Bienvenue aux UE
Cancer et Génomique

UE11: Big data moléculaire et son traitement
UE12: Big data et modèles prédictifs

université PARIS-SACLAY
FACULTÉ DES SCIENCES D'ORSAY

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Planning de la semaine

UE11: Big data moléculaire et son traitement

	Lundi 17 janvier 2021 - Salle 21, B2M
09:00-10:30	Technologies et données omiques en cancérologie. Daniel GAUTHERET
10:45-12:15	L'analyse des altérations de nombre de copies par microarrays et NGS. Bastien JOB, Ingénieur Bioinformaticien, Gustave Roussy
13:30-17:00	TP Galaxy I: Cas d'étude RNA-seq (contrôles qualité, alignements des séquences sur le génome de référence et quantification de l'expression des gènes). Gaëlle LELANDAIS

	Mardi 18 janvier 2021 - Salle 21, B2M
09:00-12:30	TP Galaxy II : Cas d'étude RNA-seq (création d'un workflow, matrice d'expression des gènes et analyse différentielle) Gaelle LELANDAIS
13:30-15:00	Problématique et applications de la détection de variants somatiques par séquençage d'exome. D. GAUTHERET
15:15-16:45	Principes et application de l'analyse d'expression en single-cell. Bastien JOB, Ingénieur Bioinformaticien, Gustave Roussy

	Mercredi 19 janvier 2021 - Salle 21, B2M
09:00-12:30	TP Galaxy: Analyse exome. Visualisation de résultats avec IGV. D. GAUTHERET

UE12: Big data et modèles prédictifs

	Mercredi 19 janvier 2021 - Salle 21, B2M
13:30-17:00	Premiers pas avec le langage R. Cours Video de G. Lelandais. Exercices corrigés par D. GAUTHERET (réservé aux débutants en R)

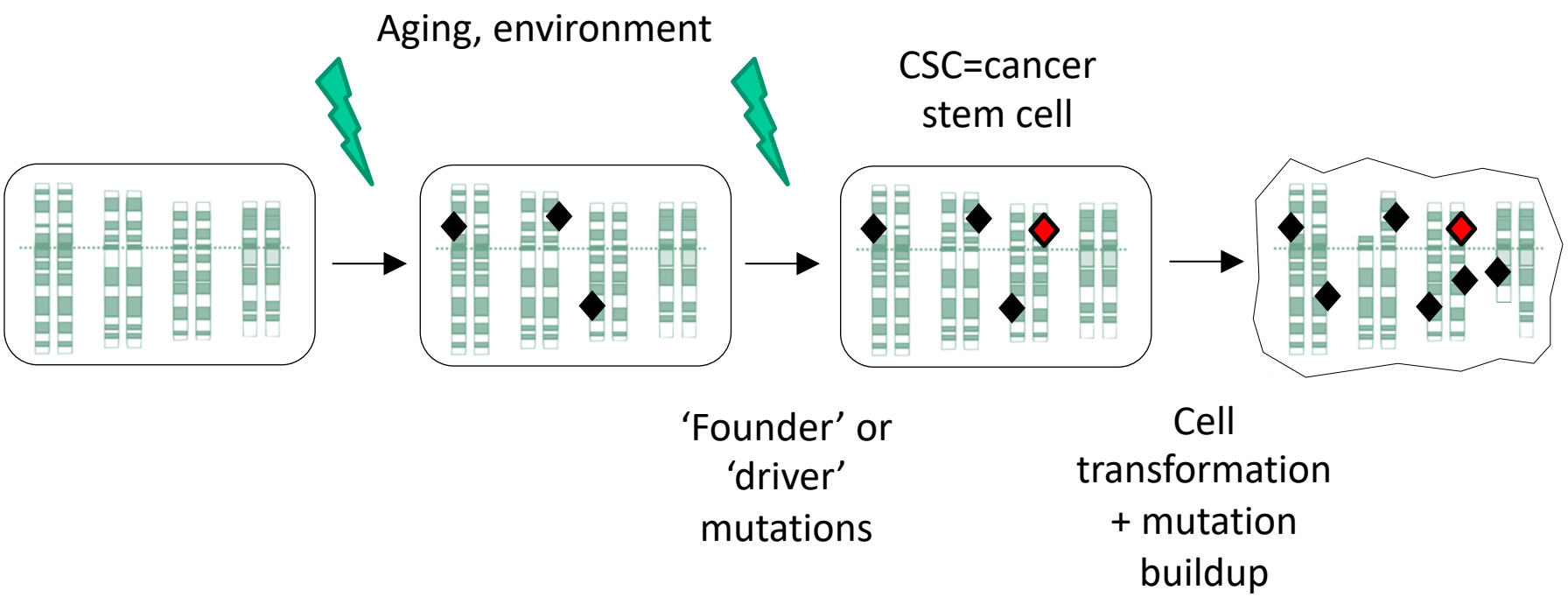
	Jeudi 20 janvier 2021 - Salle 21, B2M
9:00-10:00	Pourquoi utiliser les methodes d'apprentissage automatique en oncologie personalisée? Julien VIBERT (Gustave Roussy)
10:00-11:00	Methodes d'apprentissage automatique. Yoann PRADAT (Centrale-Supelec)
11:30-12:30	Exemple d'un projet en machine learning: Julien VIBERT (Gustave Roussy)
13:30-17:00	TP: Machine learning avec des données d'expression issues de TCGA. Julien VIBERT (Gustave Roussy) et Yoann PRADAT (Centrale-Supelec).

	Vendredi 21 janvier 2021 - Salle 21, B2M
9:00-11:00	Introduction au Deep Learning: applications en oncologie. Yoann PRADAT (Centrale Supelec)
11:30-12:30	Exemple d'un projet en Deep Learning: intervenant CentraleSupelec.
13:30-17:00	TP: Construire son premier réseau de neurones. Julien VIBERT (Gustave Roussy) et Yoann PRADAT (Centrale-Supelec).

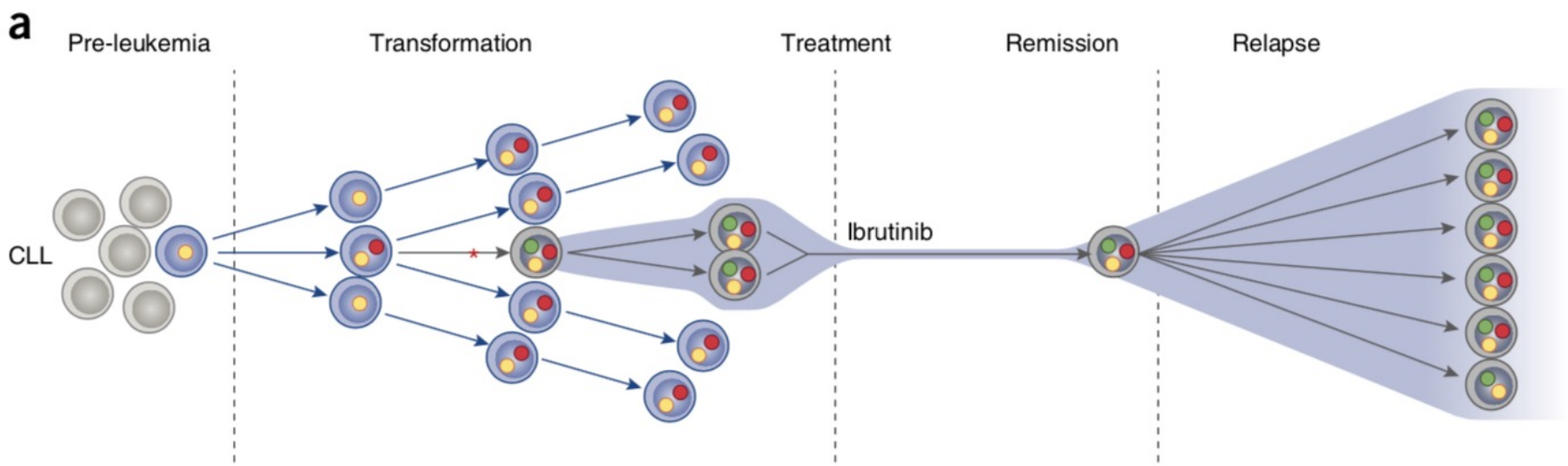
Evaluation:
UE 11: Rapport d'Etonnement
UE 12: Vos protocoles de TP

Introduction

- Le cancer: une maladie du génome



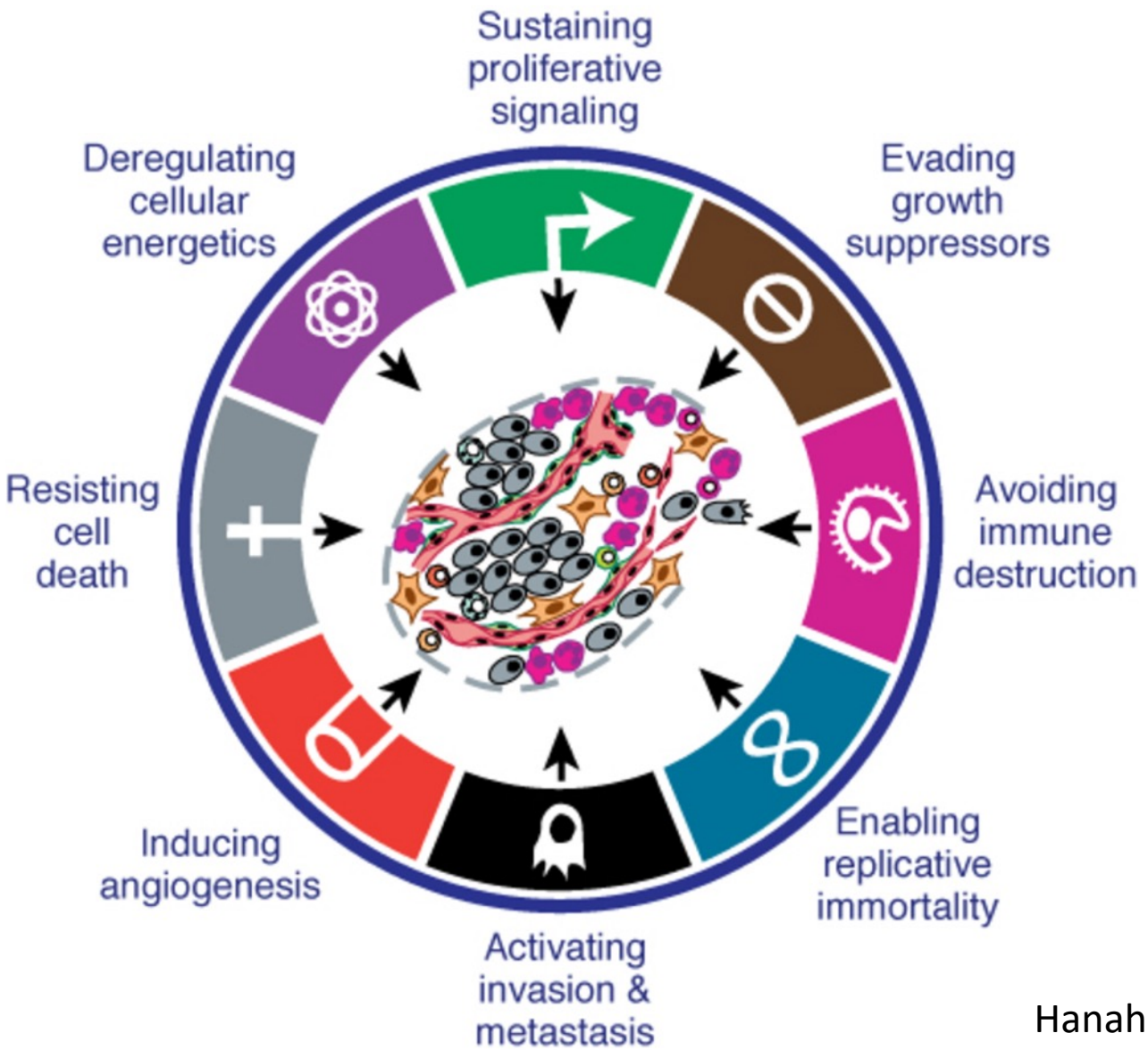
Clonal evolution of cancer



Ferrando & Lopez-Otin, Nature Med. 2017

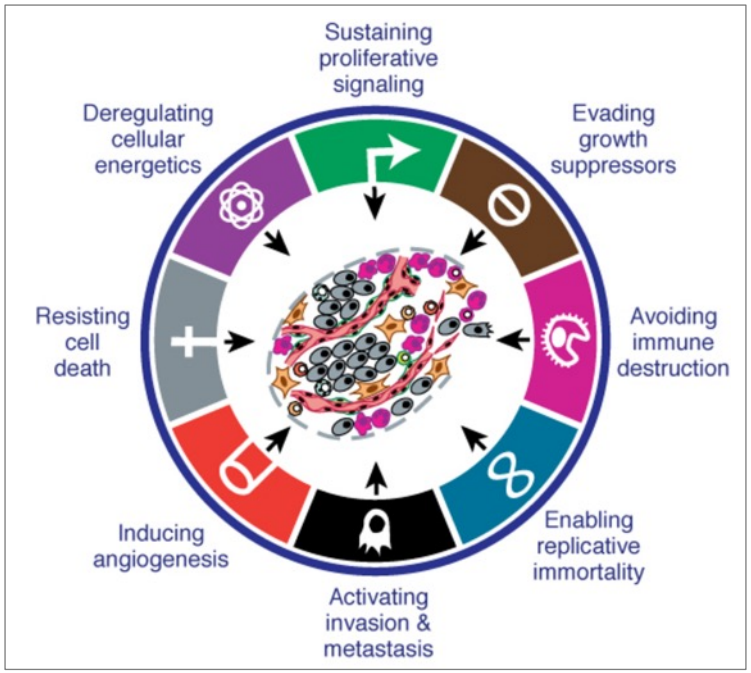
What is different in a cancer cell?

Hallmarks of cancer



Hanahan & Weinberg, 2015

Chemotherapy with <u>nonspecific</u> cancer drugs

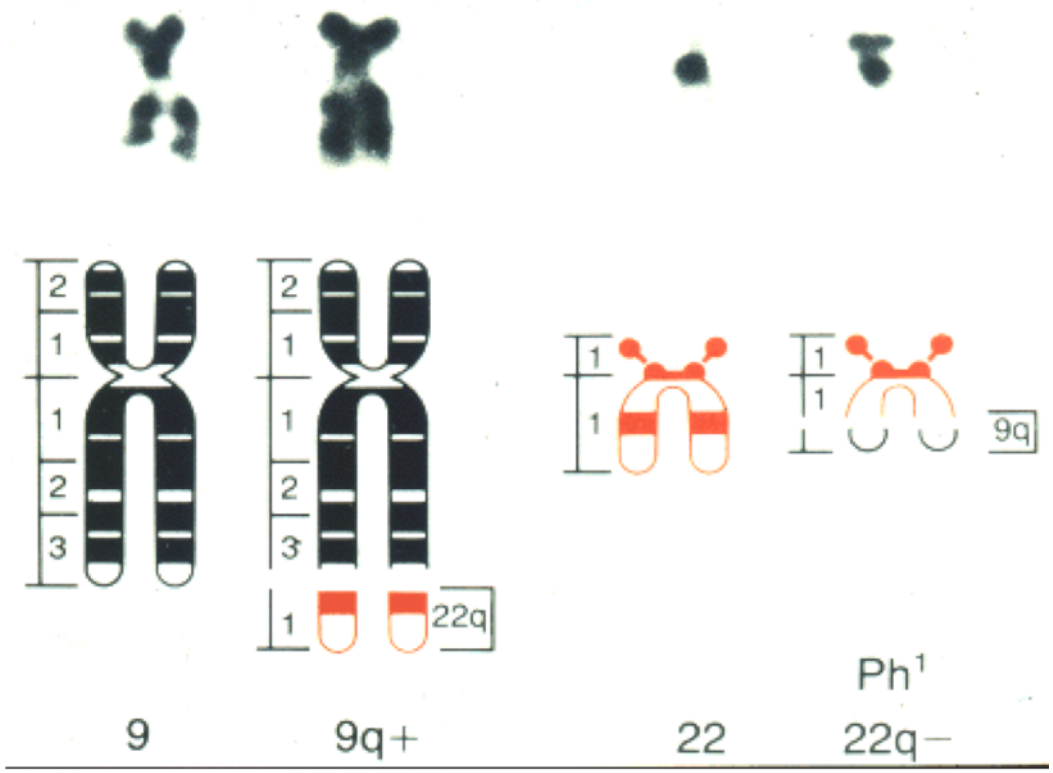


- Taxanes: block cell division
- Alkylating agents (platin salts): kill dividing cells
- Tamoxifene : block hormonal activation

