Détection de variants à partir de données de séquençage short & long reads

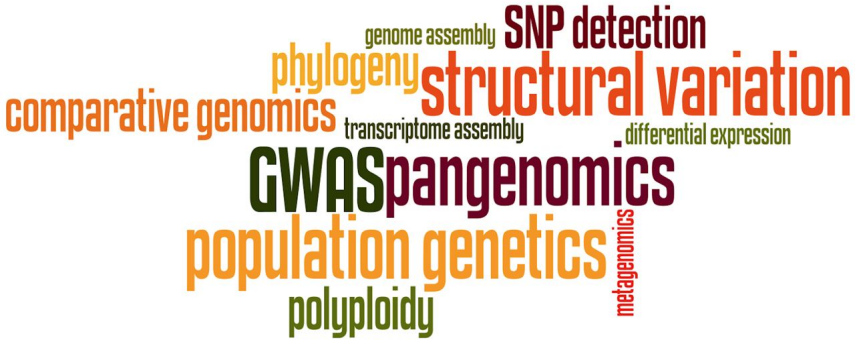
www.southgreen.fr

https://southgreenplatform.github.io/trainings

Modules de formation 2022

SouthGreen bioinformatics platform

Bioinformatics platform dedicated to the genetics and genomics of tropical and Mediterranean plants and their pathogens




Rice

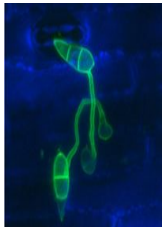

Banana


Palm


Sorghum


Coffee


Cassava


Magnaporthe

www.southgreen.fr

SouthGreen bioinformatics platform

Charte

Larmande Pierre
Orjuela-Bouniol Julie
Sabot François
Tando Ndomassi
Tranchant-Dubreuil Christine

Comte Aurore
Dereeper Alexis
Ravel Sébastien

Bocs Stephanie
Boizet Alice
De Lamotte Frédéric
Droc Gaetan
Dufayard Jean-François
Hamelin Chantal
Martin Guillaume
Pitollat Bertrand
Ruiz Manuel
Sarah Gautier
Summo Marilyne

Rouard Mathieu
Guignon Valentin
Catherine Breton

Sempere Guilhem

SouthGreen bioinformatics platform

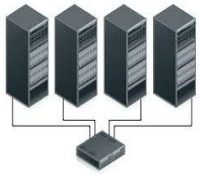
Workflow manager

TOGGLE Toolbox for generic NGS analyses

SNAKEMAKE

Galaxy

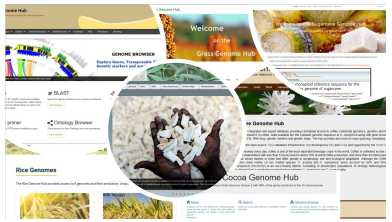
HPC and trainings….



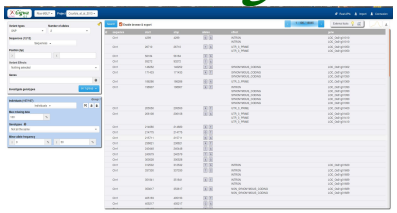
IRD Institut de Recherche pour le Développement

cirad

Genome Hubs & Information System

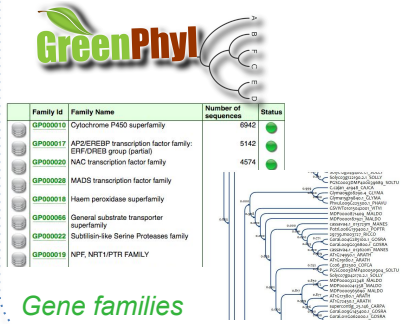


Gigwa



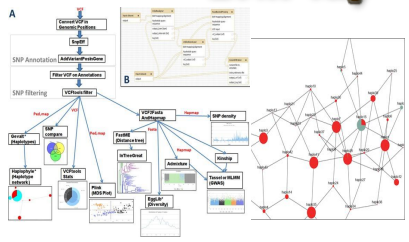
SNPs and Indels

GreenPhyl



Gene families

SNiPlay



https://github.com/SouthGreenPlatform

@green_bioinfo

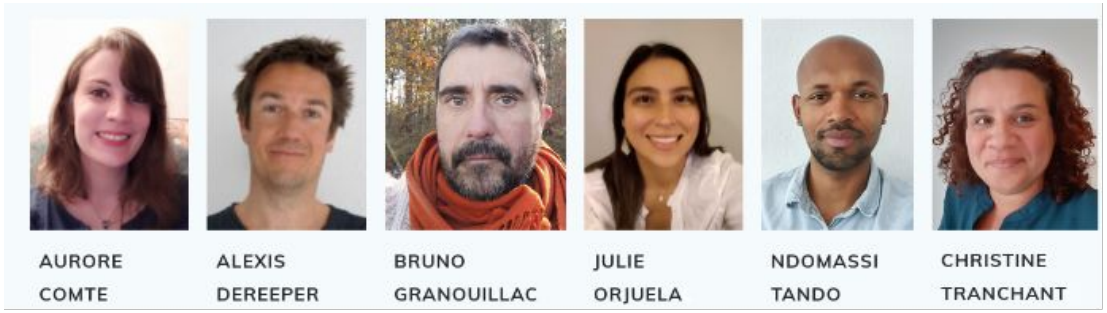
The South Green portal: a comprehensive resource for tropical and Mediterranean crop genomics, Current Plant Biology, 2016

I-Trop

Plant & Health Bioinformatics Platform

EURO-QUALITY SYSTEM
ISO 9001

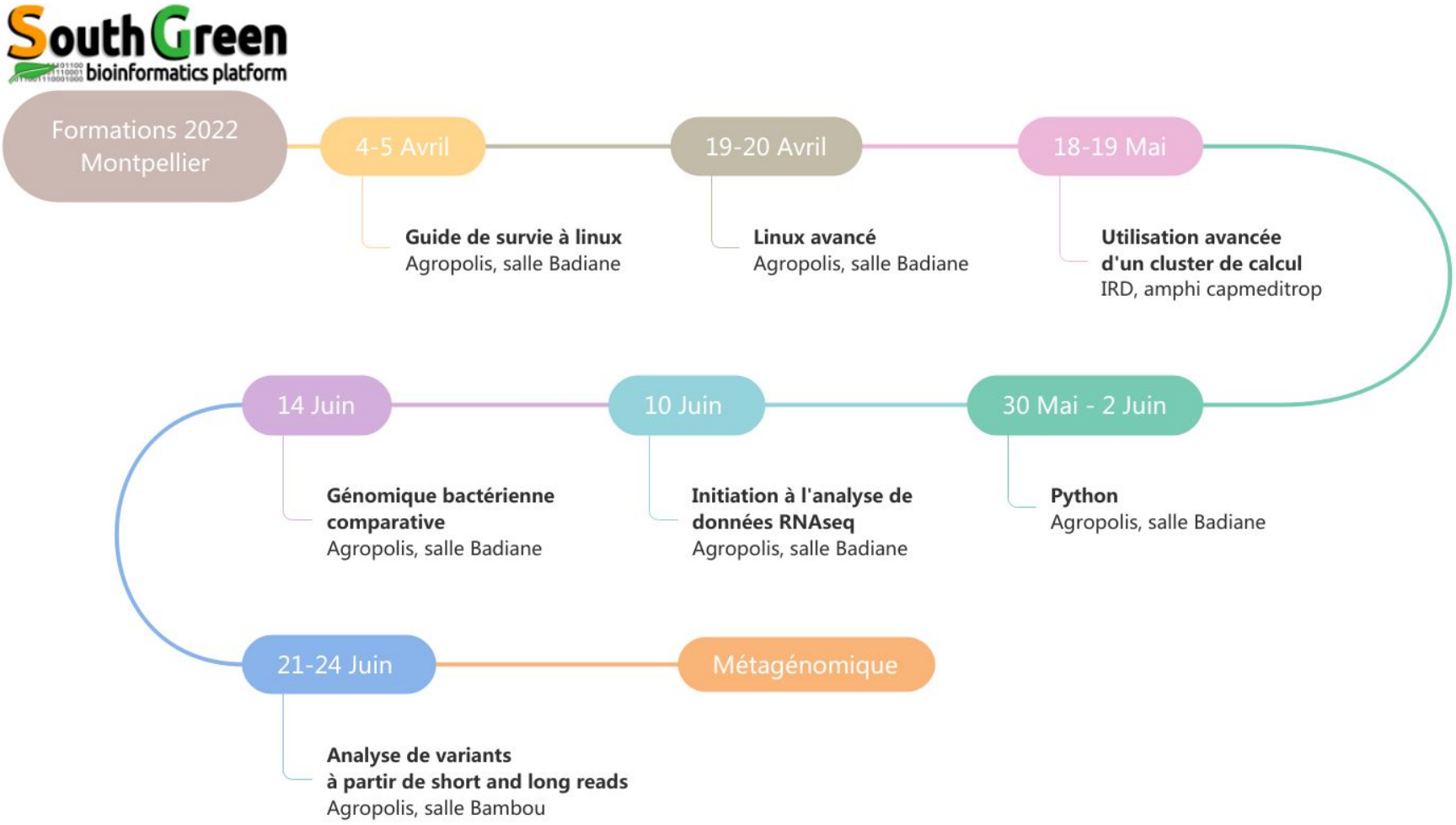
https://bioinfo.ird.fr/



AURORE COMTE	ALEXIS DEREEPER	BRUNO GRANOUILLAC	JULIE ORJUELA	NDOMASSI TANDO	CHRISTINE TRANCHANT
IE bioinfo	IE bioinfo	IE systèmes d'information	IE bioinfo	IE systèmes	IR bioinfo

bioinfo@ird.fr

@ItropBioinfo


Formations 2022
Montpellier
4-5 Avril
Guide de survie à linux
Agropolis, salle Badiane
19-20 Avril
Linux avancé
Agropolis, salle Badiane
18-19 Mai
Utilisation avancée
d'un cluster de calcul
IRD, amphi capmeditrop
30 Mai - 2 Juin
Python
Agropolis, salle Badiane
10 Juin
Initiation à l'analyse de
données RNAseq
Agropolis, salle Badiane
14 Juin
Génomique bactérienne
comparative
Agropolis, salle Badiane
21-24 Juin
Analyse de variants
à partir de short and long reads
Agropolis, salle Bambou
Métagénomique

Modules de formation 2022

- Toutes nos formations :
  https://southgreenplatform.github.io/trainings/

- Topo & TP : https://southgreenplatform.github.io/trainings/sv
  https://github.com/SouthGreenPlatform/training_SV_teaching/tree/2022

- **tablet**

Détection de variants
à partir de données de séquençage
short & long reads

www.southgreen.fr

https://southgreenplatform.github.io/trainings

Objectifs

Détecter des variants (SNP, variants structuraux) à partir de données de séquençage short et long reads.

Applications :

- Mapper des reads contre un génome ***bwa***, ***minimap2***
- Détecter des SNPs à partir du mapping de reads - ***GATK, deepvariants***
- Analyser les données SNPs brutes (ex: stats, filtres) - ***vcftools, bcftools***
- Exemples d'études possibles à partir de SNPs - ***SNIPlay***
- Détecter des variants structuraux (SV) à partir de :
  - reads mappées contre un génome - ***breakdancer, sniffle***
  - génomes entiers - ***nucmer, assemblytics, siry***

Objectifs

Détecter des variants (SNP, variants structuraux) à partir de données de séquençage short et long reads.

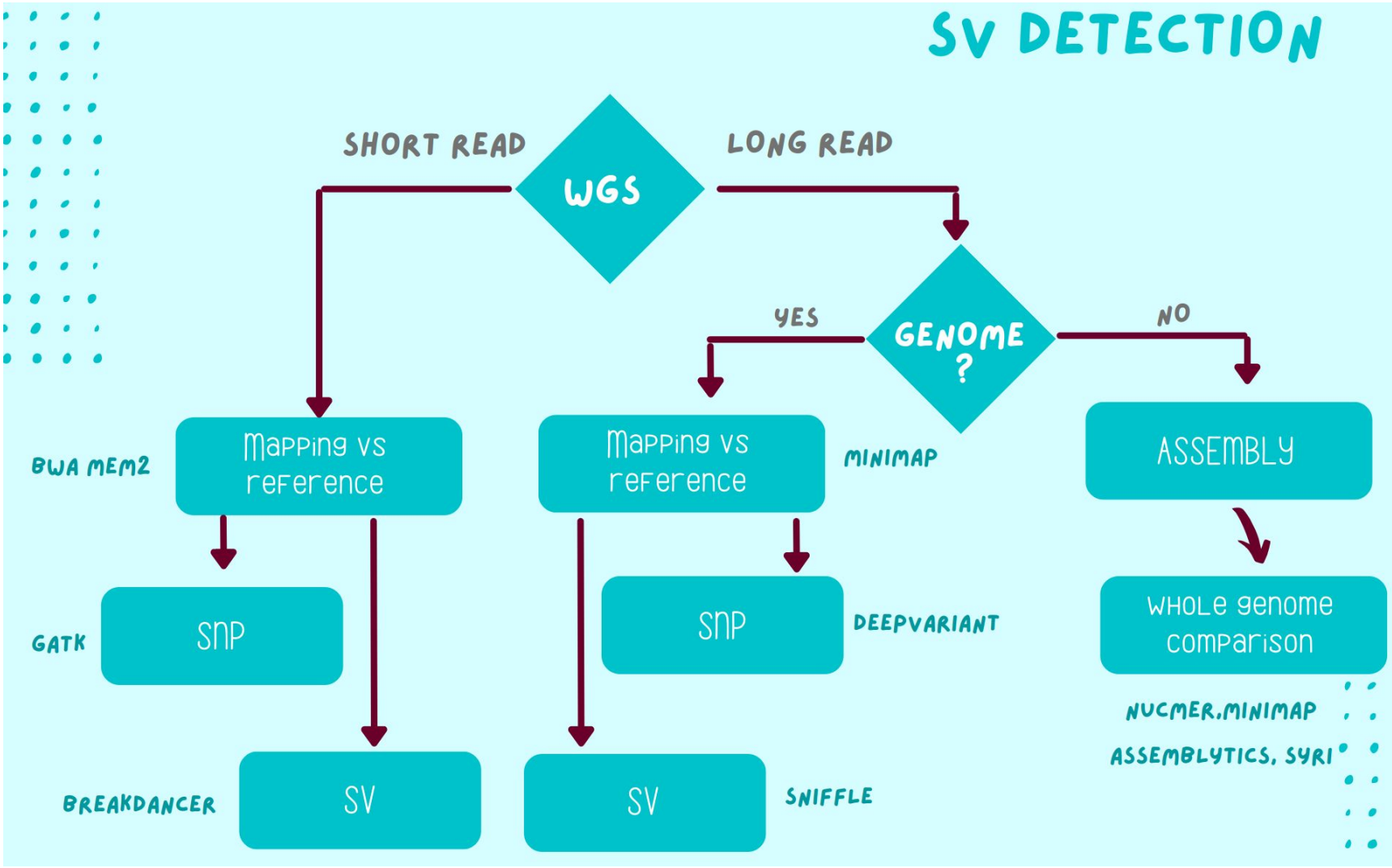
Applications :

- Mapper des reads contre un génome ***bwa***, ***minimap2***
- Détecter des SNPs à partir du mapping de reads - ***GATK, deepvariants***
- Analyser les données SNPs brutes (ex: stats, filtres) - ***vcftools, bcftools***
- Exemples d'études possibles à partir de SNPs - ***SNIPlay***
- Détecter des variants structuraux (SV)

Avec jupyter book : lancer les commandes + analyser les résultats

=> Avoir un plan de bataille opérationnel

Plan de bataille !!!



Let’s discover Jupyter through the IFB cloud

Working environment

What is jupyter book ?

- One of the most popular tool among data scientists to perform data analysis

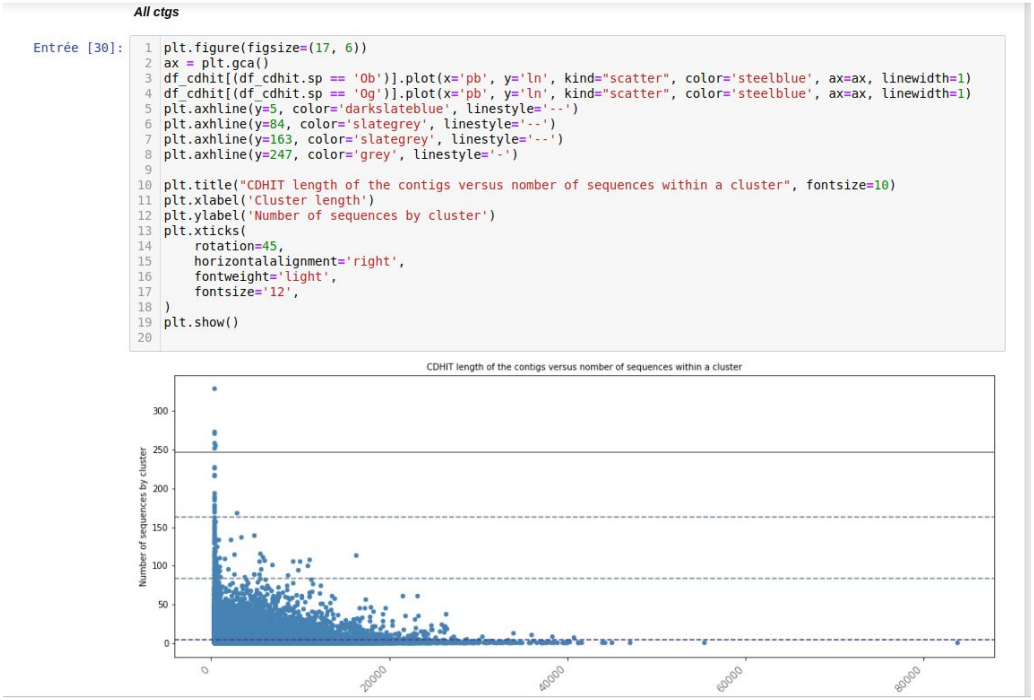
- Provides a complete environment in which numerous programming languages can be use through a simple web browser

ex : Bash (Linux), Python, Java, R, Julia, Matlab, Octave, Scheme, Processing, Scala

Why use jupyter book ?

An unique interface/file where text,code and output codes can be mixed :

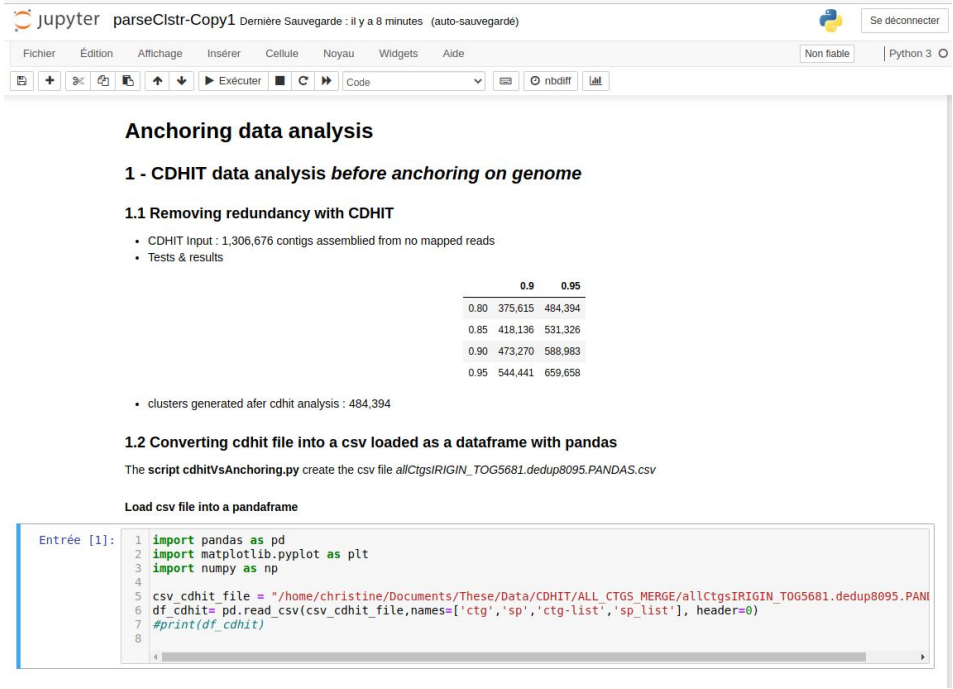
- code can be executed inside each cell of the notebook
- code output is directly displayed in the notebook



Why use jupyter book ?

An unique interface/file where text,code and output codes can be mixed :

- code can be executed inside each cell of the notebook
- code output is directly displayed in the notebook
- explanations, formulas, charts can be added



Lab notebook for science data ?

- One file to analyze data and generate reports
- Can be exported to many formats, including PDF and HTML, which makes it easy to share your project with anyone.
- Analysis are more transparent, repeatable and shareable

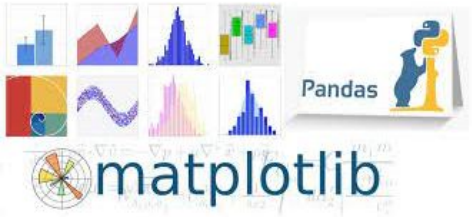
How to become a super datascientist ?

