

FreshBiostats





- *short reads*      *short read pairs*
- *Fastq*
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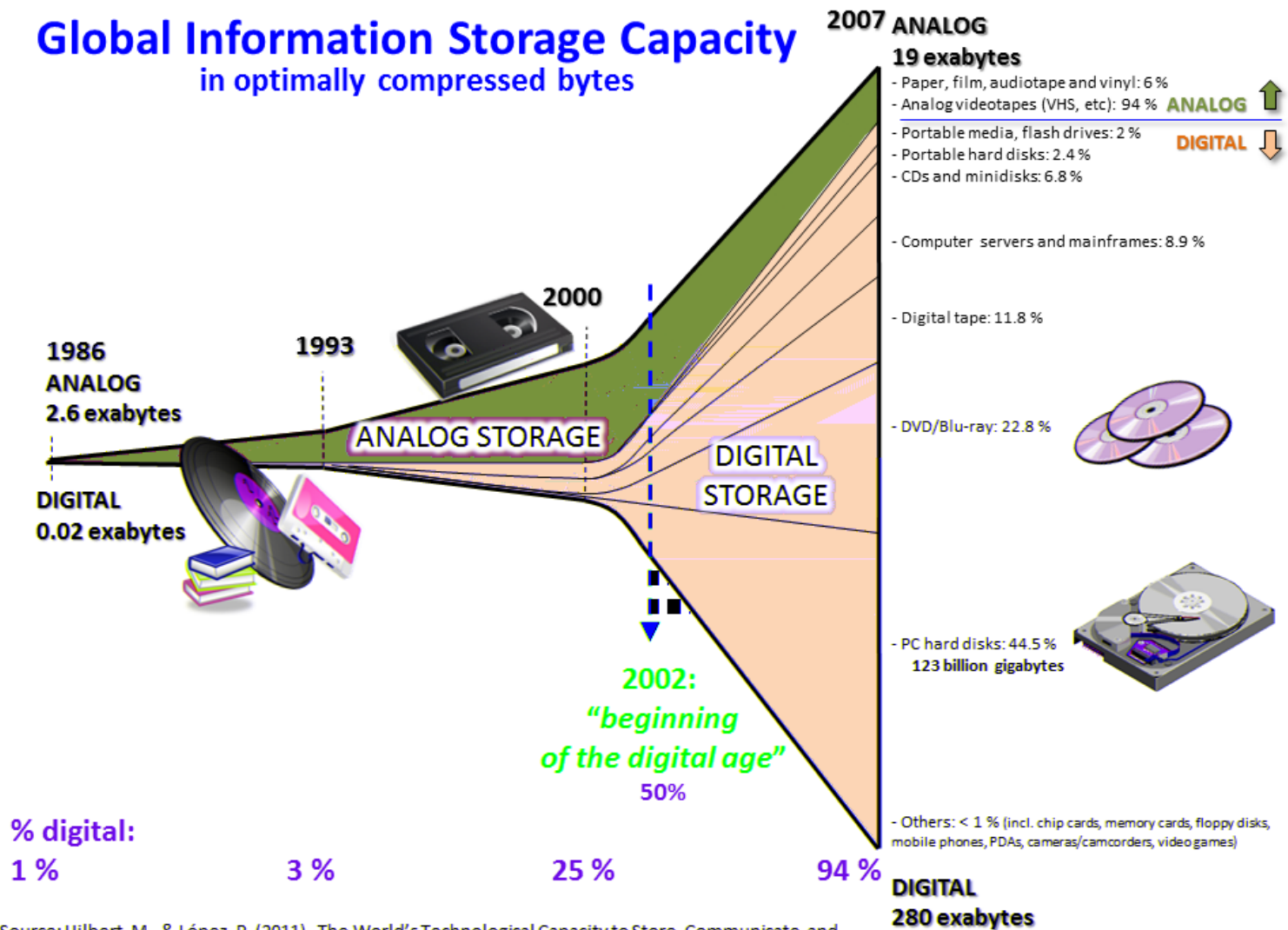
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+
@@@DDDDH8<BAHG@BHGIHIII>(<
@Read_id_2
TGATGTGCCGCCTCACTACGGTGGTG
+
FHHHHHJIJIJIJIJIJIIIJJIJJGIGII
@Read_id_3
...

```



Platform Name	Illumina HiSeq 2500	Ion Torrent-Proton II	PacBio RS II	OxFord Nanopore Minion
Instrument				
Cost (USD) **	690 k	224 k	695 k	1 k ***
Reagent cost Per run/per GB	4126/45.84	1000/20.41	100/1111.11	900/1000
Reads per run	300 millions	280 millions	0.03 millions	0.1 millions
Average Read length	2 × 150 bp	175 bp	14,000 bp	9,000 bp
Run time	10 h	5 h	2 h	6 h
Major errors	substitution	indel	indel	deletion
Error rate (%)	0.1	1	1	4
Amplification	bridgePCR	emPCR	none, SMS	none, SMS
Advantage	low cost per GB; high output	low cost	long reads; no amplification bias	long reads; no amplification bias
Disadvantage	high cost	homopolymer errors	low throughput; high cost	high error rate

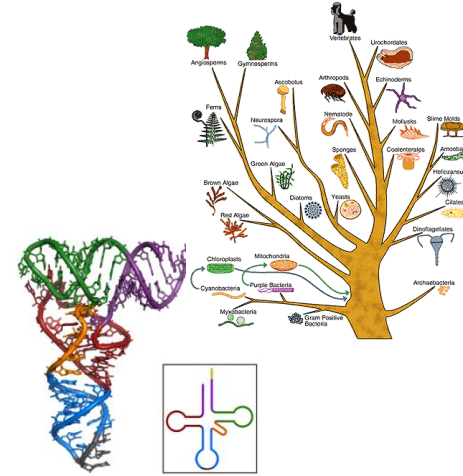
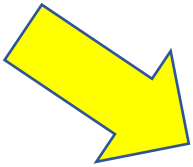
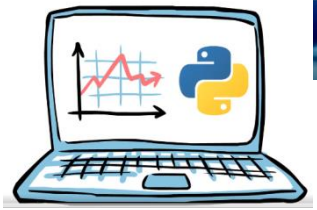
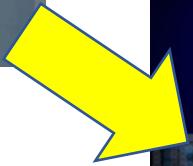
# Global Information Storage Capacity in optimally compressed bytes





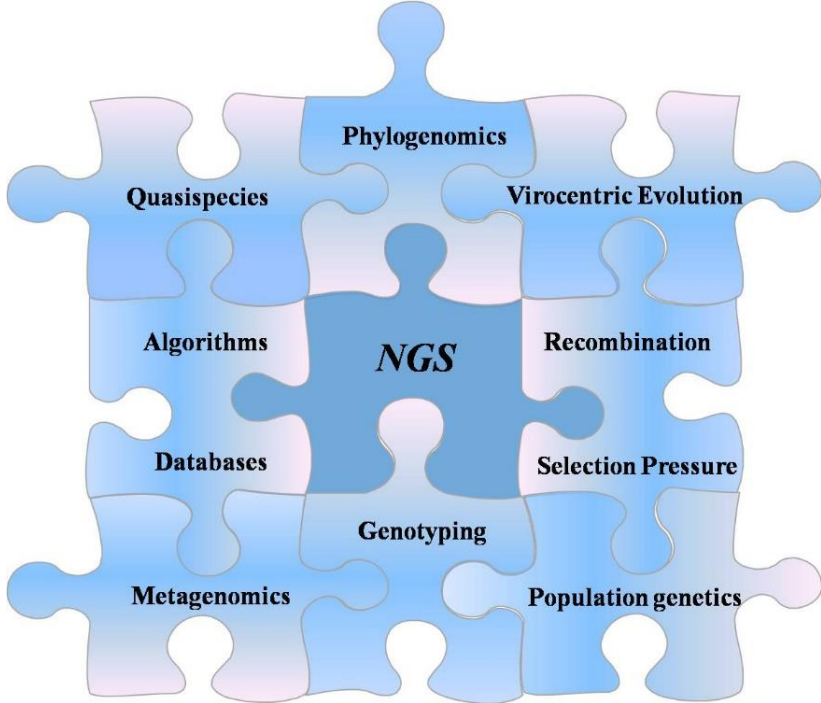
# bioinformatics

regions nlm snp secondary metabolomics homology fasta databases alignment structural  
algorithm databases assembly pathway  
dna rna protein data sequence  
information systemy  
regions nlm snp secondary metabolomics homology fasta databases alignment structural  
algorithm databases assembly pathway  
dna rna protein data sequence  
information systemy





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- *De novo*

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# *De Novo*

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- *de novo*

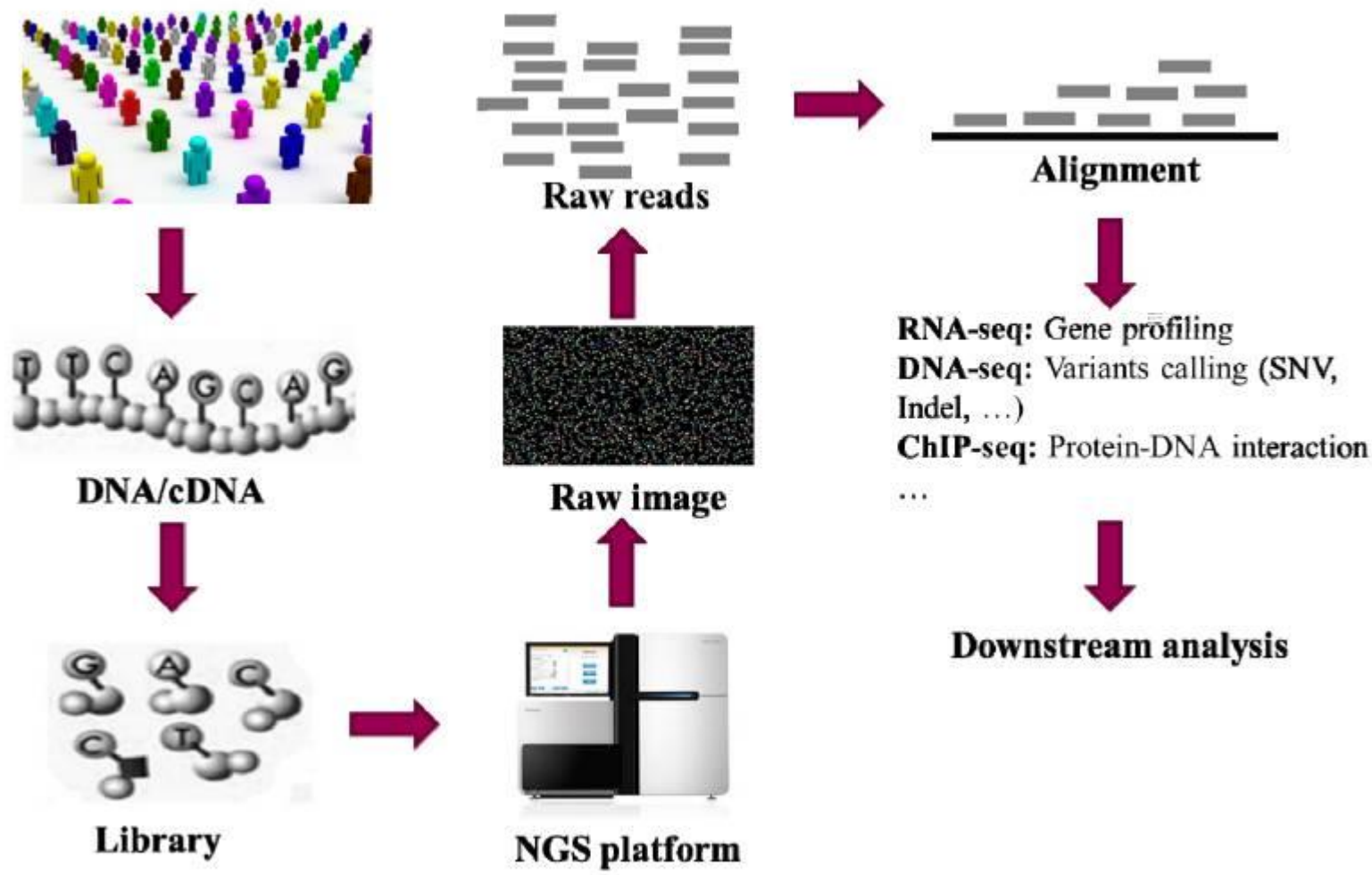
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**blastx**  
translated nucleotide ► protein

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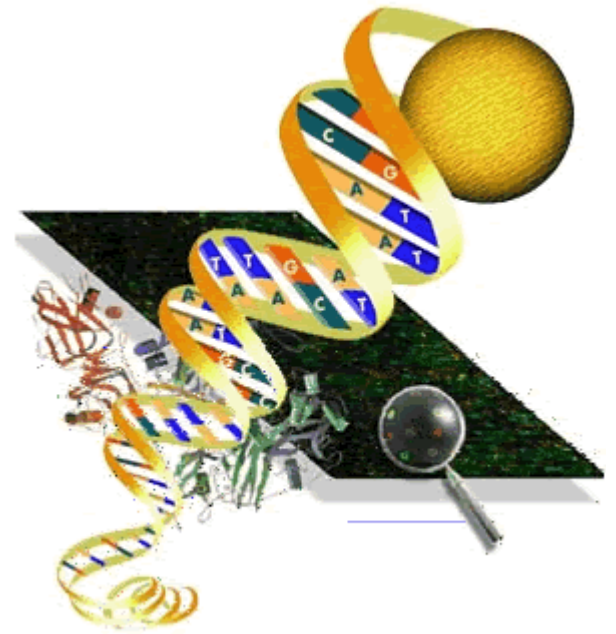
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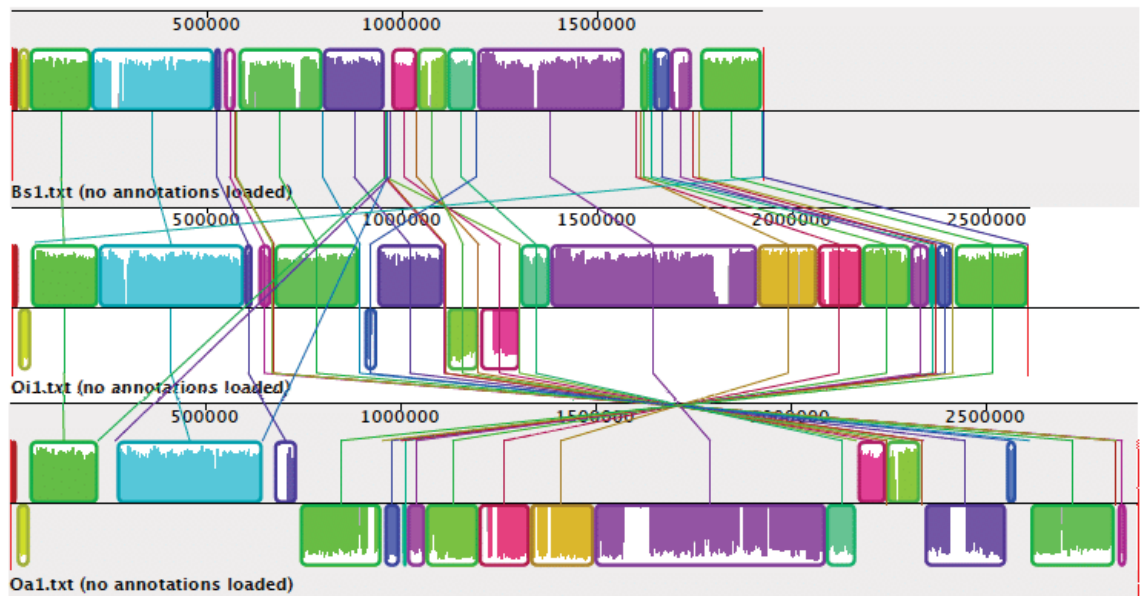


# mauve

## Multiple Genome Alignment

- rearrangement and inversion

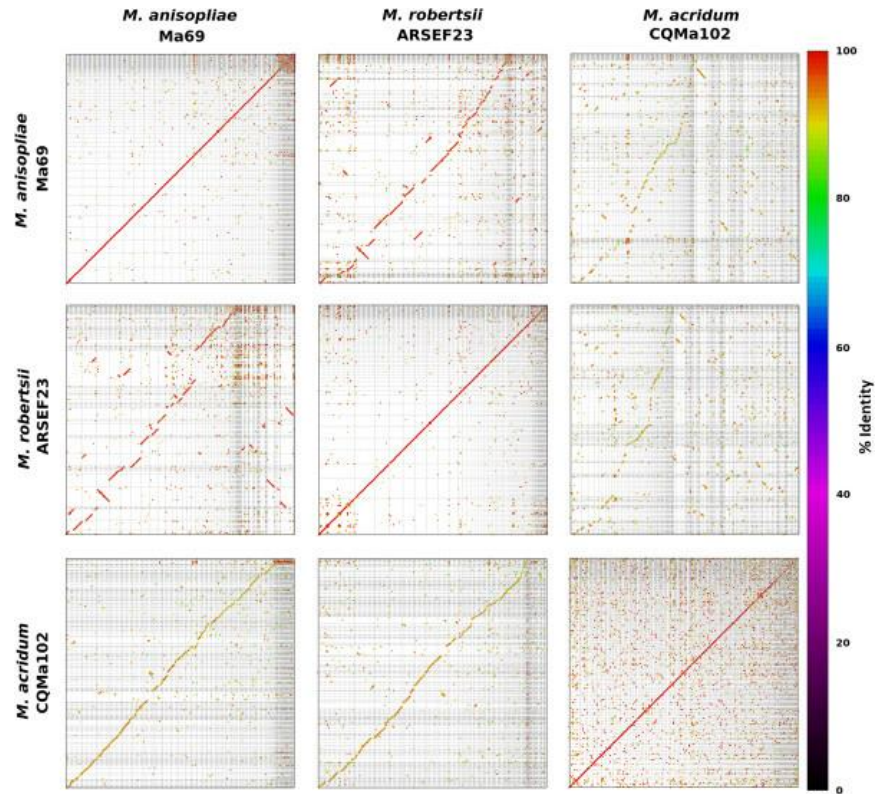
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AMUMMERA3BL

MUMMER 3.4

MUMMER 3.4K





$$C=LN/G$$

C  
L  
G  
N

## Coverage / Read Count Calculator

Calculate how much sequencing you need to hit a target depth of coverage (or vice versa).

Instructions: set the read length/configuration and genome size, then select what you want to calculate.