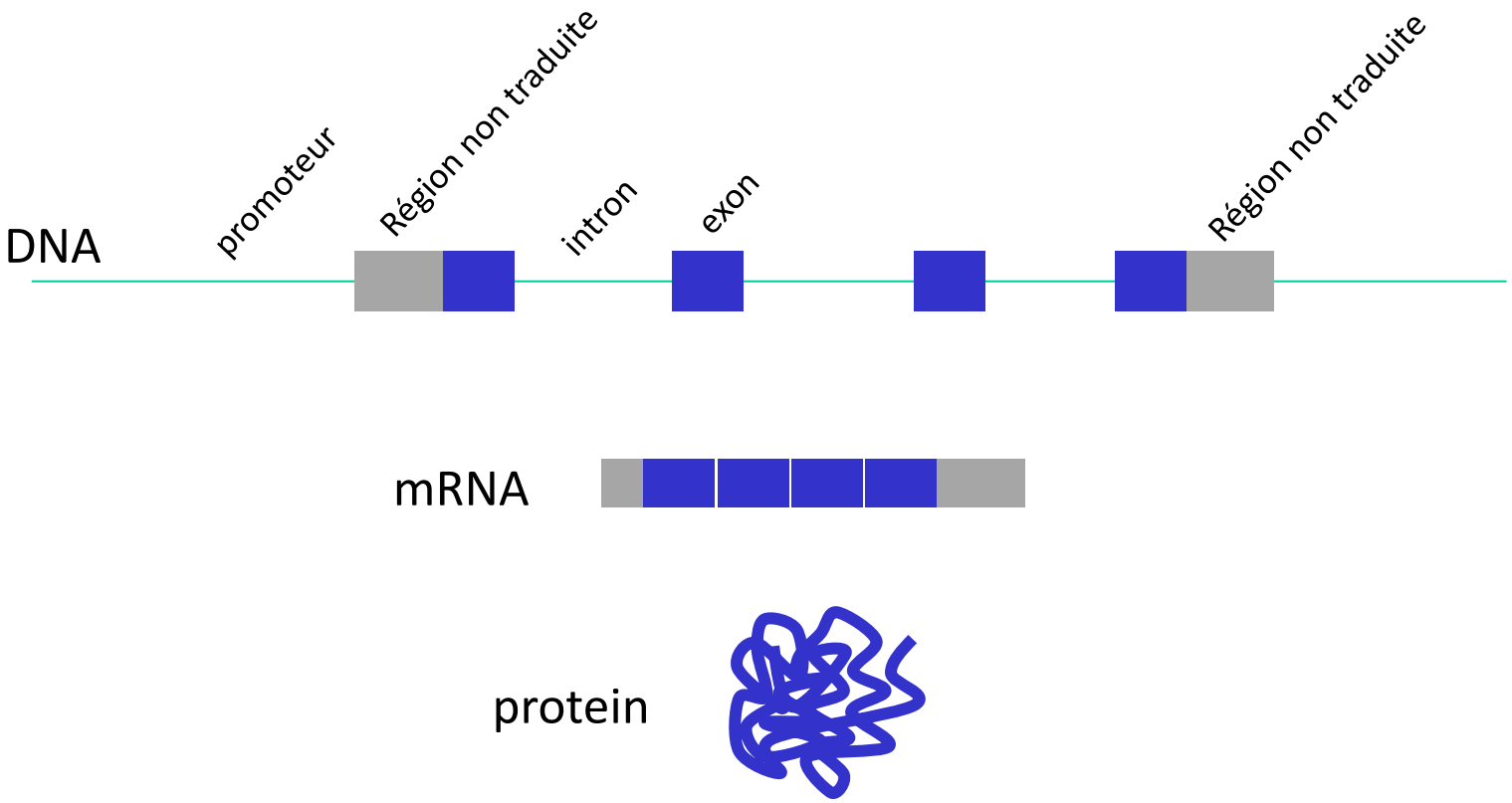
Precision medicine

Le « chromosome de Philadelphie »

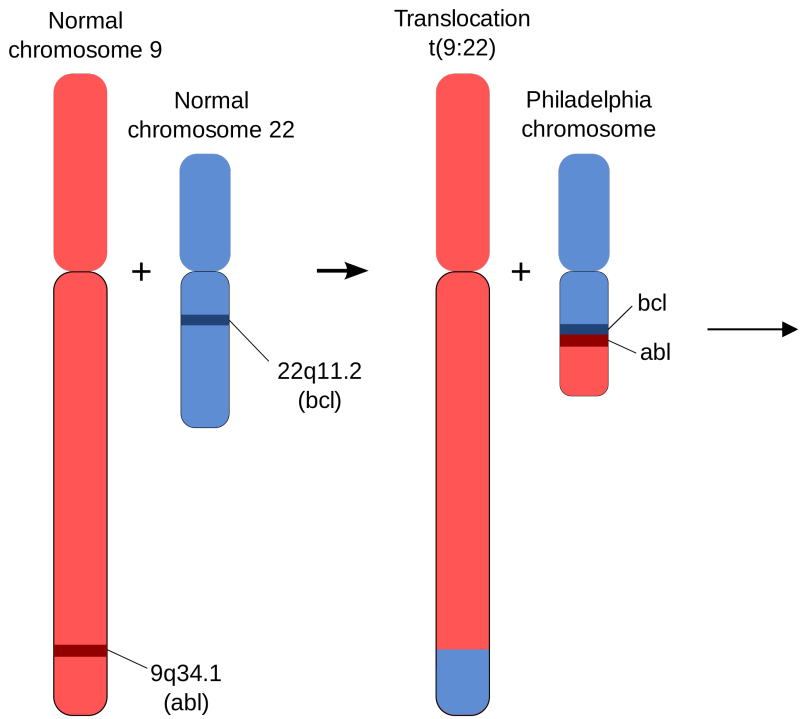
Translocation entre le chromosome 9 et le chromosome 22 dans la leucémie myéloïde chronique (LMC)



Rappel: structure d'un gène eucaryote (animaux, plantes, levures)



Conséquence de la translocation: un oncogène sur chromosome 9 fusionne avec un gène du chromosome 22 : la protéine de fusion BCR-ABL possède une activité tyrosine kinase qui active le cycle cellulaire de manière constitutive et provoque une <u>leucémie myéloide chronique (LMC).</u>

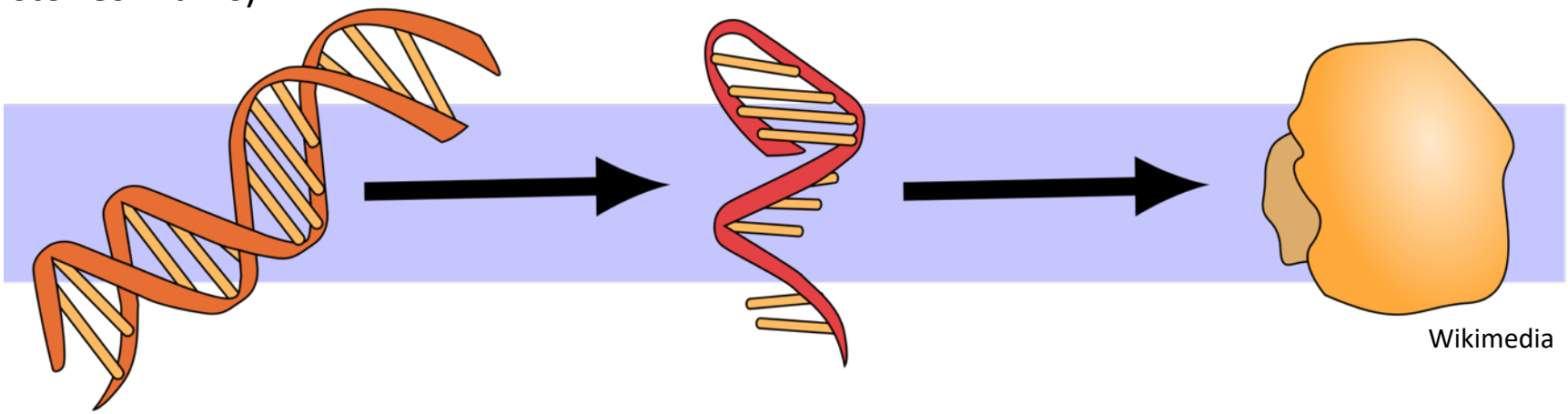


Protéine de fusion
BCR-ABL

Glivec
(inhibiteur de
tyrosine kinase)

Types of tumor-specific alterations

- Epigenetic changes (methylation, histones marks)



DNA

- Mutations, deletions
- Copy number variations
- Translocations,
- Transposon insertions

RNA

- Change in expression
- Change in processing (splicing)
- Base modifications
- Activation of noncoding genes

Protein

- Change in quantity
- AA modifications

Cancer driver genes

- Genes whose alterations can be oncogenic
- ~500 known drivers in our 20,000 protein-coding genes
  - Tumor suppressors ◄ Impairing mutations
  - Oncogenes ◄ Activating mutations

Actionable genes

- Genes that can be targeted by a known drug
- Ex:
  - BRCA2: PARP inhibitors
  - ERBB2: anti-HER2 antibody
  - KRAS: RAS inhibitor
- < 50 genes

RNA and DNA sequencing:

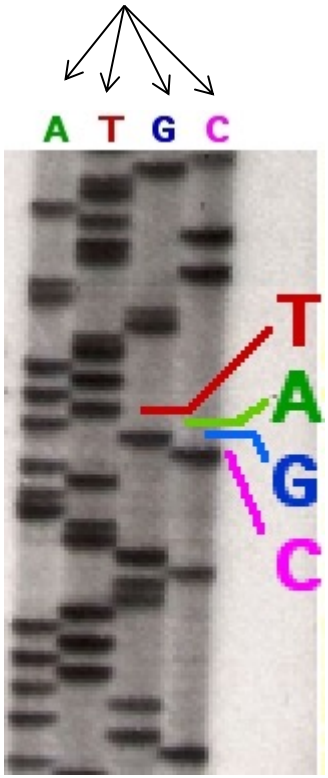
The main omics technologies in oncology

Sanger sequencing(1977)

- Chain termination sequencing or "sequencing by synthesis"
  - dideoxynucleotides are used to block synthesis at a specific base.

- Improved in 1987 with flurorescent markers (1 unique réaction), capillaries and automatisation.

dideoxynucleotides

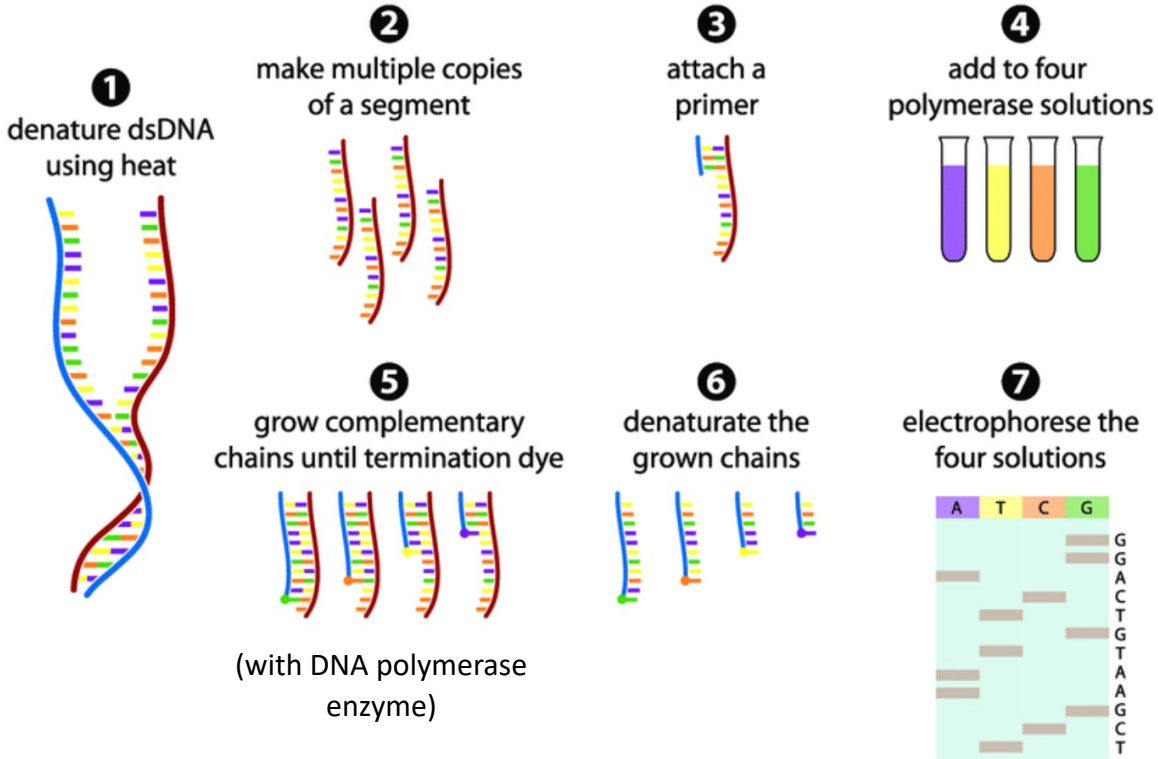


Wikipedia

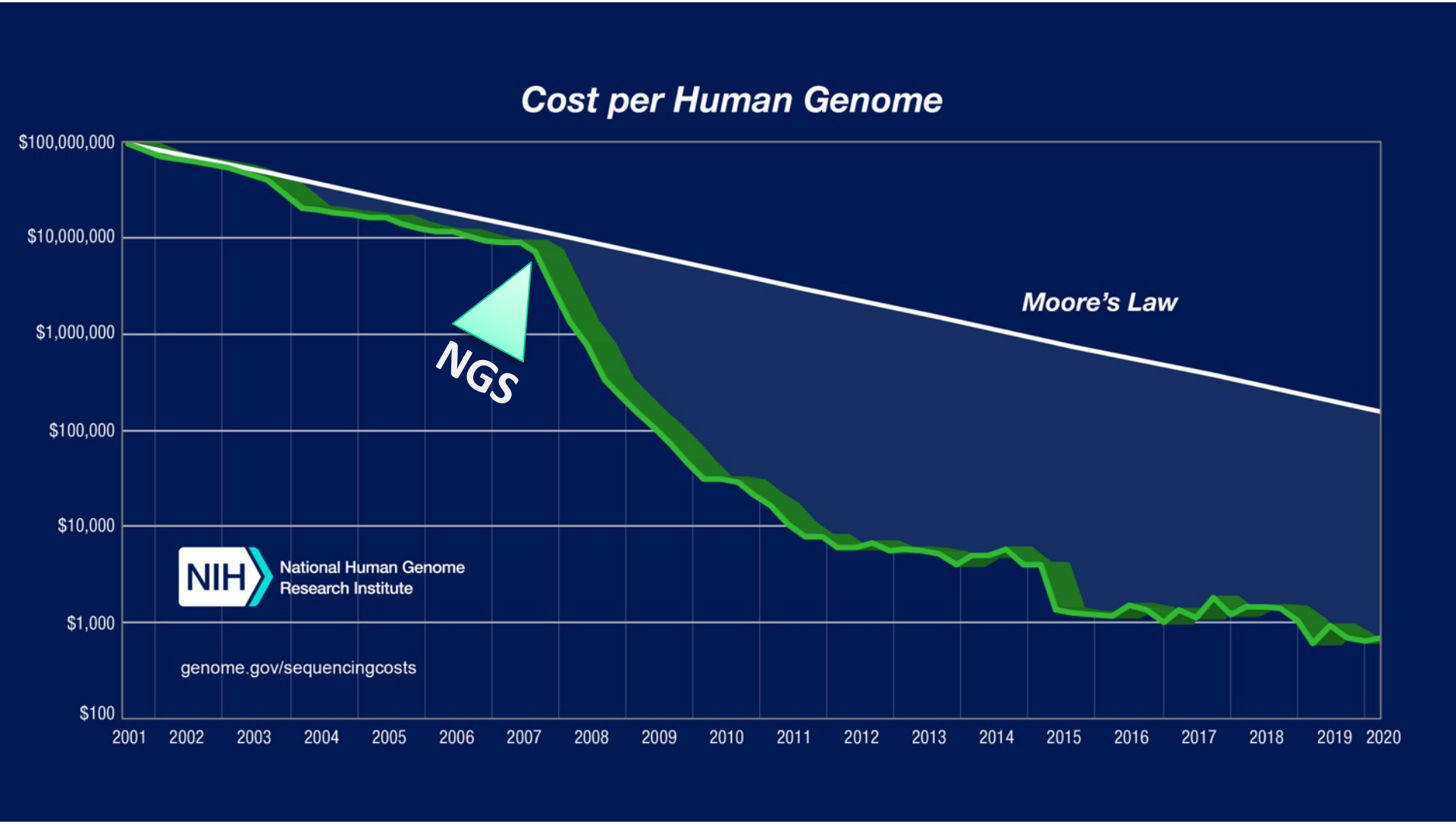
Acrylamid gel

Sanger sequencing(1977)

- Chain termination sequencing or "sequencing by synthesis"
  - Termination nucleotides are used to block synthesis at a specific base. (A, T , G or C)