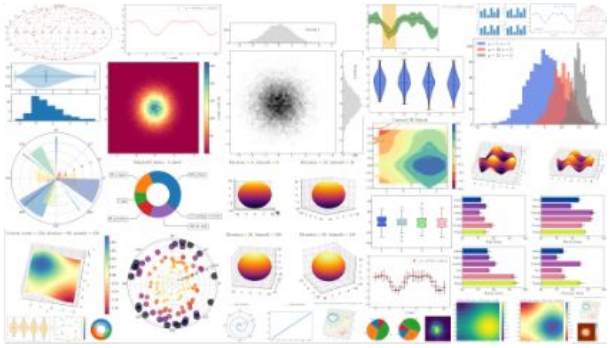
- facilement importer des fichiers tabulés dans des dataframes, similaires aux dataframes sous R.

  (et exporter)

- manipuler ces tableaux de données / DataFrames

- facilement tracer des graphes à partir de ces DataFrames grâce à matplotlib





How will you use Jupyter Notebook ?

- Launch our analyses through a jupyter book within a virtual machine launched via the IFB cloud “BIOSPHERE”

How will you use Jupyter Notebook ?

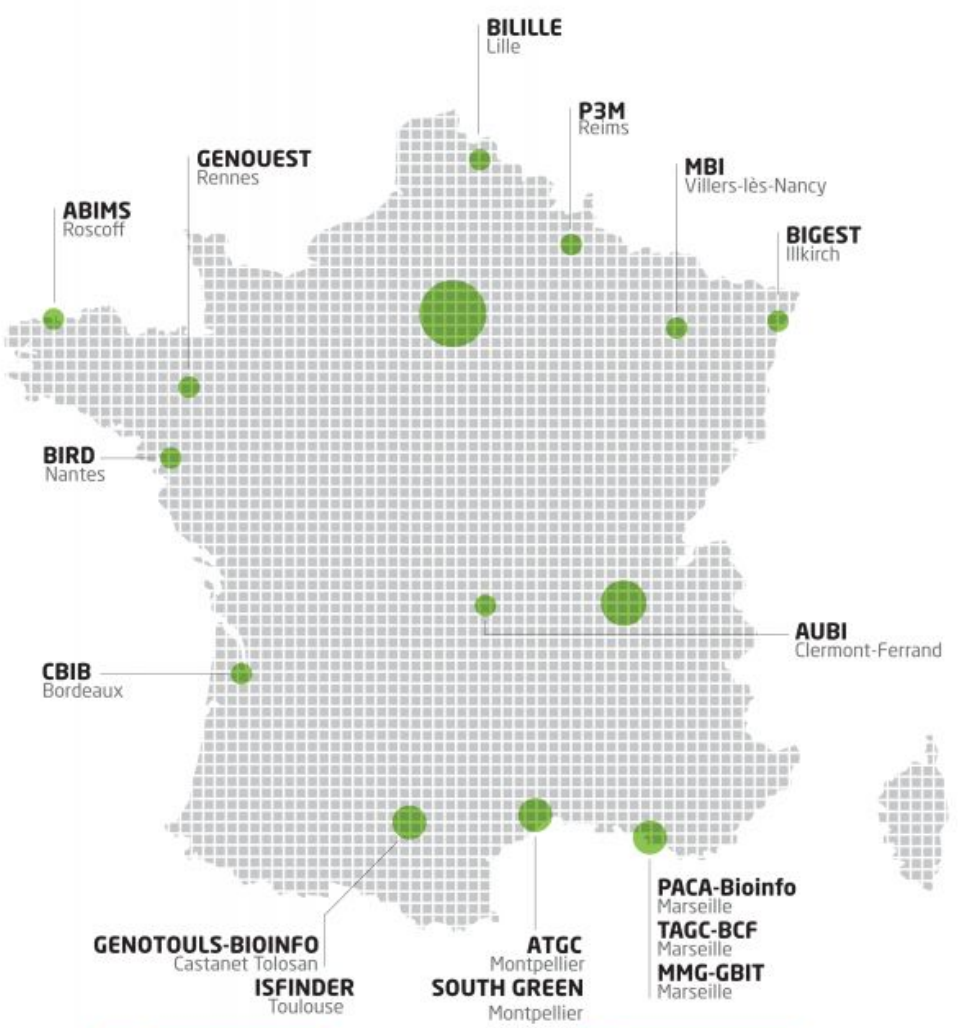
- Launch our analyses through a jupyter book within a virtual machine launched via the IFB cloud “BIOSPHERE”

- Through this virtual machine, we will create jupyter books and execute all our analysis

IFB ?

INSTITUT FRANÇAIS DE BIOINFORMATIQUE

22 plateformes-membres
7 plateformes contributrices
8 équipes associées
>400 experts (~200 FTE)



Biosphere, IFB CLOUD FOR LIFE SCIENCES

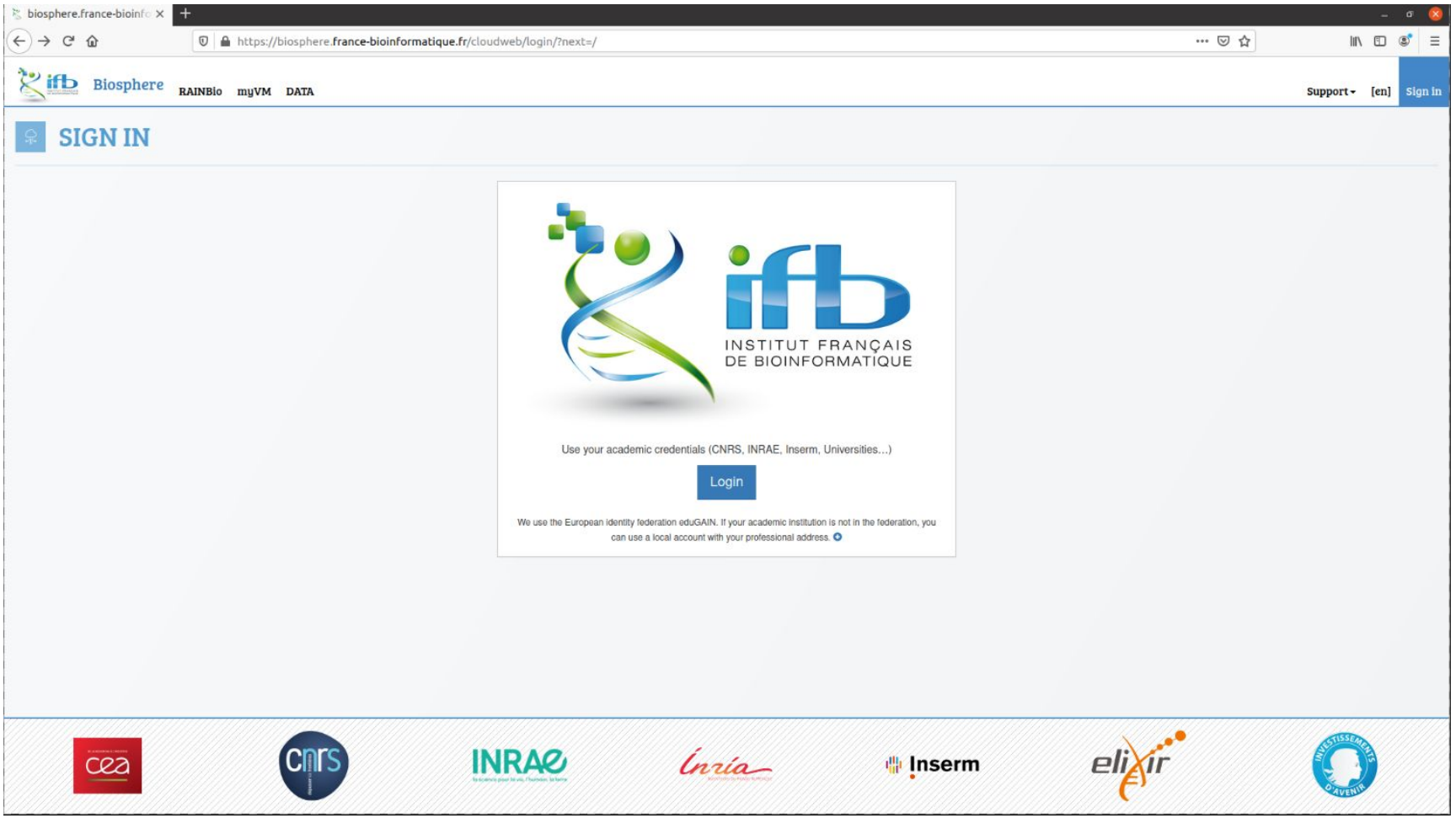
- A federation of clouds, which relies on interconnected IFB's infrastructures, providing distributed services to analyze life science data

- .Access to a large set of virtual machines (computing ressources, bioinformatics tool)

- Used for scientific production in the life sciences, developments, and also to support events like cloud and scientific training sessions, hackathons or workshops.

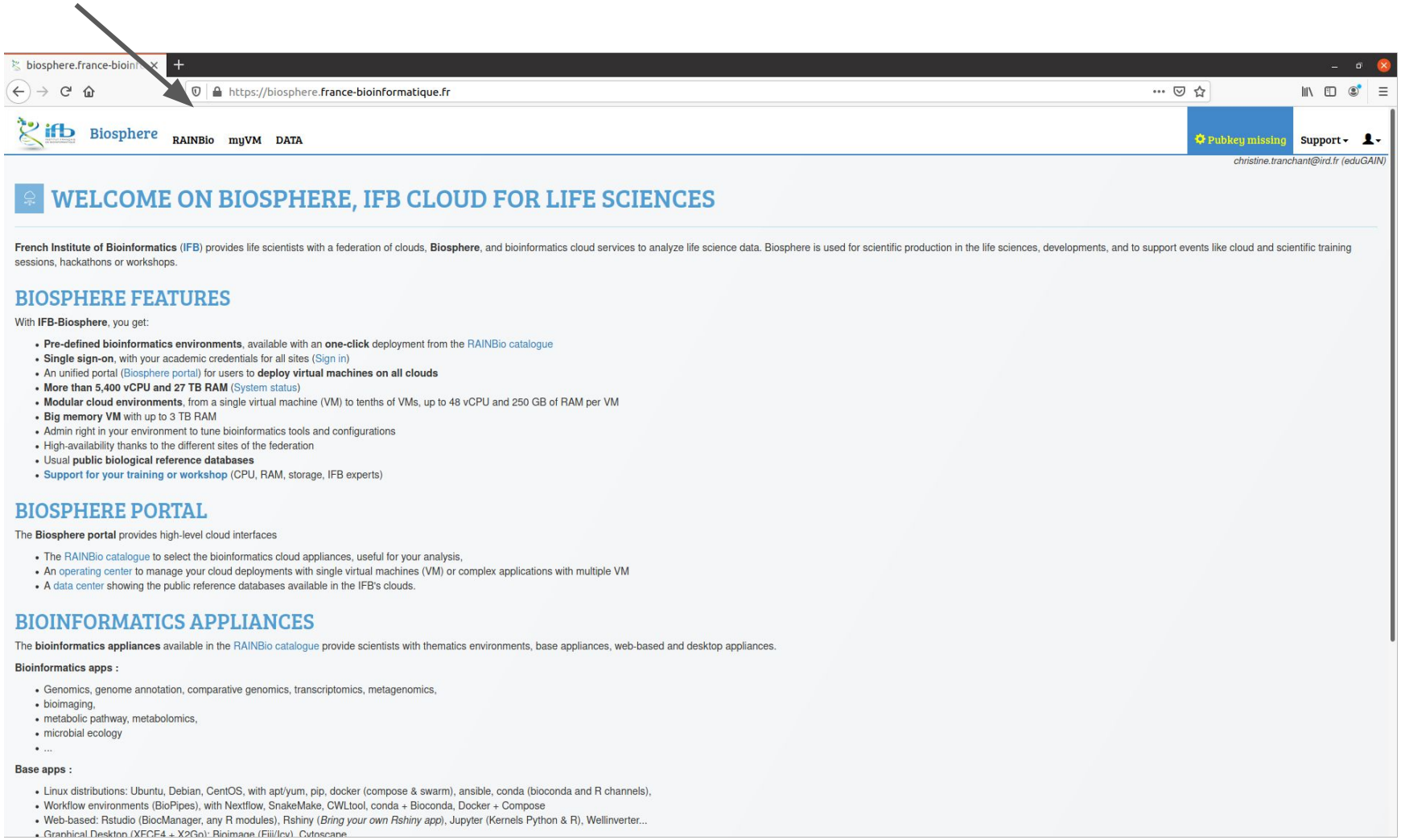
Let's start with biosphere

- Open the biosphere website : https://biosphere.france-bioinformatique.fr/cloud/ and sign in



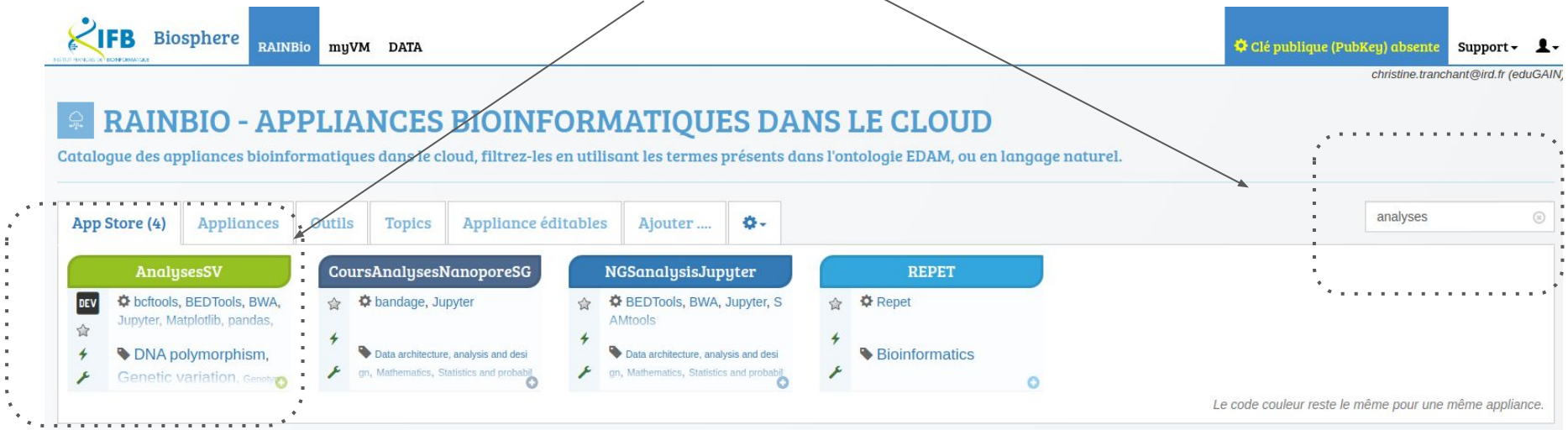
Connected / here we are

RAINBIO catalog to access our Virtual Machine (VM)