Written by Stephen Turner, based on the Lander-Waterman formula, inspired by a similar calculator written by James Hadfield. Coverage is calculated as  $C=LN/G$  and reads as  $N=CG/L$  where  $C$  = Coverage (X),  $L$  = Read length (bp),  $G$  = Haploid genome size (bp), and  $N$  = Number of reads. Source code on [GitHub](#).

### Read length (bp)

- Paired-end  
 Single-end

### Genome size

Pick a genome below or manually enter the haploid genome size in bp. You can use scientific notation (e.g., enter  $3.2e9$  for 3,200,000,000bp, or 3.2 Gb).

- Human (3.1 Gb)  
 Agilent V6 exome (60 Mb)  
 S. cerevisiae (12.2 Mb)  
 E. coli K-12 (4.6 Mb)  
 Other (Enter manually)

Selected genome size: 3,096,649,726

### What do you want to know?

- # Reads (how many reads do I need to hit a target depth of coverage?)  
 Coverage (what's my coverage depth obtained from a set number of reads)

### Number reads sequenced (millions)



**\*10X coverage\* for a 3.1GB genome obtained with 150M 2x100 sequencing reads.**

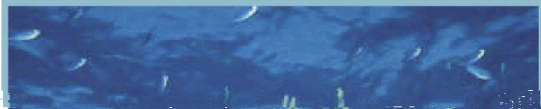


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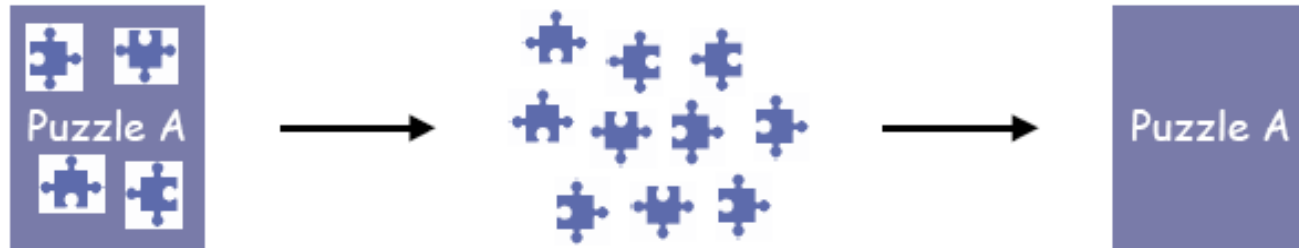
## THE METAGENOMICS PROCESS



### DETERMINE WHAT THE GENES ARE (Sequence-based metagenomics)

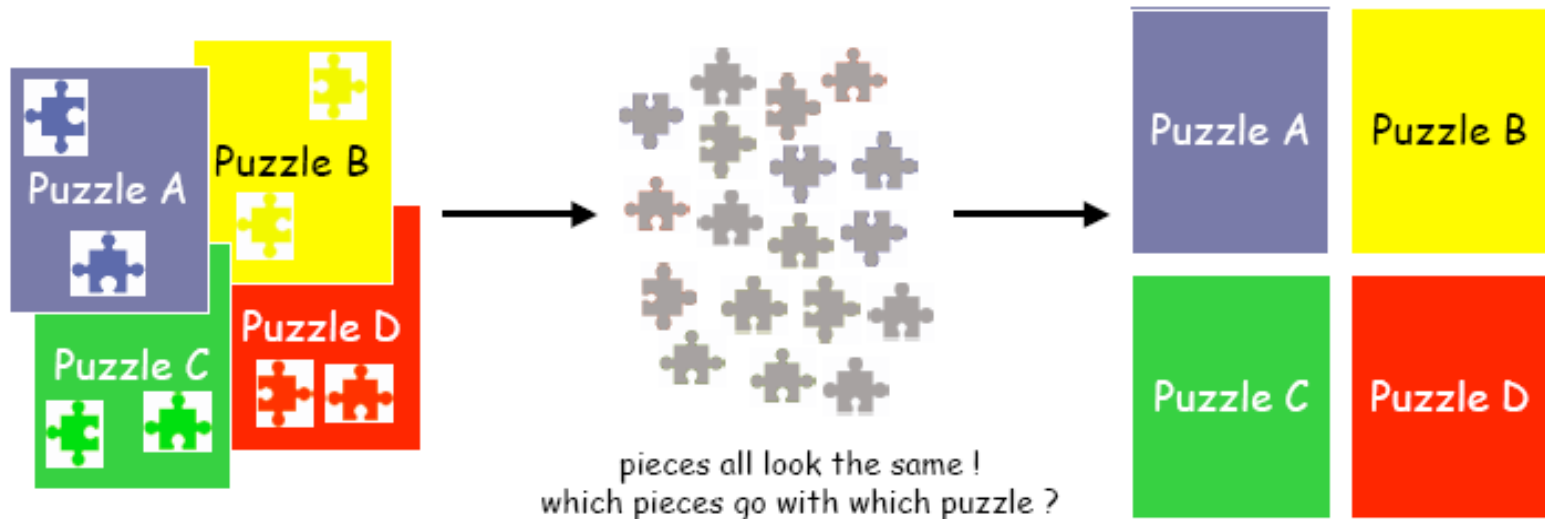
- Identify genes and metabolic pathways
- Compare to other communities

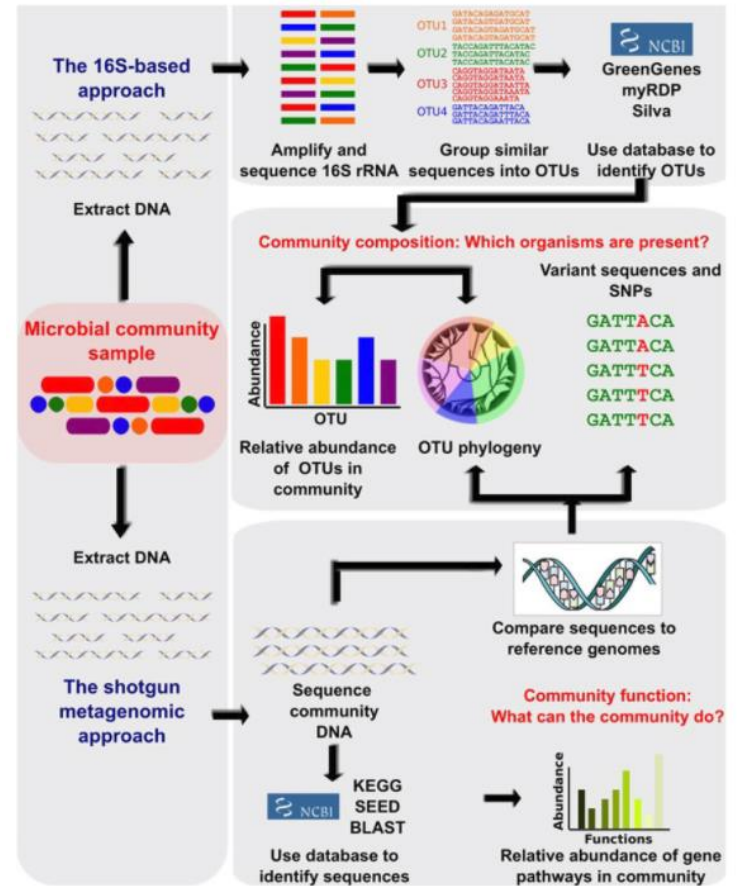
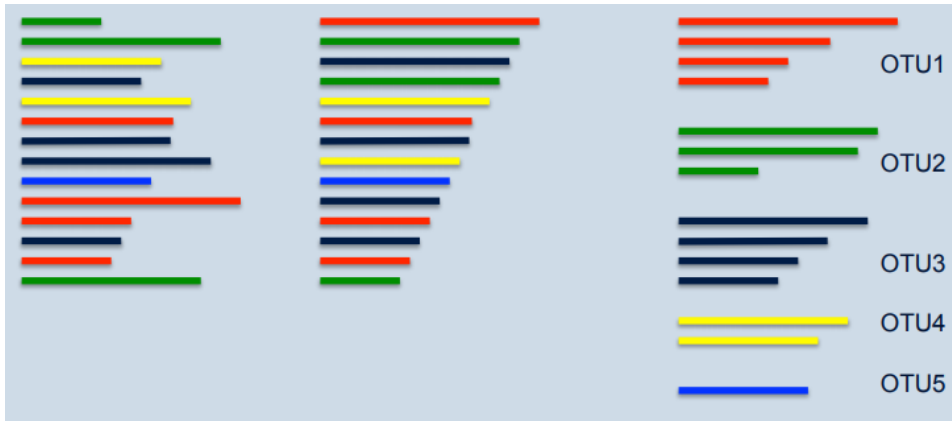
### Isolate genome - single source of DNA



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### Environmental genome - multiple sources of DNA







# EBI Metagenomics

## By selected biomes



Soil (438)



Freshwater  
(118)

Biome	Project name	Samples	Last updated
	16S amplicon based soil and leaf microbiome survey in Hungarian vineyards	19	02-May-2017
	16S metabarcoding of bacteria associated with cultured strains of the brown alga <i>Ectocarpus</i> sp.	51	12-Jan-2017
	16S rRNA amplicons (V4 region) of bacteria living on and in roots and leaves of <i>Boechera stricta</i> from field experiments in the Rocky Mountains	650	13-Dec-2016
	16S rRNA gene pyrosequencing- Secondary successional trajectories of structural and catabolic bacterial communities in oil-polluted soil planted with hybrid Poplar	34	12-Jan-2017
	A diverse array of bacteria that inhabit the rhizosphere and different plant organs play a crucial role in plant health and growth.	4	02-Dec-2016
	Accessing and Identification of Novel Environmental Alleles of the ACC Deaminase Domain Region through a Competition Assay	1	02-Dec-2016
	Agroforestry leads to shifts within the gammaproteobacterial microbiome of banana plants cultivated in Central America	48	05-Jan-2017
