
VAF profiles of SNVs – Clustering



Cell-Lineage VAF Constraint



"Possibly mutations in u happened before those in v"

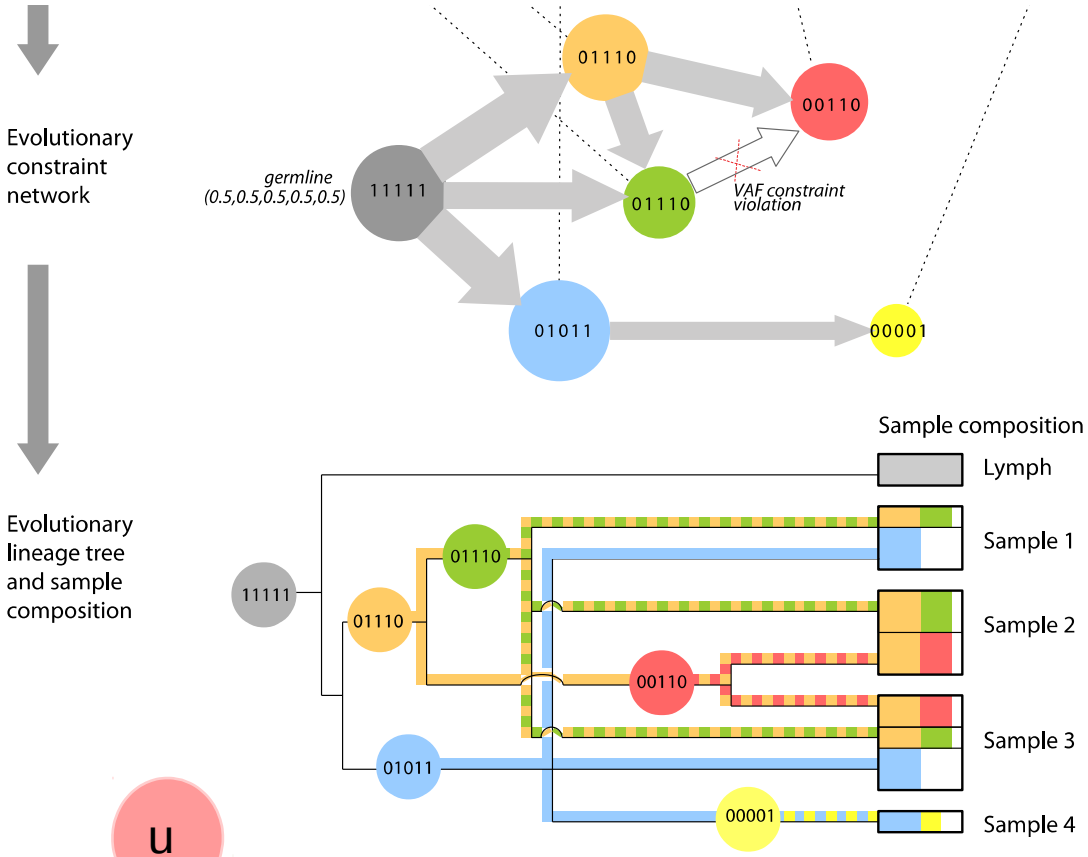


Edge u → v : $\forall i \in samples$:

(1) $u.\overline{VAF}[i] \geq v.\overline{VAF}[i] - \epsilon_{uv}$

(2) $v.\overline{VAF}[i] = 0 \; if \; u.\overline{VAF}[i] = 0$

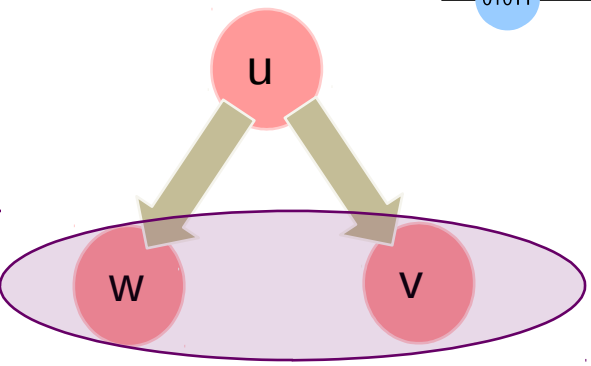
Tree Construction



Find all spanning trees that satisfy VAF constraints

(extension of Gabow&Myers spanning tree search algorithm)