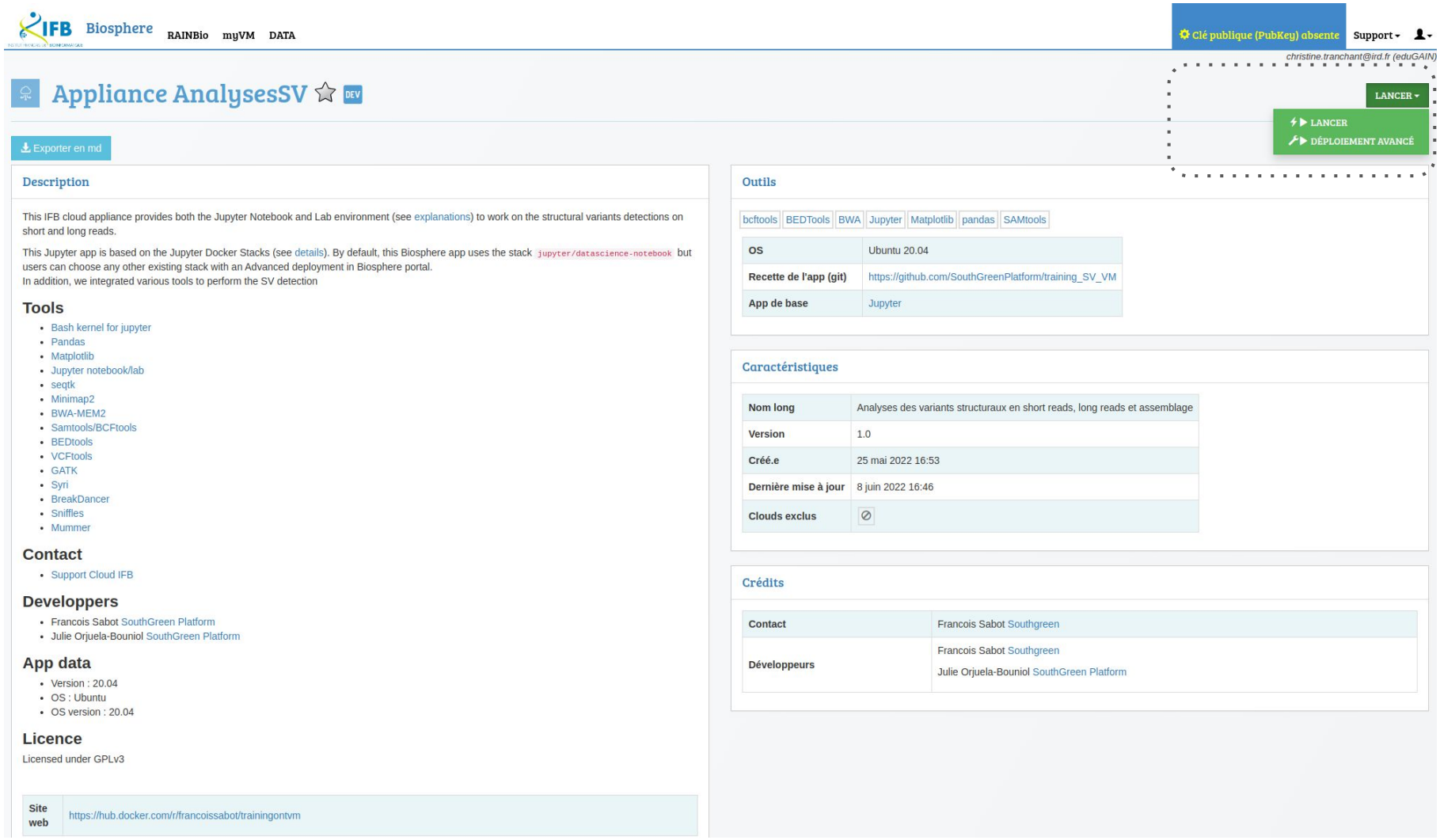


Searching for the vm we will use

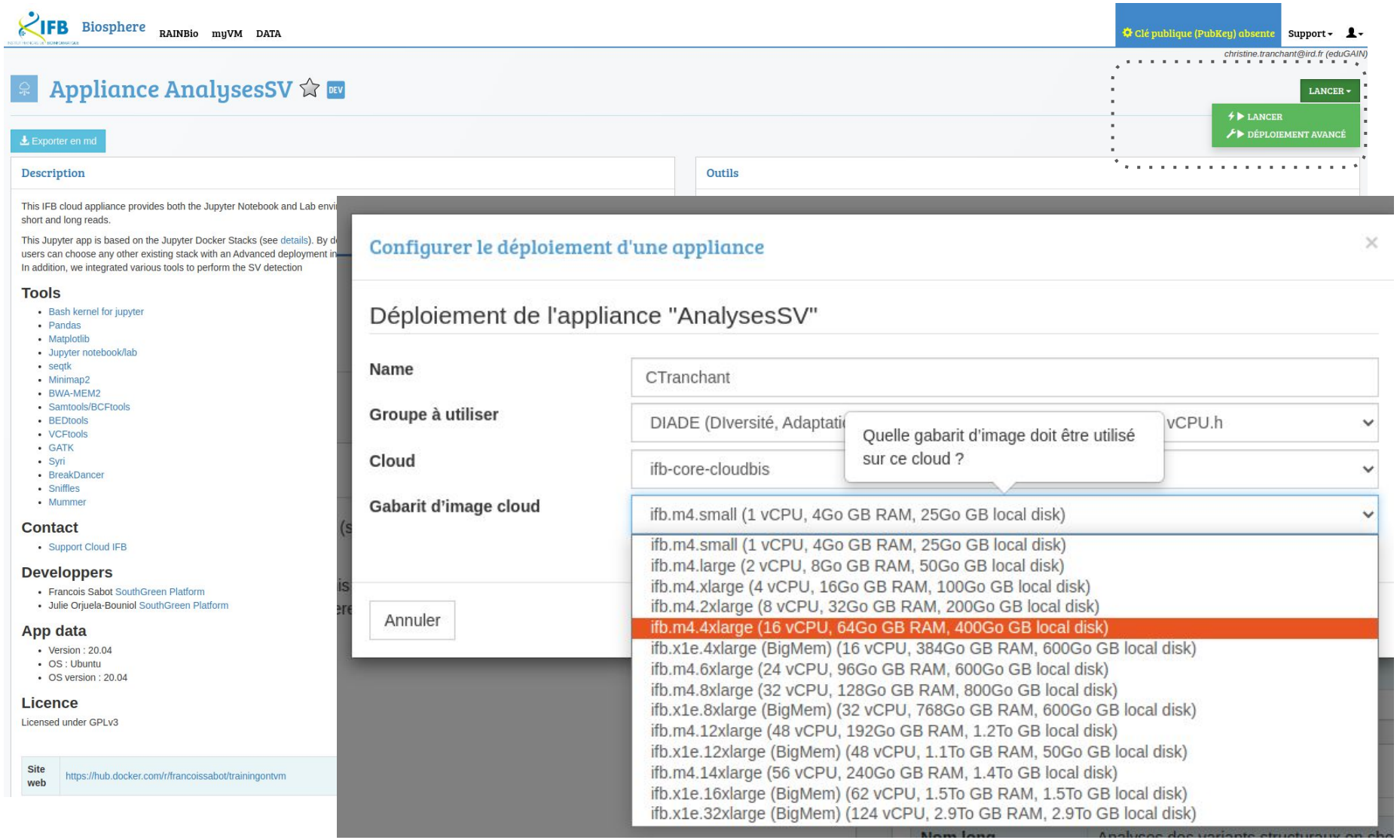
vm's name : **analysesSV**



Let’s run your vm through the cloud

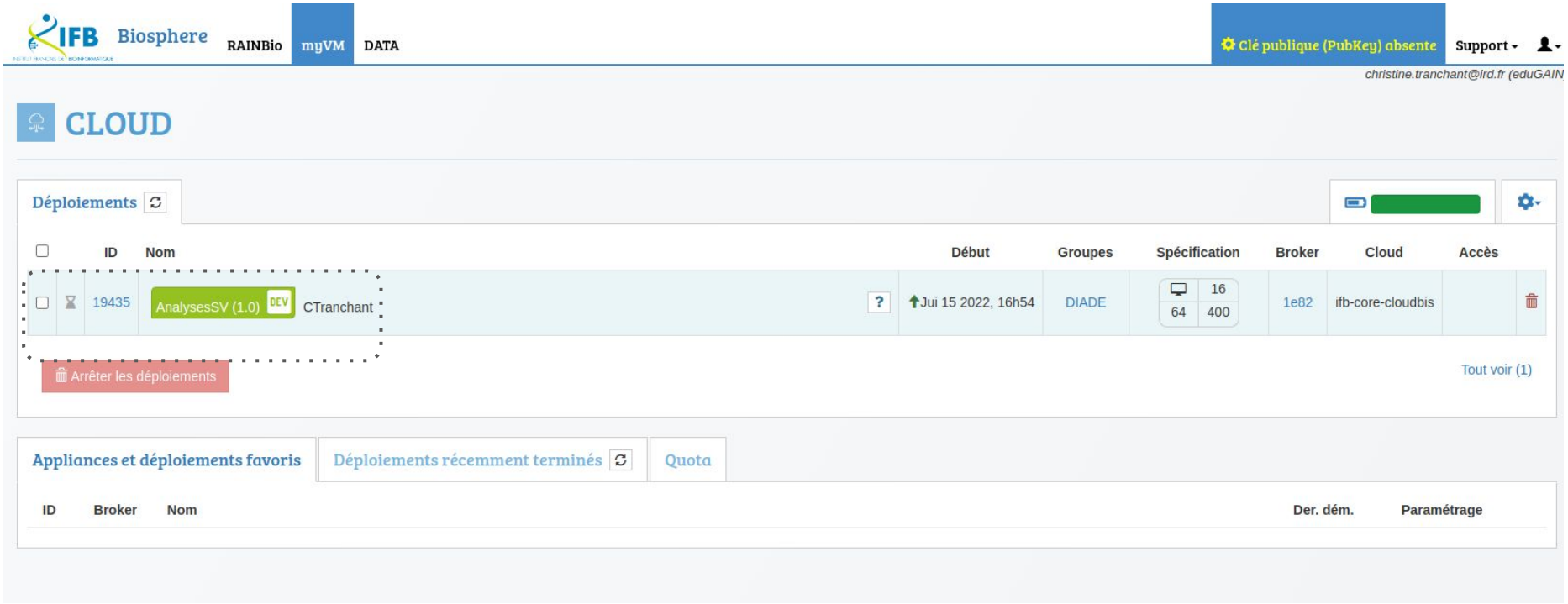


Let’s run your vm through the cloud



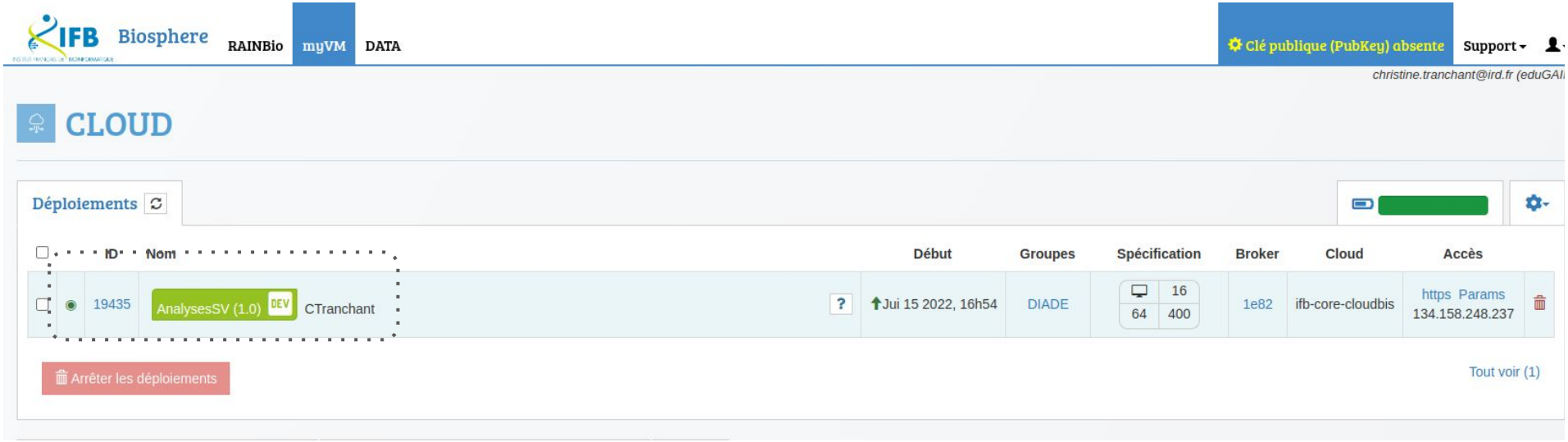
Let’s run your vm through the cloud

Loading…



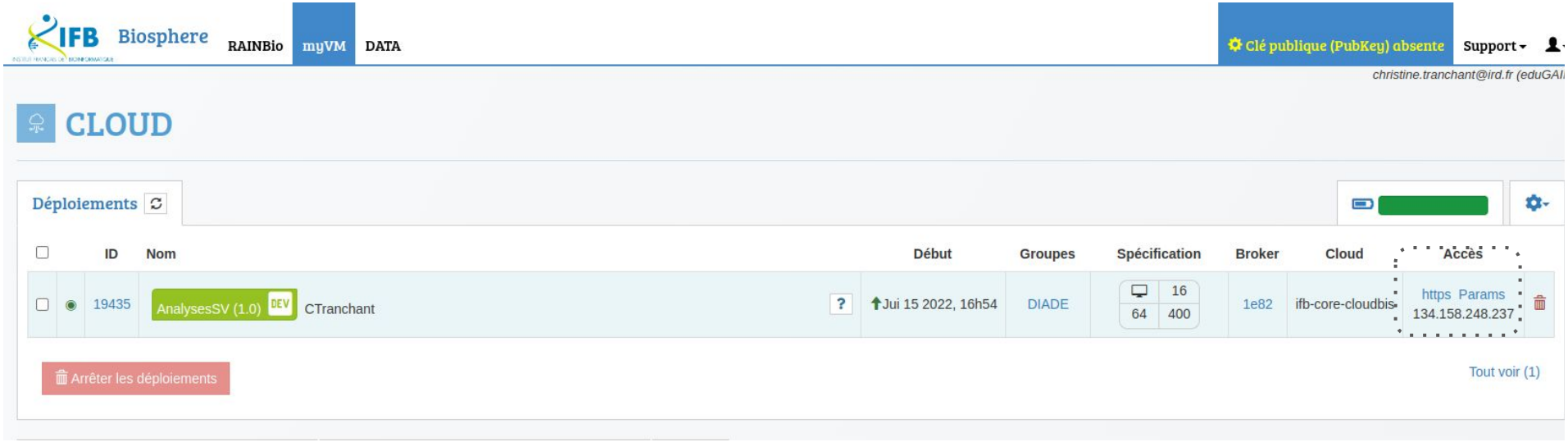
Let’s run your vm through the cloud

ready !



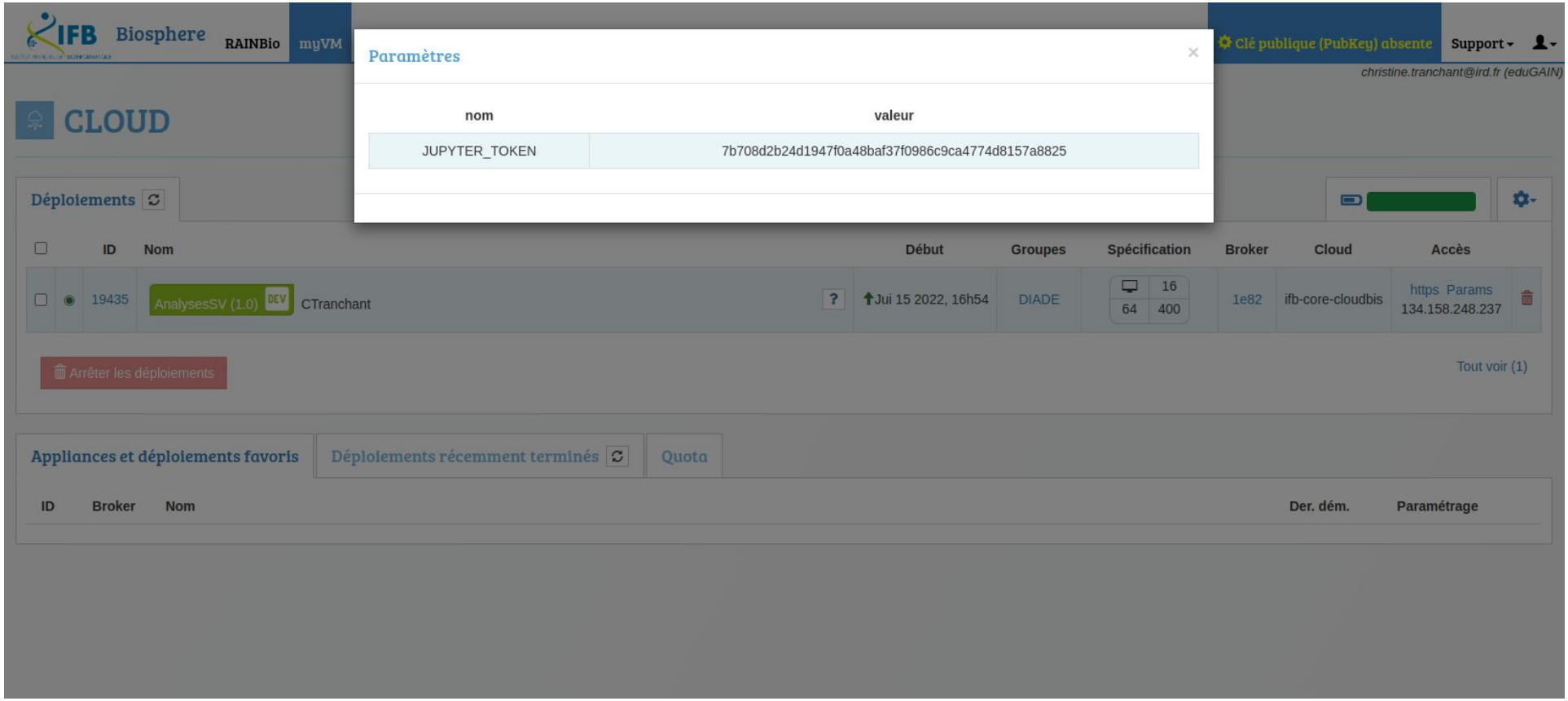
Let’s run your vm through the cloud

get the url… link “https”



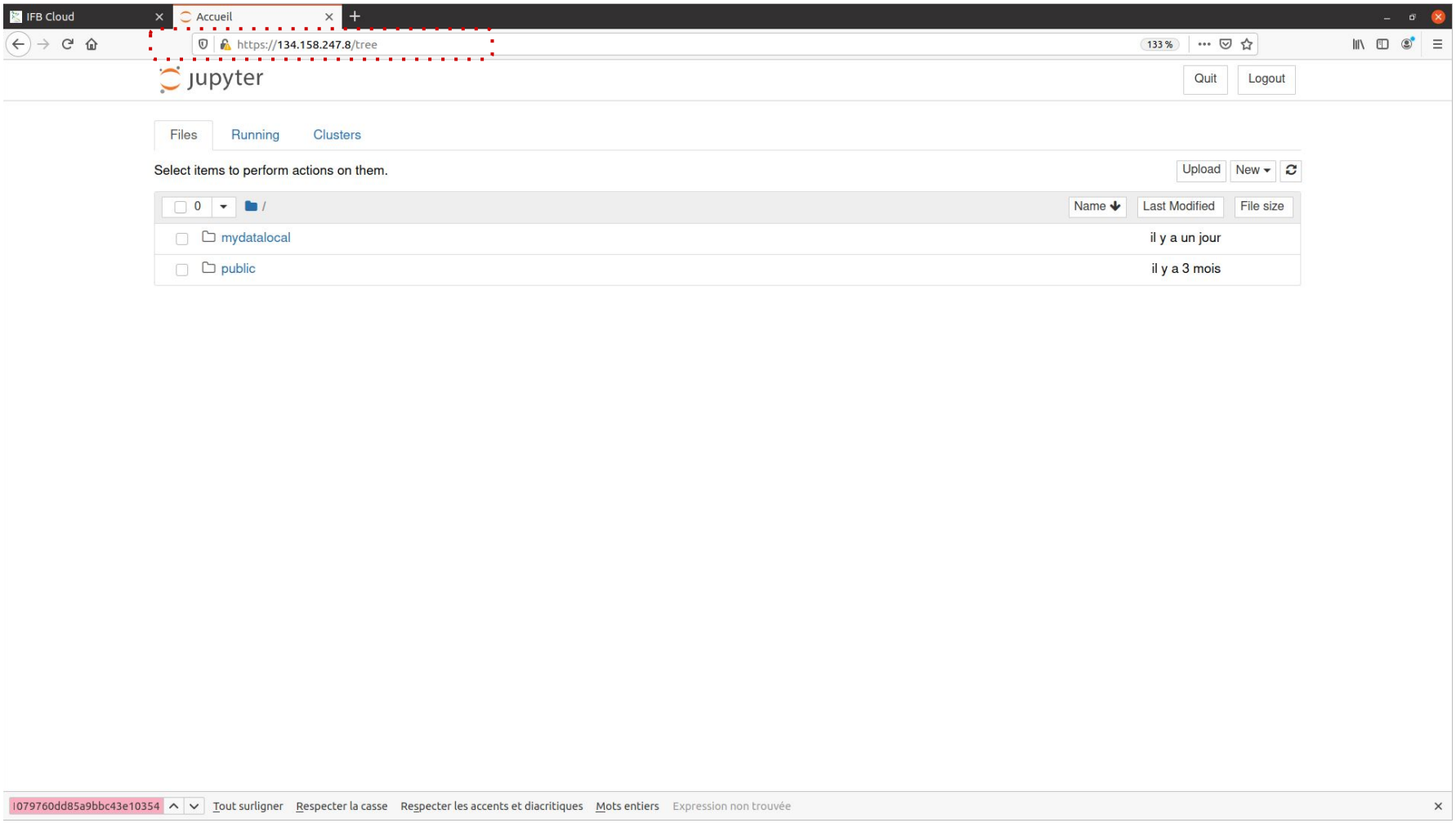
Let’s run our vm through the cloud

Get the token identifiant… link “Params”