	Alk B pyrosequencing -Secondary successional trajectories of structural and catabolic bacterial communities in oil-polluted soil planted with hybrid Poplar	34	12-Jan-2017
	AMF from contaminated and uncontaminated rhizosphere soils Metagenome	70	16-May-2016
	Amplicon-based metagenomics analysis of <i>Vitis vinifera</i> L. cv. Corvina grapes and fresh musts	39	08-Sep-2016



OPEN

## Transgenic banana plants expressing *Xanthomonas* wilt resistance genes revealed a stable non-target bacterial colonization structure

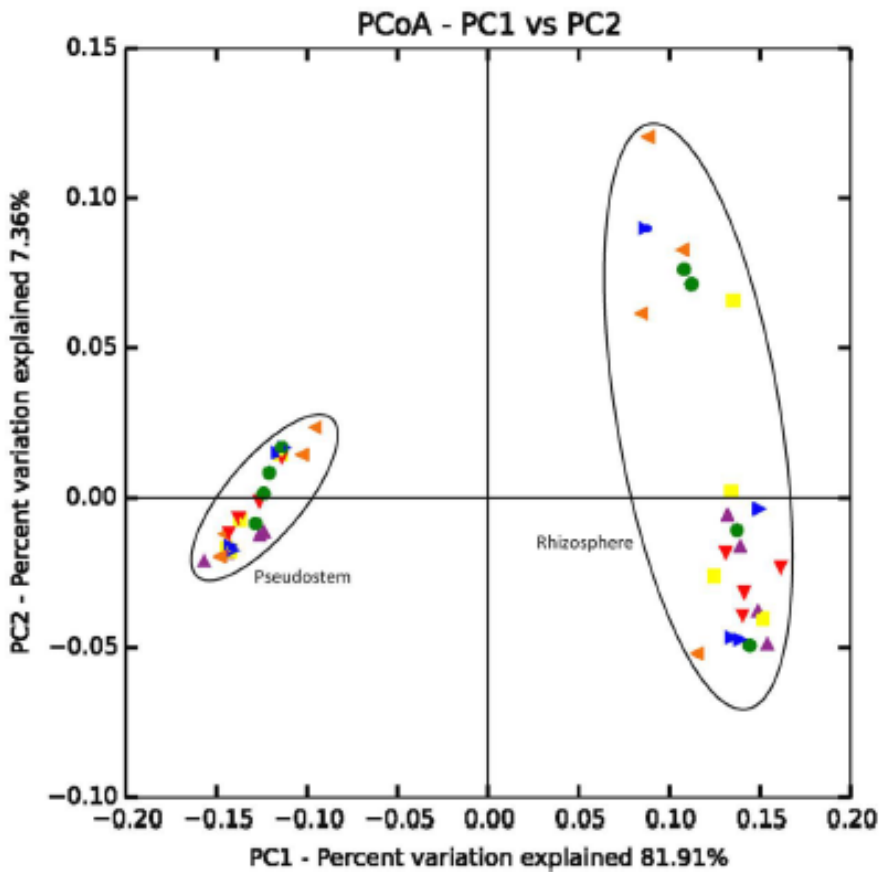
Received: 30 June 2015

Accepted: 03 November 2015

Published: 10 December 2015

Jean Nimusiima<sup>1,2,\*</sup>, Martina Köberl<sup>3,\*†</sup>, John Baptist Tumuhairwe<sup>2</sup>, Jerome Kubiriba<sup>1</sup>, Charles Staver<sup>4</sup> & Gabriele Berg<sup>3</sup>

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## Principal coordinate analysis (PCoA)





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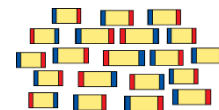
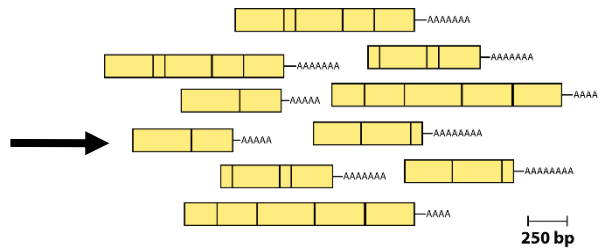
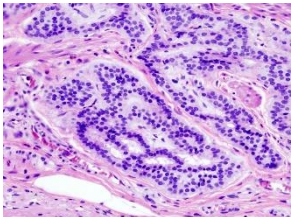
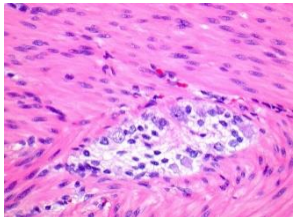
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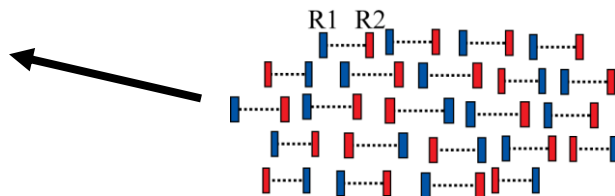
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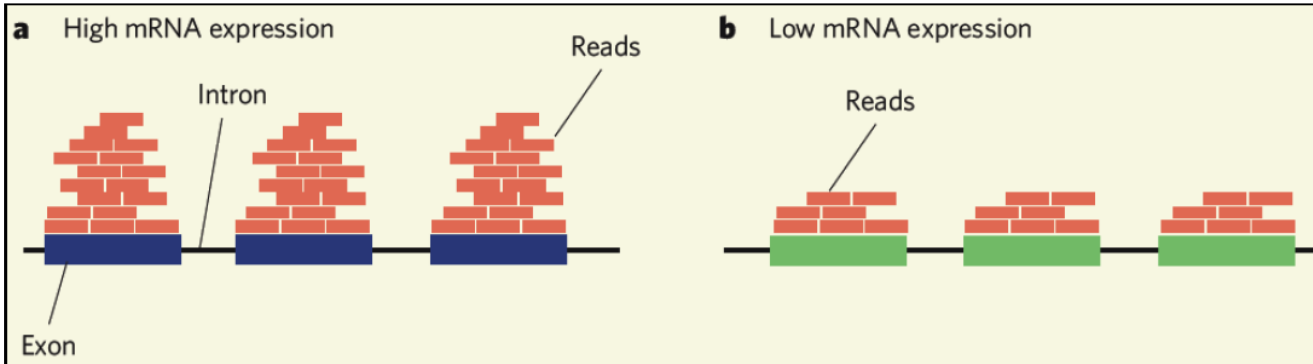
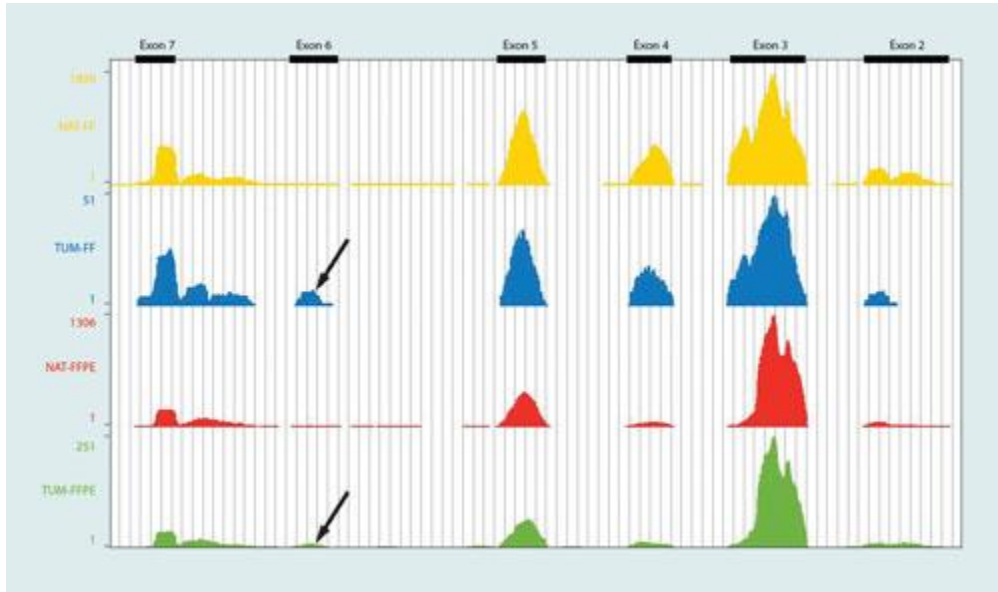
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250 bp









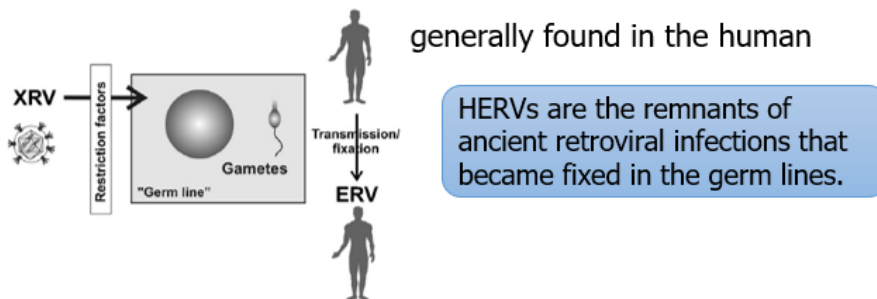
## Endogenous florendoviruses are major components of plant genomes and hallmarks of virus evolution

Andrew D. W. Geering<sup>✉</sup>, Florian Maumus, Dario Copetti, Nathalie Choisne, Derrick J. Zwickl, Matthias Zytnicki, Alistair R. McTaggart, Simone Scalabrini, Silvia Vezzulli, Rod A. Wing, Hadi Quesneville & Pierre-Yves Teycheney

*Nature Communications* 5, Article number: 5269 (2014) | Download Citation ↕

## HERV = Human Endogenous Retrovirus

- Endogenous retroviruses (ERVs) = DNA sequences within a genome that are similar to sequences of infectious retroviruses



generally found in the human

HERVs are the remnants of ancient retroviral infections that became fixed in the germ lines.





# EnHERV: Human Endogenous Retrovirus Enrichment Tool

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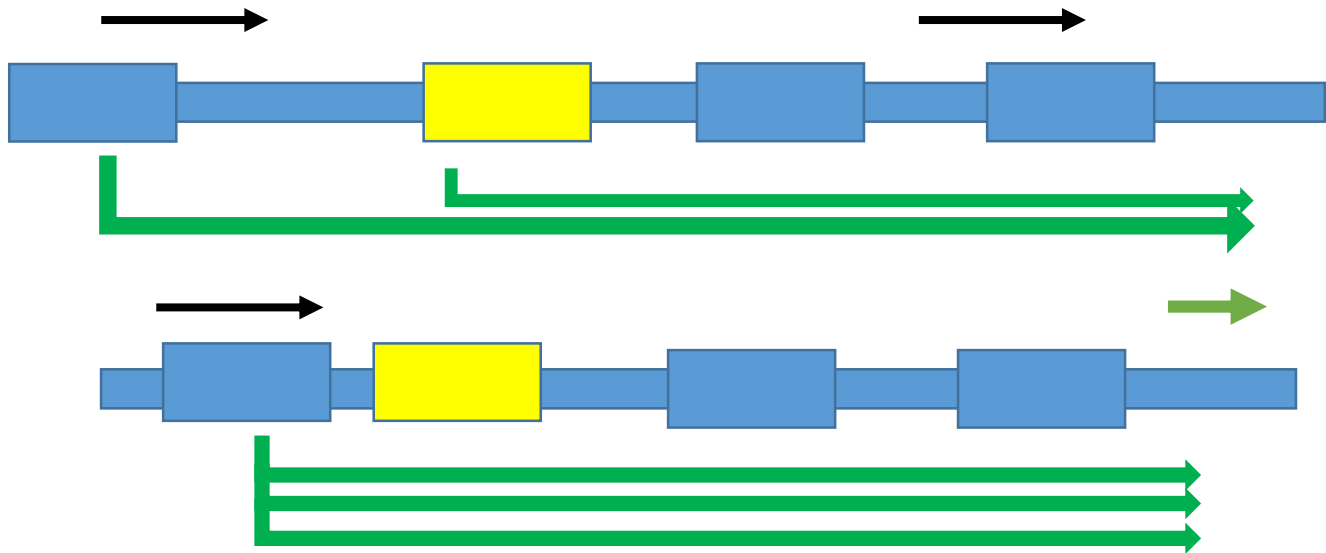
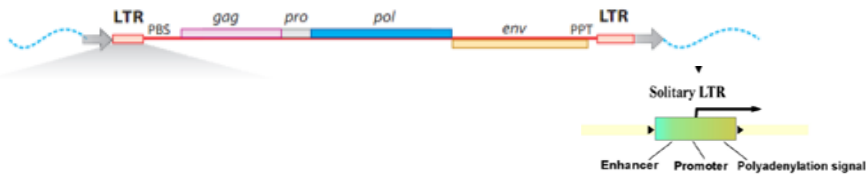
## Welcome to EnHERV.

The human genome contains a wide variety of endogenous retrovirus-like sequences. Human endogenous retroviruses (**HERVs**) comprise up to 6–8% of the human genome. From a junk DNA aspect, they become more interested in biomedical world because of their expression tend to associated with several diseases, including cancer and autoimmune diseases.