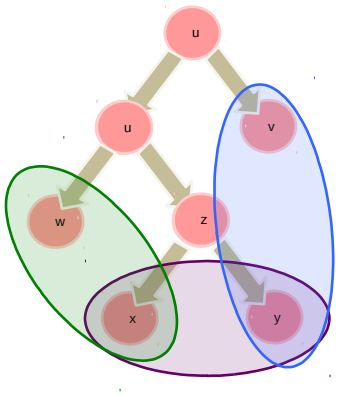
Rank trees according to their agreement with VAFs



For each node u and its children C :

$$\forall i \in samples: \sum_{v \in C} v.\overline{VAF}[i] \leq u.\overline{VAF}[i] + \epsilon$$

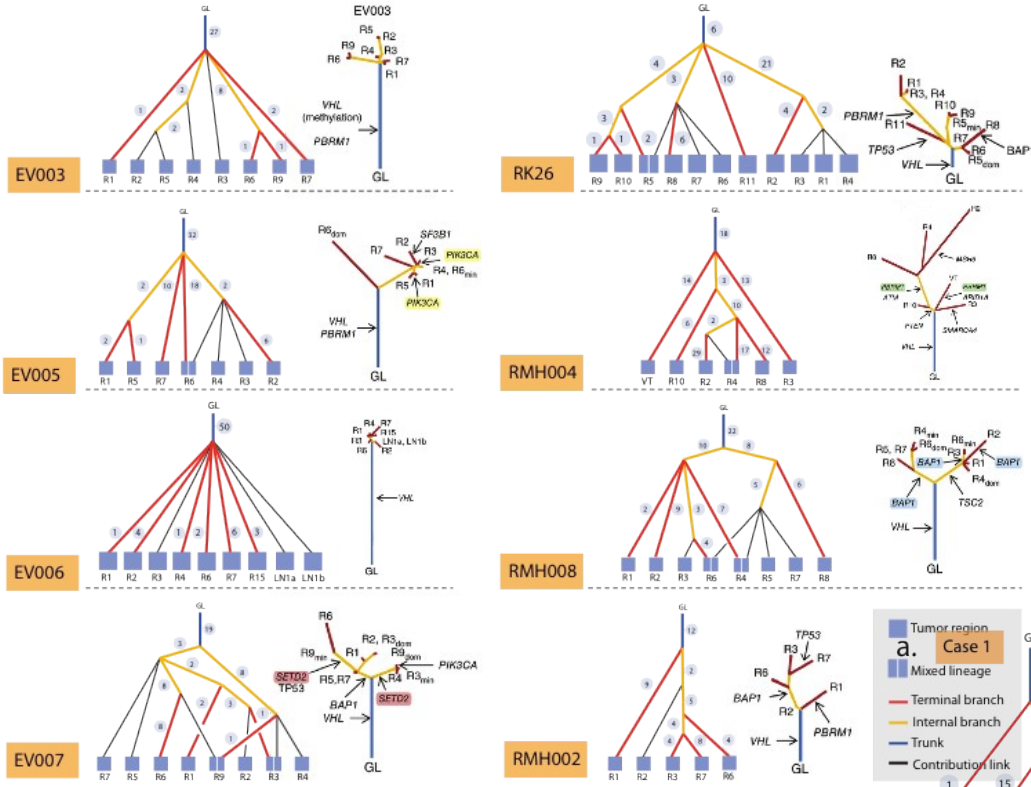
Simulation Results



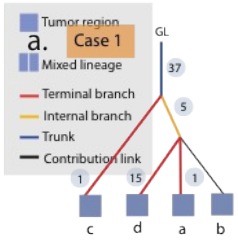
	VAF Stdev 0.03				VAF Stdev 0.1			
# Samples	Pred	Branch	Shared Edges	# Trees	Pred	Branch	Shared Edges	# Trees
5	0.95	0.91	0.81	85	0.95	0.85	0.80	43
6	0.91	0.93	0.82	77	0.91	0.91	0.79	43
7	0.89	0.91	0.83	67	0.88	0.89	0.83	38
8	0.89	0.96	0.84	64	0.93	0.95	0.87	43
9	0.88	0.95	0.81	60	0.87	0.97	0.82	34
10	0.89	0.94	0.82	50	0.87	0.95	0.85	24
11	0.89	0.94	0.85	46	0.86	0.97	0.77	26
12	0.86	0.97	0.81	39	0.85	0.97	0.80	20
13	0.91	0.97	0.85	50	0.91	0.87	0.84	26
14	0.88	0.94	0.83	42	0.89	0.90	0.89	15
15	0.88	0.95	0.85	38	0.82	0.91	0.79	16