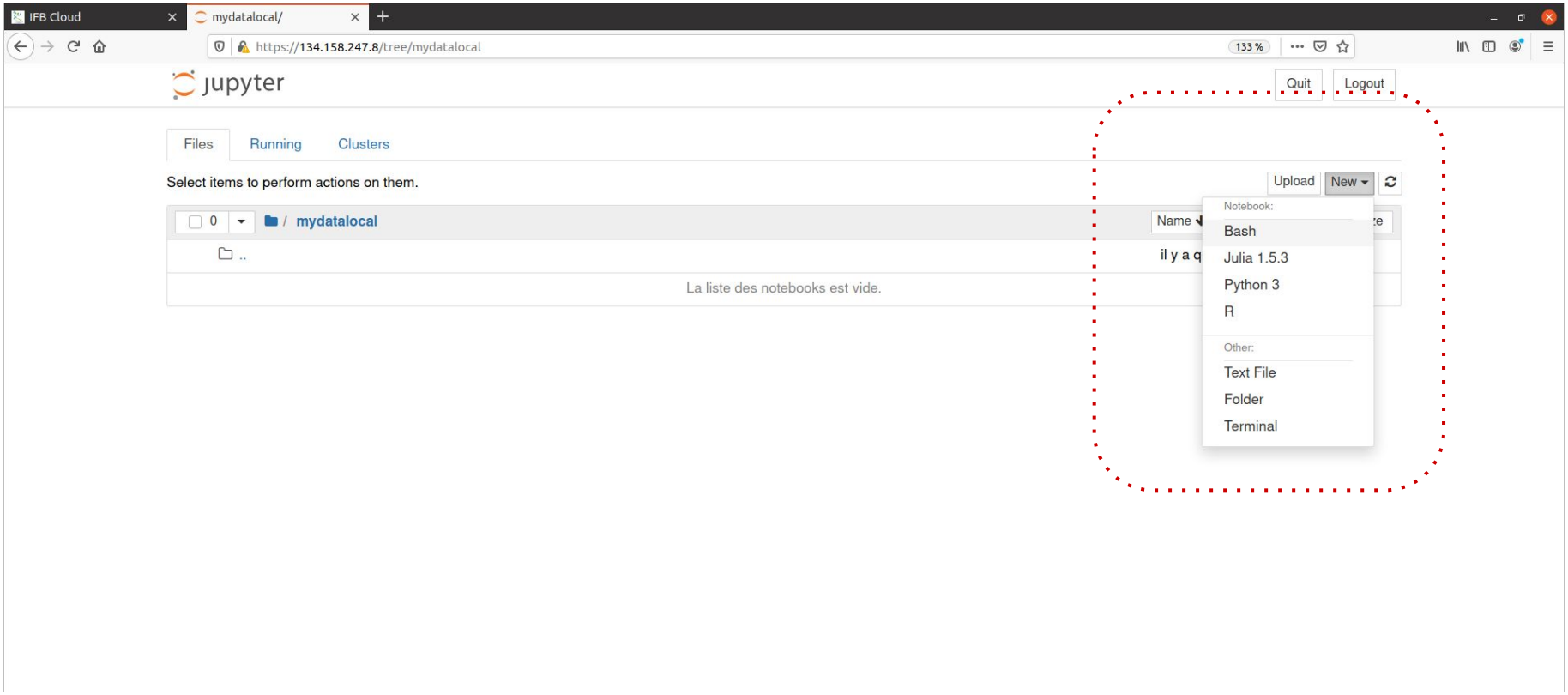
Let’s run our vm through the cloud

Open your vm (https link) to access to your own jupyter lab



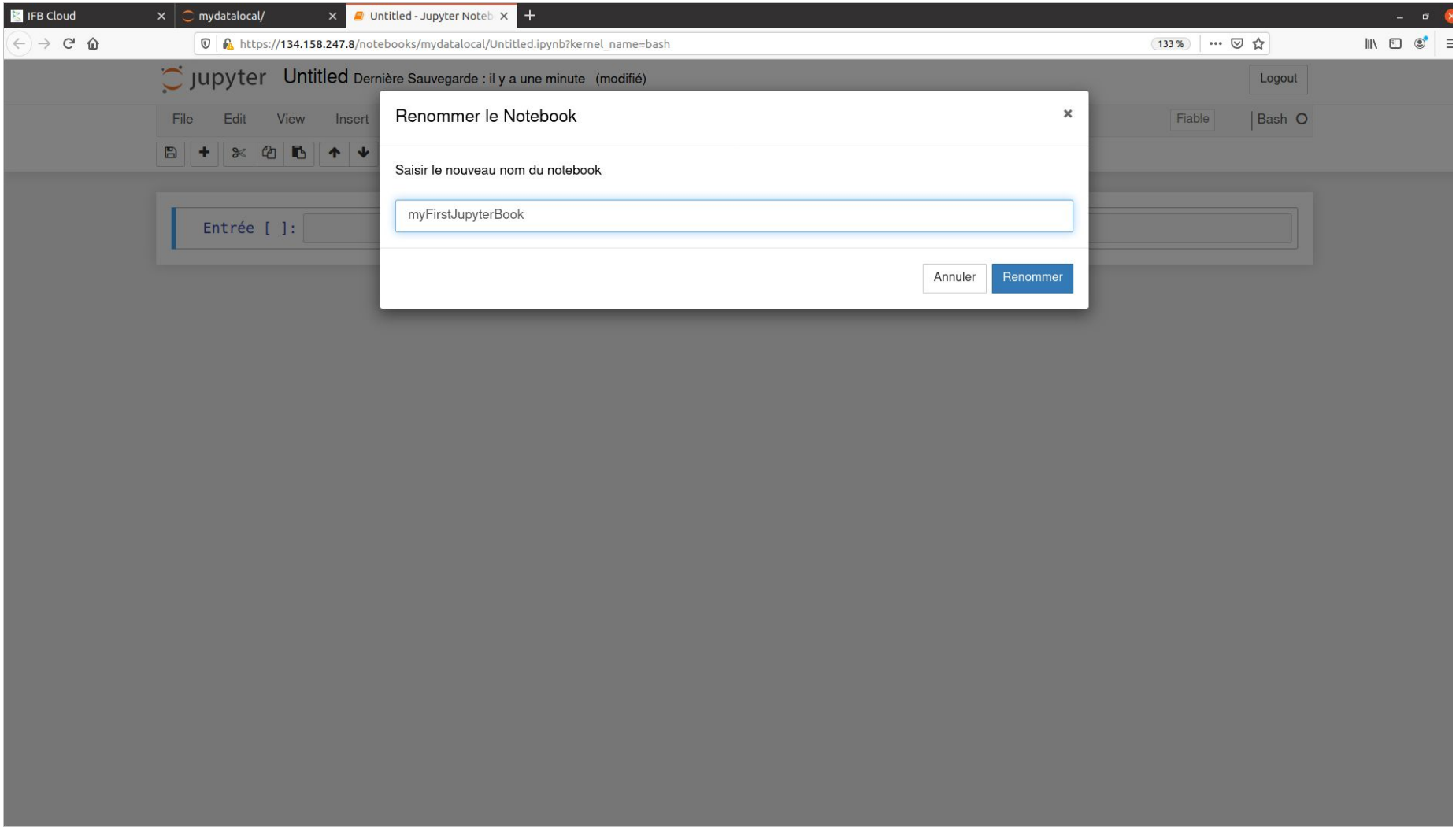
Create your first jupyter book

Go into the directory “work” and create a new jupyter book
-> kernel : bash



Rename your first jupyter book

myFirstJupyterBook

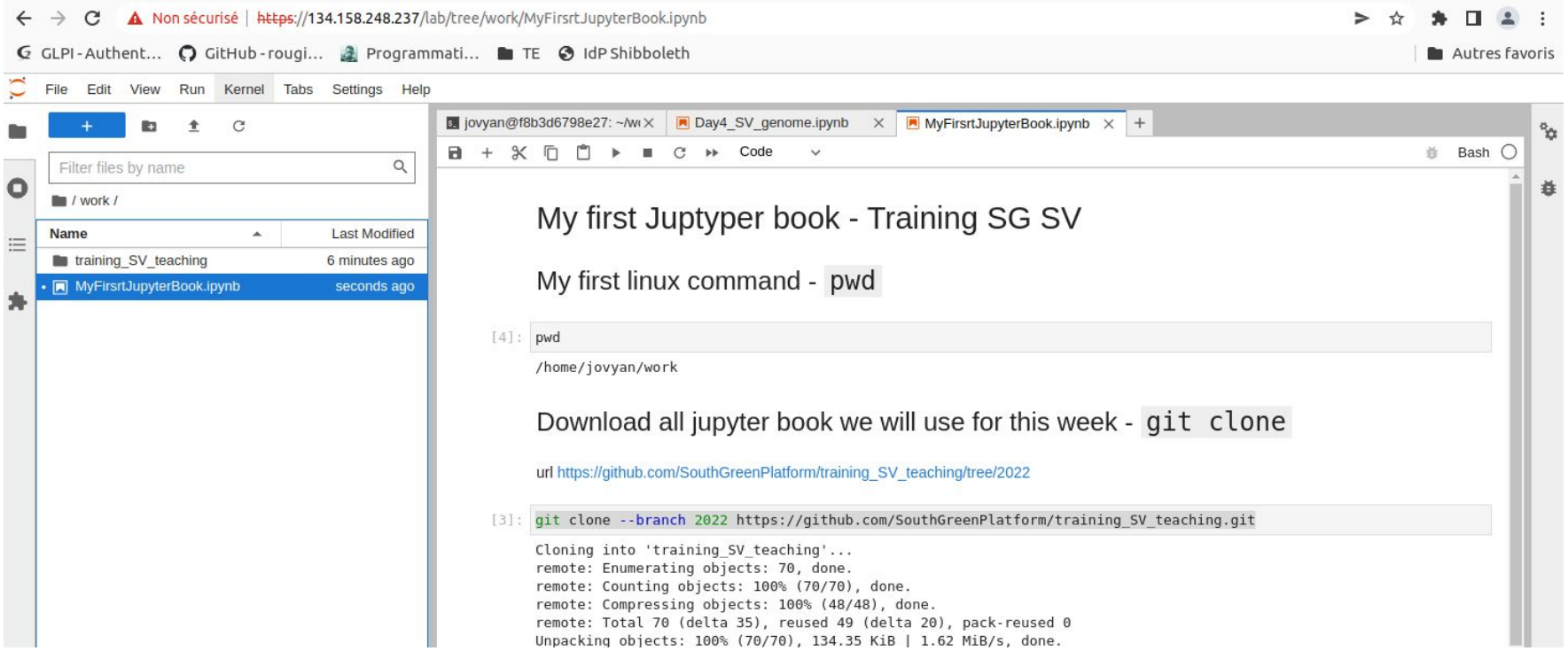


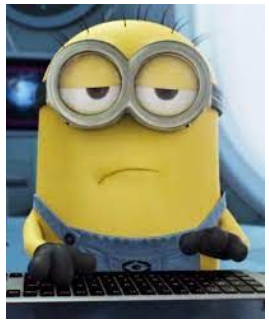
Run your first bask command - *git clone*

- All jupyterbook used for practice are here :
  https://github.com/SouthGreenPlatform/training_SV_teaching/tree/2022

- Download all the jupyter books with the command *git clone*

git clone --branch 2022 https://github.com/SouthGreenPlatform/training_SV_teaching.git

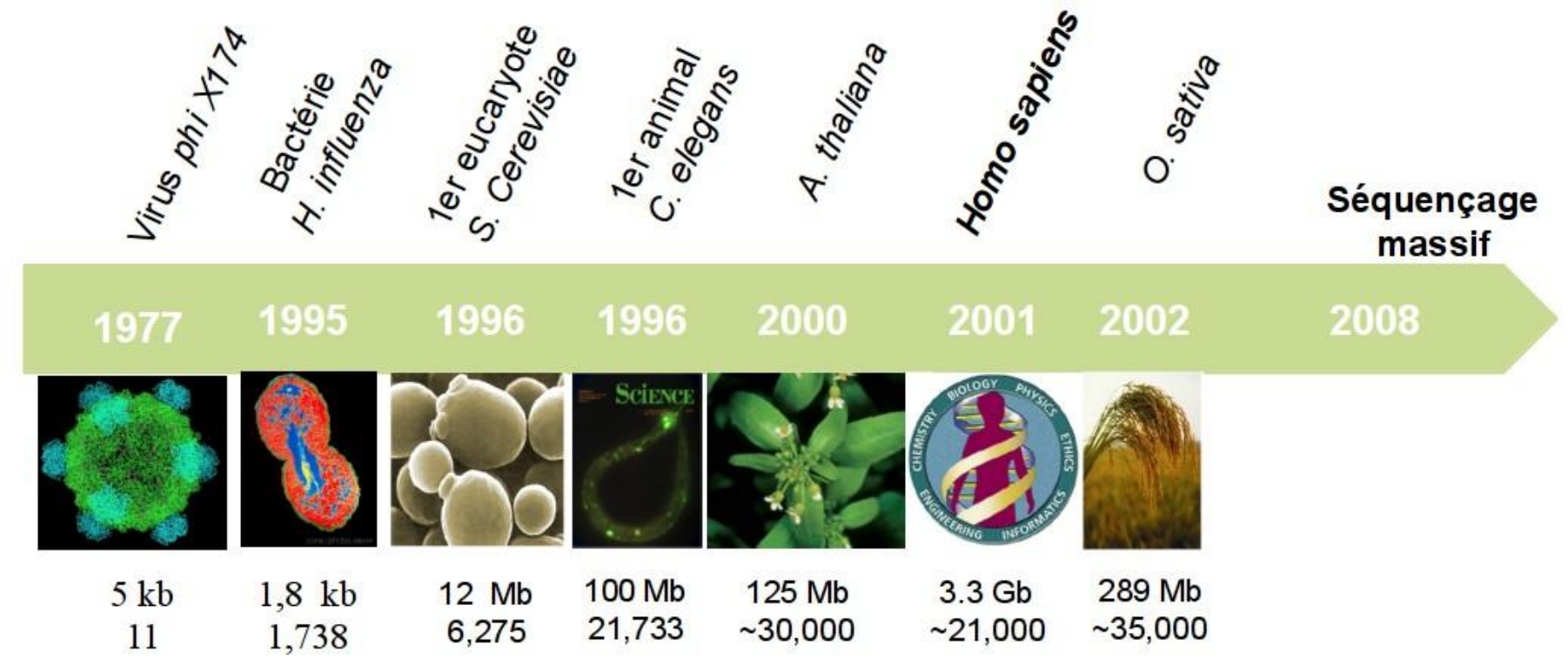




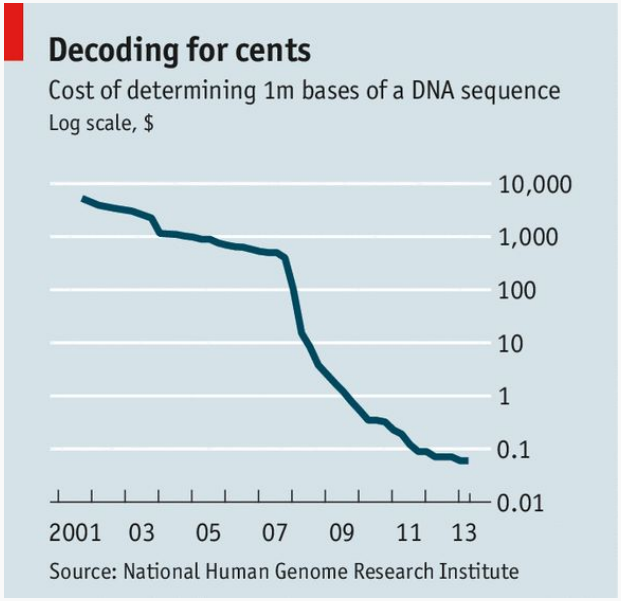
Introduction & NGS method

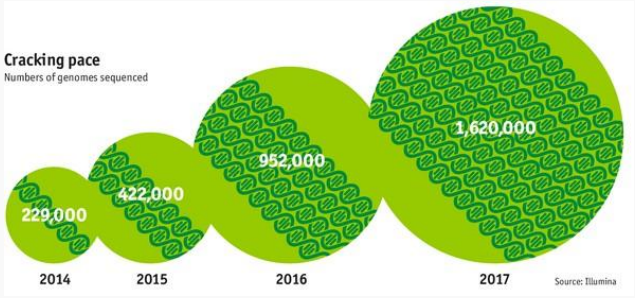
The NGS in themselves

A little history of sequencing...

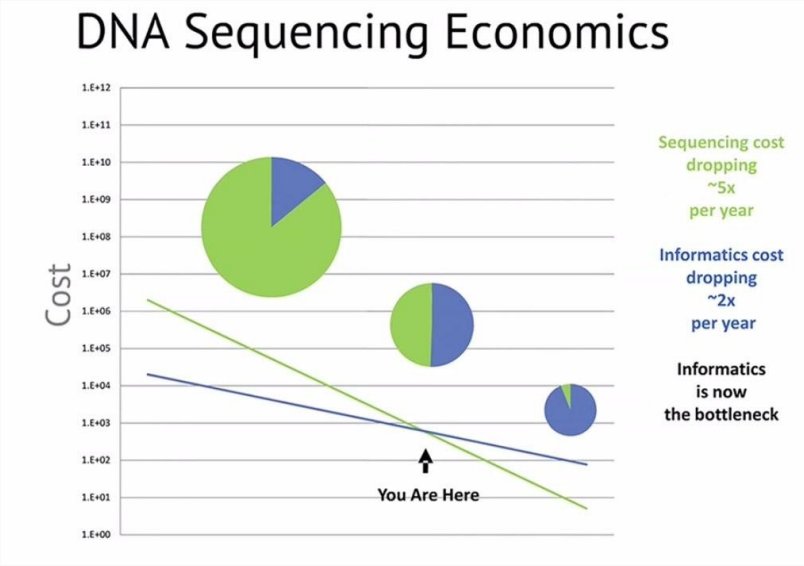


...From Data Rarity to Data Deluge



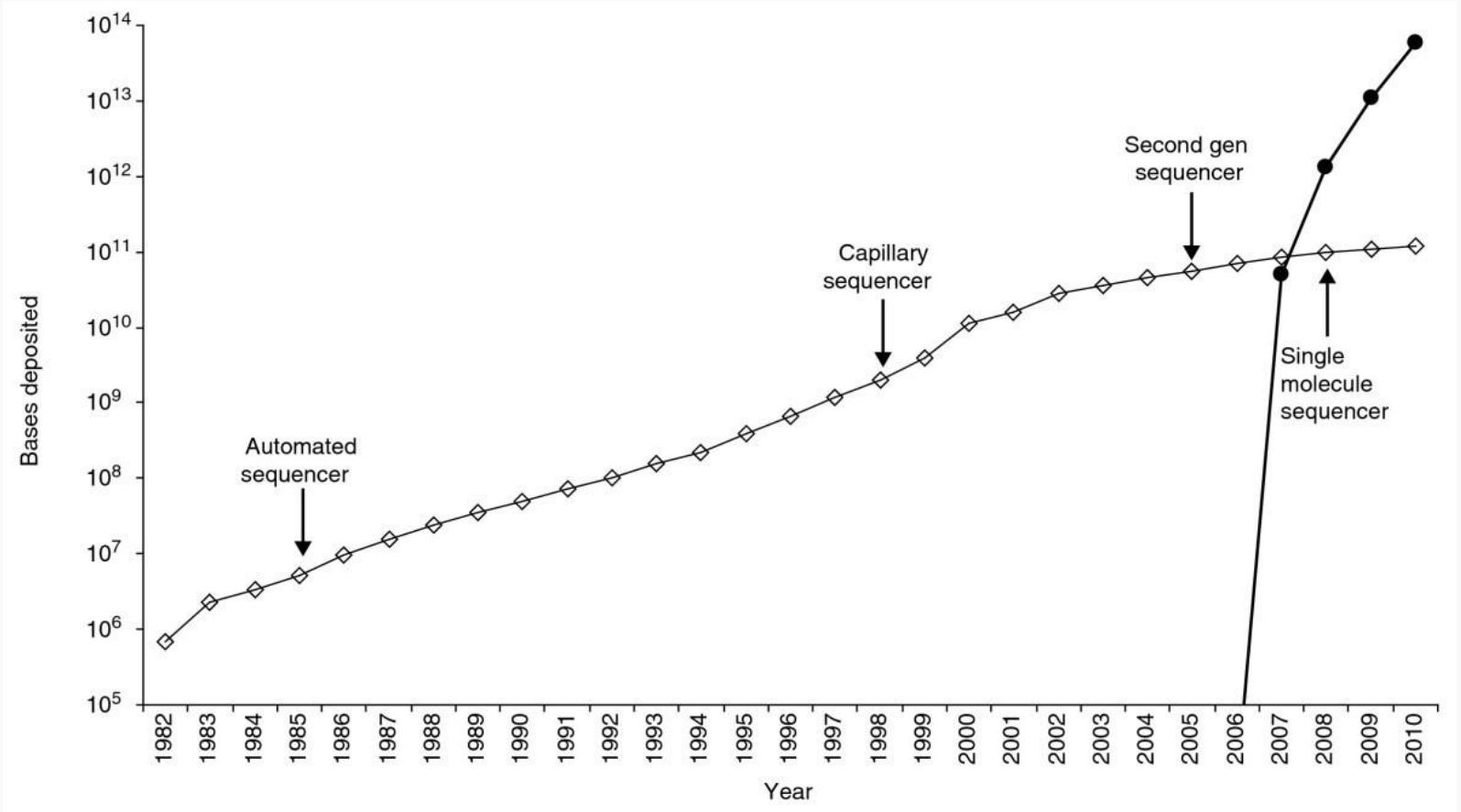


From The economist



From Bussiness Insider

...From Data Rarity to Data Deluge



What can we do with it ?

- Genetic diversity
- Gene discovery
- Genomic structure
- Contamination/pathogen detection
- Metagenomic
- Pangenomic
- And many other things...

Methods

Current Technologies

2nd Generation Sequencing

- DNA fragmentation (short)
- Matrix amplification
- Short reads
- Limited error rate
- High throughput

Current Technologies

2nd Generation Sequencing

- DNA fragmentation (short)
- Matrix amplification
- Short reads
- Limited error rate
- High throughput

3rd Generation Sequencing

- DNA fragmentation (long)
- NO MATRIX AMPLIFICATION
- Long reads
- Important error rate
- Medium throughput

Current Technologies

2nd Generation Sequencing

- DNA fragmentation (short)
- Matrix amplification
- Short reads
- Limited error rate
- High throughput

454
IonTorrent
Illumina

3rd Generation Sequencing

- DNA fragmentation (long)
- NO MATRIX AMPLIFICATION
- Long reads
- Important error rate
- Medium throughput

Current Technologies

2nd Generation Sequencing

- DNA fragmentation (short)
- Matrix amplification
- Short reads
- Limited error rate
- High throughput

454
IonTorrent
Illumina

3rd Generation Sequencing

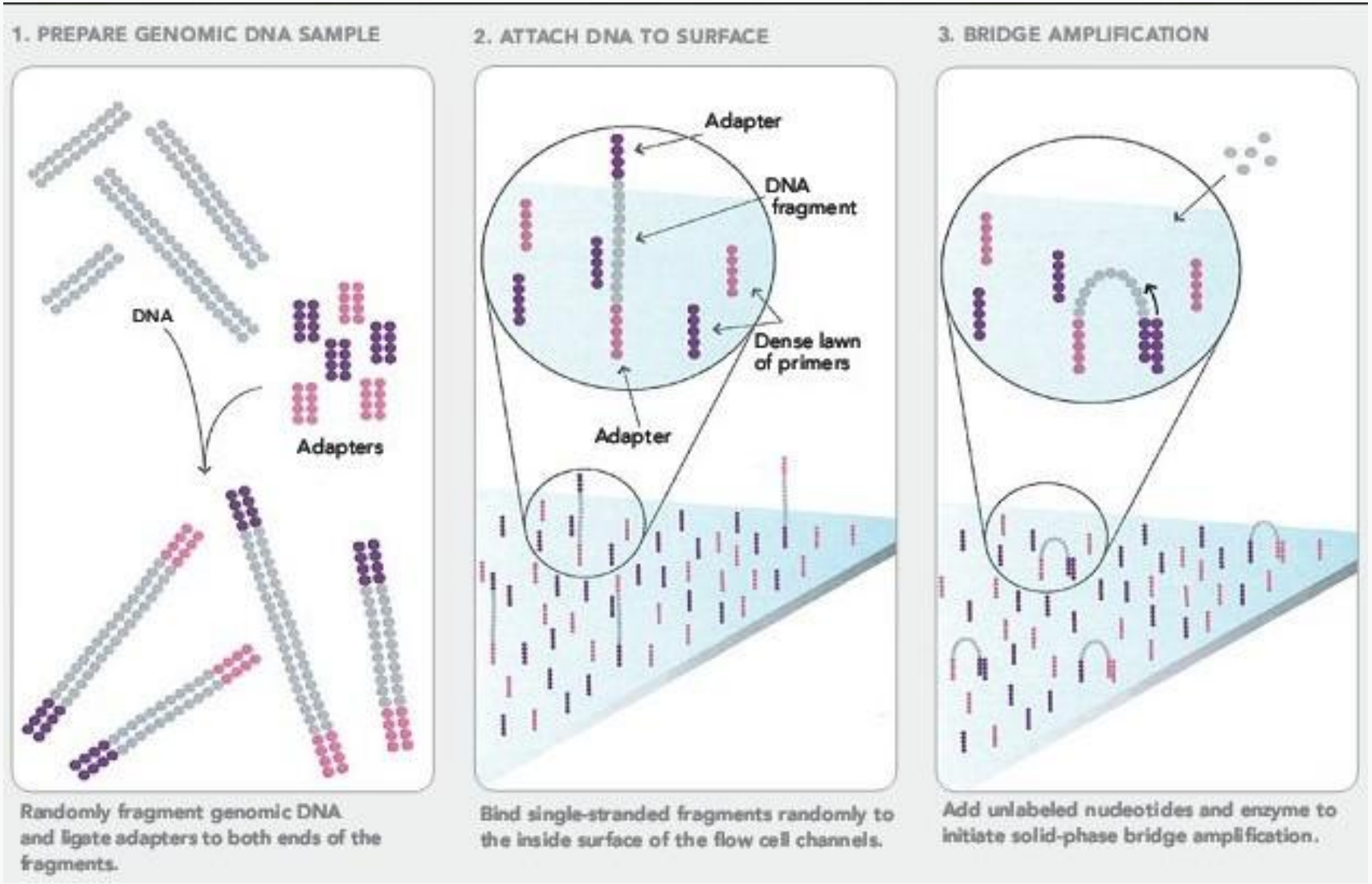
- DNA fragmentation (long)
- NO MATRIX AMPLIFICATION
- Long reads
- Important error rate
- Medium throughput