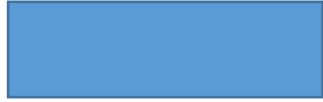
**EnHERV** is a database designed for not only searching HERV neighboring gene, this database provides enrichment analysis function of selected HERV characteristics agint genes list. This database is compiled from the human genome nucleotide analysis mainly in the repeat analysis pipeline from Repbase Update (RU). This database allows user to easily search for gene certaining HERV in a specific characteristics in a entire human genome. **EnHERV** aims to identified certain HERV characteristic that statistically significant of enrichment in specified gene list especially for gene expression data. User can start using searching function by selecting **Search** tab at navigation panel. User can search by genes name or HERV characteristics. Then user can run the Fisher's exact test for identifying by using **Enrichment Analysis** function. User also can retrieved the entire database from **Download** section.

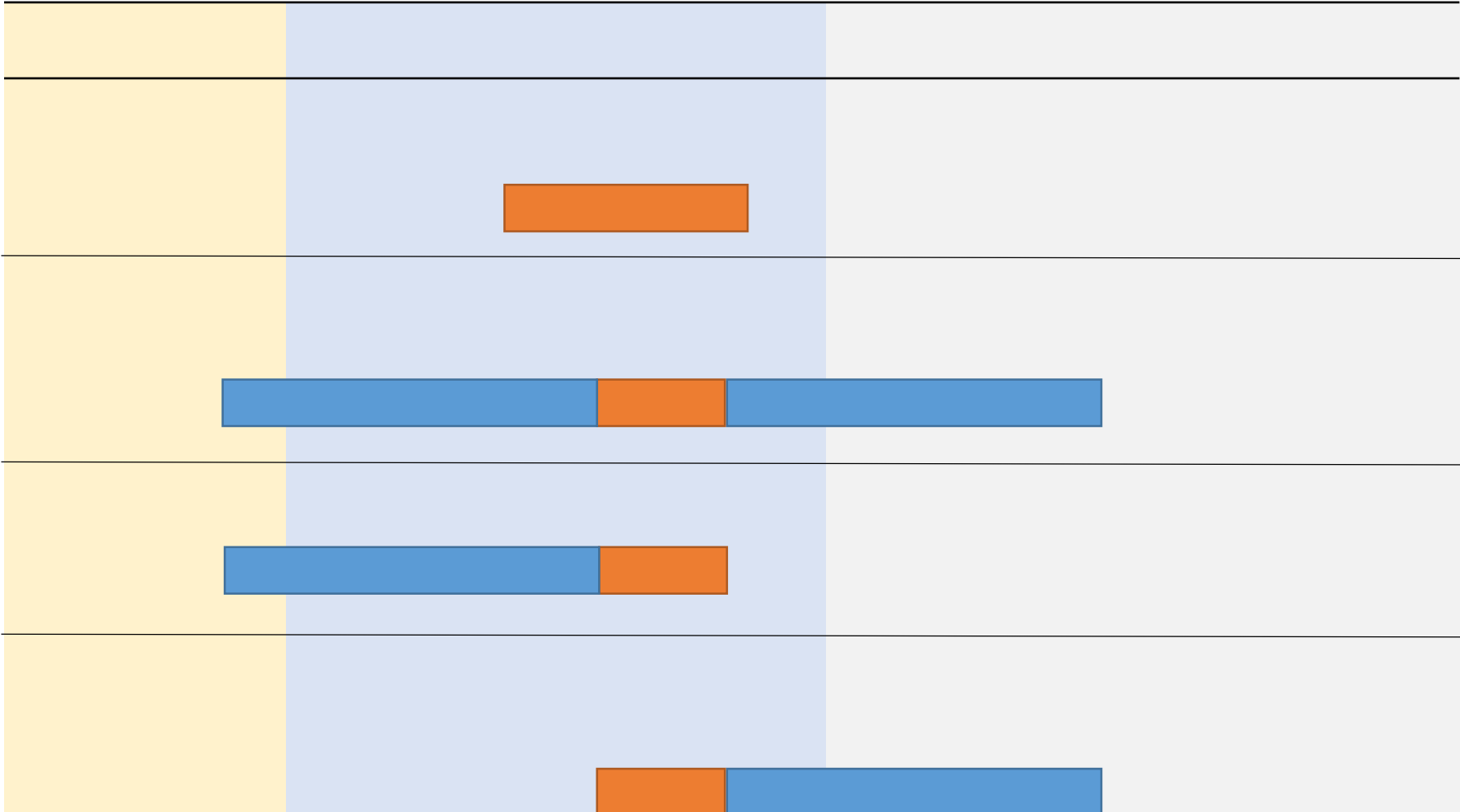
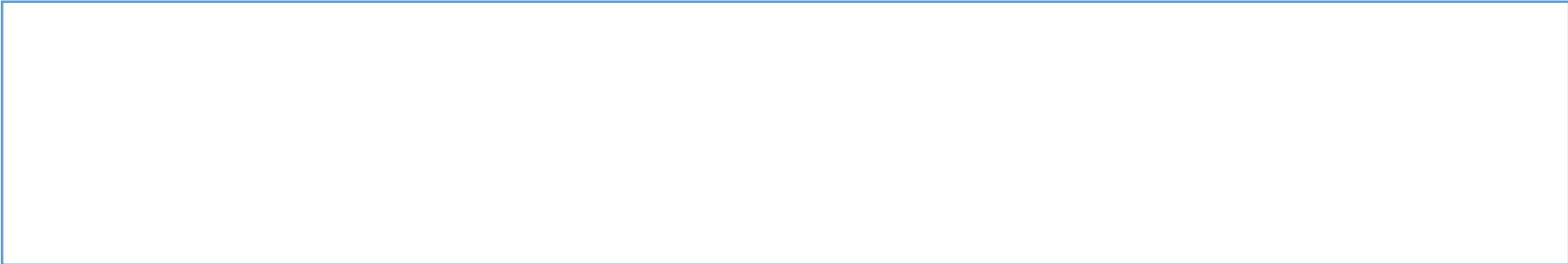


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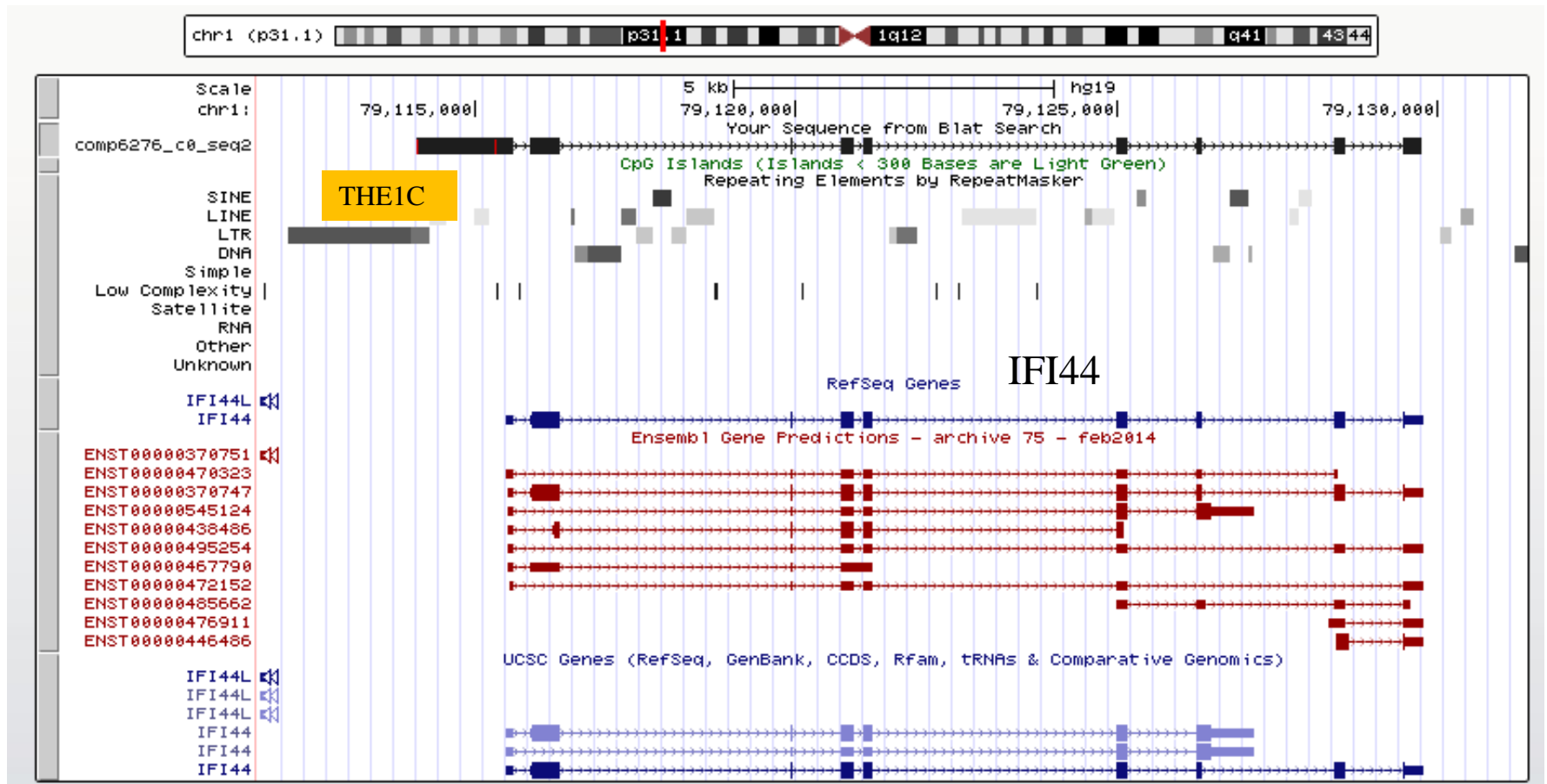


*De novo*



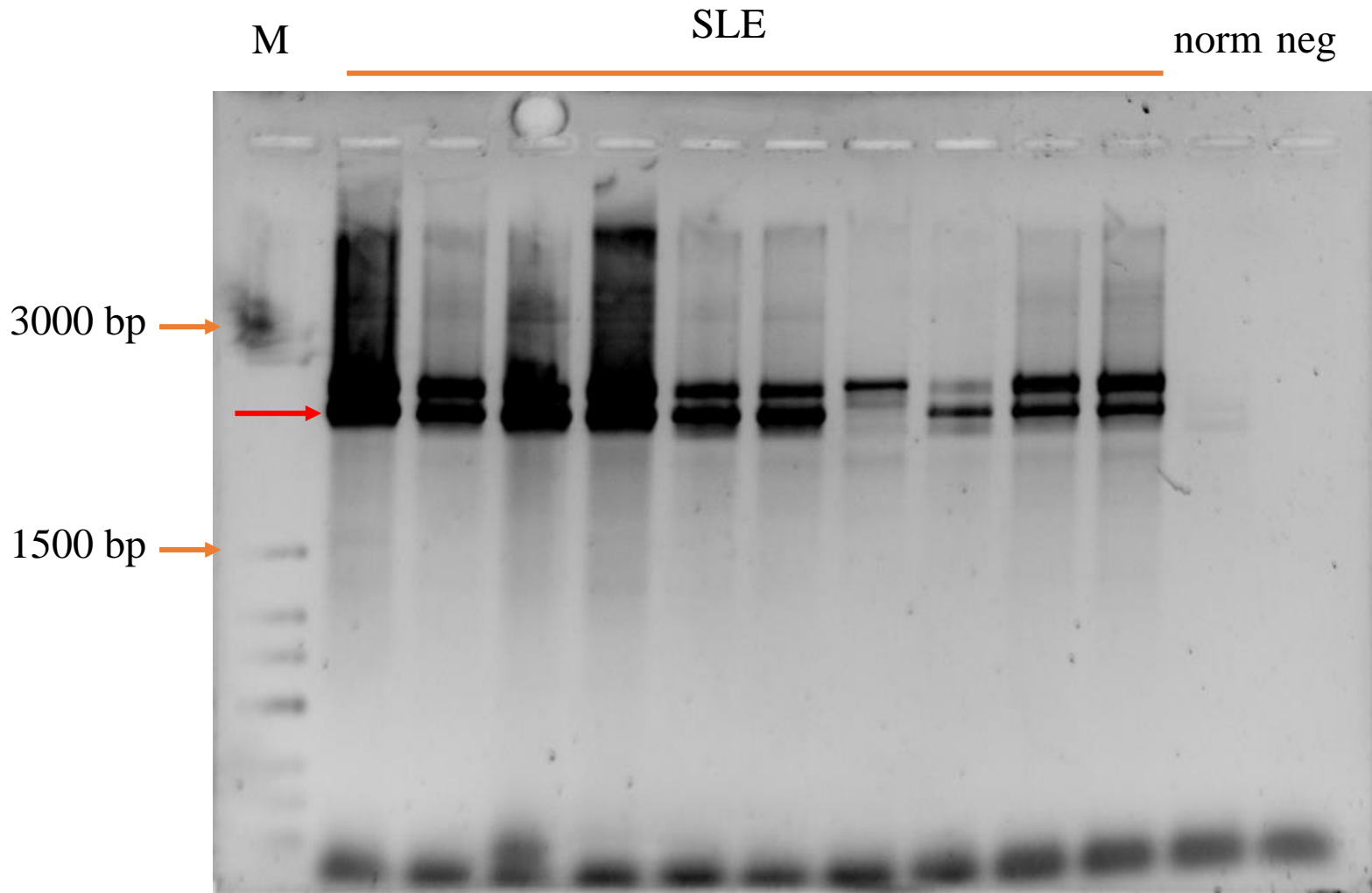


# THE1C