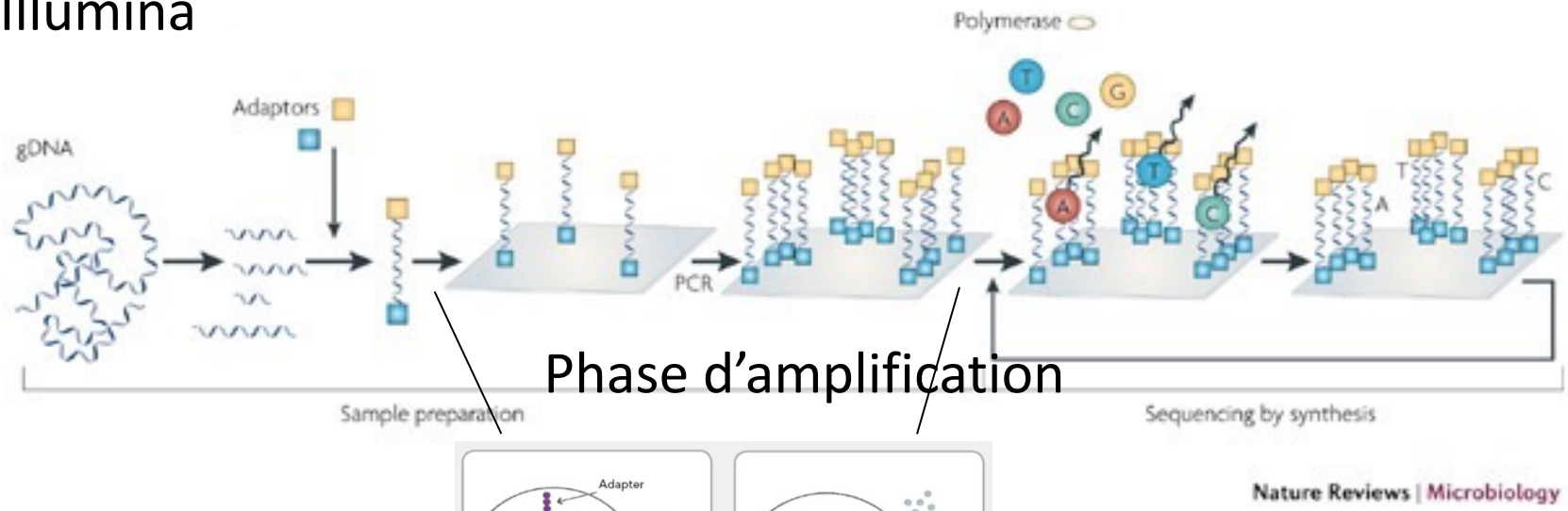
The NGS revolution



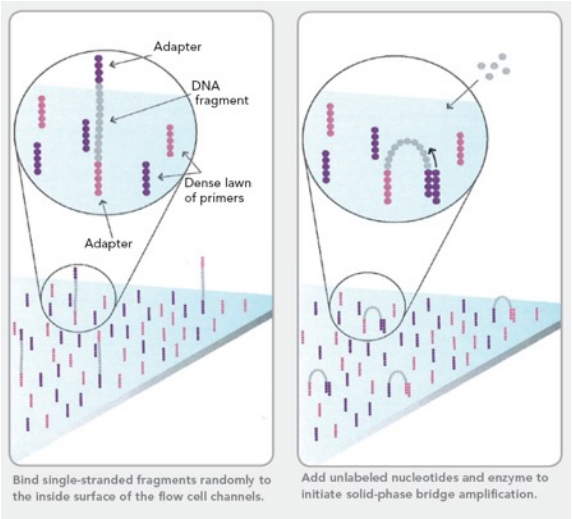
NGS :
Next Generation Sequencing
(2005-)

The most common NGS is « sequencing by synthesis » too!

Illumina



Phase d'amplification



Next Generation Sequencing

Nanopore MinIon

50Mb

Lifetech Ion torrent PGM

400 Mb

Illumina MySeq

4 Gb

Lifetech Ion proton

20 Gb

Illumina Hi-Seq 2000

300 Gb

Illumina NovaSeq

3Tb

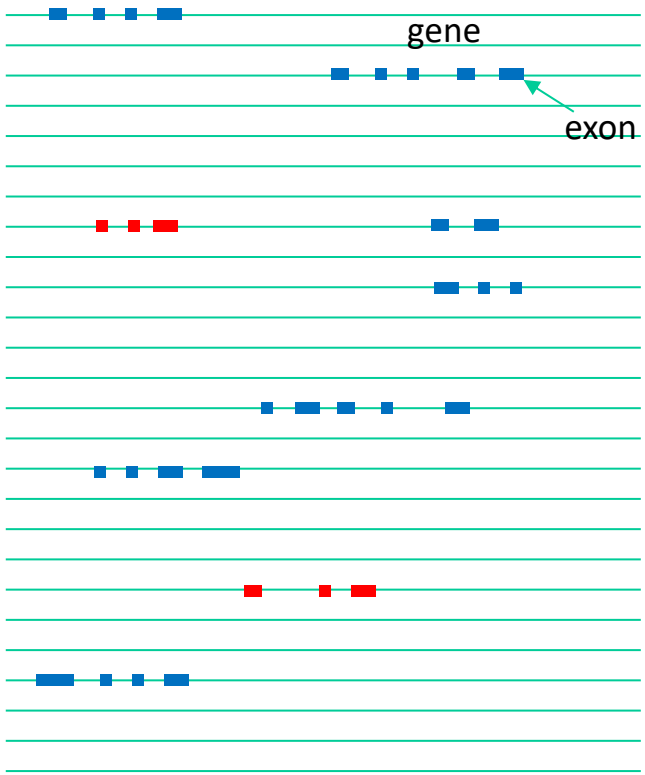
Les grandes applications des NGS

- DNA-seq (variants génomiques, de novo)
- RNA-seq (transcriptome)
- ChiP-Seq (sites de liaisons à l'ADN)
- Autres applications
  - Hi-C, clip-seq, net-seq, ribosome profiling etc.

DNA-seq: Recherche de variants génomiques

- En cancérologie, 2 grandes applications
  - Génétique constitutionnelle (recherche de prédisposition)
  - Génétique somatique (diagnostic, médecine de précision)

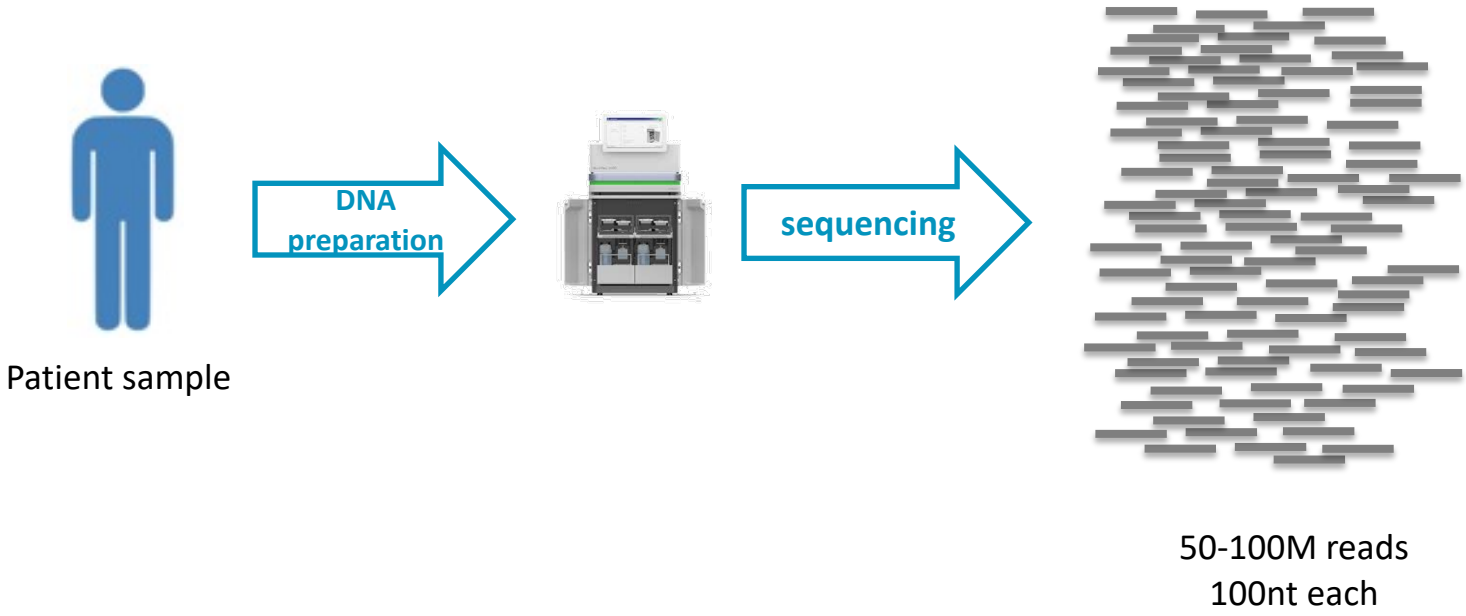
DNA sequencing types



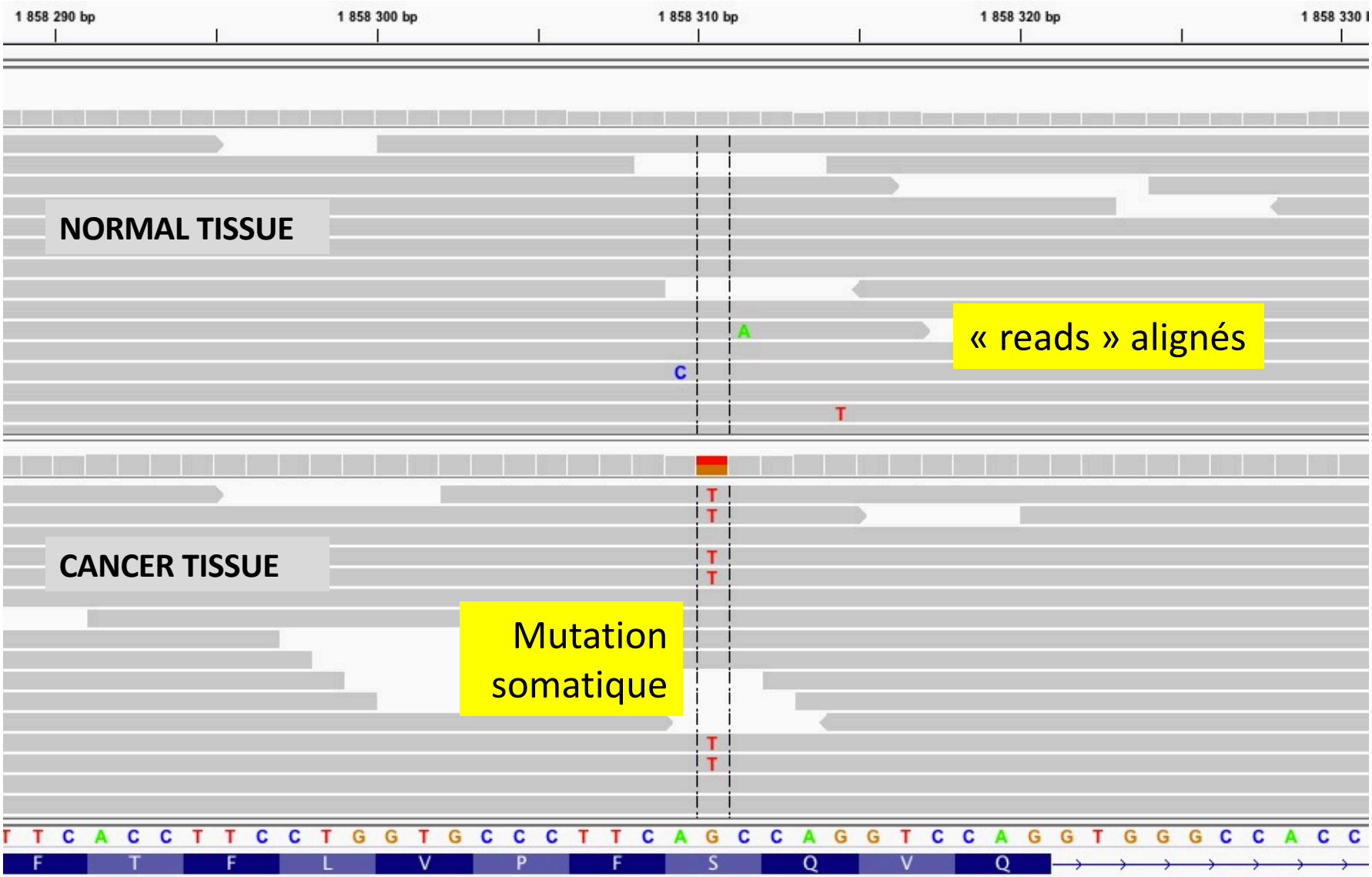
Chromosome sequence

- WGS= Whole genome (3Gb)
- WES: whole exome (50Mb)
- Panel: selected genes (200kb)

Protocole DNA-seq



Evénéments recherchés

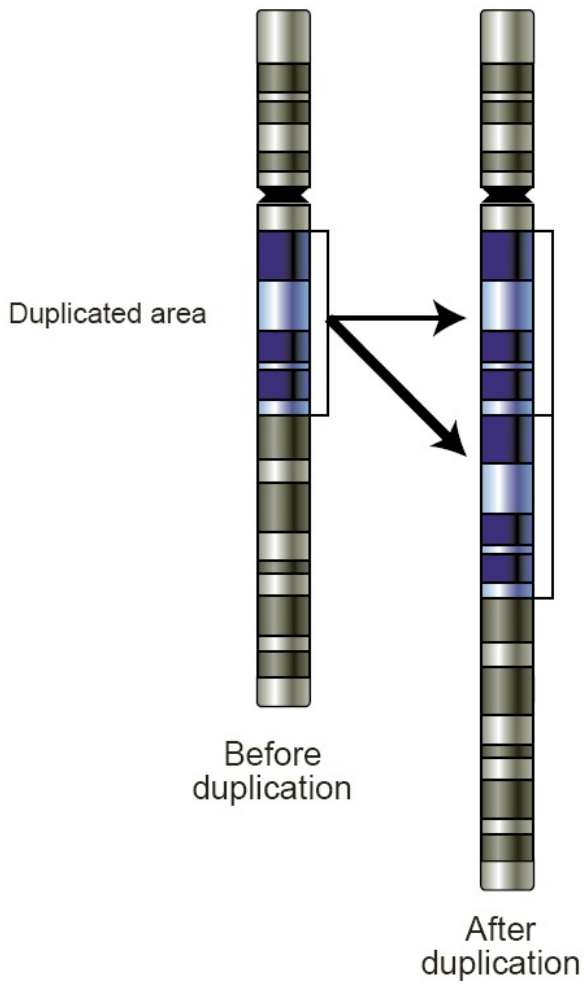


Génome de référence

Evénéments recherchés par DNA-seq

- Variations ponctuelles=SNV
- Réarrangements
- Changement de nombres de copies =CNV
- Amplification de microsatellites
- Profils mutationnels

Copy number variations



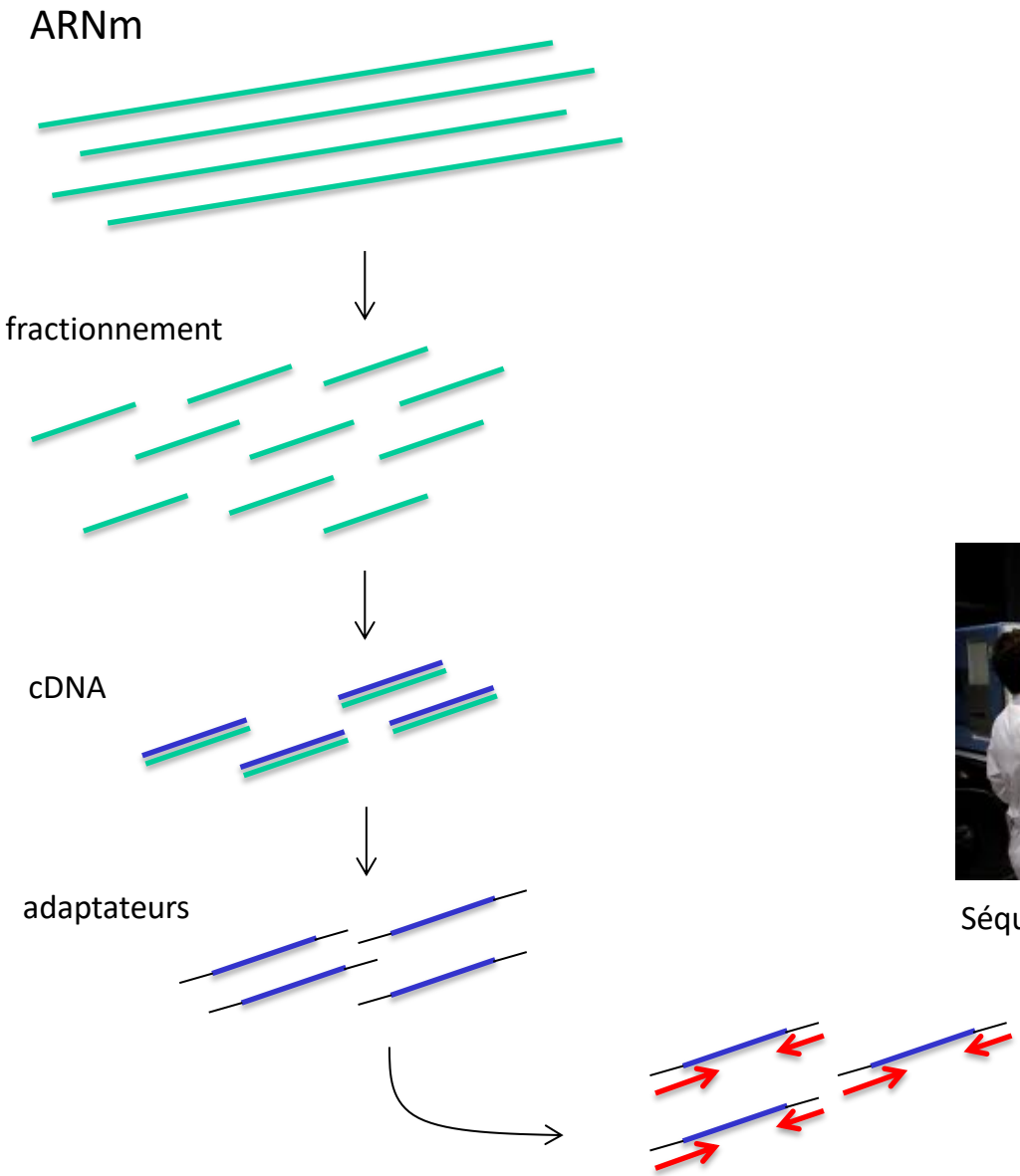
- Caused by recombination errors during DNA repair
- Can be loss or gain
- Oncogenic events= loss of a tumor suppressor or gain of a oncogene