PacificBiosciences
Oxford Nanopore

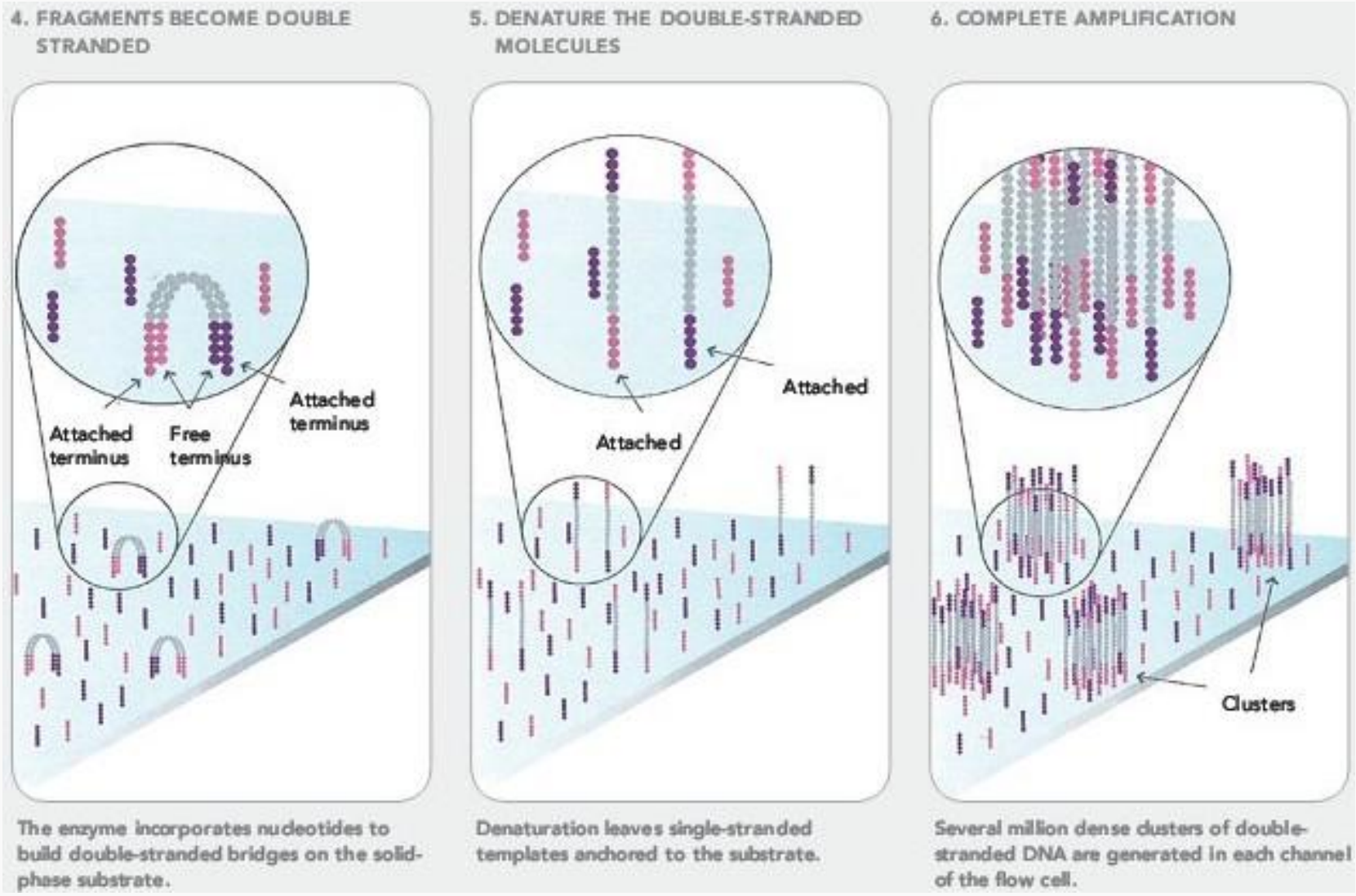
Illumina

Illumina

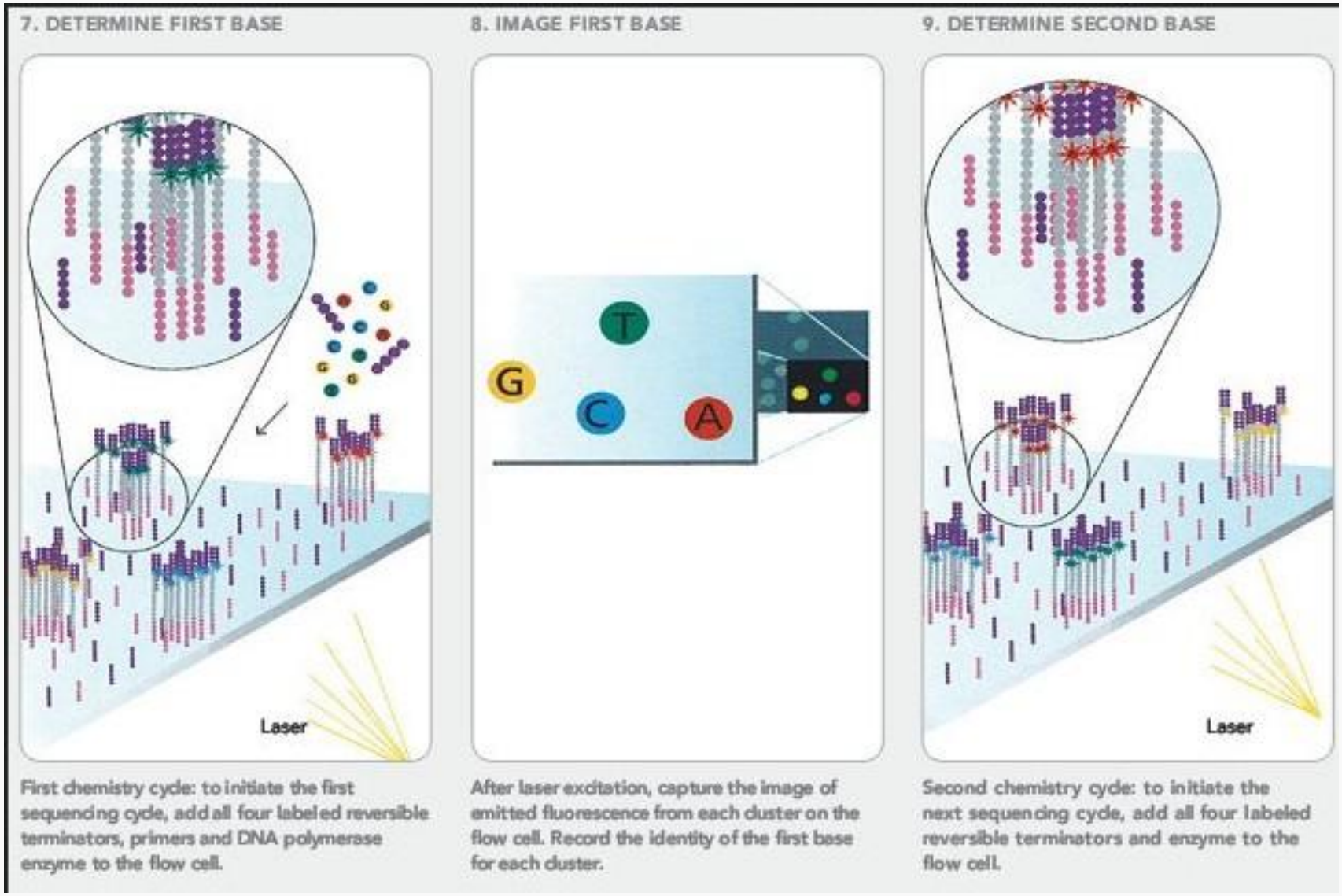
Illumina


1. PREPARE GENOMIC DNA SAMPLE
DNA
Adapters
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.
2. ATTACH DNA TO SURFACE
Adapter
DNA fragment
Dense lawn of primers
Adapter
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.
3. BRIDGE AMPLIFICATION
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

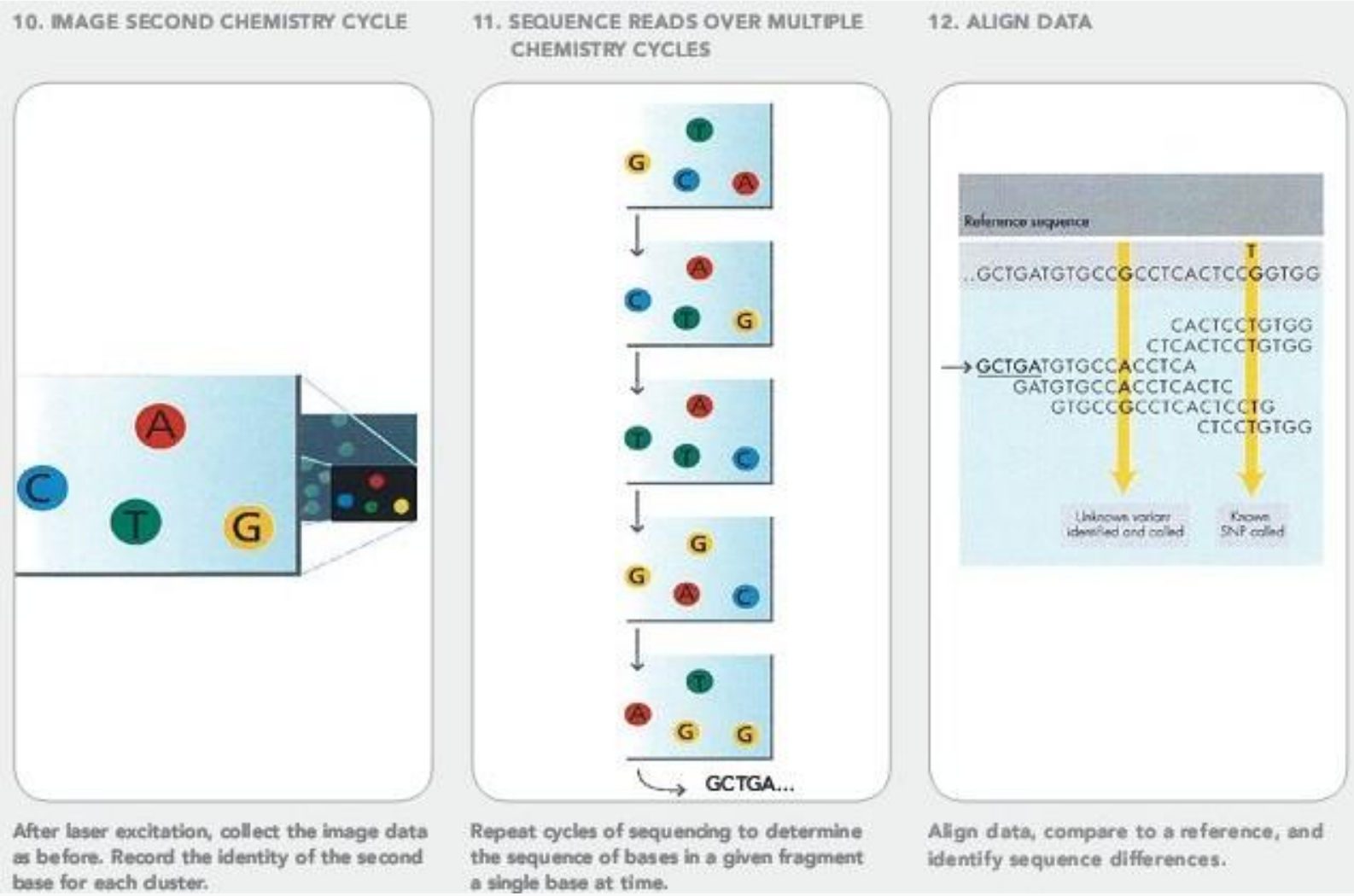
Illumina


4. FRAGMENTS BECOME DOUBLE STRANDED
5. DENATURE THE DOUBLE-STRANDED MOLECULES
6. COMPLETE AMPLIFICATION
Attached terminus
Free terminus
Attached terminus
Attached
Attached
Clusters
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.
Denaturation leaves single-stranded templates anchored to the substrate.
Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Illumina


7. DETERMINE FIRST BASE
8. IMAGE FIRST BASE
9. DETERMINE SECOND BASE
Laser
T
G
C
A
Laser
First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

Illumina


10. IMAGE SECOND CHEMISTRY CYCLE
11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES
12. ALIGN DATA
A
C
T
G
GCTGA...
Reference sequence
..GCTGATGTGCCGCCTCACTCCGGTGG
CACTCCTGTGG
CTCACTCCTGTGG
GCTGATGTGCCACCTCA
GATGTGCCACCTCACTC
GTGCCGCCTCACTCCTG
CTCCTGTGG
Unknown variant identified and called
Known SNP called
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.
Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time.
Align data, compare to a reference, and identify sequence differences.

Illumina - Advantages and Limits

Advantages :

+ Output volume (20 billions of 150b reads/6Tb, NovaSeq6000)
+ Accuracy (99.99 % - but questionable)
+ Run is cheap
+ MySeq is cheap (around 60 000 USD per machine

Limits : Size (150 + 150 in NovaSeq, but 400 for MySeq)

The FASTQ Format

```
@H4:C7C99ACXX:6:1101:1360:74584/2
CTGTTTCTTAGTATTTTTGTAGTCATTCCGTGTTGGTTTAGTTGCAAGGT
+
@@@DADFFHHFFHIIEFEIGJGGHI4FFIEIGHI<FHGAHGGGB@3?BDB9D
@H4:C7C99ACXX:6:1101:1452:19906/2
CTGAGATCAATTGGATCCTGATGATACTGTGCTTAGCTATTACCTTTGGT
+
@@@DDDD>FFFAFBEABB4C+3?:CBB@<<A?E4A???9C@CFF*9*B3D?B
@H4:C7C99ACXX:6:1101:1476:35220/2
CATGTGCTATTACCAAAAGTGCAGTAACGACCTATAAATTTTAAAGTAGC
+
@CFFFFFGGHHHHIJJJIEE<HHHIJJIGBHGGEEIIJJEIEIJIHHJFIIJJJGHJJ
@H4:C7C99ACXX:6:1101:1491:94128/2
AGAAGTCTTCGGAAAAGTTCGGGTATGGCTCTAGTAGCTTTTGTCTTAT
+
@C@FFFFFGGHHDHGIIEEHIII<CGHIJIJIIJ:?FC9DGAFGHII?DGBFIIJHBI
@H4:C7C99ACXX:6:1101:1538:34462/2
ACAAAAAGCTAAAAGAACACAGTTGCTTGAAGCAGCAAACACAAGAAC
+
B@@DFFFFGHHHHJIIIIIJJJIIGJCHHEIII>GHIG@GHIDHGJIIFIFHIJJIJJJG
@H4:C7C99ACXX:6:1101:1568:67898/2
ACAAATGGGTGTGTAAGAGTTAAAAAACAATTAATGAGCAACTGAGTTC
+
@@CFFFFFHFFHFGIJJIHHIIJJIIIHIJJJECGHIJJCHGICDGGGHJ<FGGIJJ
@H4:C7C99ACXX:6:1101:1575:18963/2
AACATGTTTGTCGGGGGTTGGGAAATTGTCACTTTCTGCTACAATGCCG
+
@<@DDDDDHFFFFDIIBDFGHHGG;FGGCHHAGGGIIH@E>AEDDEECAB>
```

1 séquence = 4 lignes

- @identifiant de la séquence
- Séquence
- + (id séquence).
- Qualité de la séquence = un caractère ASCII pour chaque base

PHRED SCORE

Séquenceur assigne à chaque base séquencée un score lié à la probabilité que la base appelée soit fausse

Ewing 1998

$$Q = -10 \log_{10} P$$

or

$$P = 10^{\frac{-Q}{10}}$$

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99,99%
50	1 in 100000	99.999 %

The QPHRED Value

Phred Quality Score

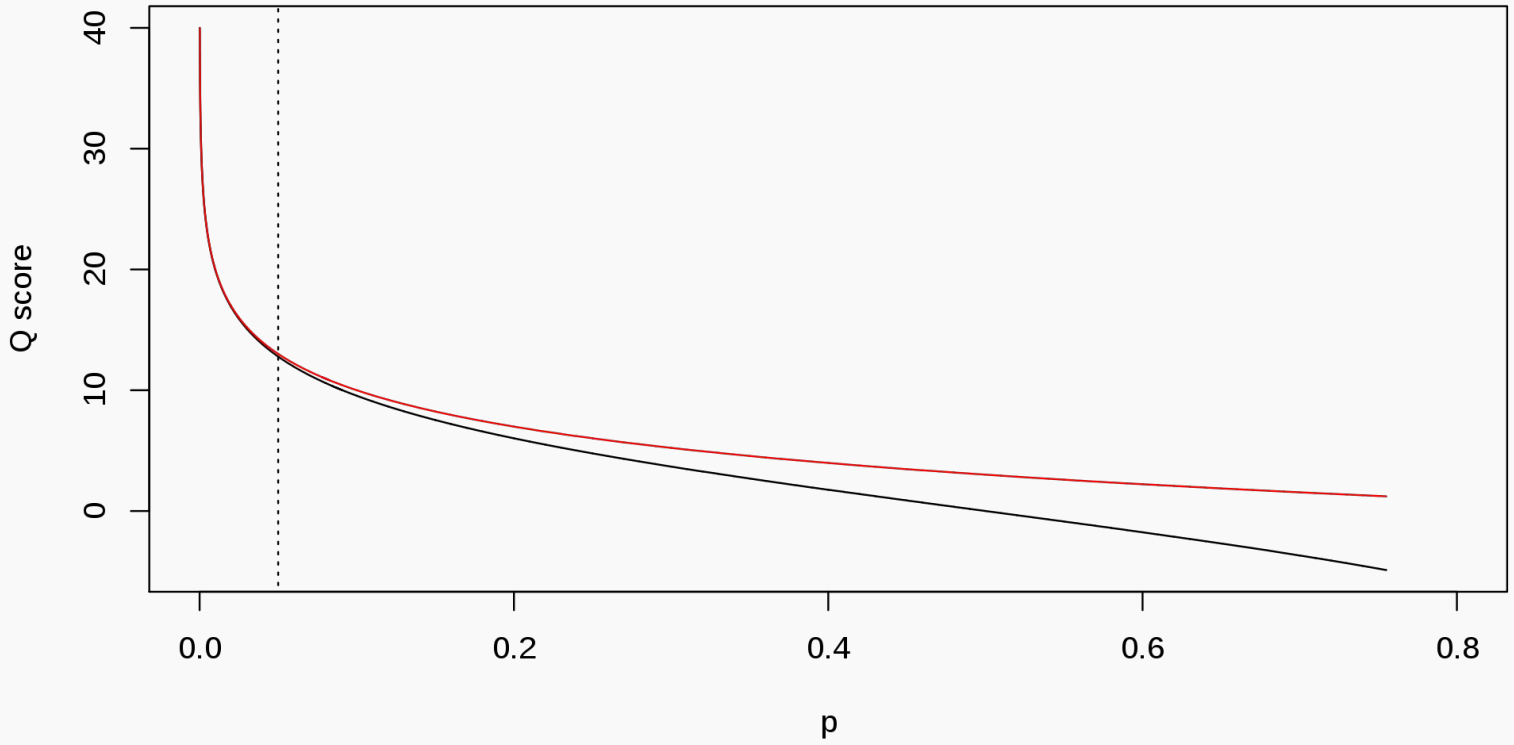
0 .. 50

Code ASCII

Dec	Hex	Oct	Chr	Dec	Hex	Oct	HTML	Chr	Dec	Hex	Oct	HTML	Chr	Dec	Hex	Oct	HTML	Chr
0	0	000	NULL	32	20	040	&#032;	Space	64	40	100	&#064;	@	96	60	140	&#096;	`
1	1	001	SoH	33	21	041	&#033;	!	65	41	101	&#065;	A	97	61	141	&#097;	a
2	2	002	SoTxt	34	22	042	&#034;	"	66	42	102	&#066;	B	98	62	142	&#098;	b
3	3	003	EoTxt	35	23	043	&#035;	#	67	43	103	&#067;	C	99	63	143	&#099;	c
4	4	004	EoT	36	24	044	&#036;	$	68	44	104	&#068;	D	100	64	144	&#100;	d
5	5	005	Enq	37	25	045	&#037;	%	69	45	105	&#069;	E	101	65	145	&#101;	e
6	6	006	Ack	38	26	046	&#038;	&	70	46	106	&#070;	F	102	66	146	&#102;	f
7	7	007	Bell	39	27	047	&#039;	'	71	47	107	&#071;	G	103	67	147	&#103;	g
8	8	010	Bsp	40	28	050	&#040;	(	72	48	110	&#072;	H	104	68	150	&#104;	h
9	9	011	HTab	41	29	051	&#041;	)	73	49	111	&#073;	I	105	69	151	&#105;	i
10	A	012	LFeed	42	2A	052	&#042;	*	74	4A	112	&#074;	J	106	6A	152	&#106;	j
11	B	013	VTab	43	2B	053	&#043;	+	75	4B	113	&#075;	K	107	6B	153	&#107;	k
12	C	014	FFeed	44	2C	054	&#044;	,	76	4C	114	&#076;	L	108	6C	154	&#108;	l
13	D	015	CR	45	2D	055	&#045;	-	77	4D	115	&#077;	M	109	6D	155	&#109;	m
14	E	016	SOut	46	2E	056	&#046;	.	78	4E	116	&#078;	N	110	6E	156	&#110;	n
15	F	017	SIn	47	2F	057	&#047;	/	79	4F	117	&#079;	O	111	6F	157	&#111;	o
16	10	020	DLE	48	30	060	&#048;	0	80	50	120	&#080;	P	112	70	160	&#112;	p
17	11	021	DC1	49	31	061	&#049;	1	81	51	121	&#081;	Q	113	71	161	&#113;	q
18	12	022	DC2	50	32	062	&#050;	2	82	52	122	&#082;	R	114	72	162	&#114;	r
19	13	023	DC3	51	33	063	&#051;	3	83	53	123	&#083;	S	115	73	163	&#115;	s
20	14	024	DC4	52	34	064	&#052;	4	84	54	124	&#084;	T	116	74	164	&#116;	t
21	15	025	NAck	53	35	065	&#053;	5	85	55	125	&#085;	U	117	75	165	&#117;	u
22	16	026	Syn	54	36	066	&#054;	6	86	56	126	&#086;	V	118	76	166	&#118;	v
23	17	027	EoTB	55	37	067	&#055;	7	87	57	127	&#087;	W	119	77	167	&#119;	w
24	18	030	Can	56	38	070	&#056;	8	88	58	130	&#088;	X	120	78	170	&#120;	x
25	19	031	EoM	57	39	071	&#057;	9	89	59	131	&#089;	Y	121	79	171	&#121;	y
26	1A	032	Sub	58	3A	072	&#058;	:	90	5A	132	&#090;	Z	122	7A	172	&#122;	z
27	1B	033	Esc	59	3B	073	&#059;	;	91	5B	133	&#091;	[	123	7B	173	&#123;	{
28	1C	034	FSep	60	3C	074	&#060;	<	92	5C	134	&#092;	\	124	7C	174	&#124;	|
29	1D	035	GSep	61	3D	075	&#061;	=	93	5D	135	&#093;	]	125	7D	175	&#125;	}
30	1E	036	RSep	62	3E	076	&#062;	>	94	5E	136	&#094;	^	126	7E	176	&#126;	~
31	1F	037	USep	63	3F	077	&#063;	?	95	5F	137	&#095;	_	127	7F	177	&#127;	Del