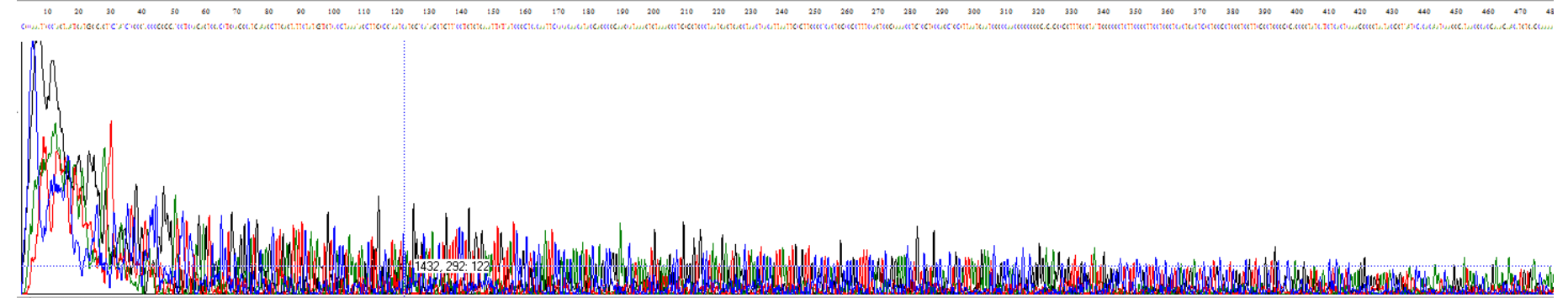
# IFI44-THE1C chimeric amplicons.

The expected size is 1740 bps.

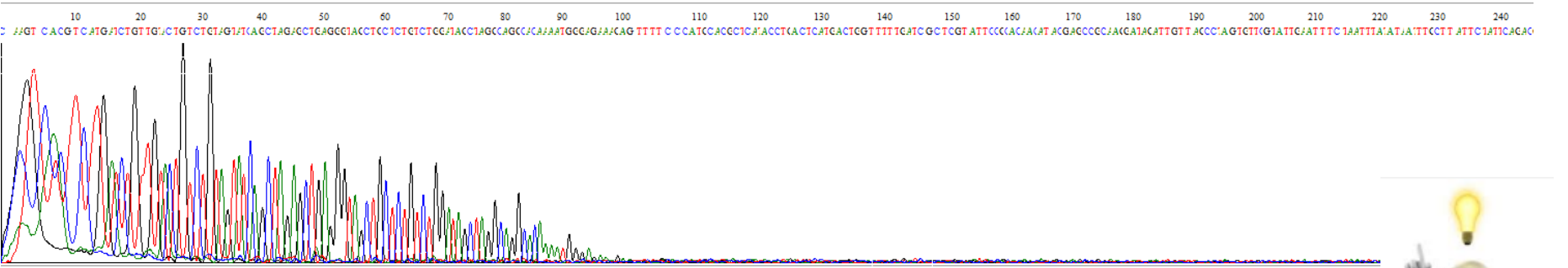


# IFI44-THE1B chimeric amplicons.

## THE1C-forward amplicon

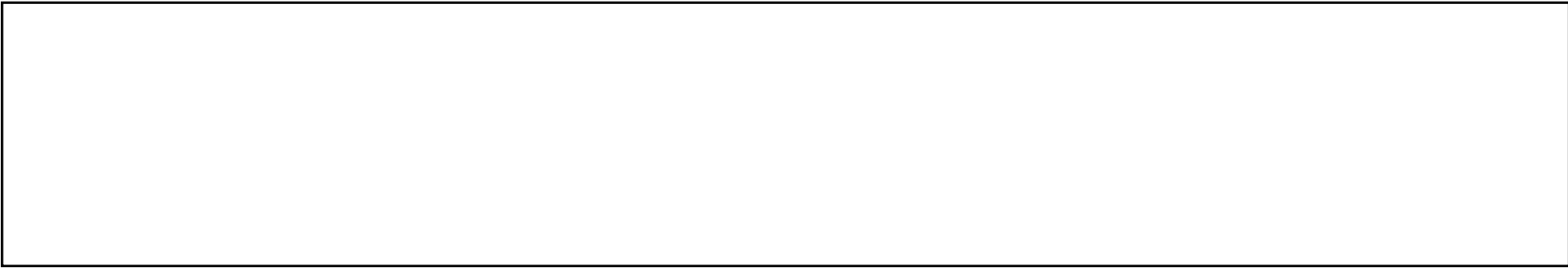


## IFI44-reverse amplicon

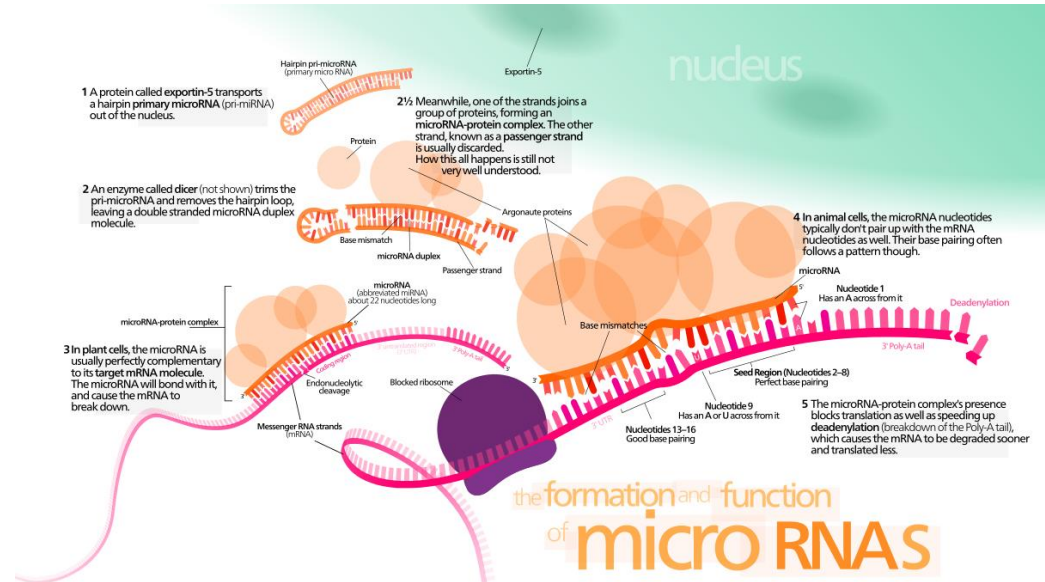
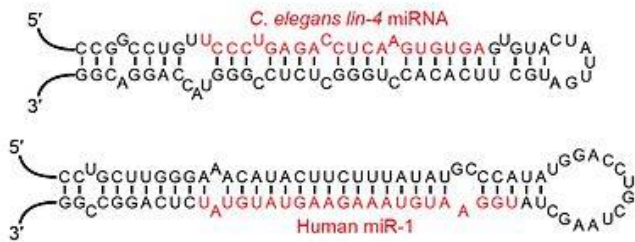


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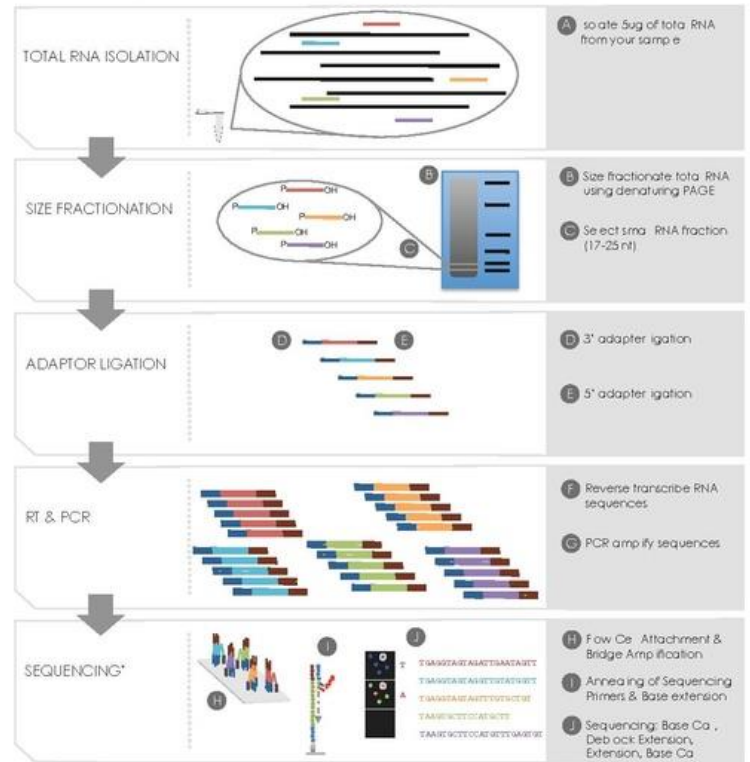
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### MIRNA-SEQ LIBRARY PREPARATION



\* Illumina sequencing method depicted however other sequencing platforms can also be used.