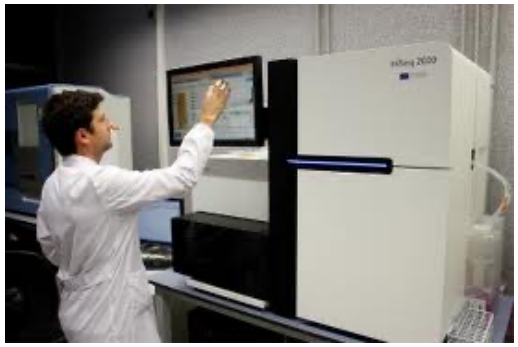
Cf cours Bastien Job

RNA-seq

- Pour l'étude du transcriptome
  - Mesure de l'expression de tous les gènes, simultanément

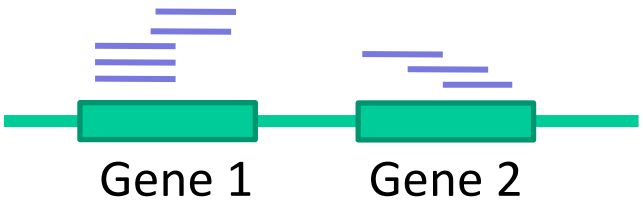
RNA-Seq



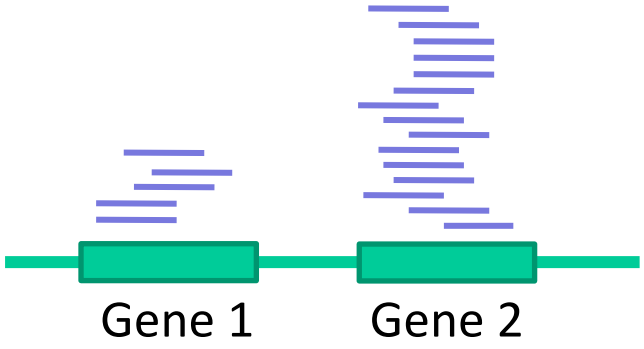


Séquençage

Mesure d'expression par RNA-seq

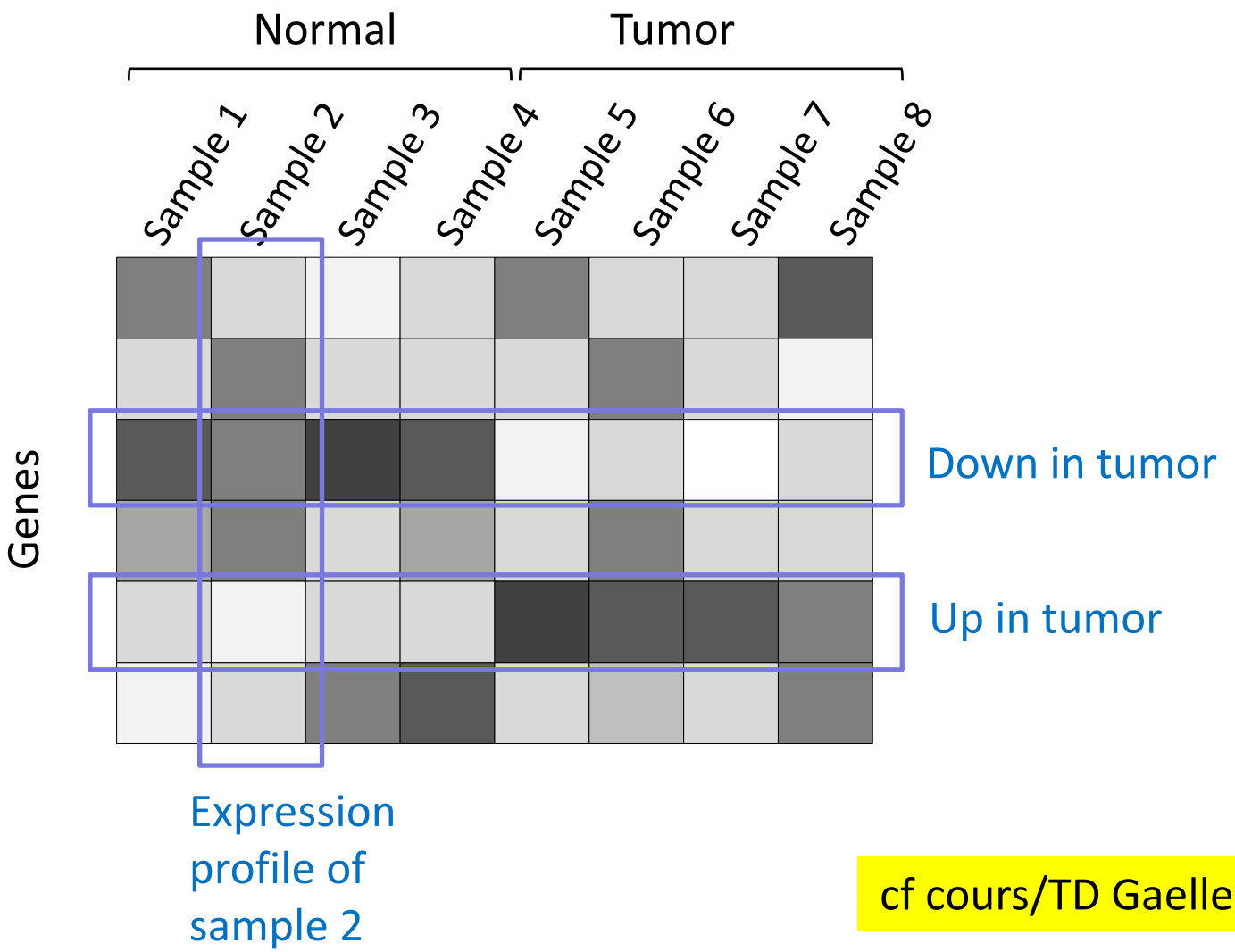


Sample 1



Sample 2

Differential expression analysis & expression profiling



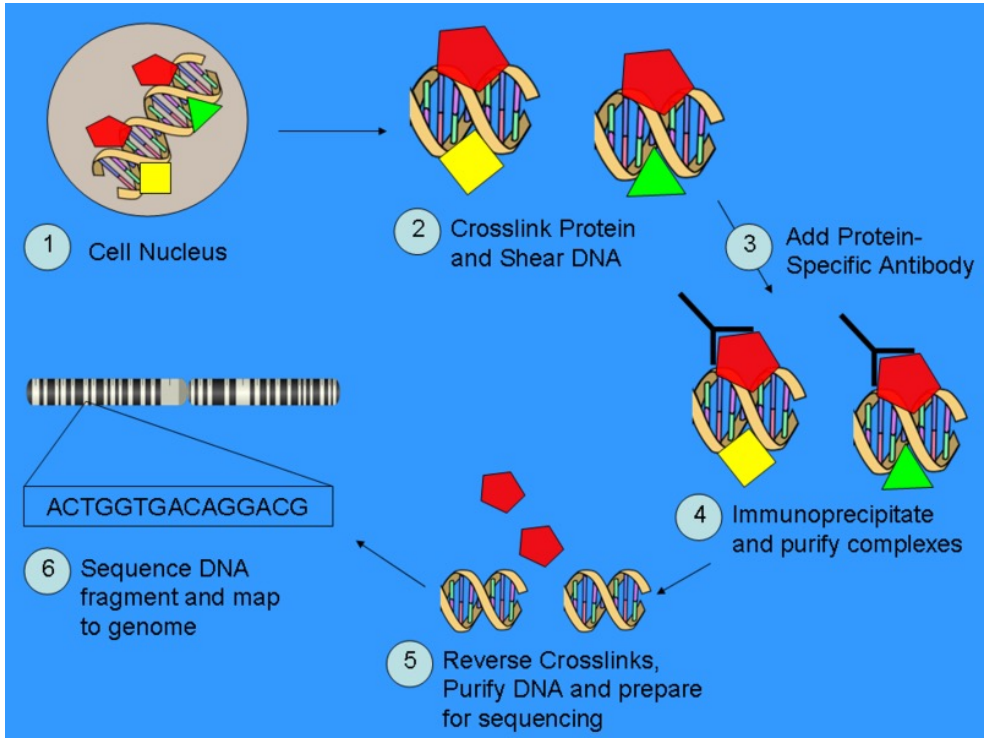
cf cours/TD Gaelle Lelandais

ChIP-Seq

- Chromatin ImmunoPrecipitation & Sequencing

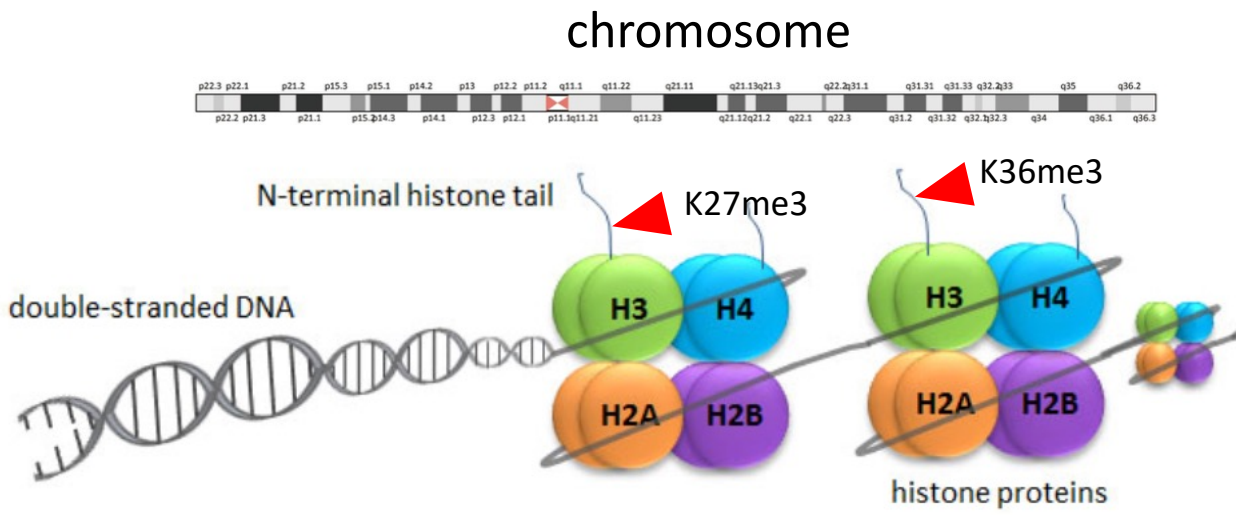
ChIP-Seq

- Permet d'identifier les sites de liaison de protéines (histones, facteurs de transcription, represseurs, enhancers, etc.) sur l'ADN génomique

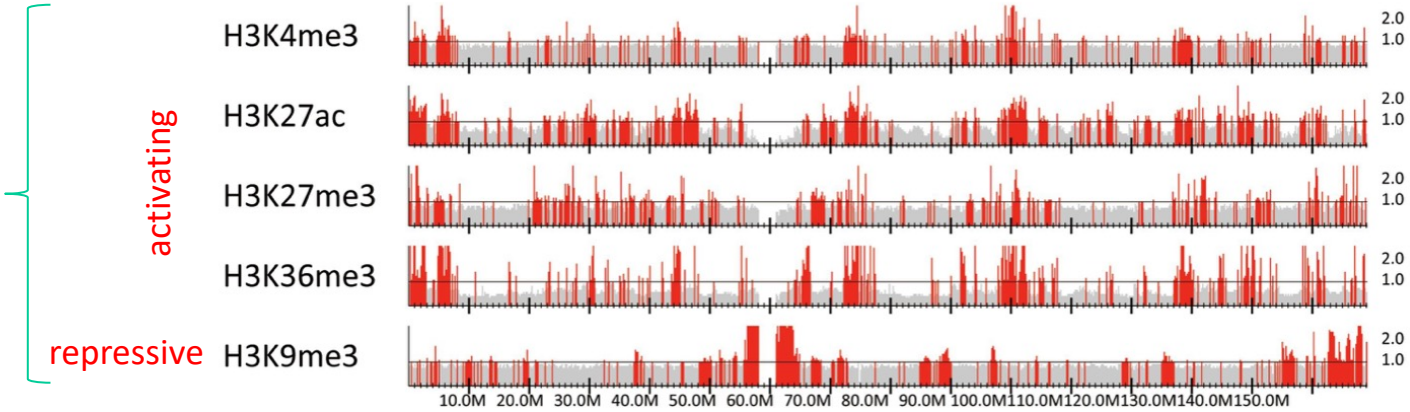


Wikipedia

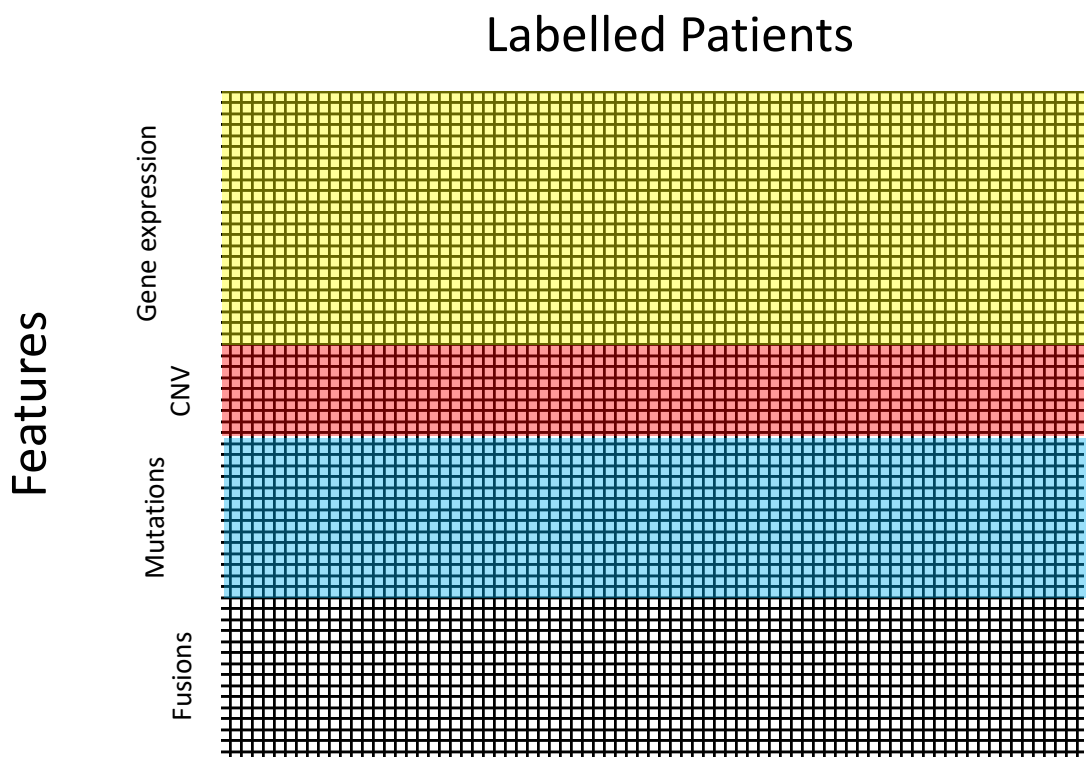
ChIP-seq in oncology



Different types of histones bound to DNA



Big data in oncology



cf UE12: cours/TD Yoann Pradat & Julien Vibert

Cohortes avec données "omiques" (WGS, WES, RNA-seq)