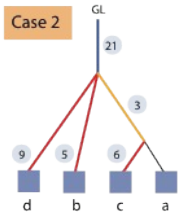
Pred: pairs of nodes ordered correctly
Branch: pairs of nodes correctly assigned to separate branches
Shared edges: edges shared between true and reconstructed trees

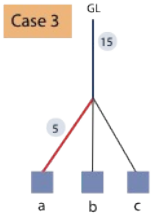
Reconstruction of Lineage Trees in Recent Literature

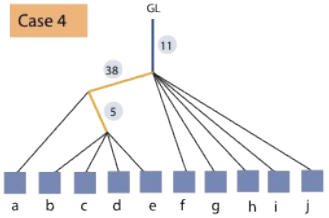


ccRCC Study of Renal Carcinoma by Gerlinger *et. al* (2014)

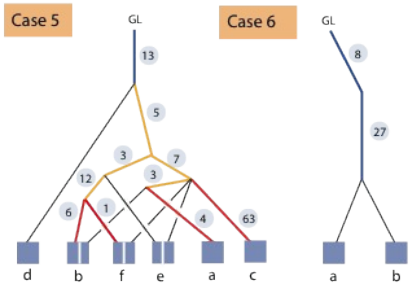


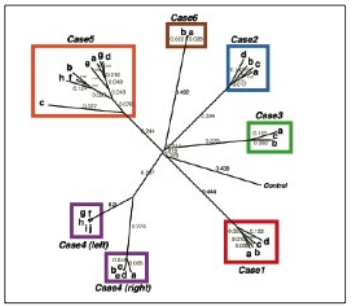




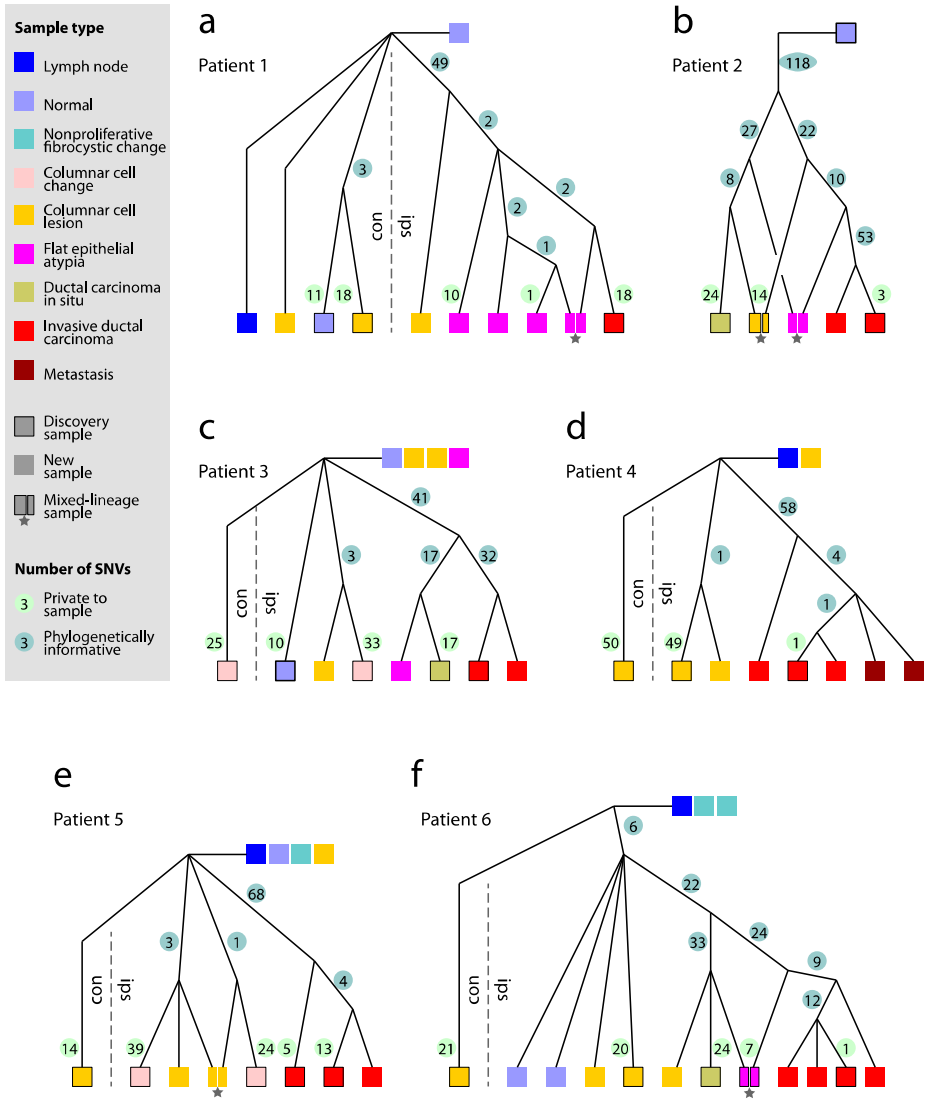


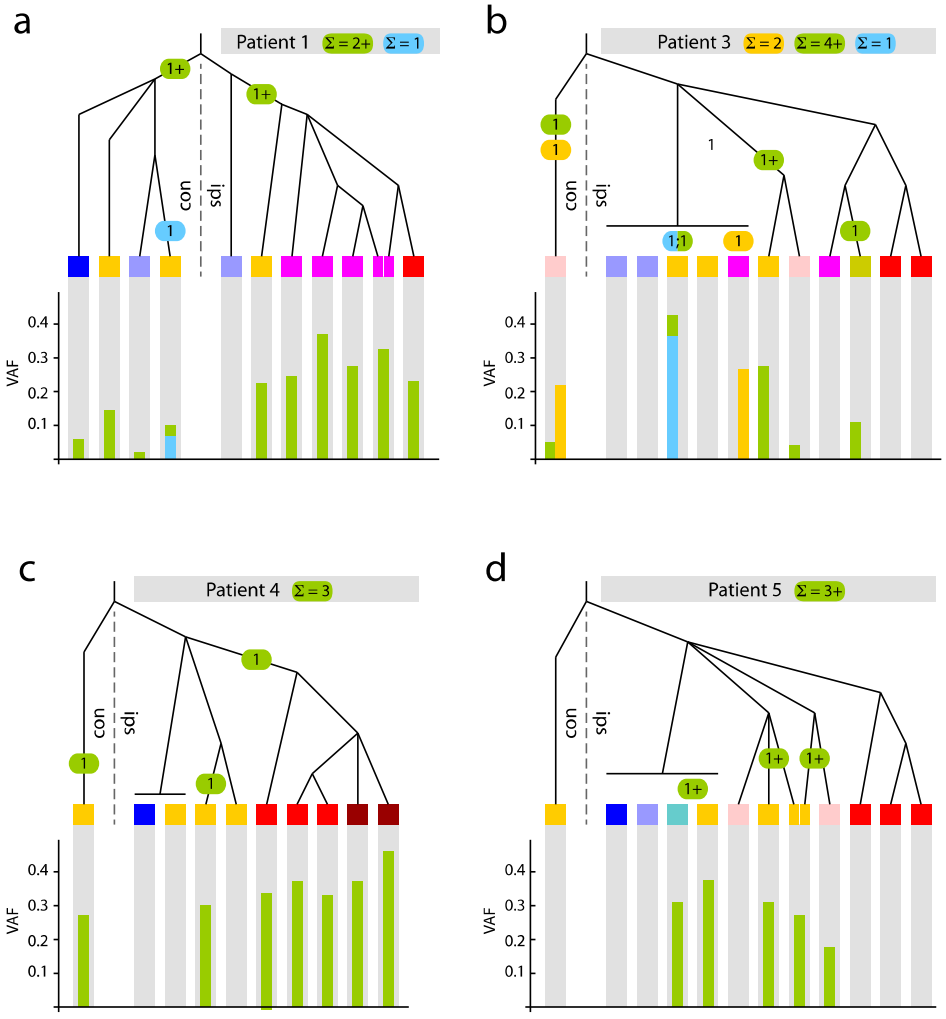
HGSC Study of Ovarian Cancer Bashashati *et. al* (2013)