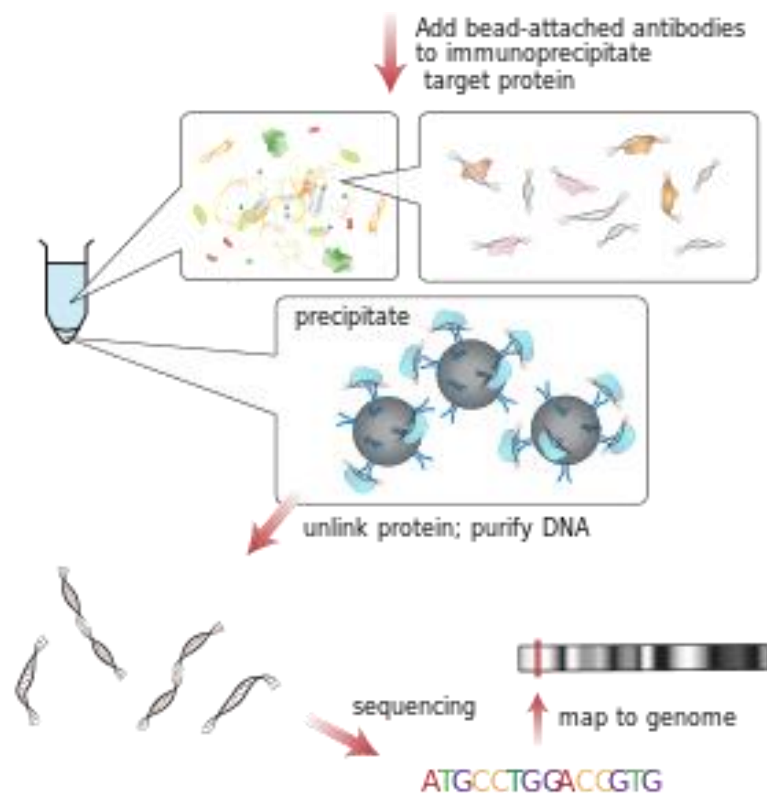
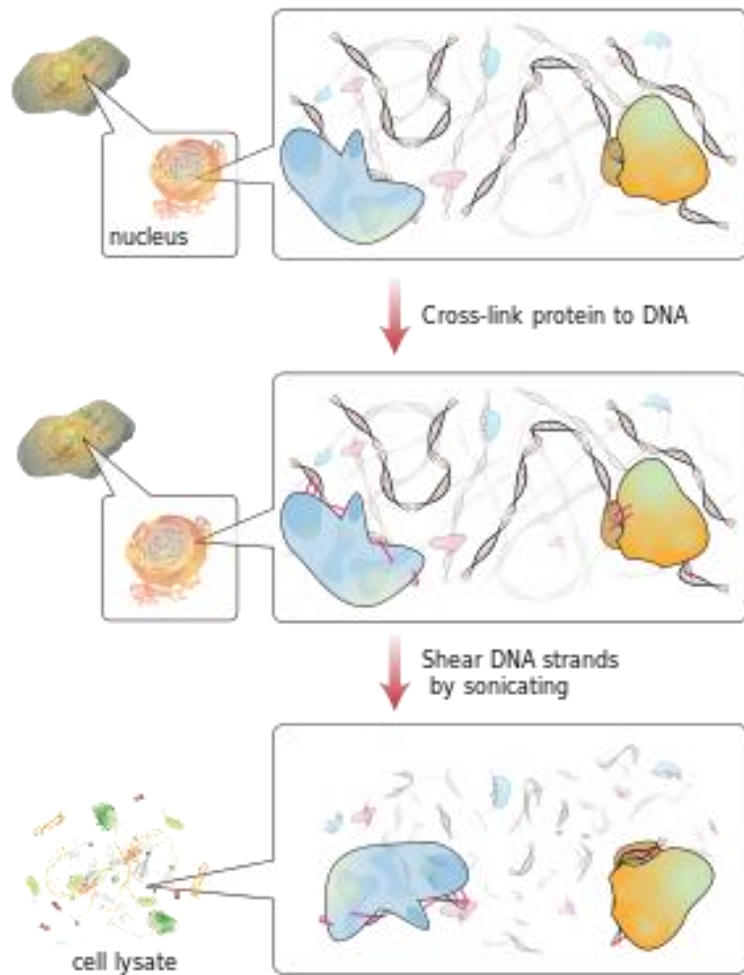


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**a**

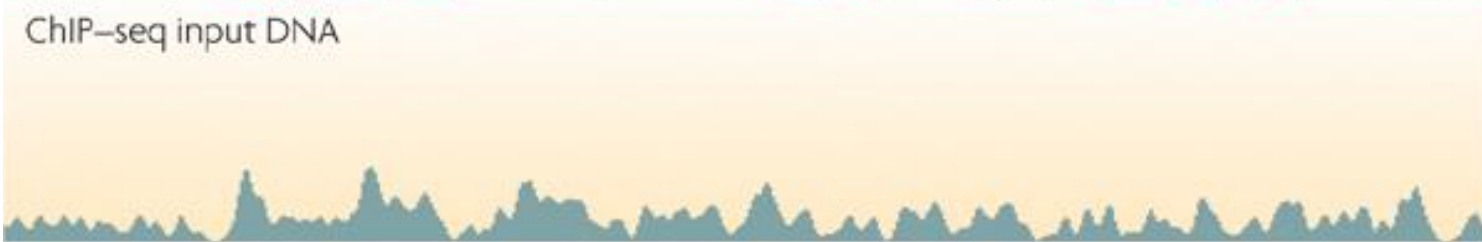
ChIP-chip



ChIP-seq



ChIP-seq input DNA



*Pros35* CG4908



*eEF1δ*



*NPC1*

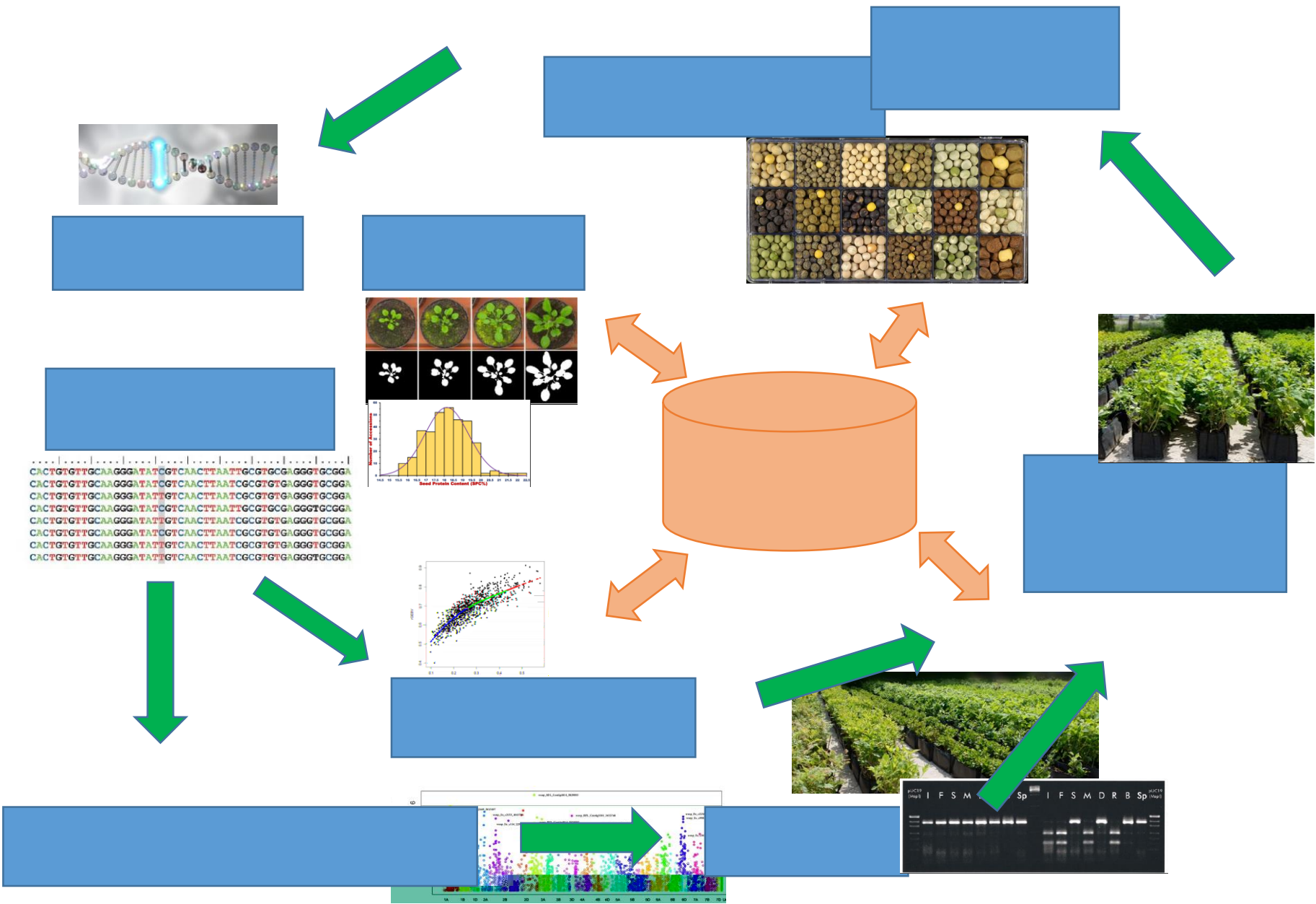


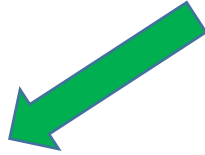
*CG5708*



*CG5694*







**THAIST**  
THAILAND ADVANCED INSTITUTE  
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**AG-BIO**  
AGRI-BIO

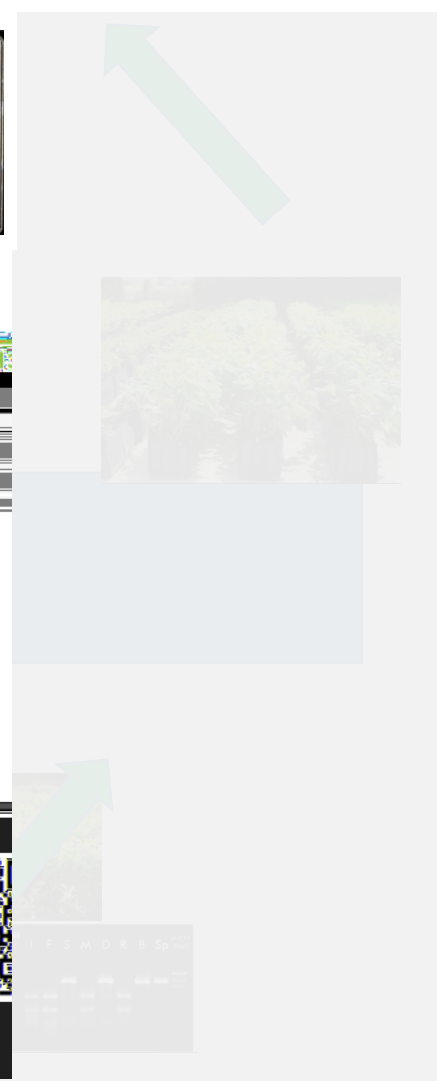
สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยี  
(Thailand Advanced Institute of Science and Technology=THAIST)  
สำนักงานคณะกรรมการนโยบายวิทยาศาสตร์-เทคโนโลยีและนวัตกรรมแห่งชาติ (สทวน)  
ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์

**ขอเชิญเข้าร่วม**  
**โครงการจัดอบรมถ่ายทอดเทคโนโลยีเชิงปฏิบัติการ**  
**“Genome assembly and annotation”**  
วันที่ 6 - 9 สิงหาคม 2561  
ณ ห้อง A106 ศูนย์เทคโนโลยีชีวภาพเกษตร  
มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม

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Breedserve.cab.kps.ku.ac.th

.....  
CACTGTGTTGCAAGGGATATCGTCAACTTAATTCGCGTGCAGGGTGC GGA  
CACTGTGTTGCAAGGGATATCGTCAACTTAATTCGCGTGCAGGGTGC GGA  
CACTGTGTTGCAAGGGATATCGTCAACTTAATTCGCGTGCAGGGTGC GGA  
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CACTGTGTTGCAAGGGATATCGTCAACTTAATTCGCGTGCAGGGTGC GGA  
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*De novo*





- *de novo*

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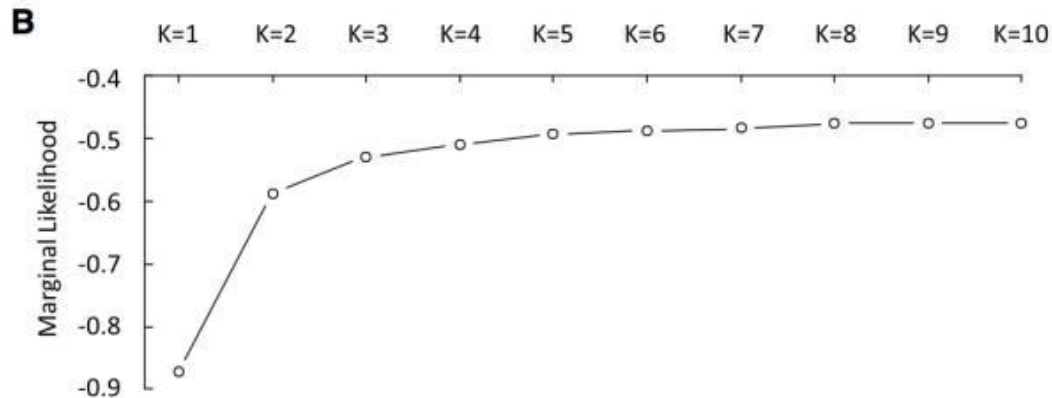
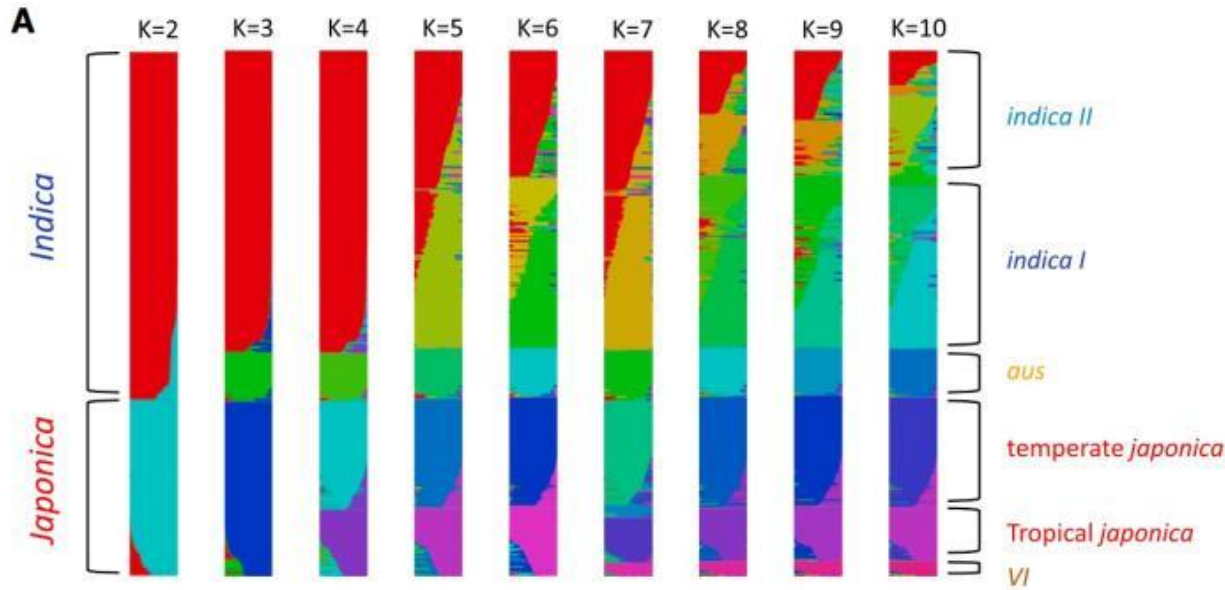
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AATGCTAGCTAGCTGACTAATGCTAGCTA



TACGATCGA**A**CGA**G**TGATT**ANN**TCG

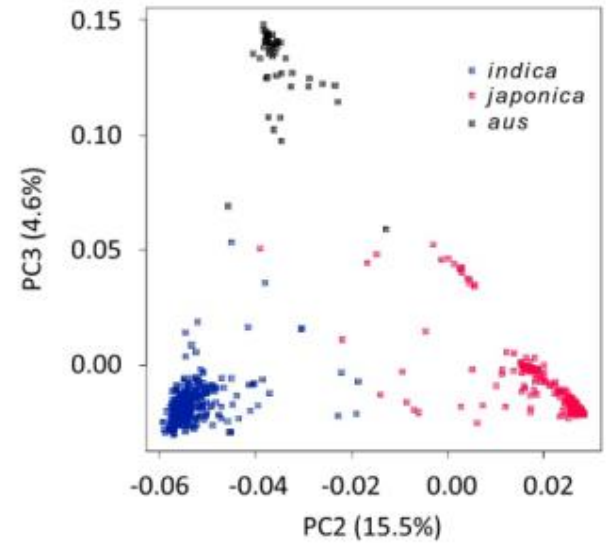
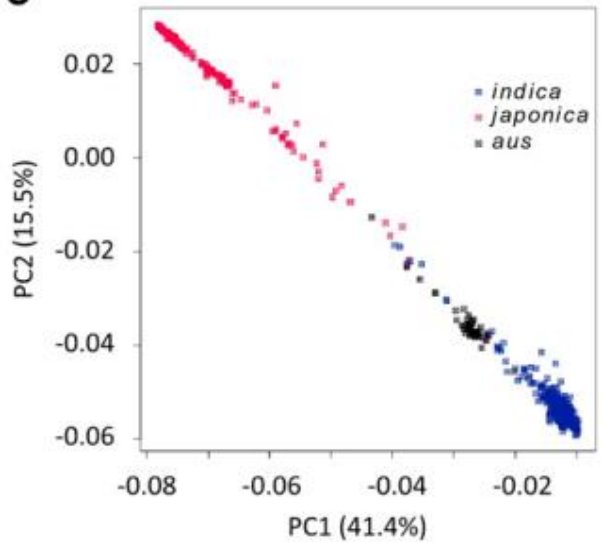
# K



- Detects the underlying genetic population among a set of individuals genotyped at multiple markers
- Computes the proportion of the genome of an individual originating from each inferred population (quantitative clustering method)