Genomics
england

About Us | 100,000 Genomes Project

Cohorte "population générale"

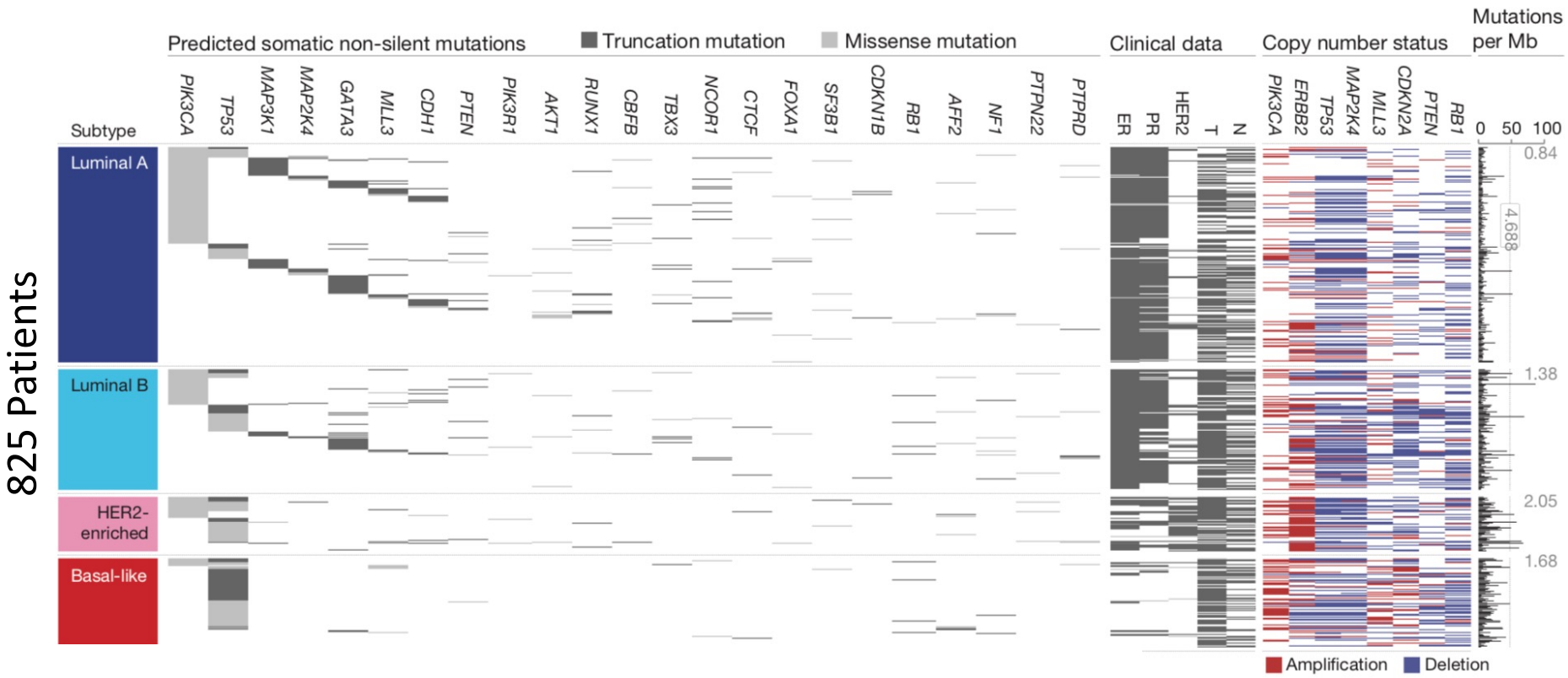
Médecine France génomique 2025

mise à jour : **19.07.17**

A+ A-

Le plan « Médecine France génomique 2025 », se concrétise. En effet, le ministère chargé de la santé a lancé en décembre 2016 un appel à projets national amorçant le financement des 2 premières plateformes génomiques à visée diagnostique et de suivi thérapeutique, sur les 12 attendues dans les 5 ans. Ces équipements d'excellence illustrent le soutien constant des pouvoirs publics vis-à-vis de l'innovation médicale, en l'occurrence du séquençage à très haut débit du génome humain qui fonde la médecine génomique, dite aussi « personnalisée ».

Omics cohorts in cancer
TCGA project: 10,000 patients

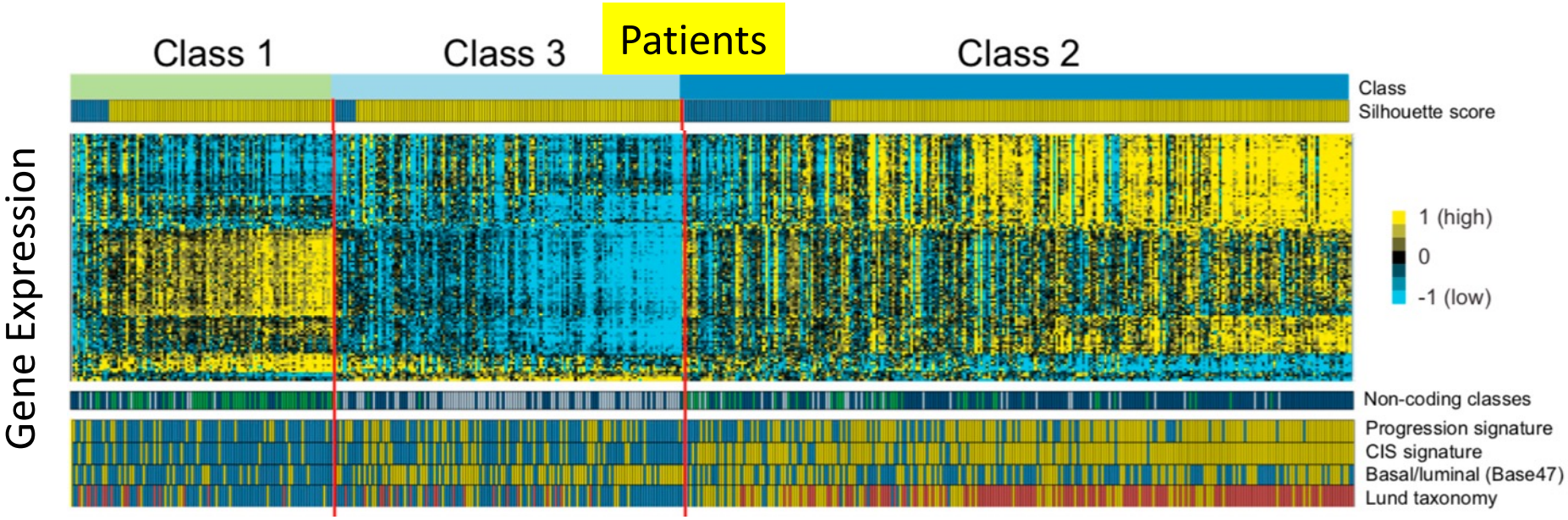


TCGA Breast cancer cohort (2012): 825 patients.
Stratified by cancer type

TCGA consortium, Nature, 2012

Subtype discovery (unsupervised classification)

Gene expression profiling in 460 Urothelial carcinoma:
A 117-gene signature.



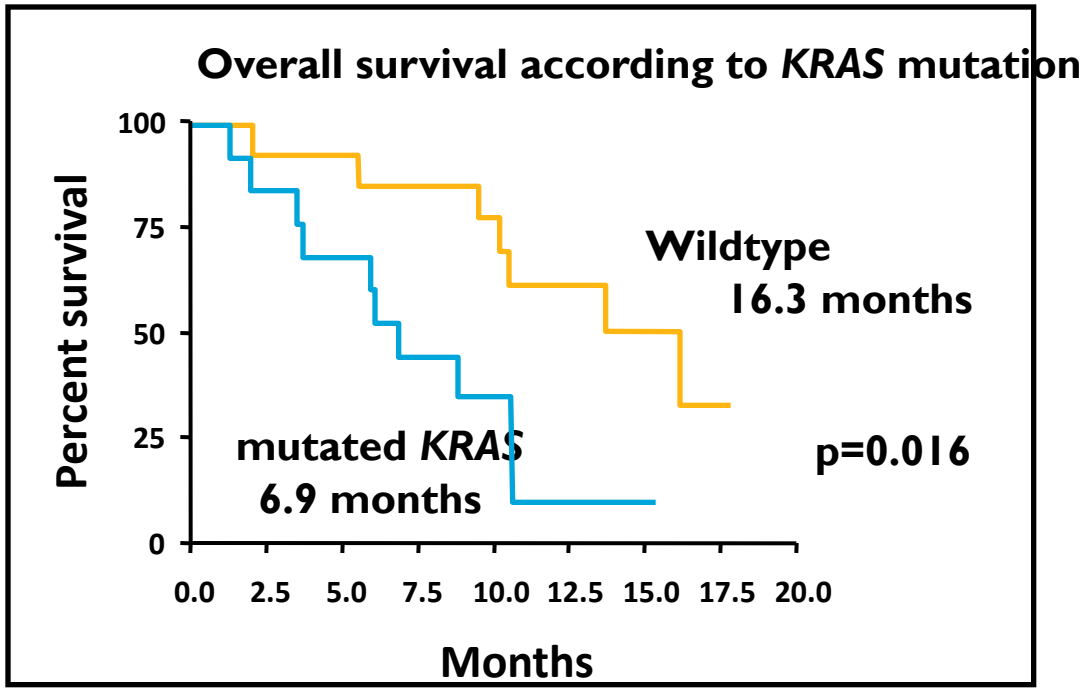
Hedegaard et al., Cancer Cell, 2016

Discovery of outcome-related mutations

KRAS Mutation and Anti-EGFR therapy in advanced colorectal cancer

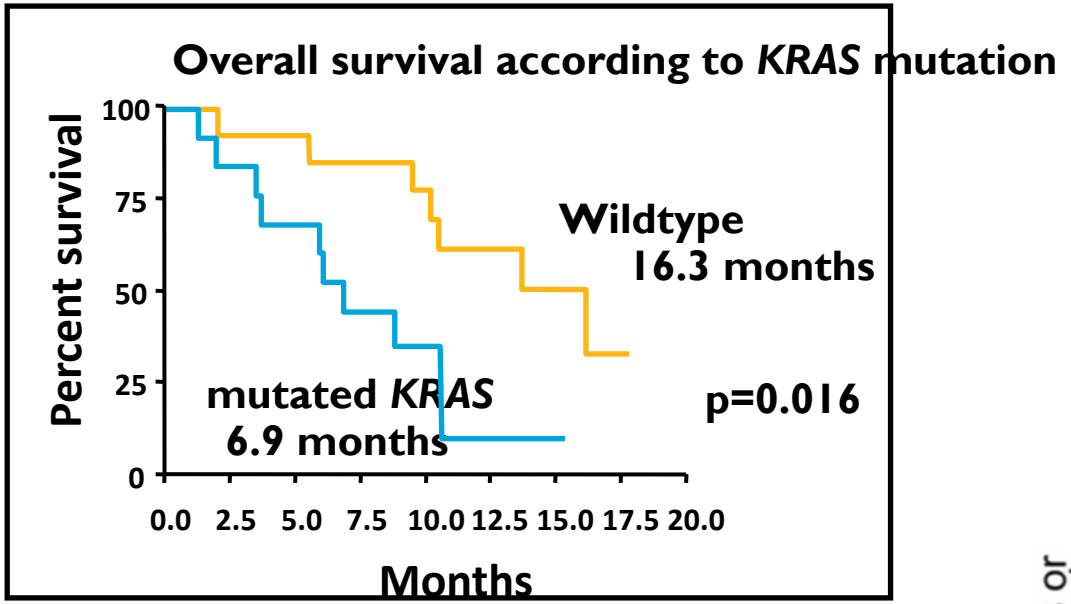
KRAS Status	Responders*	Non responders*	Total
KRAS mutation (%)	0 (0)	13 (100)	13
Wildtype (%)	11 (65)	6 (35)	17

p=0.0003



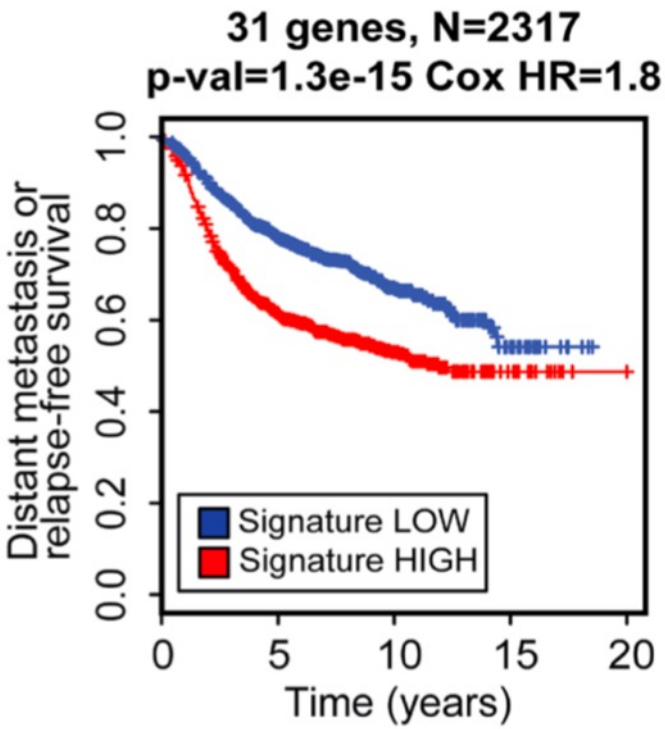
Lièvre A et al. Cancer Res 2006.

Modèles prédictifs de survie



DNA-based

Expression-based



Transcriptional signatures: current applications

Diagnostic

- Tumor vs normal
- Tumor subtyping

Precision medicine

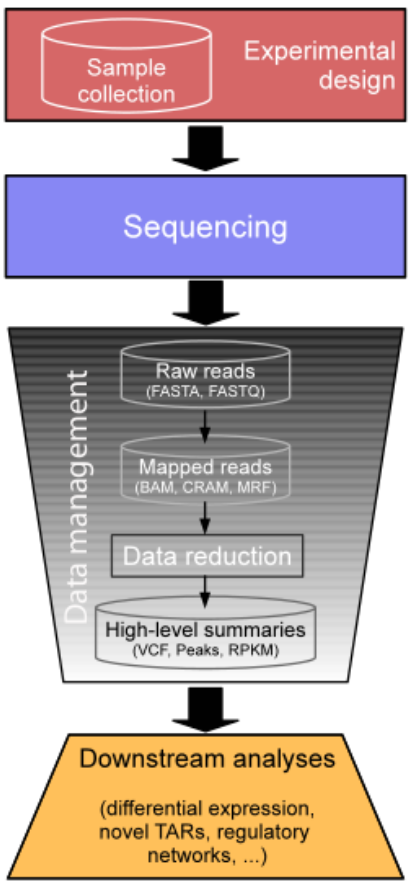
- Response to treatment
- Relapse prediction

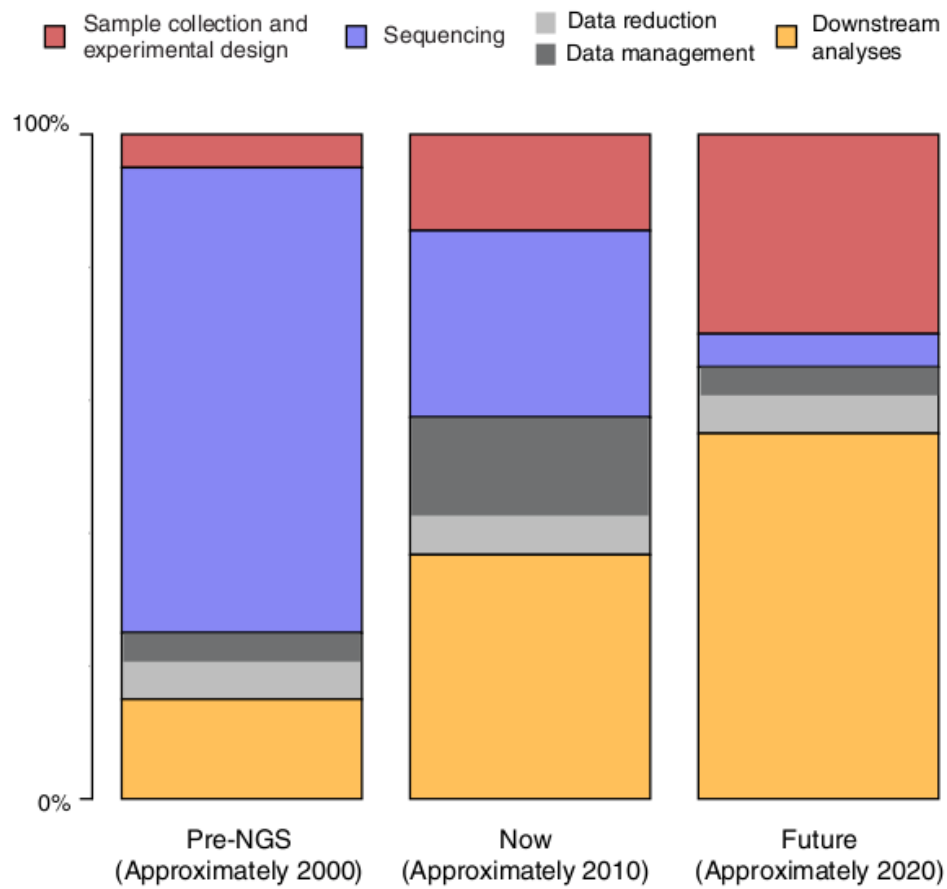
Les données NGS

Volume des données NGS

- Un exome humain (N+T) avec fichiers de mapping et analyse: 70 Go
  - (~5 fois le volume des séquences brutes)
- Données génomiques produites annuellement dans un hôpital universitaire: >500 To
- La banque TCGA complete: 1 Po