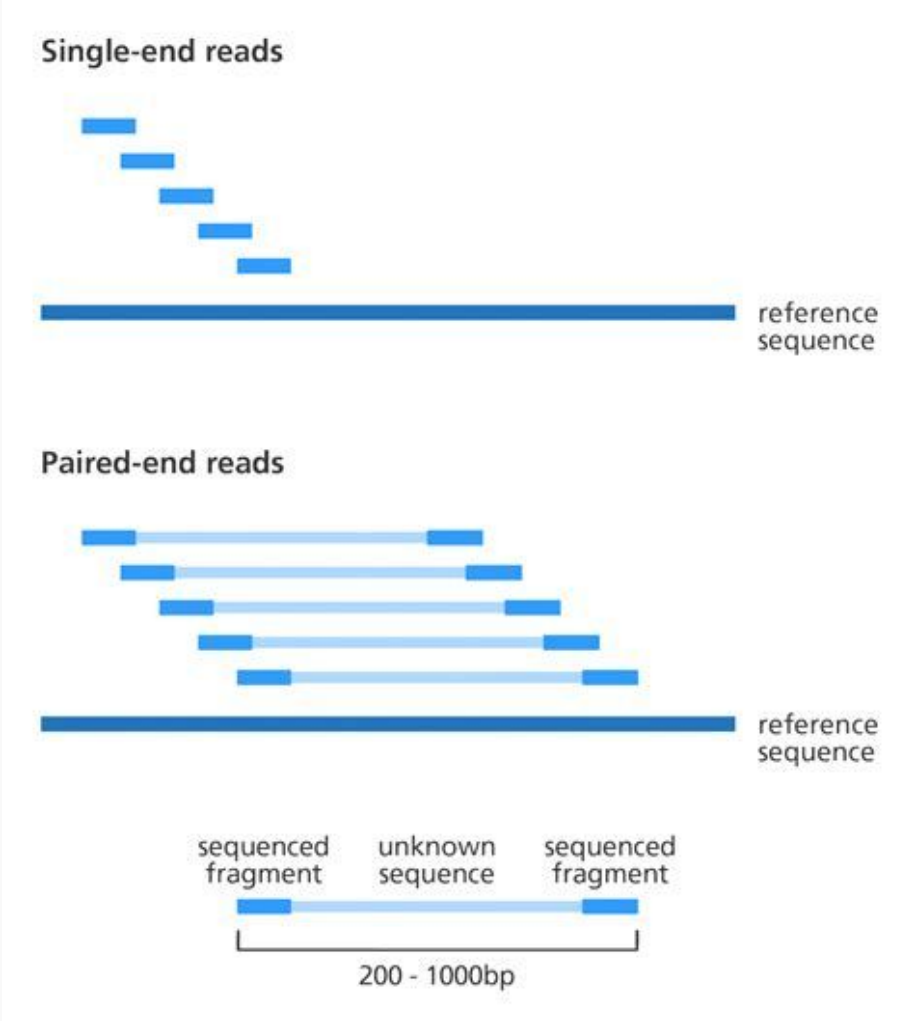
charstable.com

The QPHRED Value



The QPHRED Scale

Mapping


Single-end reads
reference sequence
Paired-end reads
reference sequence
sequenced fragment
unknown sequence
sequenced fragment
200 - 1000bp

SAM Format

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAA
r003   0 ref  9 30 5H6M       *  0   0 AGCT
r004   0 ref 16 30 6M14N5M    *  0   0 ATAG
r003  16 ref 29 30 6H5M       *  0   0 TAGG
r001  83 ref 37 30 9M         =  7 -39 CAGC
```

Header

- Ligne commençant par @

Type	Tag	Description
HD - header	VN*	File format version.
	SO	Sort order. Valid values are: *unsorted*, *queryname* or *coordinate*.
	GO	Group order (full sorting is not imposed in a group). Valid values are: *none*, *query* or *reference*.
SQ - Sequence dictionary	SN*	Sequence name. Unique among all sequence records in the file. The value of this field is used in alignment records.
	LN*	Sequence length.
	AS	Genome assembly identifier. Refers to the reference genome assembly in an unambiguous form. Example: HG18.
	M5	MD5 checksum of the sequence in the uppercase (gaps and space are removed)
	UR	URI of the sequence
	SP	Species.
RG - read group	ID*	Unique read group identifier. The value of the ID field is used in the RG tags of alignment records.
	SM*	Sample (use pool name where a pool is being sequenced)
	LB	Library
	DS	Description
	PU	Platform unit (e.g. lane for Illumina or slide for SOLiD); should be a full, unambiguous identifier
	PI	Predicted median insert size (maybe different from the actual median insert size)
	CN	Name of sequencing center producing the read.
	DT	Date the run was produced (ISO 8601 date or date/time).
	PL	Platform/technology used to produce the read.
PG - Program	ID*	Program name
	VN	Program version
	CL	Command line
CO - comment		One-line text comments

SAM Format

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA     *
r003   0 ref  9 30 5H6M       *  0   0 AGCTAA    *   NM:i:1
r004   0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       *  0   0 TAGGC     *   NM:i:0
r001  83 ref 37 30 9M         =  7 -39 CAGCGCCAT         *
```

alignement

Format tabulé

SAM format : http://samtools.sourceforge.net/samtools.shtml

SAM Format

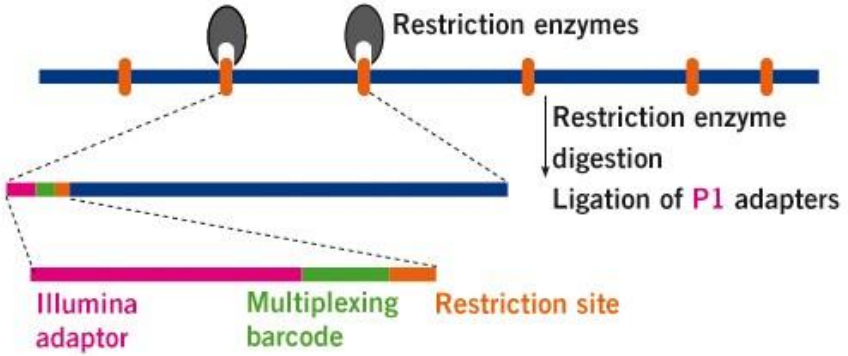
```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003   0 ref  9 30 5H6M       *  0   0 AGCTAA   *   NM:i:1
r004   0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC
r003  16 ref 29 30 6H5M       *  0   0 TAGGC    *   N
r001  83 ref 37 30 9M         =  7 -39 CAGCGCCAT
```

alignement

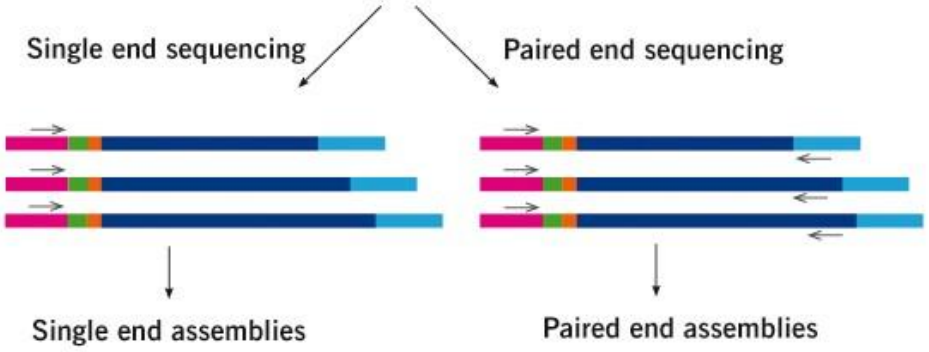
Format tabulé

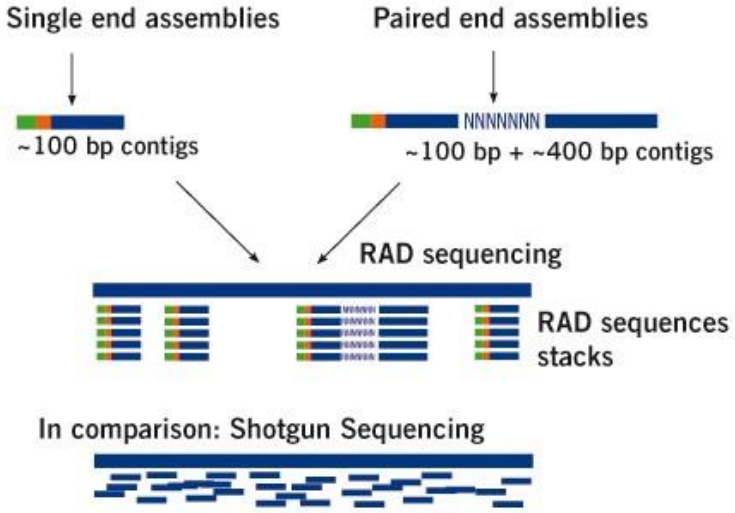
Col	Name	Description
1	QNAME	Query NAME of the read or the read pair
2	FLAG	bitwise FLAG (pairing, strand, mate strand, etc.)
3	RNAME	Reference sequence NAME
4	POS	1-based leftmost POSition of clipped alignment
5	MAPQ	MAPping Quality (Phred-scaled)
6	CIGAR	extended CIGAR string (operations: MIDNSHP)
7	NRNM	Mate Reference NaMe (`=' if same as RNAME)
8	MPOS	1-based leftmost Mate POSition
9	ISIZE	inferred Insert SIZE
10	SEQ	query SEQuence on the same strand as the reference
11	QUAL	query QUALity (ASCII-33=Phred base quality)

RAD-Seq



Pooling of samples
Shearing, Size Selection
Adapter ligation (P2)
PCR enrichement





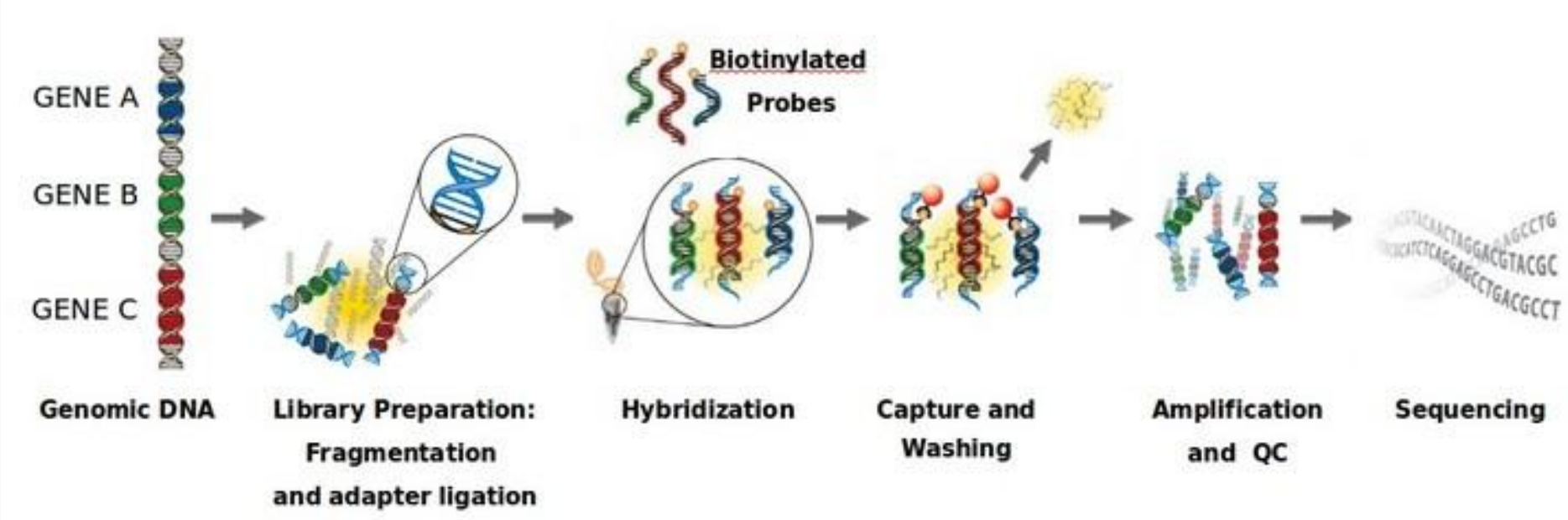
DNA is digested with a specific set of restriction enzymes and two adaptors ligated to the fragments afterwards.

Sequencing the fragments from either one side or both sides results in RAD sequences stacks.

The stacks are the perfect starting point to identify and analyse genetic markers.

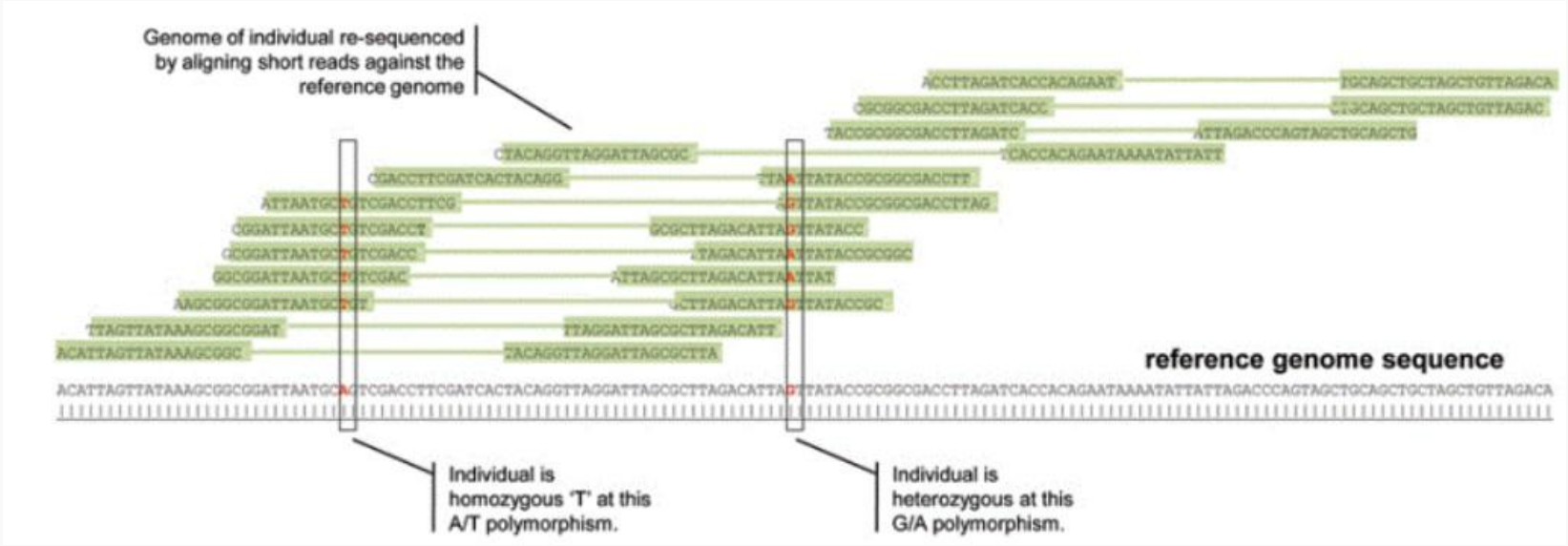
From Eurofins

CaptureSeq



From CGFB, Bordeaux, France

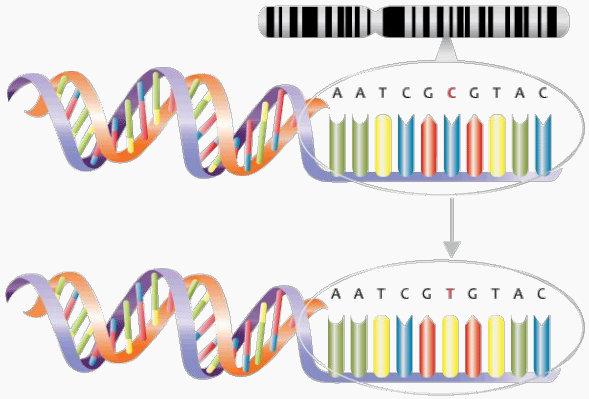
SNP and InDel Detection



SNP and InDel Detection

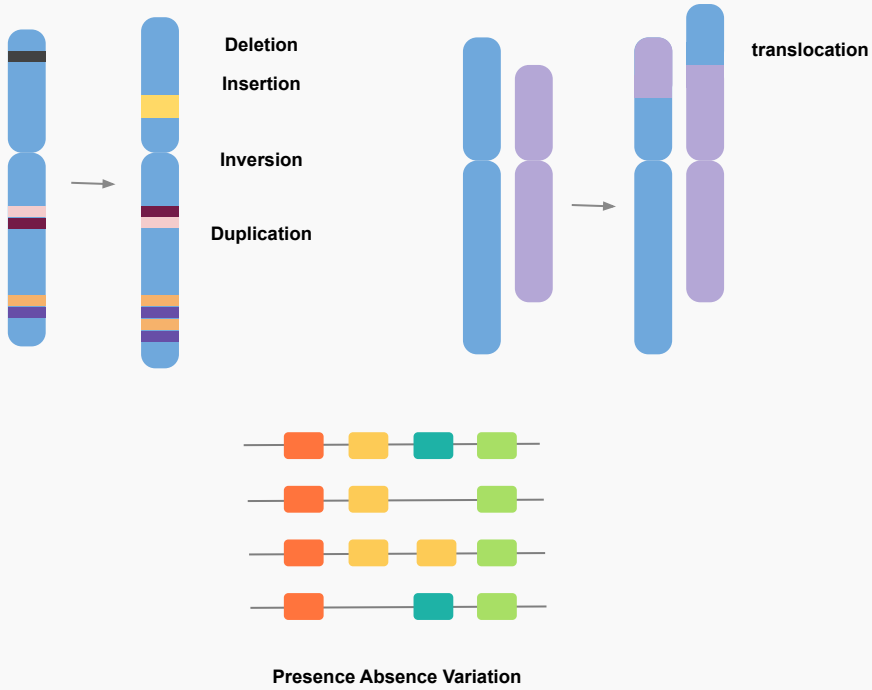
Mutations & Variations as main source of genetic diversity

SNP

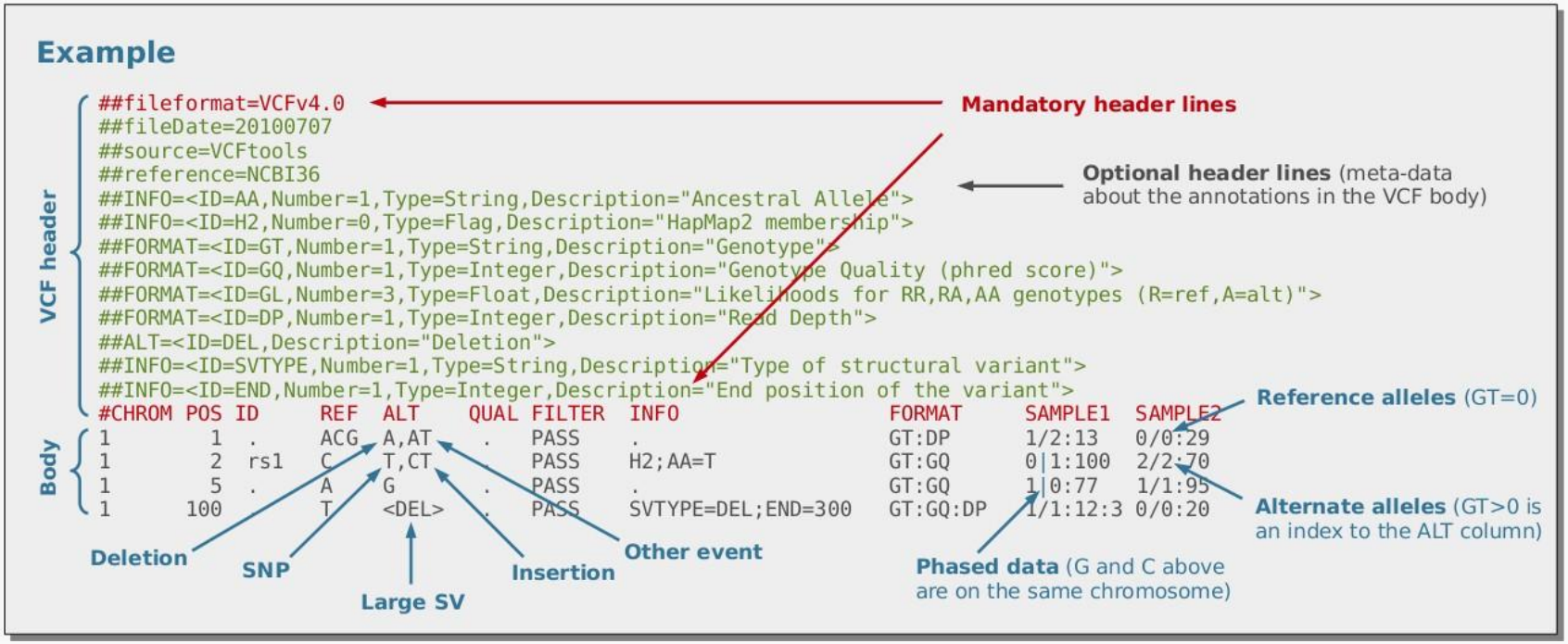


From NHS National Genetics and Genomics Education Centre, CC BY 2.0, via Wikimedia Commons