Expanded Breast Cancer Lineage Trees

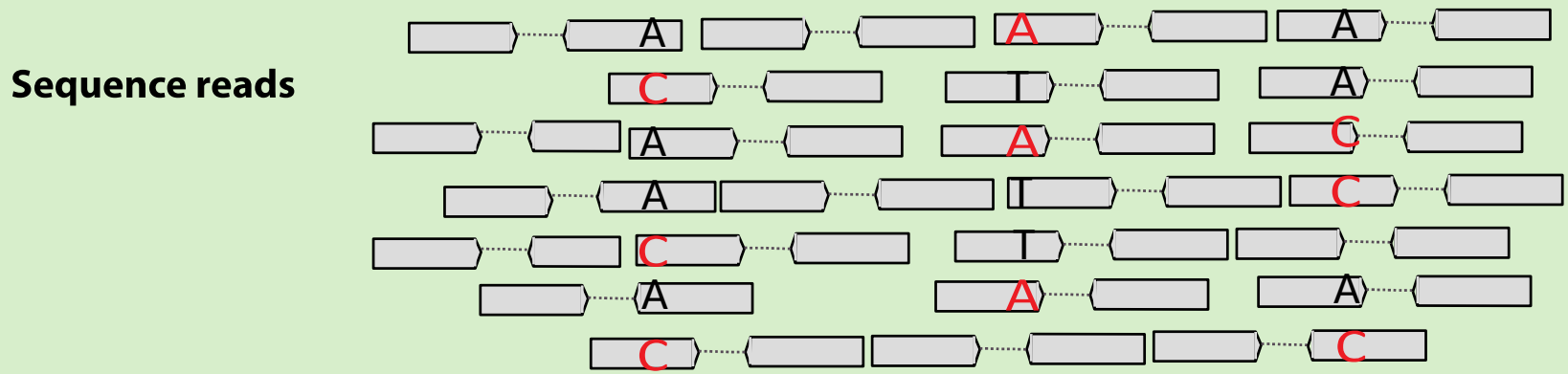




PIK3CA H1047R

PIK3CA H1047L

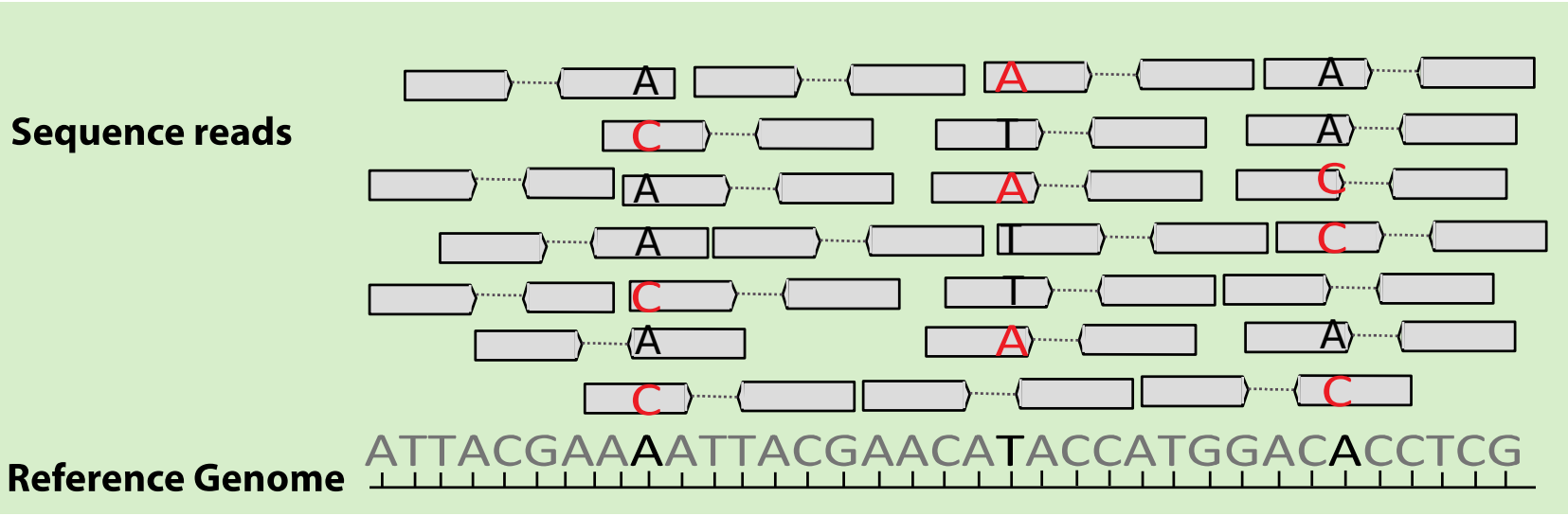
What is a haplotype?