Costs of sequencing vs analysis





Sboner et al. 2011. Genome Biology, **12**:125

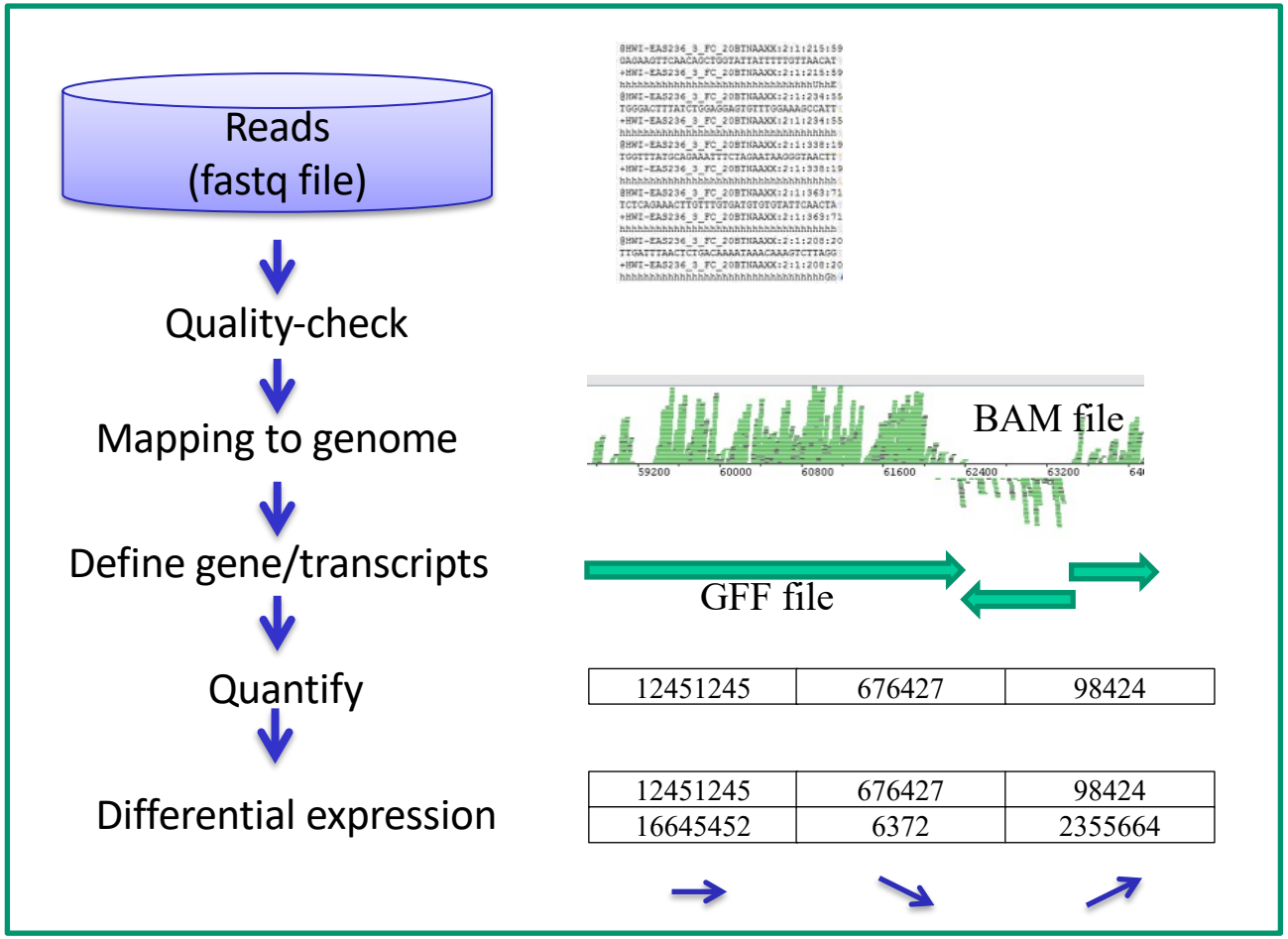
Les outils

« Pipelines » & « workflows »

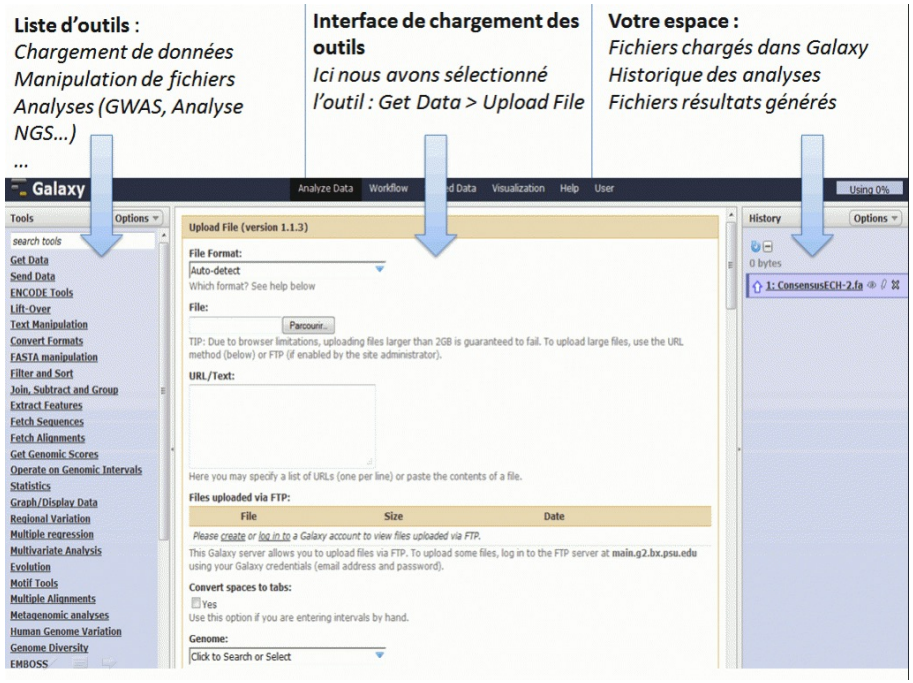
« Bricks » from Unix open source programs

Combined into pipelines (typically a few hours to days to run)

Example: an RNA-seq pipeline



Galaxy: user-friendly interface to NGS pipelines



Credit: Biorigami

- Interest: avoiding Unix command line + traçability
- But: running NGS workflow on real human data often requires a computer cluster (will not run on a single-node Galaxy server)

Les bases de données en génomique du cancer

Cancer Genomics Databases

- TCGA: the Cancer Genome Atlas
- COSMIC
- cBioPortal
- CCLE: Cancer Cell Lines Encyclopedia
- GDSC: Genomics of Drug Sensitivity in Cancer
- dbGaP: database of Genotypes and Phenotypes
- GEO: Gene Expression Omnibus
- ArrayExpress

The Cancer Genome Atlas

Understanding genomics to improve cancer care

NCI, NHGRI, USA

The Cancer Genome Atlas *Understanding genomics to improve cancer care*

TCGA

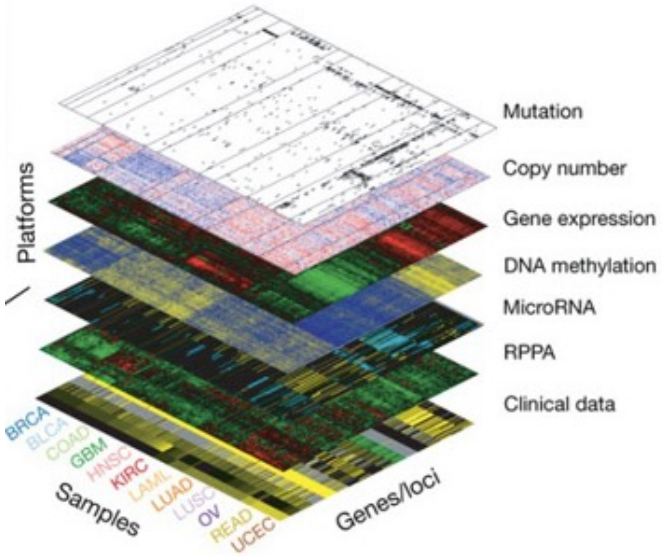
- launched in 2006
- 33 tumor types
- 11,000 patients
- whole-genome sequencing (WGS) for 1,000 tumors

TCGA data types and levels

Controlled access ← Free access

- Clinical Data
- Images
- Microsatellite Instability (MSI)
- DNA Sequencing
- miRNA Sequencing
- Protein Expression
- mRNA Sequencing
- Total RNA Sequencing
- Array-based Expression
- DNA Methylation
- Copy Number

Level 1 | Level 2 | Level 3



Example of access levels

	Level 1	Level 2	Level 3
RNA-seq	mRNA sequence for each participant's tumor sample		The calculated expression signal of a particular composite exon of a gene, per sample
DNA-seq	Whole exome sequence for both tumor and normal sample for each participant	Somatic mutation calls for each participant	

Controlled access

Main data types

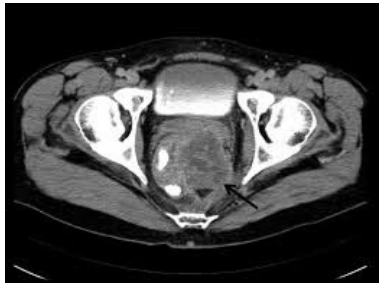
- DNA sequencing
  - Whole exome or whole genome DNA sequence
  - Platform: Illumina HiSeq
- mRNA sequencing / miRNA sequencing
  - PolyA+ RNA / small RNA expression from RNA-seq
  - Platform: Illumina HiSeq or similar
- Array-based expression
  - mRNA expression levels (1 or 2 colors)
  - Illumina or Agilent DNA microarrays

Main data types

- DNA Methylation
  - covalent modification of cytosine bases at the C-5 position, generally within a CpG sequence context
  - platforms: Illumina Methyl arrays
- Protein expression
  - protein expression & concentration
  - Platform: custom antibody array (5ABx1000 samples/slide)
- Copy number
  - Loss and gain of DNA fragments
  - Platform: Agilent CGH array

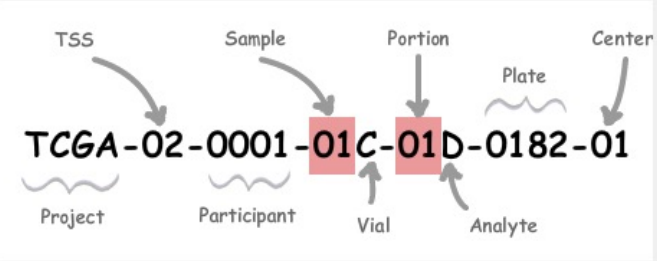
Main data types

- Microsatellite instability
  - MSI-Mono-Dinucleotide Assay: panel of 4 mononucleotide and 3 dinucleotide repeat loci



- Image
  - Images of tissue samples
  - CT (computed tomography), DX (digital radiography), CR (computed radiography)
- Clinical data
  - Available clinical information for each participant (demographic, treatment, survival, etc)
  - Biospecimen data: how specimen was processed

1 sample = 1 TCGA barcode



This figure of an aliquot barcode shows how it can be broken down into its components and translated into its metadata. The barcode metadata are further described in the following table.

Label	Identifier for	Value	Value description	Possible values
Project	Project name	TCGA	TCGA project	TCGA
TSS	Tissue source site	02	GBM (brain tumor) sample from MD Anderson	See Code Tables Report
Participant	Study participant	0001	The first participant from MD Anderson for GBM study	Any alpha-numeric value
Sample	Sample type	01	A solid tumor	Tumor types range from 01 - 09, normal types from 10 - 19 and control samples from 20 - 29. See Code Tables Report for a complete list of sample codes
Vial	Order of sample in a sequence of samples	C	The third vial	A to Z
Portion	Order of portion in a sequence of 100 - 120 mg sample portions	01	The first portion of the sample	01-99
Analyte	Molecular type of analyte for analysis	D	The analyte is a DNA sample	See Code Tables Report
Plate	Order of plate in a sequence of 96-well plates	0182	The 182nd plate	4-digit alphanumeric value
Center	Sequencing or characterization center that will receive the aliquot for analysis	01	The Broad Institute GCC	See Code Tables Report

TCGA **Clinical Data** (patient or sample XML file)

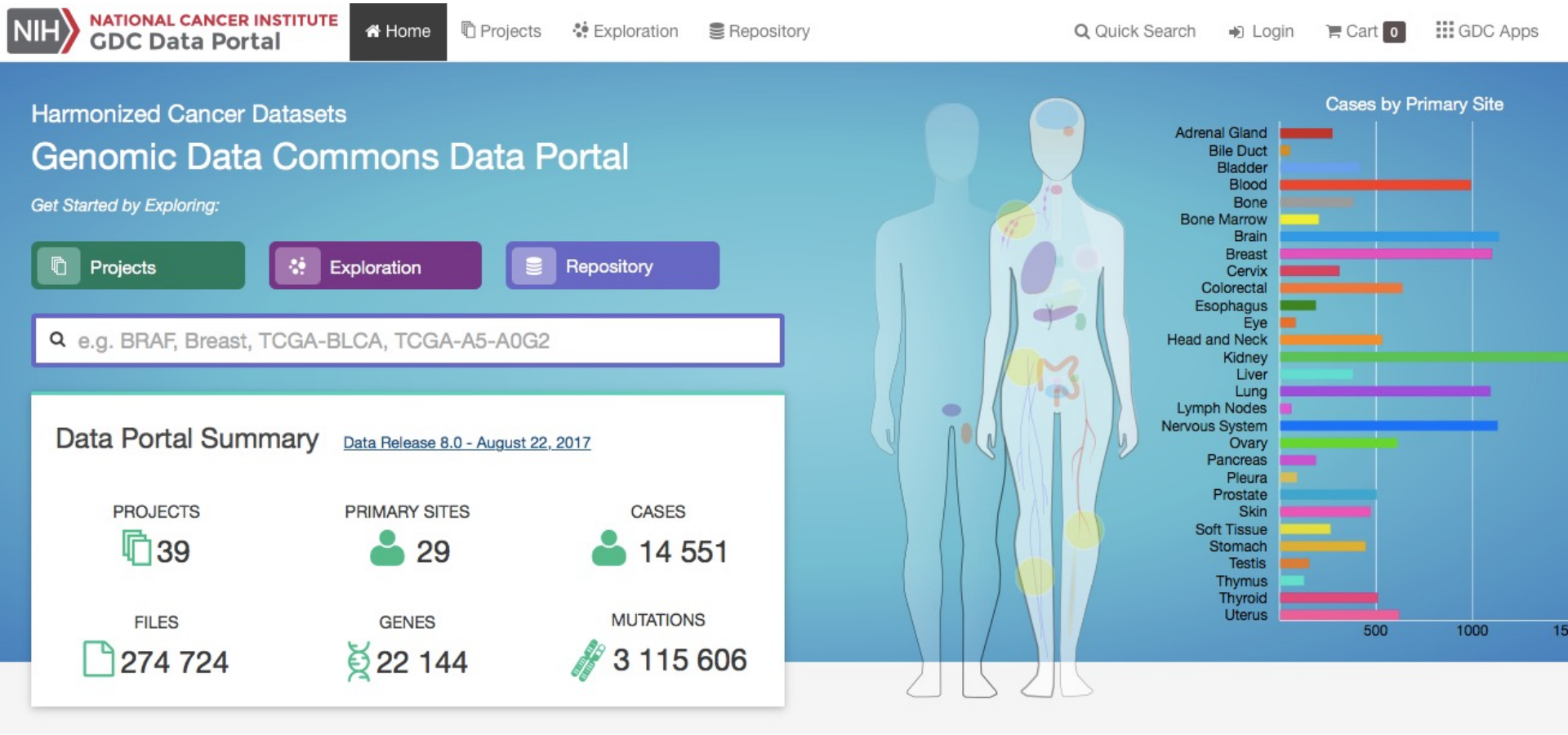
Clinical — emacs — 179×47


```xml
<?xml version="1.0" encoding="UTF-8"?>
<brca:tcga_bcr>
    <admin:admin>
        <admin:bcr xsd_ver="1.17">Nationwide Children's Hospital</admin:bcr>
        <admin:batch_number xsd_ver="1.17">80.48.0</admin:batch_number>
        <admin:disease_code xsd_ver="2.3">BRCA</admin:disease_code>
        <admin:day_of_dcc_upload xsd_ver="1.17">27</admin:day_of_dcc_upload>
        <admin:month_of_dcc_upload xsd_ver="1.17">2</admin:month_of_dcc_upload>
        <admin:year_of_dcc_upload xsd_ver="1.17">2014</admin:year_of_dcc_upload>
        <admin:patient_withdrawal>
            <admin:withdrawn>false</admin:withdrawn>
        </admin:patient_withdrawal>
    </admin:admin>
    <brca:patient>
        <shared:tumor_tissue_site>Breast</shared:tumor_tissue_site>
        <shared:gender>FEMALE</shared:gender>
        <shared:vital_status>Alive</shared:vital_status>
        <shared:race>WHITE</shared:race>
        <shared:bcr_patient_barcode>TCGA-BH-A0B2</shared:bcr_patient_barcode>
...
        <shared:history_of_neoadjuvant_treatment>No</shared:history_of_neoadjuvant_treatment>
        <shared:informed_consent_verified>YES</shared:informed_consent_verified>
...
        <shared:age_at_initial_pathologic_diagnosis>43</shared:age_at_initial_pathologic_diagnosis>
...
        <shared:histological_type>Infiltrating Ductal Carcinoma</shared:histological_type>
        <brca_shared:breast_carcinoma_progesterone_receptor_status>Positive</brca_shared:breast_carcinoma_progesterone_receptor_status>
...
        <brca_shared:breast_carcinoma_estrogen_receptor_status>Positive</brca_shared:breast_carcinoma_estrogen_receptor_status>
        <brca_shared:lab_proc_her2_neu_immunohistochemistry_receptor_status>Negative</brca_shared:lab_proc_her2_neu_immunohistochemistry_receptor_status>
...
        <brca_nte:new_tumor_events>
            <nte:new_tumor_event_after_initial_treatment>
        </brca_nte:new_tumor_events>
    </brca:patient>
</brca:tcga_bcr>
```