Structural Variations



Common File for all Variations, the VCF



VCF = Variant Call Format From 1000 Genomes Project

Limits, Wetlab

- Amount of original samples
- Choice of Sample
- Purity of Sample
- Size of sequenced unit
- Error rate
- Volume of Outputted data

All linked to technical constraints

Limits, Technical

- Cleaning data level
- Mapping Conditions
- Mapping Cleaning Conditions
- Variation Calling level

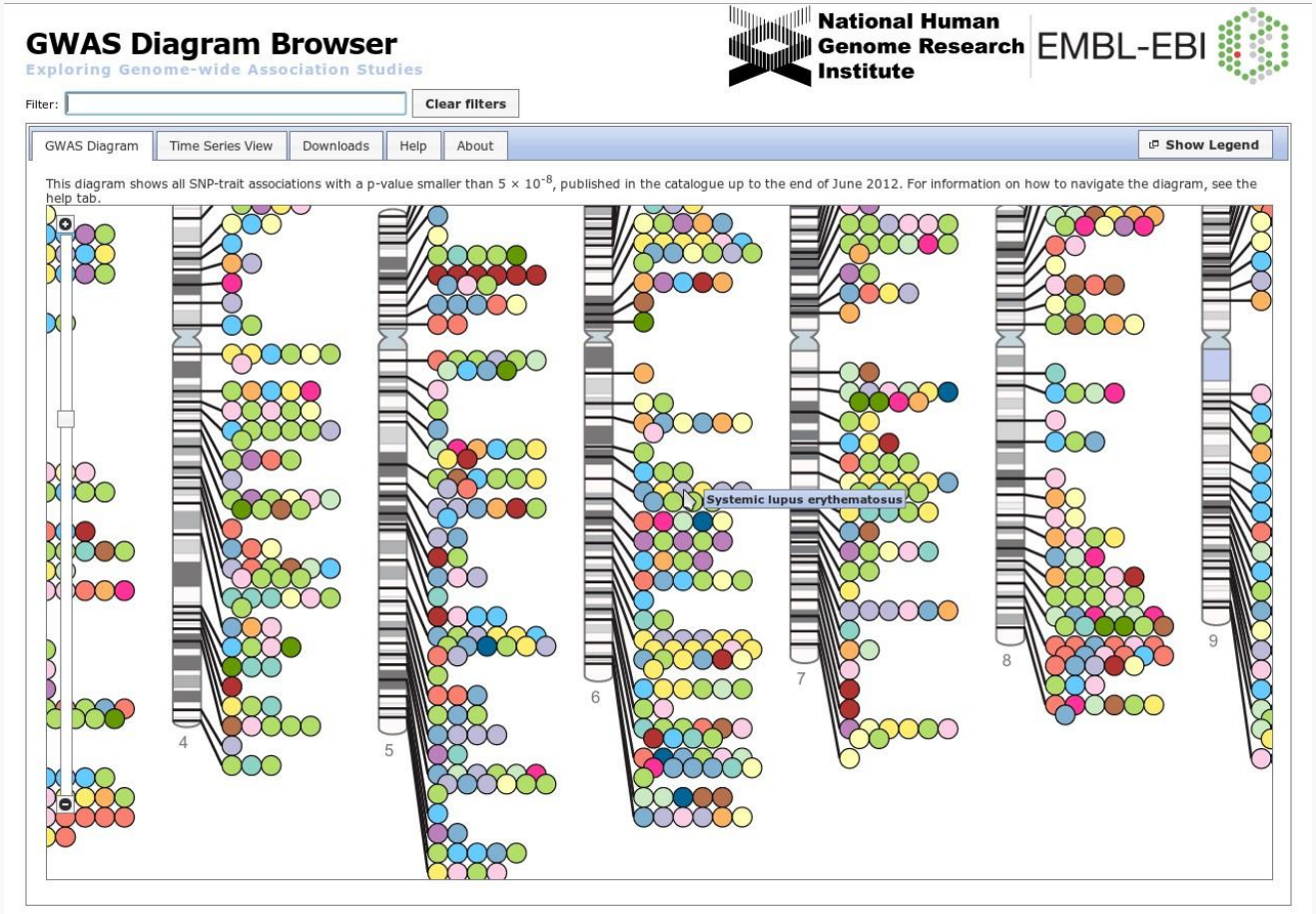
All linked to the Specificity/Sensitivity Informatics Paradox

Applications

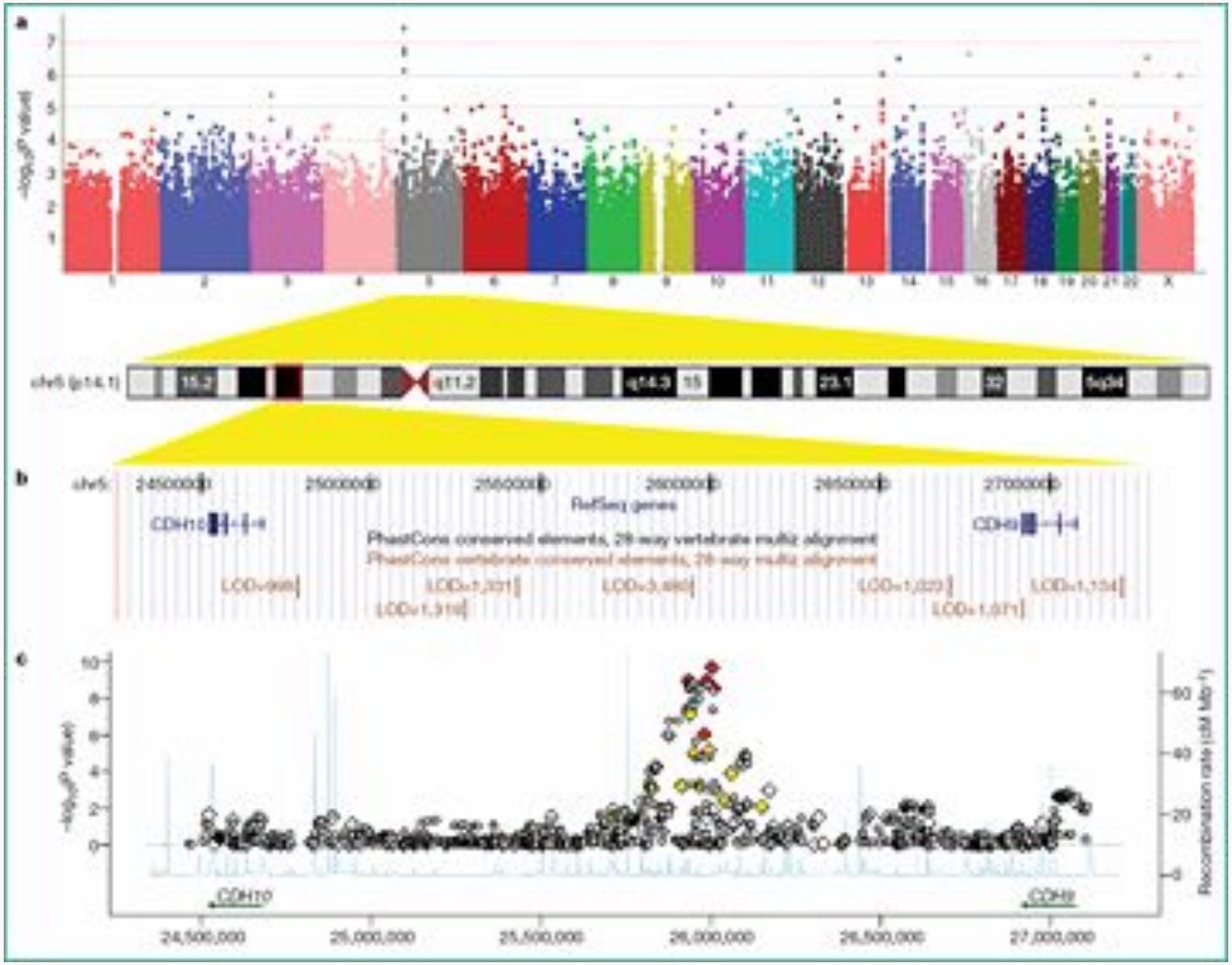
Population Genomics/Genomics

- Gene discovery/GWAs
- Species Definition
- Subspecies/specific subgroup definition
- Global genotyping (for breeding in agriculture e.g.)
- Genomic Ecology (Transposable elements, etc...)

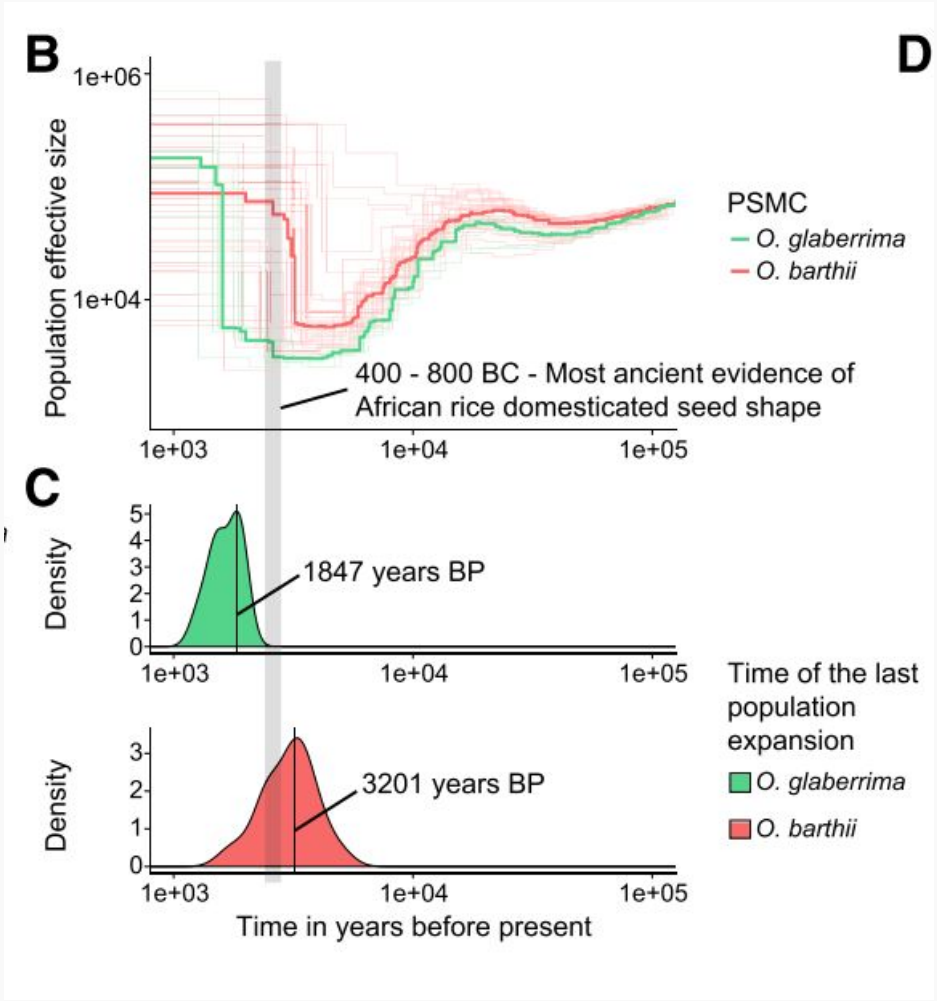
Example in GWAs & Population Genomics

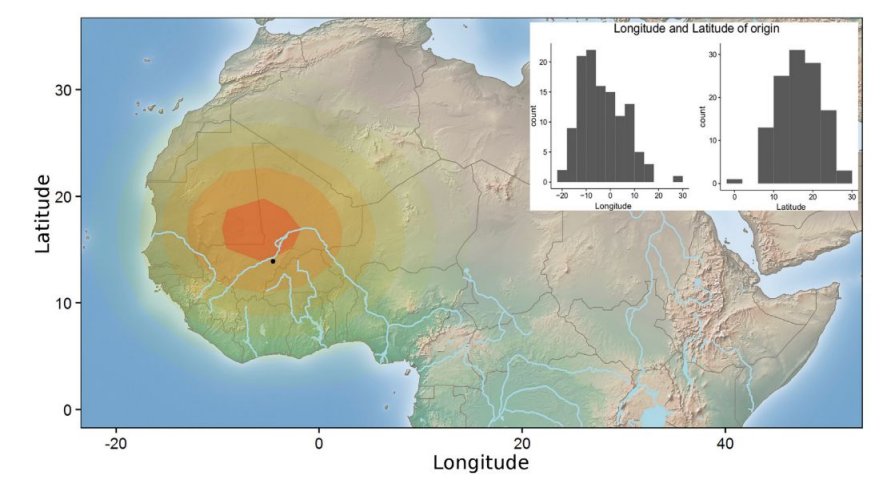


Example in GWAs & Population Genomics



Example in Global Genotyping & Population Genomics





The Rise and Fall of African Rice Cultivation Revealed by Analysis of 246 New Genomes

From Cubry et al, 2018

Large Projects

Sample types

- DNA from plant, animal, microbial...
- Organite DNA (mitochondria, chloroplast)
- Subsample DNA (exon capture, 16S capture for Barcoding)
- Viral sample from infected tissue
- Environmental sample: water, feces, cloud...

Possibilities in the next 5-10 years (From a presentation in 2013)

- Real-time Transcriptomics

Possibilities in the next 5-10 years (From a presentation in 2013)

- Real-time Transcriptomics
- Single-Cell Genomics -> DONE in 2014

Possibilities in the next 5-10 years (From a presentation in 2013)

- Real-time Transcriptomics
- Single-Cell Genomics -> DONE in 2014
- Single-Cells Transcriptomics (and smallRNA) -> DONE in 2015

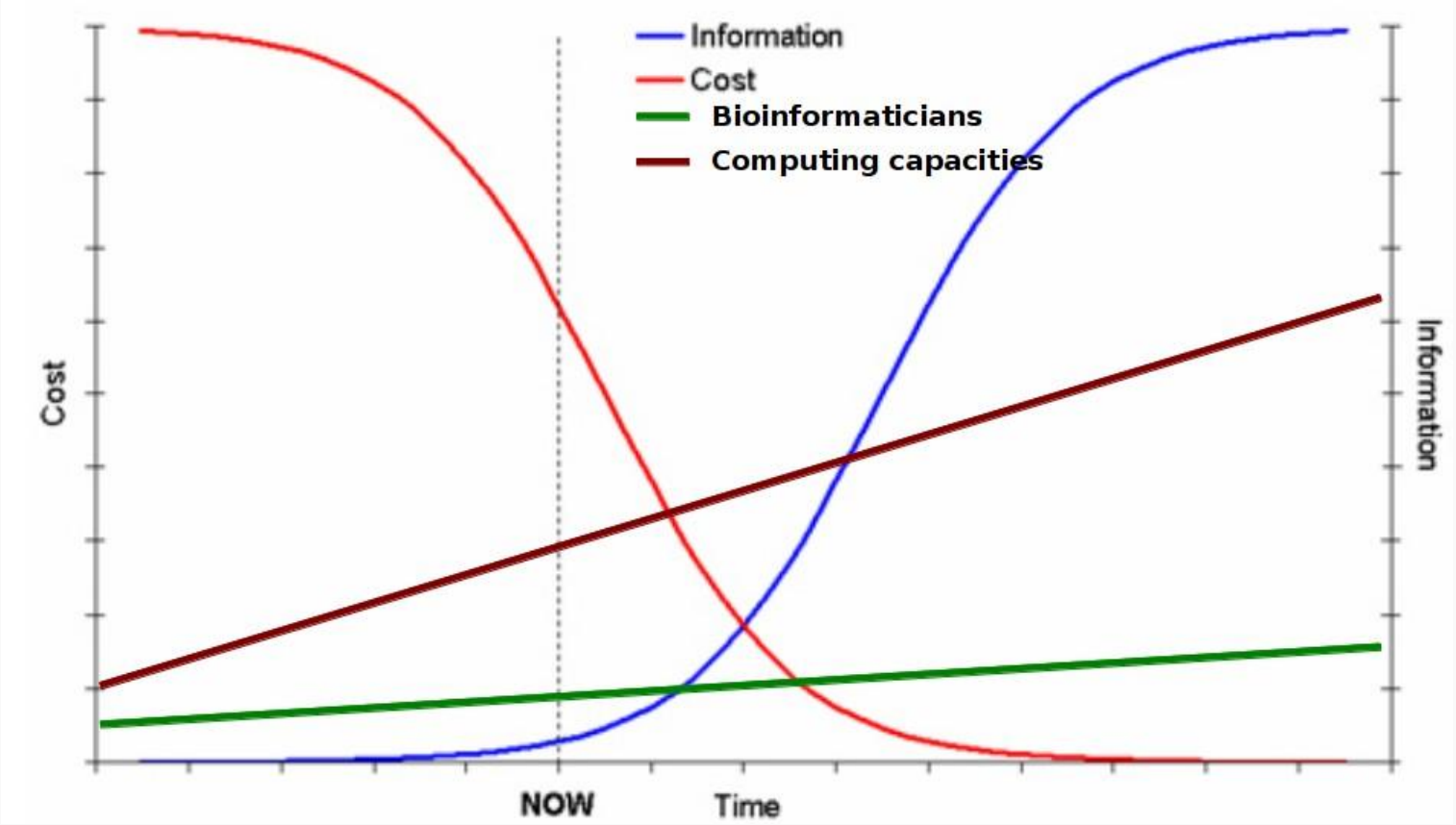
Possibilities in the next 5-10 years (From a presentation in 2013)

- Real-time Transcriptomics
- Single-Cell Genomics -> DONE in 2014
- Single-Cells Transcriptomics (and smallRNA) -> DONE in 2015
- Personal Genomics medicine (ethical problems...) -> Available

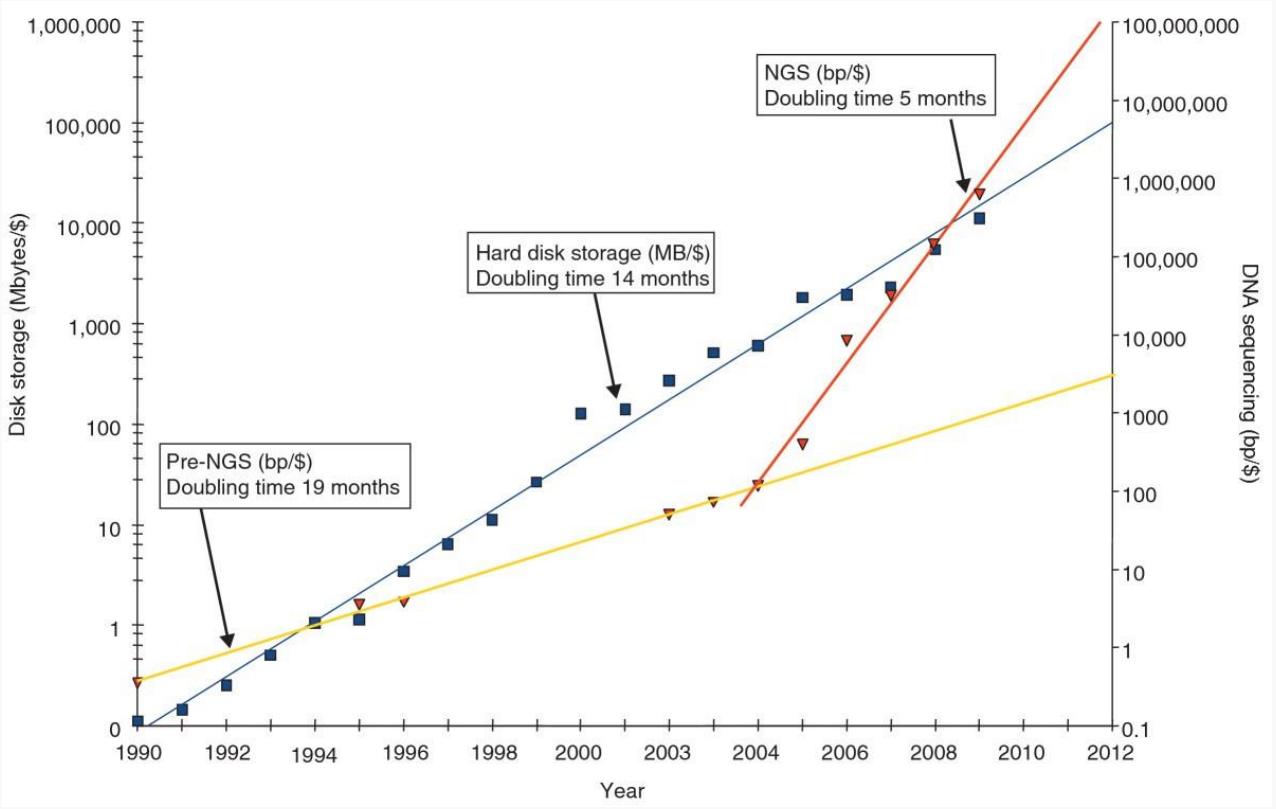
Possibilities in the next 5-10 years (From a presentation in 2013)

- Real-time Transcriptomics
- Single-Cell Genomics -> DONE in 2014
- Single-Cells Transcriptomics (and smallRNA) -> DONE in 2015
- Personal Genomics medicine (ethical problems...) -> Available
- And any new ideas you will have...