- Calculate K: when approaching a plateau or continues increasing slightly
- ***For the TRUE value of K, find the smallest value of K that captures the major structure in the data***

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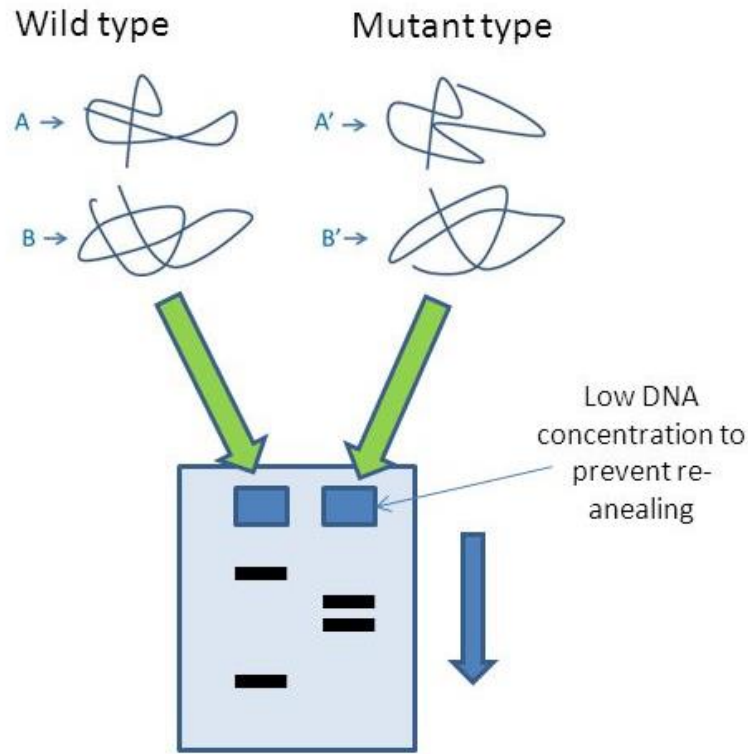
**C**



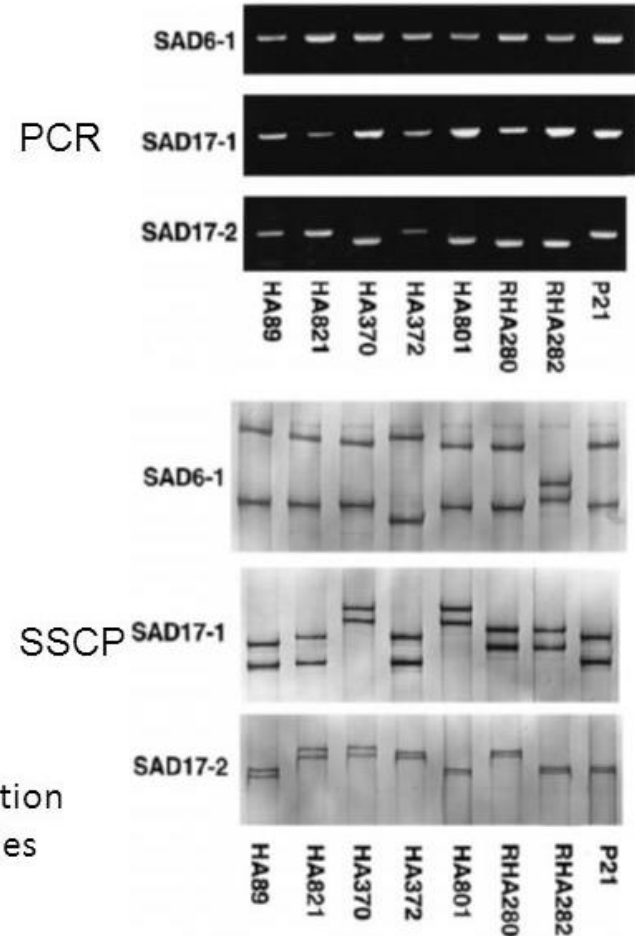




# single-strand conformation polymorphism (SSCP)

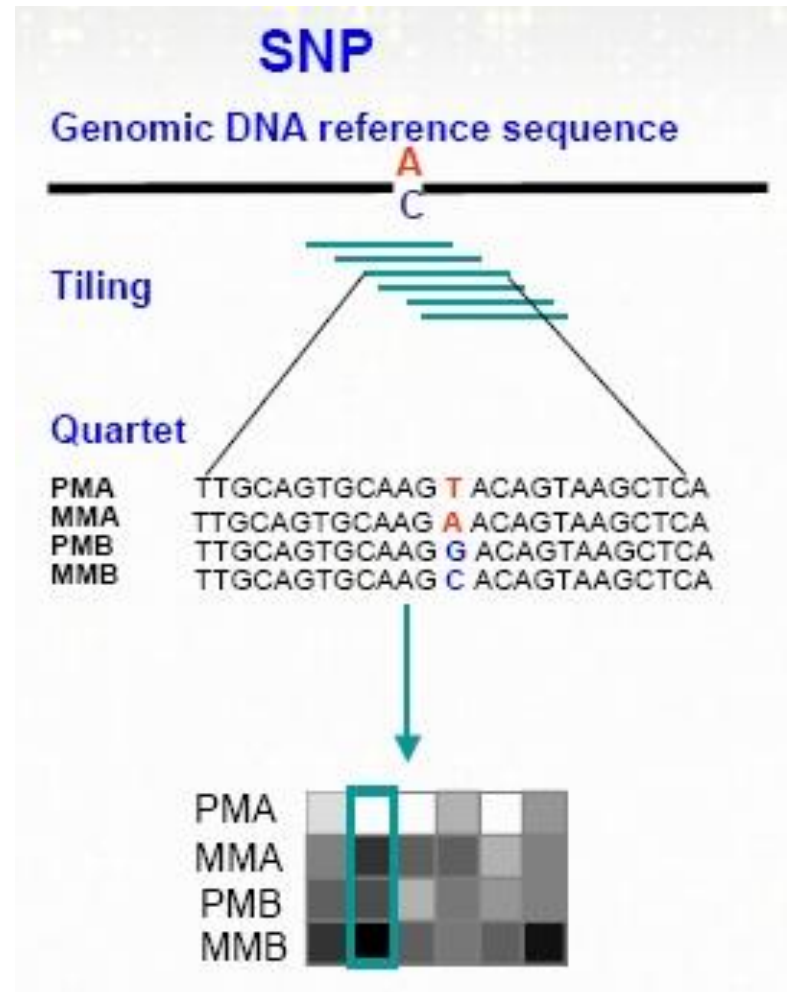


The electrophoretic mobility of separation is a function of the shape of the folded, single-stranded molecules

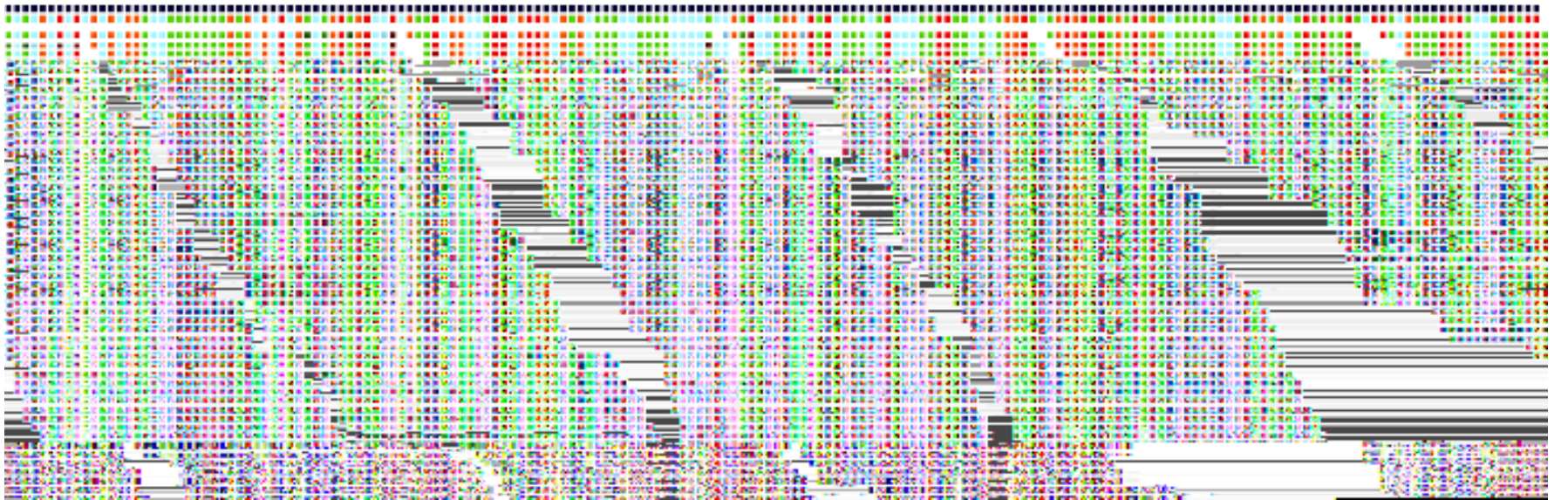


(Hongtrakul et al. 1998)

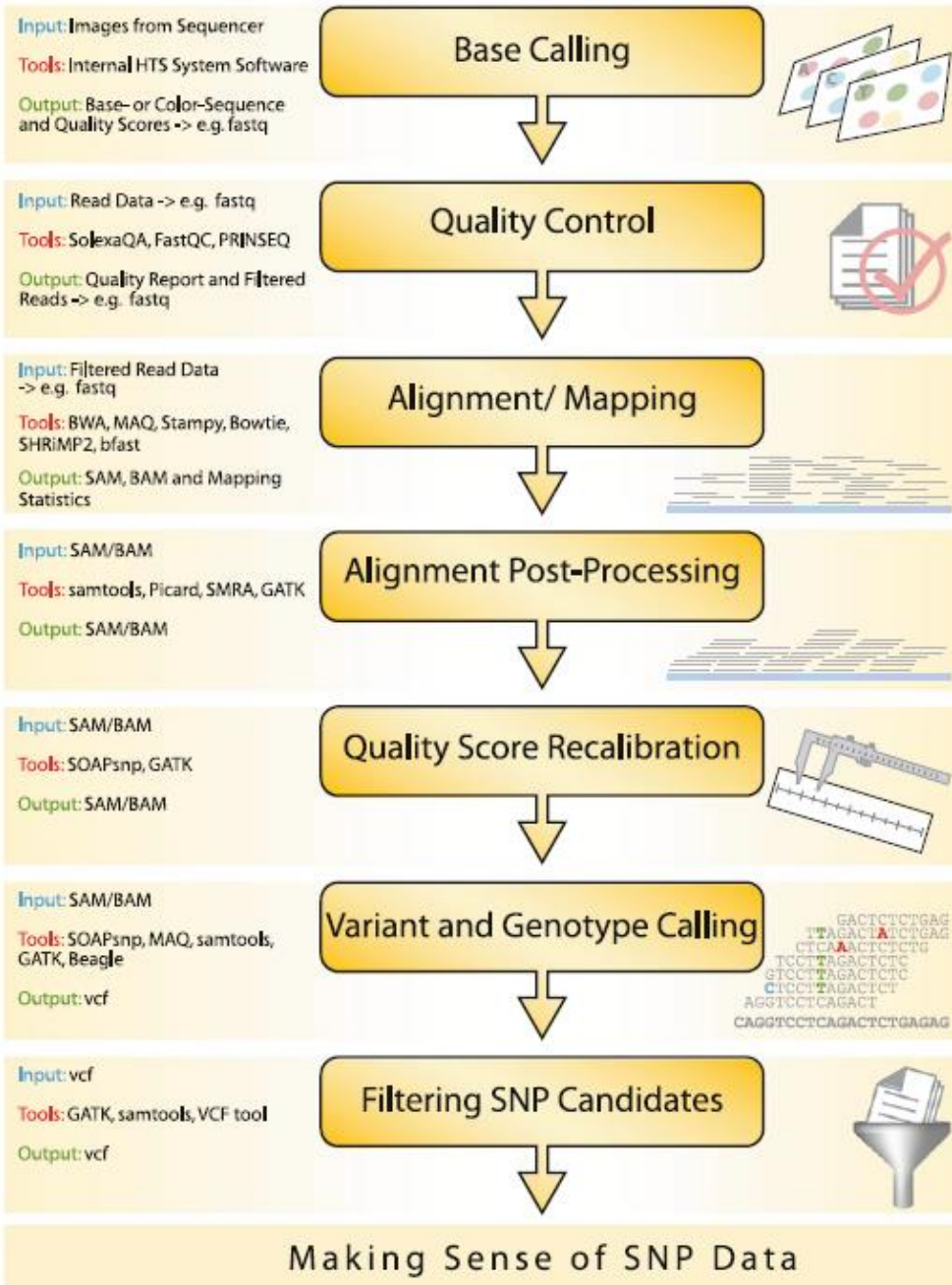
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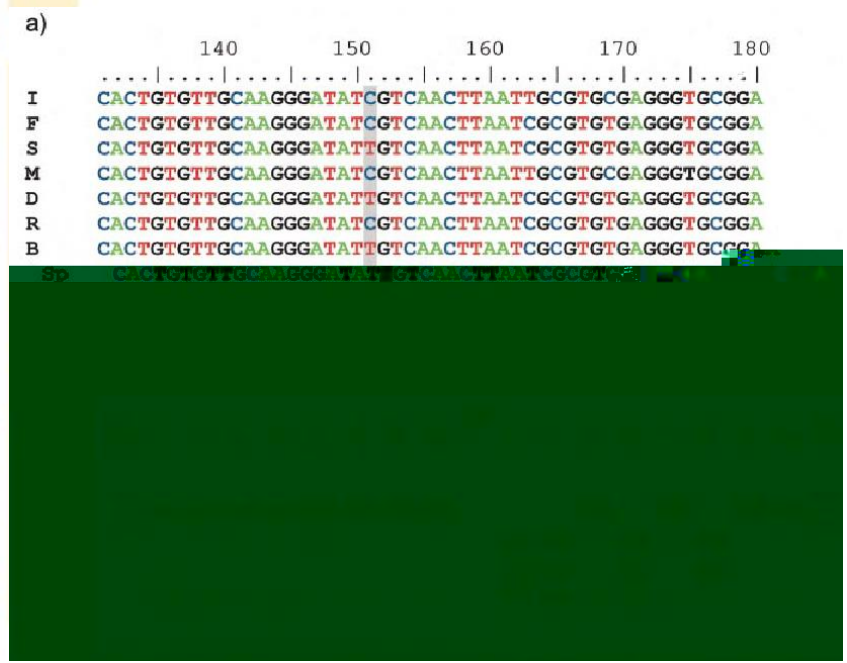








Common for all HTS pipelines



Making Sense of SNP Data



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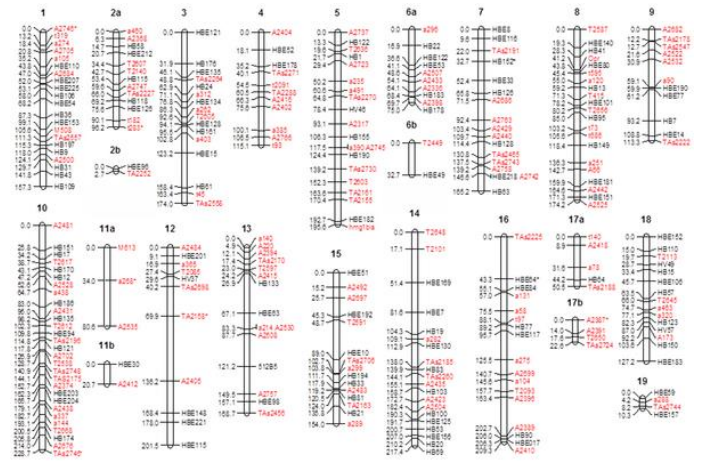
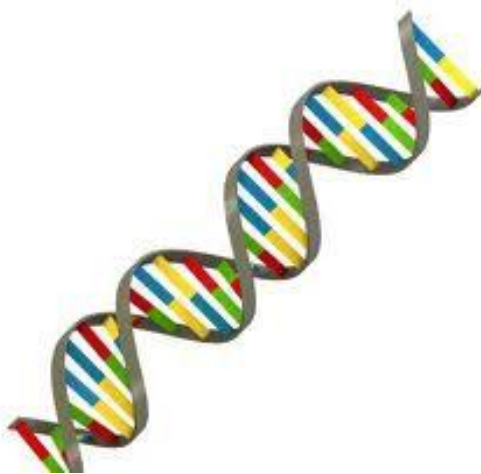
# Quantitative Trait Loci (QTL)

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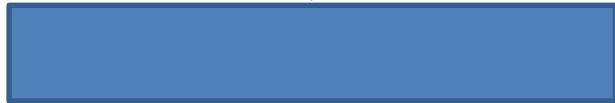
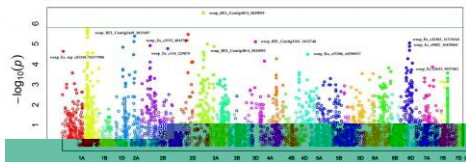


Souza LM, et al. 2013.

# Association Analysis



C A C T G T G T T G C A A G G G A T A T C G T C A A C T T A A T T G C G T G C G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T C G T C A A C T T A A T T G C G T G T G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T T G T C A A C T T A A T T G C G C G T G T G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T C G T C A A C T T A A T T G C G T G C G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T T G T C A A C T T A A T T G C G C G T G T G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T C G T C A A C T T A A T T G C G C G T G T G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T T G T C A A C T T A A T T G C G C G T G T G A G G G T G C G G A  
C A C T G T G T T G C A A G G G A T A T T G T C A A C T T A A T T G C G C G T G T G A G G G T G C G G A



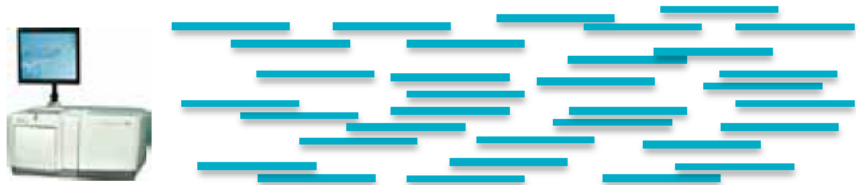
## Marker Assisted Selection



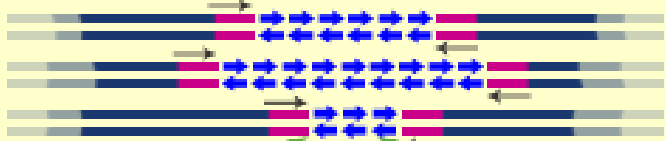




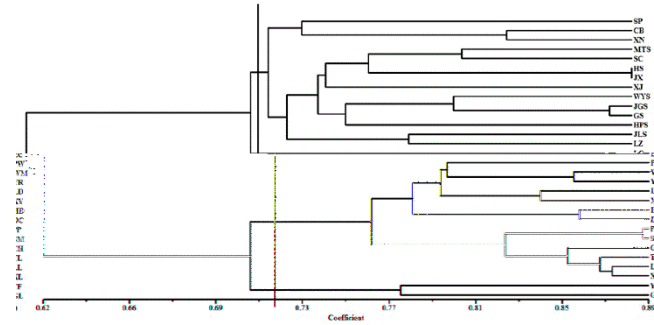