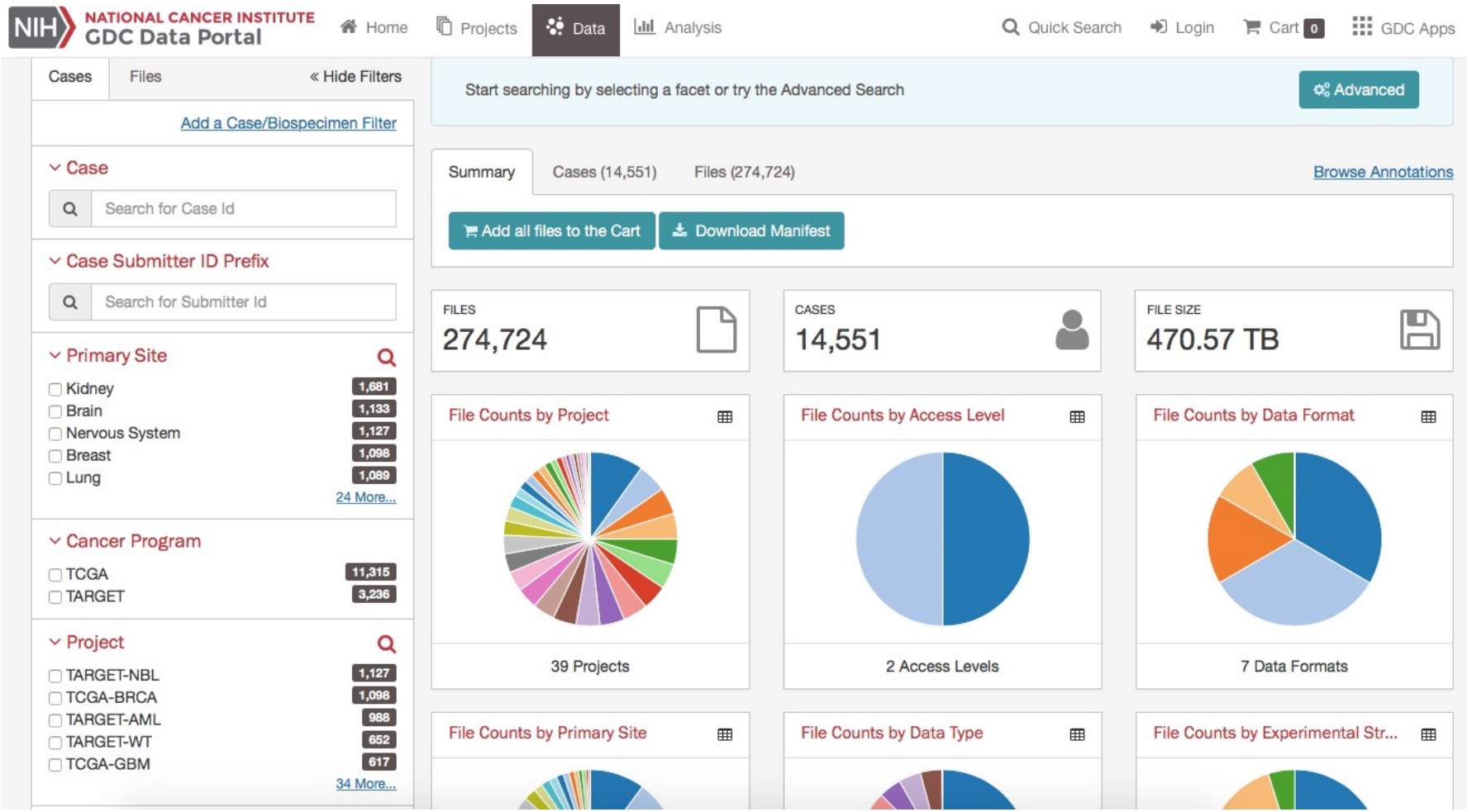

200/300 lines per file

Extract of patient xml clinical file

TCGA access via the GDC portal (Genomics Data Commons)



TCGA data access via the GDC Data portal



From TCGA to PCAWG

- PCAWG[1]: a collaboration TCGA-ICGC[2] to analyze whole genome data from 2,800 pairs of tumor and normal samples and integrate the results with clinical and other molecular data available on those same cases.

1. PCAWG: Pan-Cancer Analysis of Whole Genomes
2. ICGC: International Cancer Genome Consortium

Sanger Institute, UK

COSMIC Curation

- Manual curation
  - >25000 articles analyzed
- Automated curation
  - 1.4M samples (incl. 31k WGS) (TCGA & ICGC)
  - Annotation pipeline (Variant effect predictor

« Most [mutations] have no effect on the development of disease. We are adapting our curation processes to reduce this noise and highlight high-value information. »

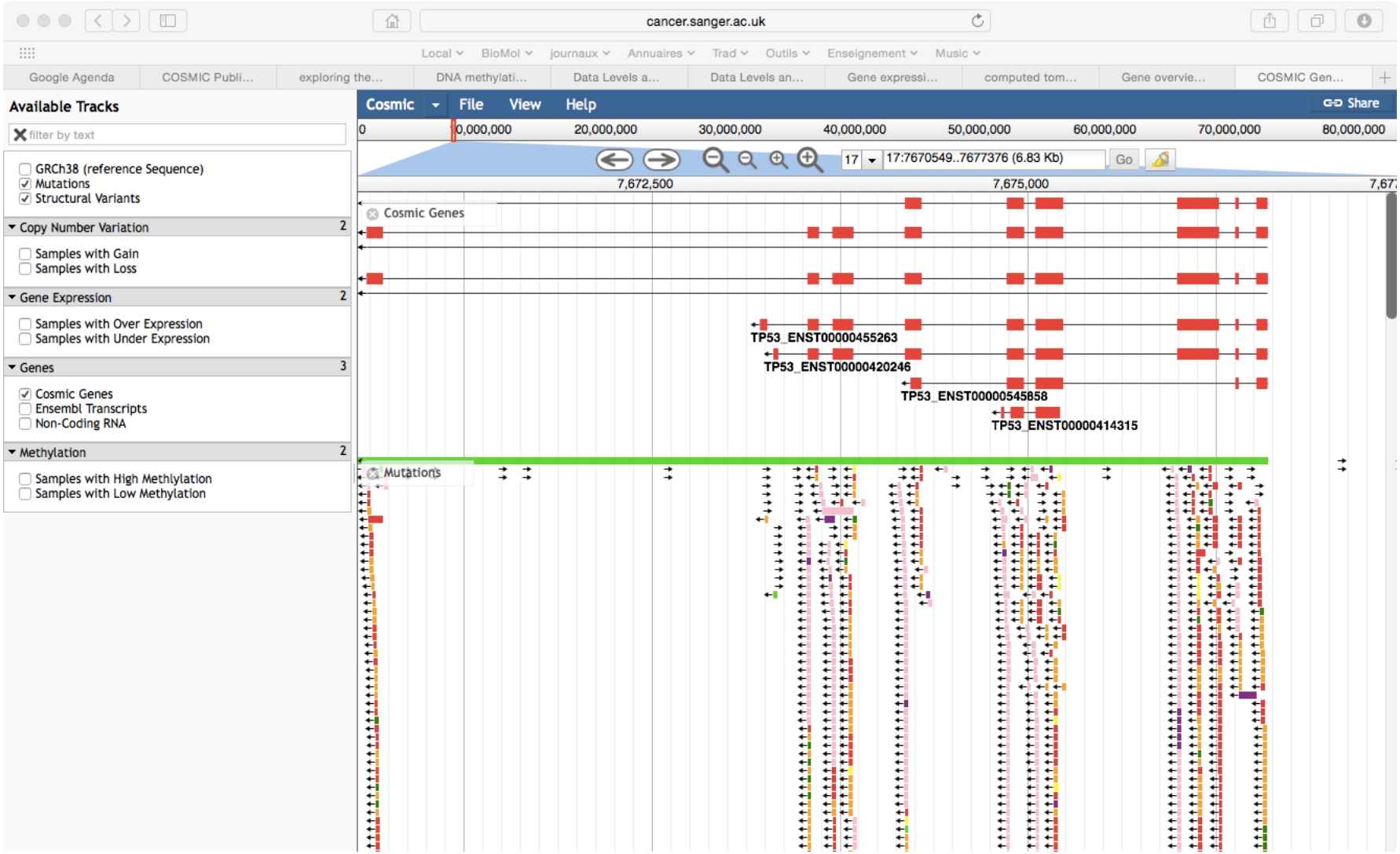
« Samples with over 20 000 point mutations, none of which have been validated are excluded from curation as their noise vastly outweighs their signal. »

COSMIC
Catalogue of somatic mutations in cancer

- Expert-curated database of cancer somatic mutations & other events
- 2019 (V90):
  - 29M coding point mutations
  - 13M non-coding variants
  - 19k gene fusions
  - 1.2 M CNV
  - 9M gene expression variants
  - 576 cancer genes

(2022: V95)

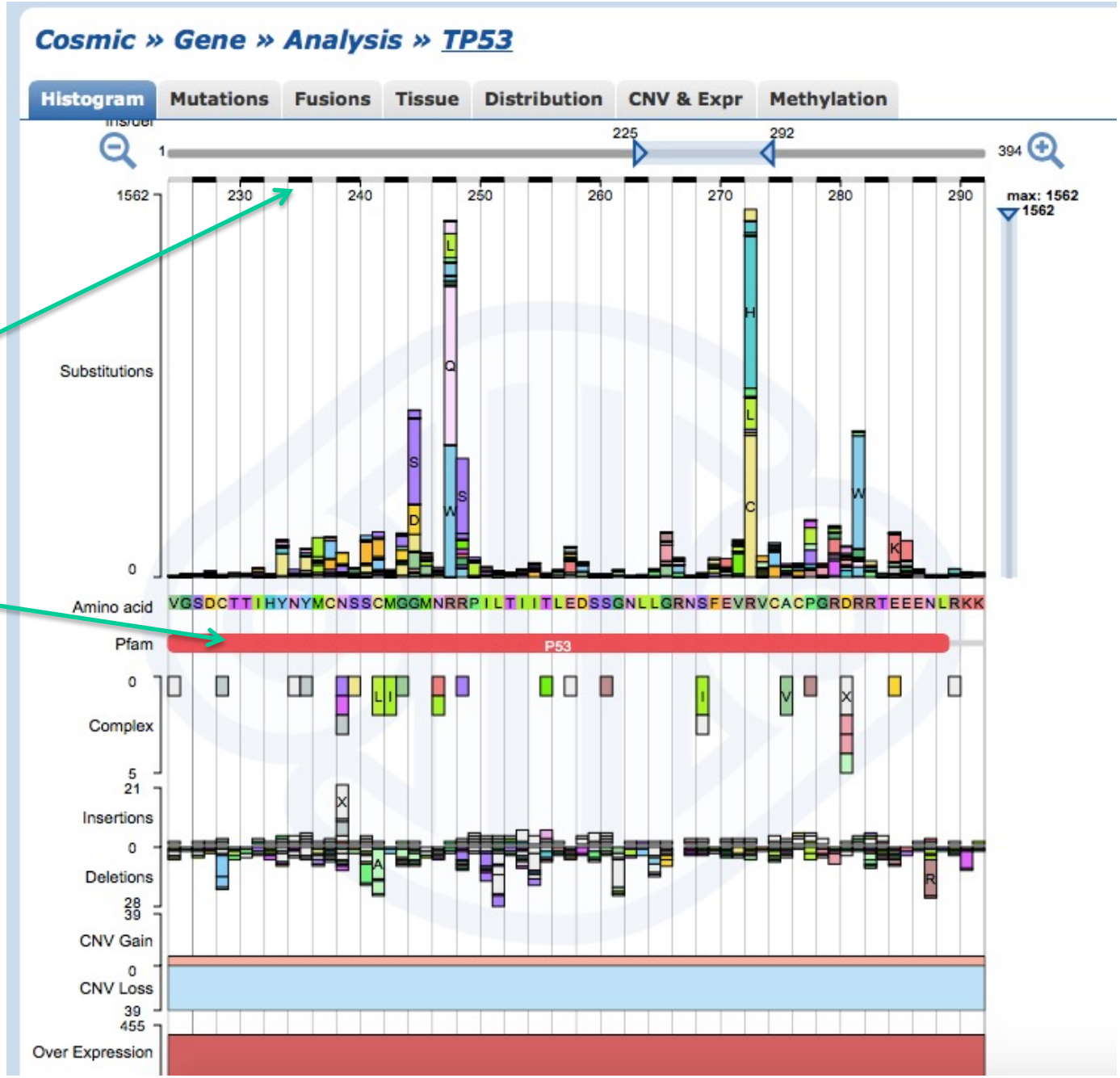
COSMIC genome browser



Histogram view

Protein coordinates

Protein domain



Tissue-distribution of mutations

Cosmic » Gene » Analysis » TP53

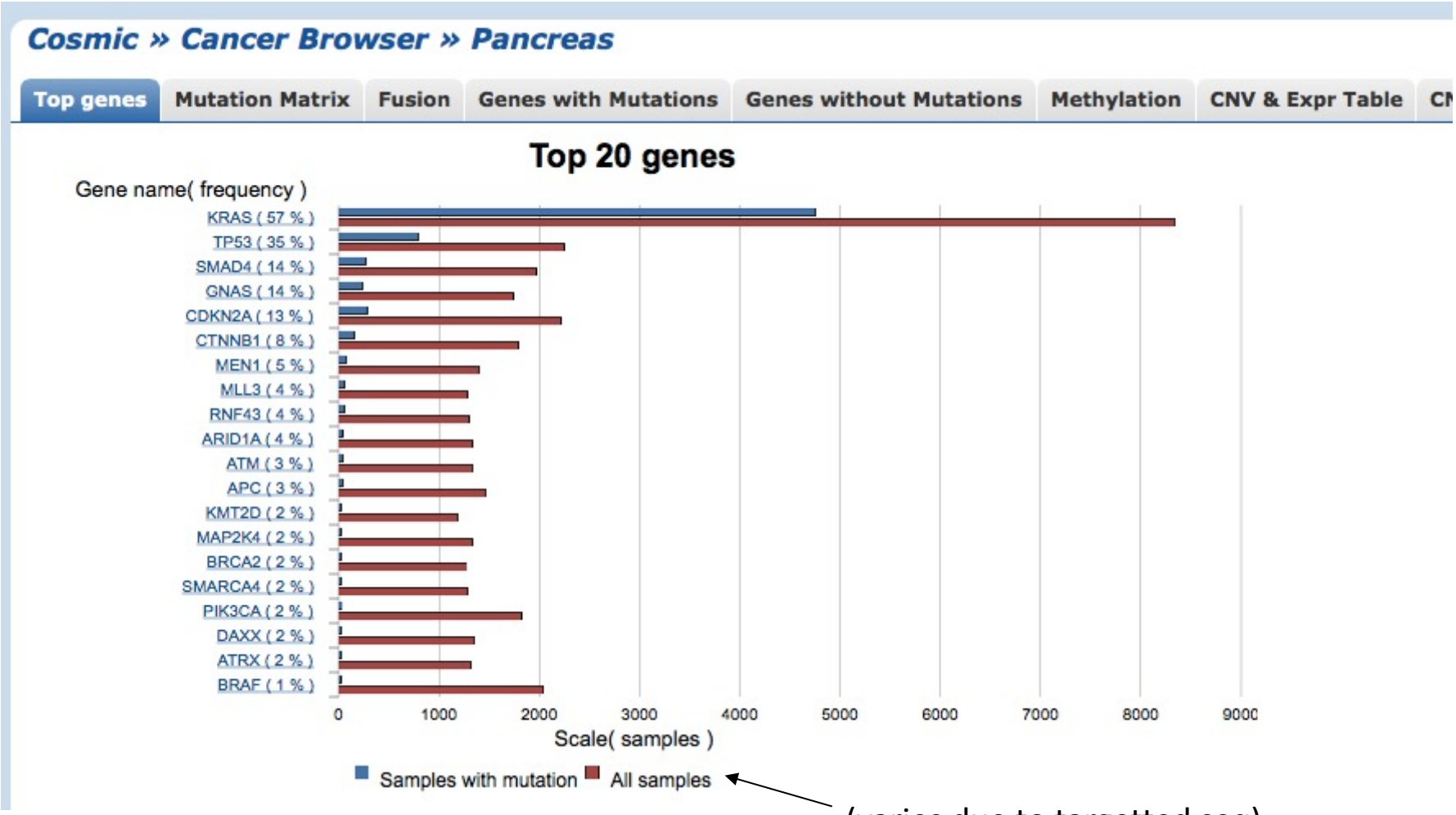
View in GRCh37 Archive

Histogram | Mutations | Fusions | Tissue | Distribution | CNV & Expr | Methylation