Keep in mind!

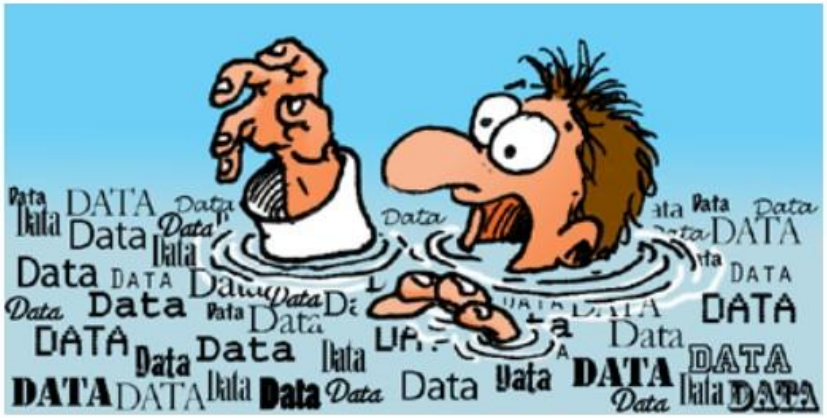

Information
Cost
Bioinformaticians
Computing capacities
Cost
Information
NOW
Time

...From Data Rarity to Data Deluge

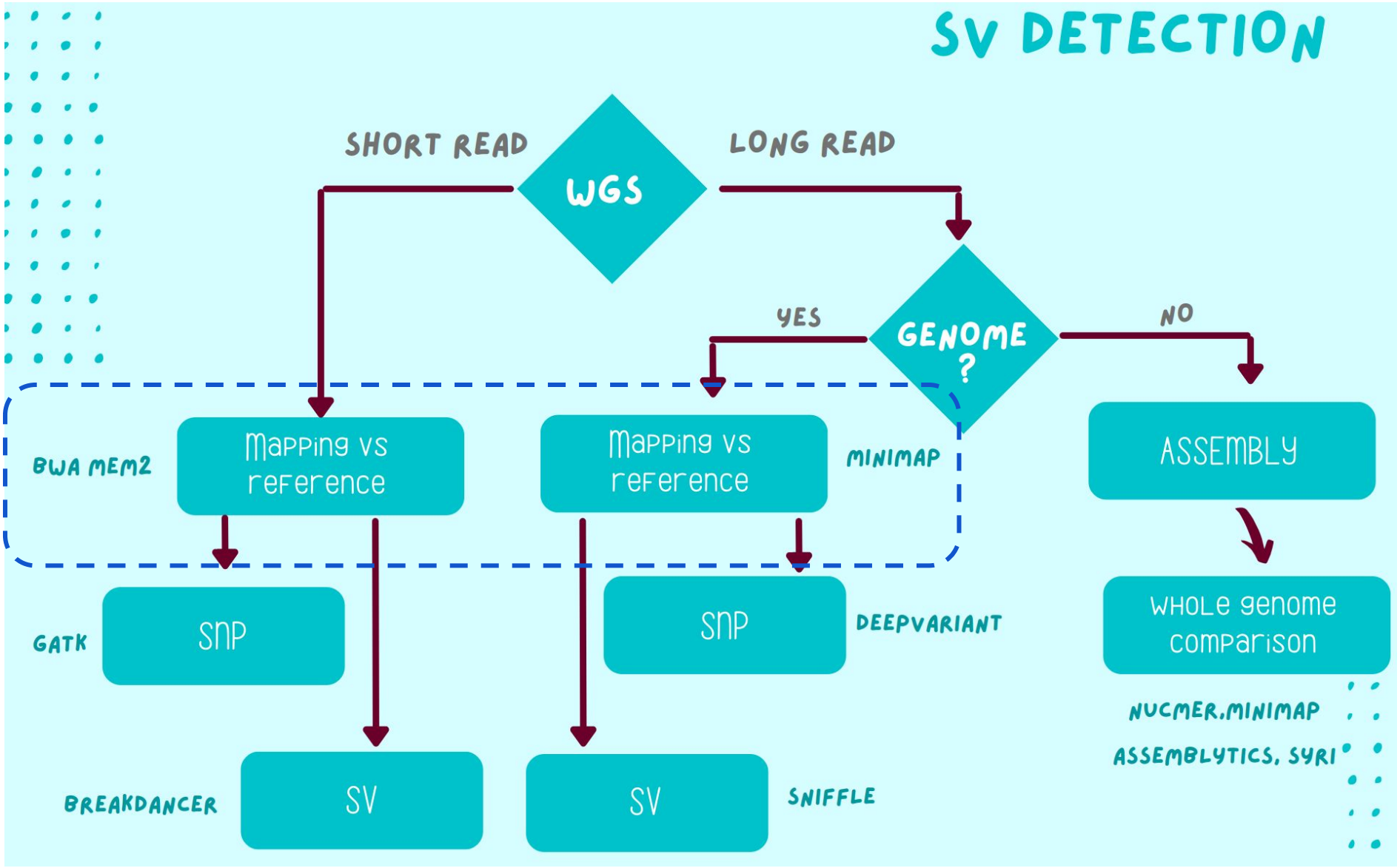


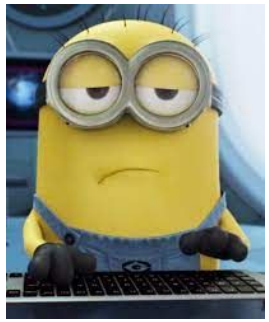
From L. Stein, 2010

Be Careful to data drowning!



Training plan - day 1





Mapping and SNP

TP goal

- Quality control of NGS data
- Learn to manipulate NGS data
- Having a critical look on *Mapping*
- Learn to launch a *Calling* and having a critical look

The data

Diploid Asian Rice, *Oryza*

From
Wikimedia

The data

Diploid Asian Rice, *Oryza*

From
Wikimedia

1. Select/Cut 1 Mb on Chromosome 10

The data

Diploid Asian Rice, *Oryza*

From
Wikimedia

1. Select/Cut 1 Mb on Chromosome 10
2. Create 20 exact clones

The data

Diploid Asian Rice, *Oryza*

From
Wikimedia

1. Select/Cut 1 Mb on Chromosome 10
2. Create 20 exact clones
3. Introduce
    - SNP (1-10%),
    - indel (10b-10kb),
    - duplications...

The data

Diploid Asian Rice, *Oryza*

From
Wikimedia

1. Select/Cut 1 Mb on Chromosome 10
2. Create 20 exact clones
3. Introduce
    - SNP (1-10%),
    - indel (10b-10kb),
    - duplications...
4. Generate short & long reads for each clone...

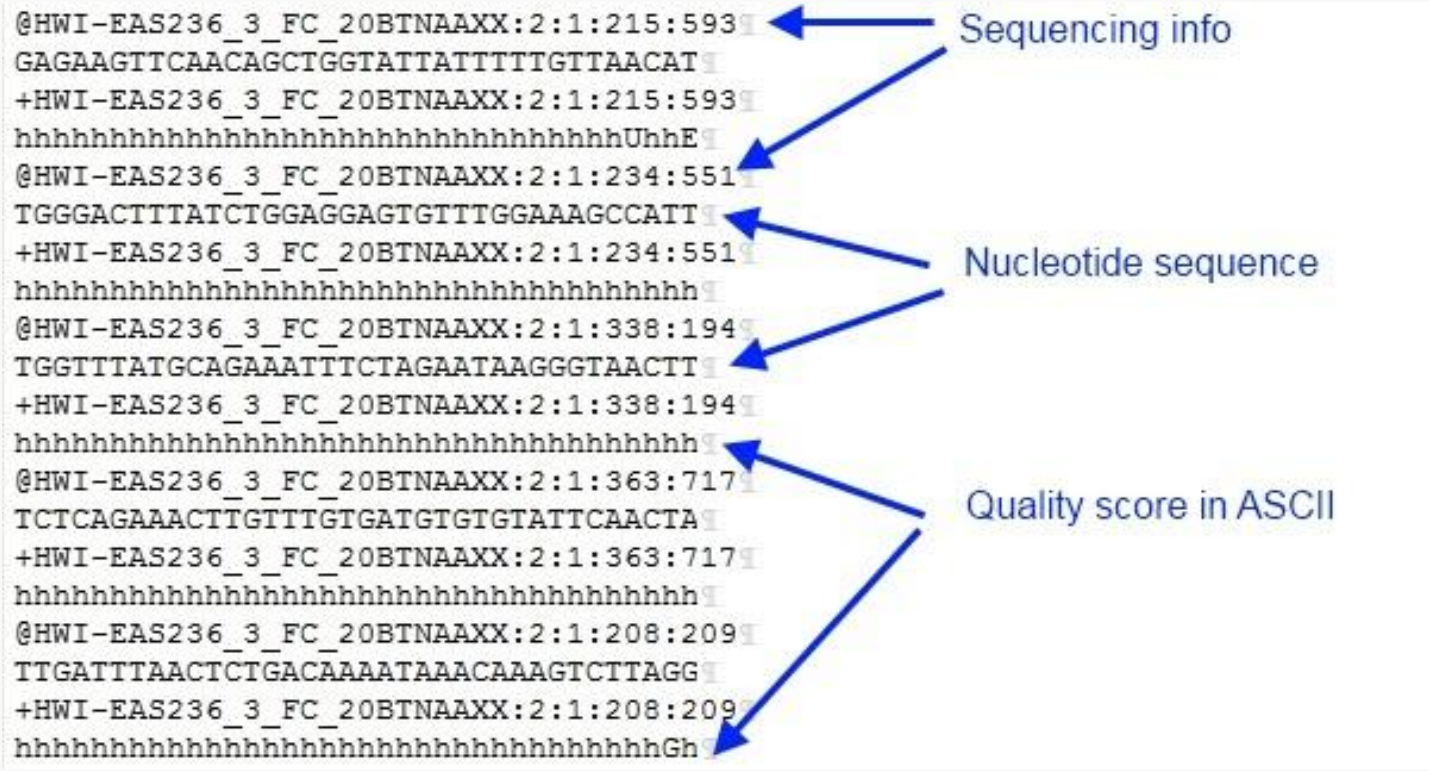
The data

Diploid Asian Rice, *Oryza*

From
Wikimedia

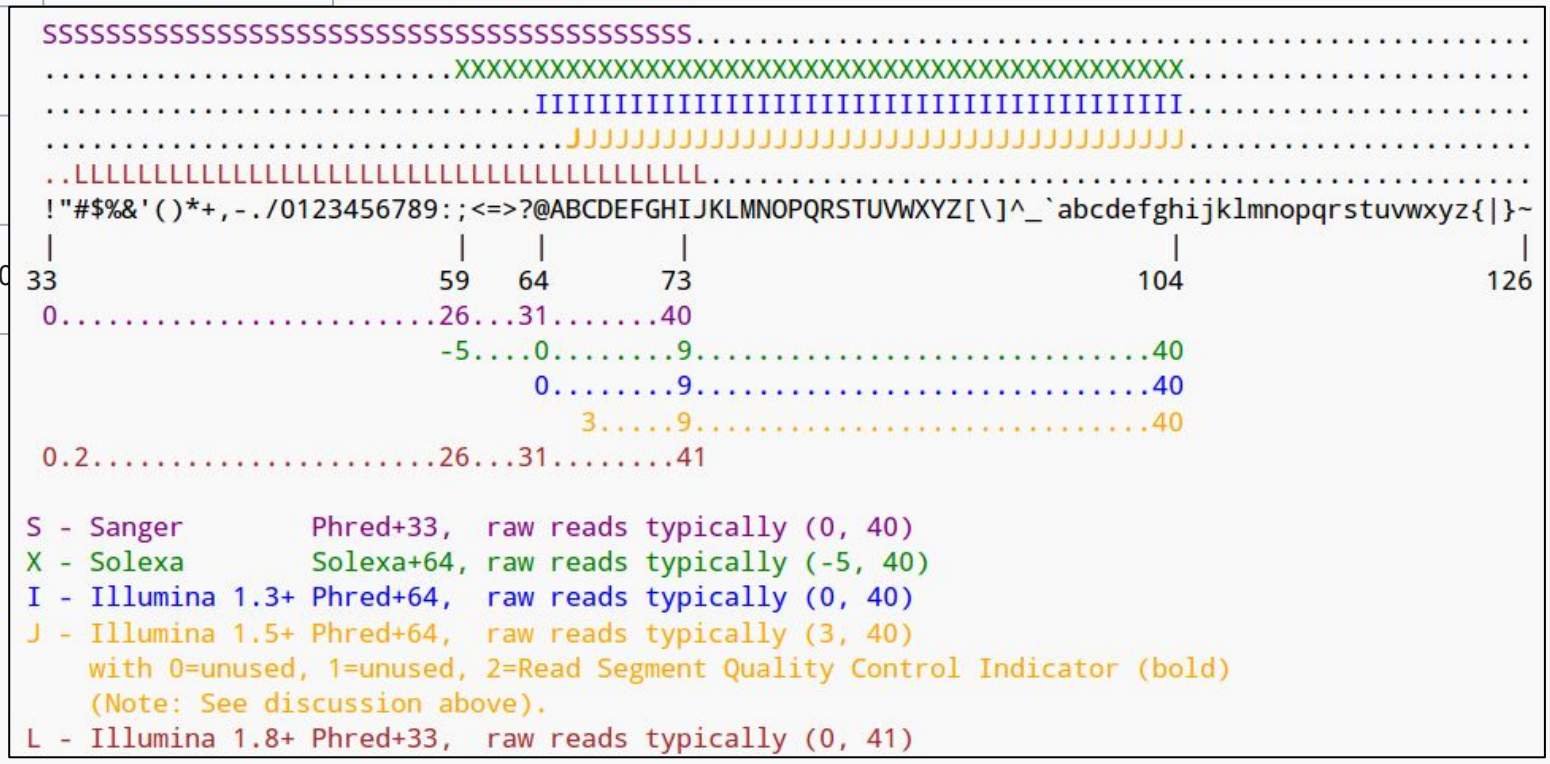
1. Select/Cut 1 Mb on Chromosome 10
2. Create 20 exact clones
3. Introduce
    - SNP (1-10%),
    - indel (10b-10kb),
    - duplications...
4. Generate short & long reads for each clone...
5. ~~Torturing students with these data~~

The FASTQ Format



The QPHRED Scale

Score qualité phred	Proba. d'une identification incorrecte	Précision de l'identification
10	1 pour 10	90 %
20	1 pour 100	99 %
30	1 pour 1000	
40	1 pour 10000	
50	1 pour 100000	



List of analyses

"Classic" launch

1. *Mapping*: bwa aln/sampe, bwa mem, bowtie2, ...

List of analyses

"Classic" launch

1. *Mapping*: bwa aln/sampe, bwa mem, bowtie2, ...
2. *Cleaning mapping*: samtools, picard-tools,...

List of analyses

"Classic" launch

1. *Mapping*: bwa aln/sampe, bwa mem, bowtie2, ...
2. *Cleaning mapping*: samtools, picard-tools,...
3. *Realigning and Duplicates*: GATK, picard-tools,...
   OPTIONAL!

List of analyses

"Classic" launch

1. *Mapping*: bwa aln/sampe, bwa mem, bowtie2, ...
2. *Cleaning mapping*: samtools, picard-tools,...
3. *Realigning and Duplicates*: GATK, picard-tools,...
   OPTIONAL!
4. *SNP calling and Cleaning*: GATK,...

List of analyses

"Classic" launch

1. *Mapping*: bwa aln/sampe, bwa mem, bowtie2, ...
2. *Cleaning mapping*: samtools, picard-tools,...
3. *Realigning and Duplicates*: GATK, picard-tools,...
   OPTIONAL!
4. *SNP calling and Cleaning*: GATK,...

Between 8 and 15 different commands...

Let’s do it by hands...

We will

1. Map the data of Clone 1 on *reference.fasta* using *bwa*

Let's do it by hands...

We will

1. Map the data of Clone 1 on *reference.fasta* using *bwa*
2. Look at the SAM file
3.
4.
5.
6.

SAM file format for Sequence Alignment Map

SAM format :

http://samtools.sourceforge.net/samtools.shtml

Type	Tag	Description
HD - header	VN*	File format version.
	SO	Sort order. Valid values are: *unsorted*, *queryname* or *coordinate*.
	GO	Group order (full sorting is not imposed in a group). Valid values are: *none*, *query* or *reference*.
SQ - Sequence dictionary	SN*	Sequence name. Unique among all sequence records in the file. The value of this field is used in alignment records.
	LN*	Sequence length.
	AS	Genome assembly identifier. Refers to the reference genome assembly in an unambiguous form. Example: HG18.
	M5	MD5 checksum of the sequence in the uppercase (gaps and space are removed)
	UR	URI of the sequence
	SP	Species.
RG - read group	ID*	Unique read group identifier. The value of the ID field is used in the RG tags of alignment records.
	SM*	Sample (use pool name where a pool is being sequenced)
	LB	Library
	DS	Description
	PU	Platform unit (e.g. lane for Illumina or slide for SOLiD); should be a full, unambiguous identifier
	PI	Predicted median insert size (maybe different from the actual median insert size)
	CN	Name of sequencing center producing the read.
	DT	Date the run was produced (ISO 8601 date or date/time).
	PL	Platform/technology used to produce the read.
PG - Program	ID*	Program name
	VN	Program version
	CL	Command line
CO - comment		One-line text comments

SAM file format for Sequence Alignment Map

SAM format :

http://samtools.sourceforge.net/samtools.shtml

Col	Name	Description
1	QNAME	Query NAME of the read or the read pair
2	FLAG	bitwise FLAG (pairing, strand, mate strand, etc.)
3	RNAME	Reference sequence NAME
4	POS	1-based leftmost POSition of clipped alignment
5	MAPQ	MAPping Quality (Phred-scaled)
6	CIGAR	extended CIGAR string (operations: MIDNSHP)
7	NRNM	Mate Reference NaMe (`=' if same as RNAME)
8	MPOS	1-based leftmost Mate POSition
9	ISIZE	inferred Insert SIZE
10	SEQ	query SEQuence on the reference
11	QUAL	query QUALity (ASCII-33

```
@HD VN:1.3 SO:coordinate
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002   0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003   0 ref  9 30 5H6M       *  0   0 AGCTAA    *   NM:i:1
r004   0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       *  0   0 TAGGC     *   NM:i:0
r001  83 ref 37 30 9M         =  7 -39 CAGCGCCAT         *
```

the SAM bitwise FLAG

SAM format: FLAG field

numeric	binary	description
1	00000001	template has multiple fragments in sequencing
2	00000010	each fragment properly mapped according to aligner
4	00000100	fragment is unmapped
8	00001000	mate is unmapped
16	00010000	sequence is reverse complemented
32	00100000	sequence of mate is reversed
64	01000000	is first fragment in template
128	10000000	is second fragment in template

From J. Aerts, in SlideShare

Let’s do it by hands...

We will

1. Map the data of Clone 1 on *reference.fasta* using *bwa*
2. Look at the SAM file
3. Compress SAM in BAM and reorder it

Let’s do it by hands...

We will

1. Map the data of Clone 1 on *reference.fasta* using *bwa*
2. Look at the SAM file
3. Compress SAM in BAM and reorder it
4. Remove wrong mapping

Let’s do it by hands...

We will

1. Map the data of Clone 1 on *reference.fasta* using *bwa*
2. Look at the SAM file
3. Compress SAM in BAM and reorder it
4. Remove wrong mapping
5. Mark the duplicates

Let’s do it by hands...

We will

1. Map the data of Clone 1 on *reference.fasta* using *bwa*
2. Look at the SAM file
3. Compress SAM in BAM and reorder it
4. Remove wrong mapping
5. Mark the duplicates
6. Call SNP on this individual

Practice

Let’s work with the jupyter book :

Day1_Mapping_Practice_EMPTY.ipynb

Observing mapping

- Download Tablet (use Google and Tablet+NGS)

Observing mapping

- Download Tablet (use Google and Tablet+NGS)
- Transfer the BAM from Clone 10 and the reference from the machine to your local computer (use scp or direct download from the browser)

Observing mapping

- Download Tablet (use Google and Tablet+NGS)

Observing mapping

- Download Tablet (use Google and Tablet+NGS)
- Transfer the BAM from Clone 10 and the reference from the machine to your local computer (use scp or direct download from the browser)

Observing mapping

- Download Tablet (use Google and Tablet+NGS)
- Transfer the BAM from Clone 10 and the reference from the machine to your local computer (use scp or direct download from the browser)
- Open Tablet, load an assembly

Observing mapping

- Download Tablet (use Google and Tablet+NGS)
- Transfer the BAM from Clone 10 and the reference from the machine to your local computer (use scp or direct download from the browser)
- Open Tablet, load an assembly
- Look at the mapping and try to find SNPs

Training plan - day 1