### Simple Sequence Repeats (SSRs)

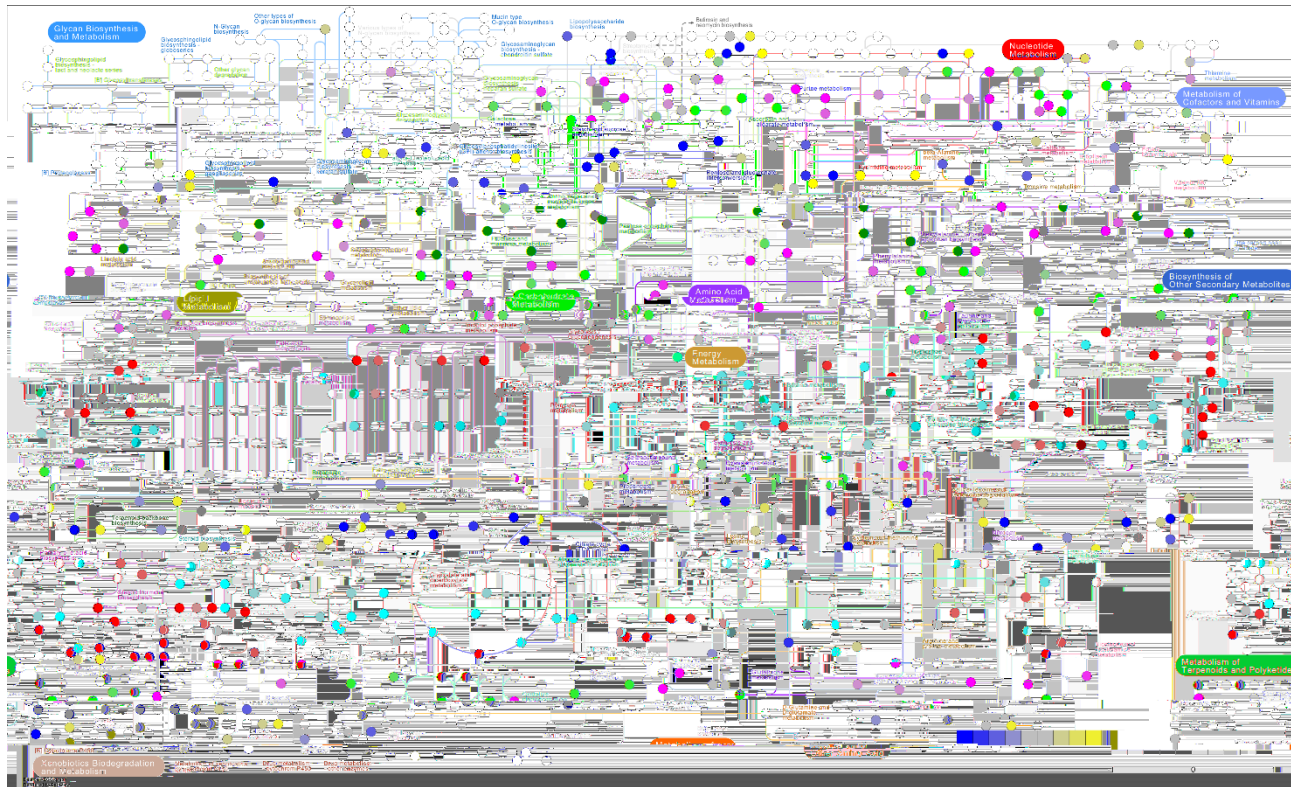


unique flanking regions  
(primer design)



ACACCATAATTTTATCGGTAATGGTTCATGTCGCTTATAAAAACCTATCTCAAGCTC  
 CCGTAGAAATTGTTCCCTGGCATAGAGAACTAGCATGTCCATATATTTTCATTAATTG  
 STAATAGTTCACAATCCTT ATCAAAGCAATGGTAAGGTGCACAACAATTTTACCA  
 AGATAACTTATTTTGATAA ACATCAAACCCATTATATTGTATACAGCGCCATACCT  
 ACTTGAGAGCAACCTAGAG CTCTCTCTCTCTCTCACATATATACTGCTGTAAGA  
 CACACACATATATACTGCT CCATTAAATTCTCGATCATAGAGTTCACACACACACA  
 CGATCATAGAGTTCACTAA GTAAGAACTTGAGAGCAACCTAGAGCCATTAAATTCT  
 ATGATAGCTTTTATAAATC TTCTTACTGCAACAATAATCCCAATCTTACACATGGC  
 CATGCGACATGCACGTCAA TTTAGTTTGCTTATCTGAACACATAGATAATGAAAAC  
 CATGAGGCCCTCCAAGGAT TAACCGTTGGATCAATGGTCAAGAAACAACCTACAAAC  
 CATCAGCGGTAATTCAAAT ATTGGTGCGCTTTCCTTATTTGCTTTCCATATAAACA








Sequences in Red: repeat motif - (CT)<sup>8</sup>(CA)<sup>2</sup>(TA)<sup>3</sup>; Sequences in Blue: repeat motif –  
 (CA)<sup>10</sup>(TA)<sup>3</sup>





## Applications Forums

Platform agnostic discussions about scientific applications of sequencing data

	<b><a href="#">Sample Prep / Library Generation</a></b> (6 Viewing) Techniques and protocol discussions on sample preparation, library generation, methods and ideas
	<b><a href="#">Genomic Resequencing</a></b> (1 Viewing) Variant discovery in previously sequenced genomes/regions
	<b><a href="#">De novo discovery</a></b> (1 Viewing) Wandering without a reference? Post here
	<b><a href="#">Metagenomics</a></b> (3 Viewing) Ever wonder what's growing in that hot spring or glacier?
	<b><a href="#">Epigenetics</a></b> (1 Viewing) Any non-primary sequence heritable modification of genetic material. ChIP-SEQ, DNA methylation (Bisulfite-SEQ), chromatin modifications (methylation, acetylation, etc), non coding RNA.
	<b><a href="#">RNA Sequencing</a></b> (20 Viewing) Application of sequencing to RNA analysis (RNA-Seq, whole transcriptome, SAGE, expression analysis, novel organism mining, splice variants)
	<b><a href="#">Clinical Sequencing</a></b> Discuss issues unique to clinical sequencing.