Show All entries Search:

Tissue	Point Mutations		Copy Number Variation		Gene Expression		Methylation	
	% Mutated	Tested	Variant %	Tested	% Regulated	Tested	% Diff. Methylated	Tested
Adrenal gland		508		-		79		-
Autonomic ganglia		586		-		-		-
Biliary tract		872		-		-		-
Bone		955		83		-		-
Breast		11869		966		1032		707
Central nervous system		6949		787		615		-
Cervix		1439		-		241		-
Endometrium		1464		405		564		-
Eye		206		-		-		-
Fallopian tube		5		-		-		-
Gastrointestinal tract (site indeterminate)		1		-		-		-
Genital tract		94		-		-		-
Haematopoietic and lymphoid		12075		277		216		-
Kidney		2149		411		585		305
Large intestine		13101		585		587		-
Liver		4177		452		235		-
Lung		7681		986		894		294
Meninges		228		-		-		-
NS		343		261		-		-
Oesophagus		4213		95		125		-
Ovary		4095		708		266		-

Cancer browser



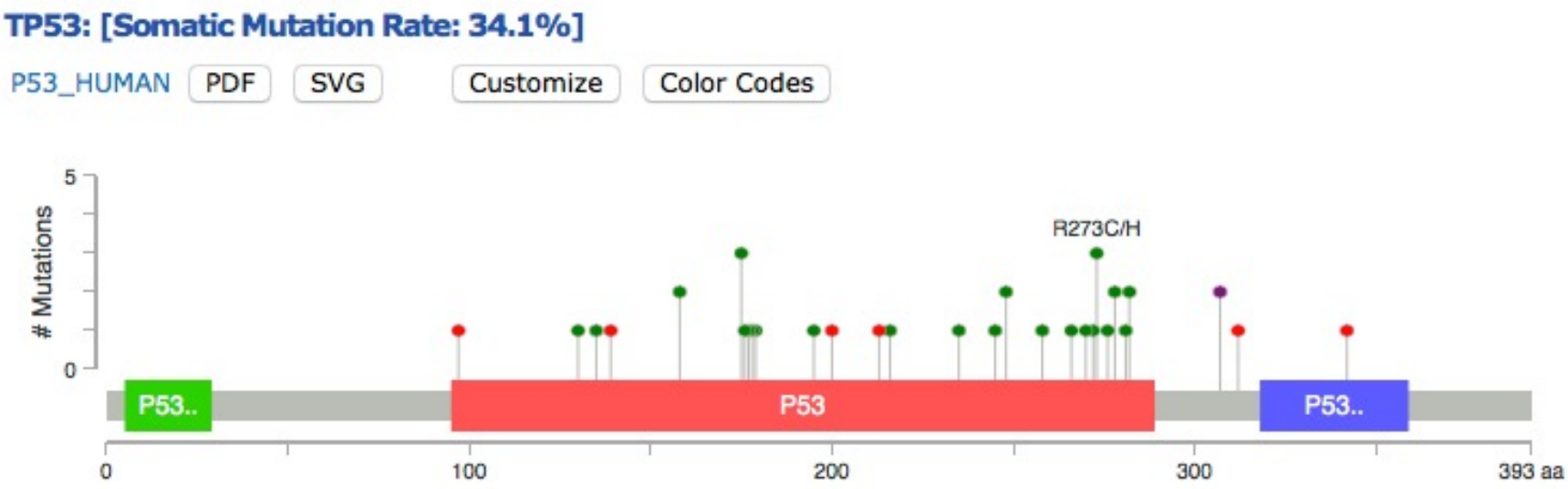
(varies due to targetted seq)

Memorial Sloan-Kettering
Cancer Center, USA

cBioPortal for Cancer Genomics

- Integration of Data from 89 cancer genomics studies.
- Focus on analysis tools
  - Mutual exclusivity
  - Gene networks

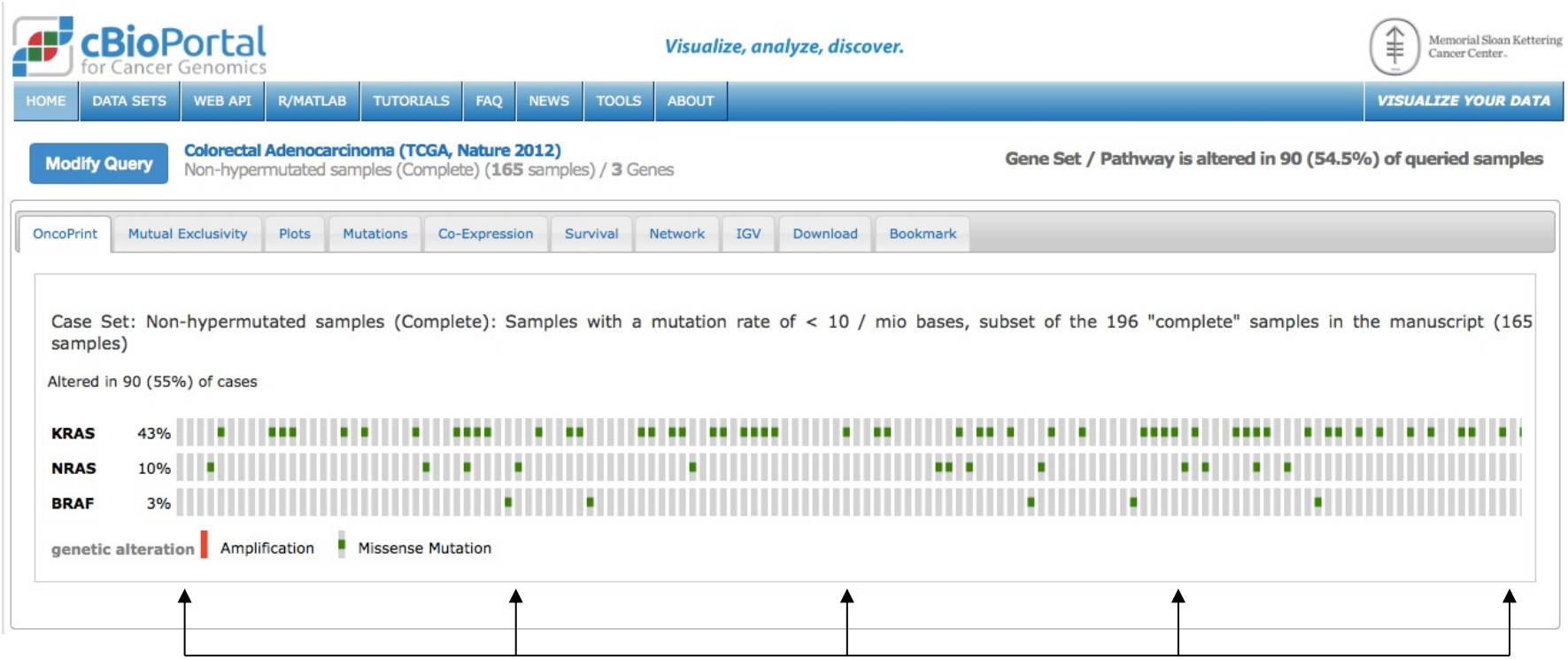
Mapped mutations on proteins



Mutations mapped on TP53 in Glioblastoma dataset (TCGA, Nature 2008)

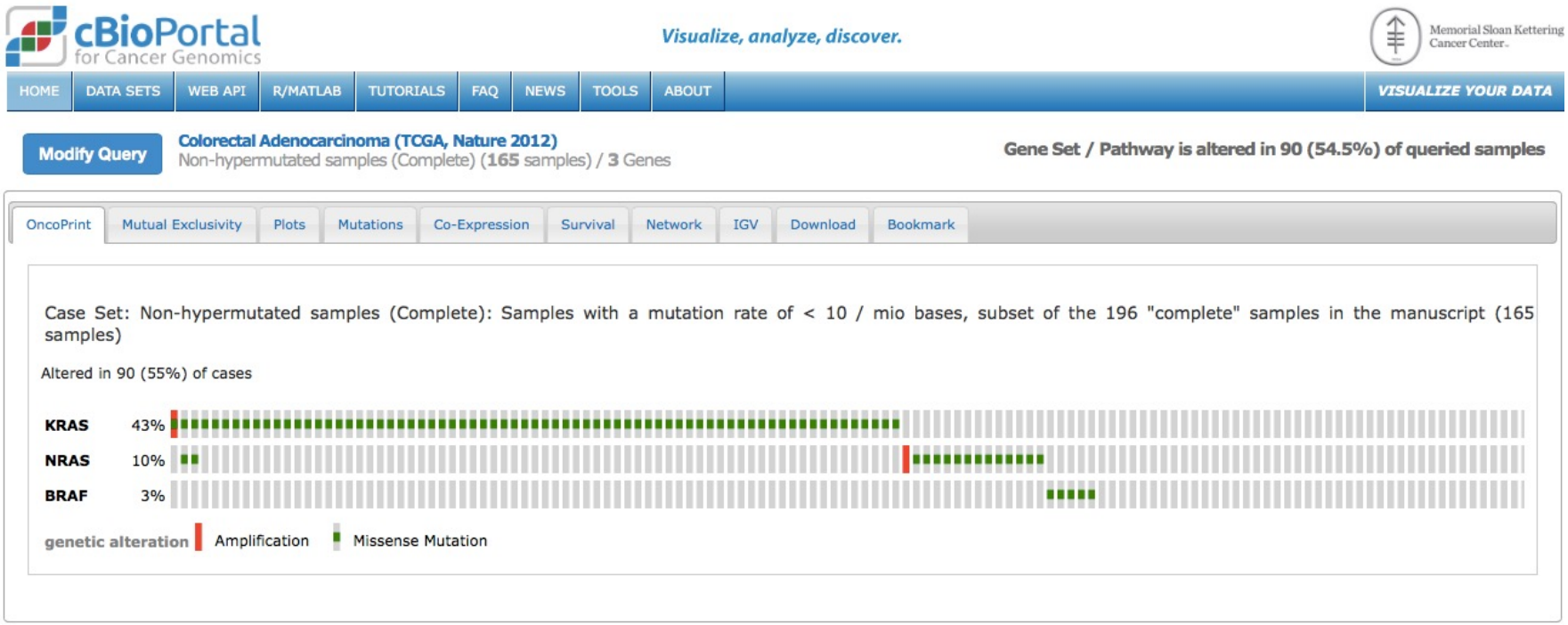
See also « MutationMapper » tool

« Oncoplot » view

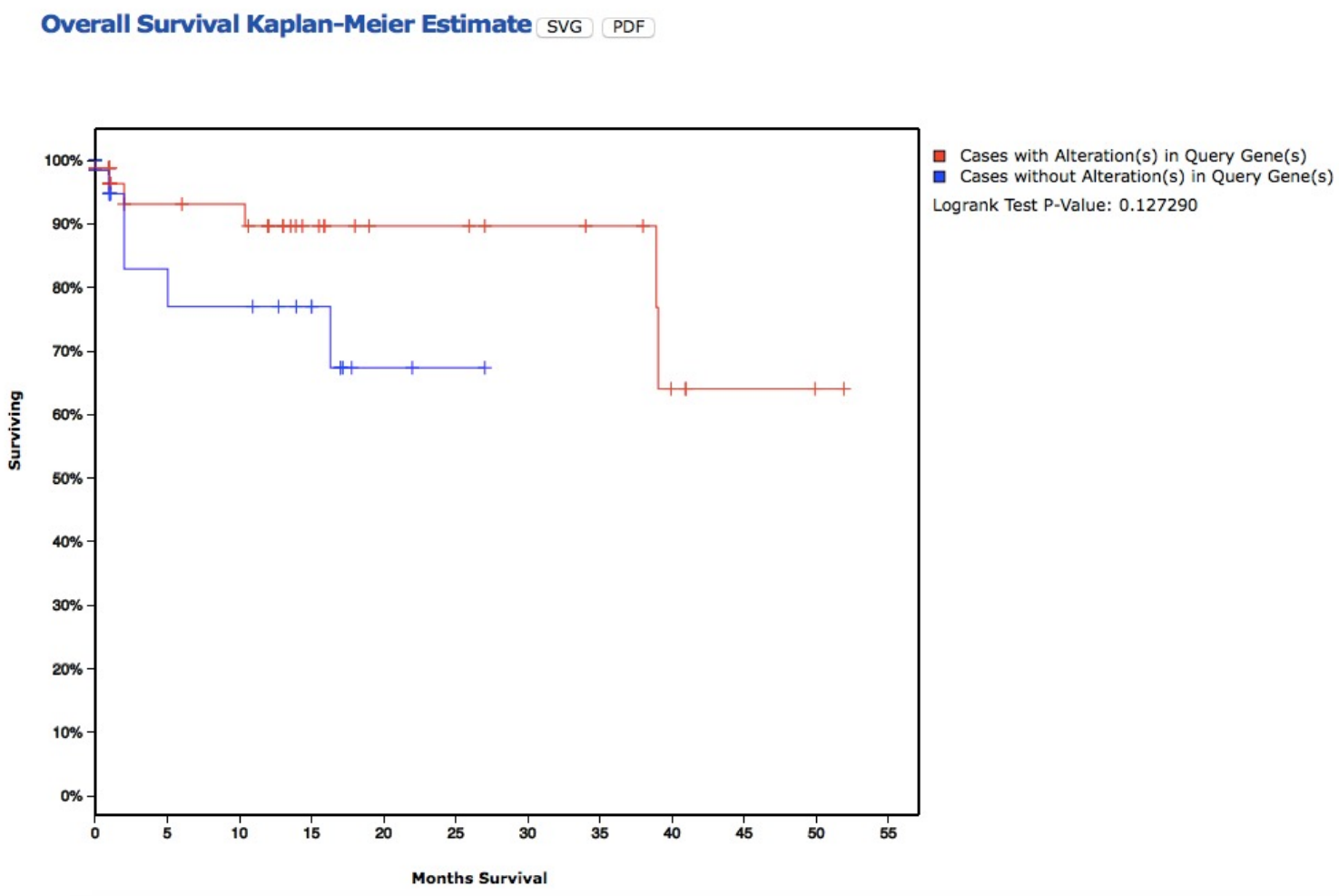


patients

Mutual exclusivity



Kaplan-Meier Curves



Programmatic Interfaces to cBioPortal

- Webservice (via URL)
- R library
  - CGDS package (CRAN)
- Matlab Library
  - CGDS toolbox @ MatLab Central

Web service example

http://www.cbioportal.org/webservice.do?cmd=getCaseLists&cancer_study_id=gbm_tcga

```
case_list_id    case_list_name  case_list_description   cancer_study_id case_ids
gbm_tcga_all    All samples     All samples (842 samples)       1863    TCGA-02-0001-01 TCGA-02-0003-01 TCGA-02-0006-01 TCGA-02-0007-01
0010-01 TCGA-02-0011-01 TCGA-02-0014-01 TCGA-02-0021-01 TCGA-02-0024-01 TCGA-02-0027-01 TCGA-02-0028-01 TCGA-02-0033-01 TCGA-02-0034-01
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```

Thank you

- Today:
  - Bastien Job on genome alterations
  - G Lelandais on Gene Expression Analysis via Galaxy