Genotype A/C A/T A/C

A	A	C	OR	A	T	C	OR	A	T	A	OR	A	A	A	?
C	T	A		C	A	A		C	A	C		C	T	A	
correct				wrong				wrong				wrong			

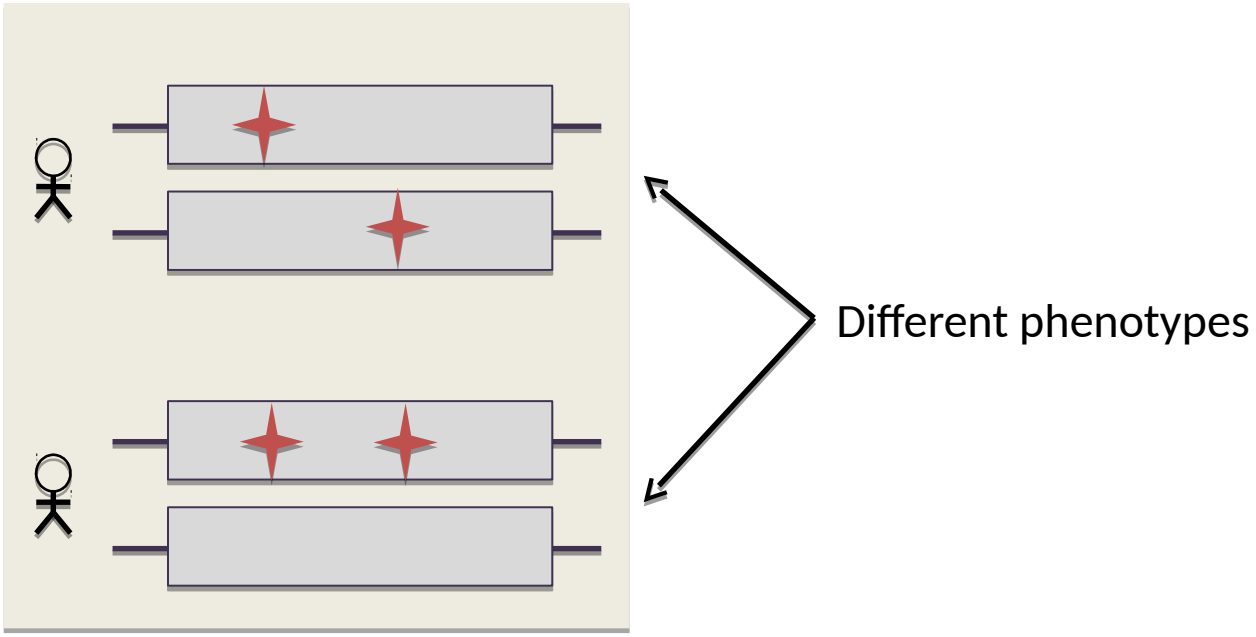
What is a haplotype?



Phasing is the process of recovering haplotypes from genotype data

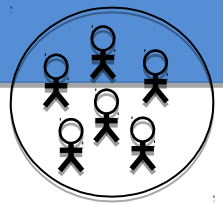
The importance of phase information

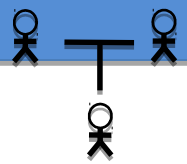
- Compound Heterozygosity



- Two-hit cancer model

Different Approaches for Phasing





Population	Genetic	Molecular
Unrelated duos, trios	Pedigrees Trios	Each physical read is a single molecule that can be directly phased
Population inference	IBD analysis, inheritance state analysis	Read Assembly

	Population	Genetic	Molecular
Common variants	Yes	Yes	Yes
Private variants	**No**	Yes	Yes
Somatic variants	**No**	**No**	Yes