Single Nucleotide Polymorphism

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Introduction

DNA

In 1870, the Swiss chemist Miescher discovered inside the nucleus of a cell a giant molecule: **deoxyribonucleic acid**.

Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many vi-ruses. DNA is a nucleic acid; together with proteins and carbohydrates, nucleic acids compose the three major macromolecules essential for all known forms of life.

DNA components

Most DNA molecules consist of *two biopolymer strands coiled around each other to form a double helix*. The two DNA strands are known as **polynucleotides** since they are composed of simpler units called **nucleotides**. Each nucleotide is composed of a **nitrogen-containing nucleobase**—either **guanine** (G), **adenine** (A), **thymine** (T), or **cytosine** (C)—as well as a monosaccharide sugar called **deoxyribose** and a **phosphate** group.

The nucleotides are joined to one another in a chain by *covalent bonds between the sugar of one nucleotide and the phosphate of the next*, resulting in an *alternating sugar-phosphate backbone*.

Rules: A with T and C with G.

DNA structure

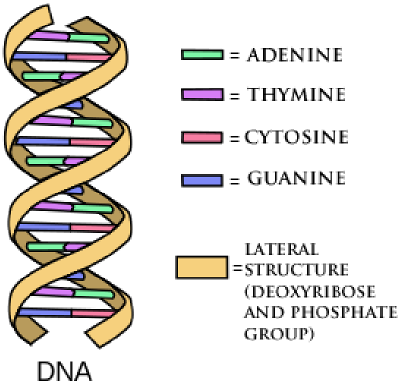


Figure 1: DNA structure

Mutations

That said, it is easy to understand how DNA is important for life. For this reason, even a small mutation (a change of the nucleotide sequence of the genome of an or-ganism) can be decisive and cause diseases.

We will discuss a particular case of genomic mutation, the **Single Nucleotide Polymorphism**.

Single Nucleotide Polymorphism

Single Nucleotide Polymorphism: what is it?

A **Single Nucleotide Polymorphism (SNP)** is a DNA sequence variation occurring commonly within a population (e.g. 1 per cent) in which a Single Nucleotide — A, T, C or G — in the genome differs between members of a biological species or paired chromo-somes.

SNP example

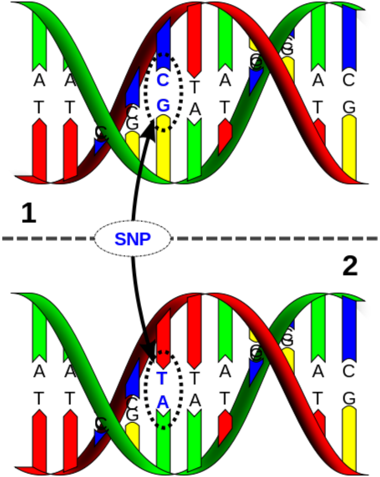


Figure 2: SNP example

What can cause a SNP?

The main causes of a SNP are:

1. natural selection, acting and fixating the allele of the SNP that constitutes the most favorable genetic adaptation
2. like genetic recombination
3. mutation rate

The different possible types of SNPs

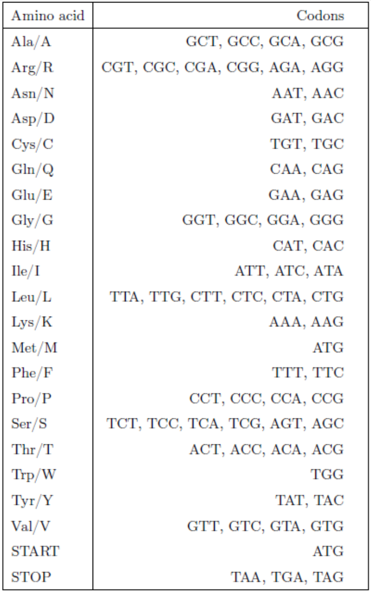
What is a coding?

Genetic Code: it is the *set of rules* by which information encoded within genetic material (DNA or even mRNA sequences) is *translated* into proteins by living cells.

During the translation, the sequence of nitrogenous bases is treated in groups of three at a time (**codon**). The code defines how codons specify which amino acid will be added next during protein synthesis.

The “original sin”

Generally, three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. On the other hand, **a single amino acid can be specified by more than one codon**.



Amino acid	Codons
Ala/A	GCT, GCC, GCA, GCG
Arg/R	CGT, CGC, CGA, CGG, AGA, AGG
Asn/N	AAT, AAC
Asp/D	GAT, GAC
Cys/C	TGT, TGC
Gln/Q	CAA, CAG
Glu/E	GAA, GAG
Gly/G	GGT, GGC, GGA, GGG
His/H	CAT, CAC
Ile/I	ATT, ATC, ATA
Leu/L	TTA, TTG, CTT, CTC, CTA, CTG
Lys/K	AAA, AAG
Met/M	ATG
Phe/F	TTT, TTC
Pro/P	CCT, CCC, CCA, CCG
Ser/S	TCT, TCC, TCA, TCG, AGT, AGC
Thr/T	ACT, ACC, ACA, ACG
Trp/W	TGG
Tyr/Y	TAT, TAC
Val/V	GTT, GTC, GTA, GTG
START	ATG
STOP	TAA, TGA, TAG

Figure 3: Amino acids

Types of SNPs

SNPs may fall within *coding* sequences of genes, *non-coding* regions of genes, as well as in the *intergenic* regions (regions between genes).

SNPs in the coding sequences

SNPs that fall in this category can be divided into two subcategories:

1. Synonymous
2. Nonsynonymous
    - Missense
    - Nonsense

Missense mutation - Example

Original DNA code for the amino acid sequence:

CAT	CAT	CAT	CAT	CAT	CAT	CAT

Resulting amino acids:

His	His	His	His	His	His	His

If we had, for example, a replacement of the eleventh nucleotide:

CAT	CAT	CAT	C**C**T	CAT	CAT	CAT

Resulting amino acids will be:

His	His	His	**Pro**	His	His	His

Nonsense mutation - Example

Original DNA code for the amino acid sequence:

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

Resulting amino acids:

Met	Thr	His	Arg	Ala	Arg	Ser

If we had, for example, a replacement of the tenth nucleotide:

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

Resulting amino acids will be:

Met	Thr	His	**Stop**			

SNPs not in coding regions

SNPs that are not in protein-coding regions may still affect:

1. gene splicing
2. transcription factor binding
3. messenger RNA degradation
4. . . .

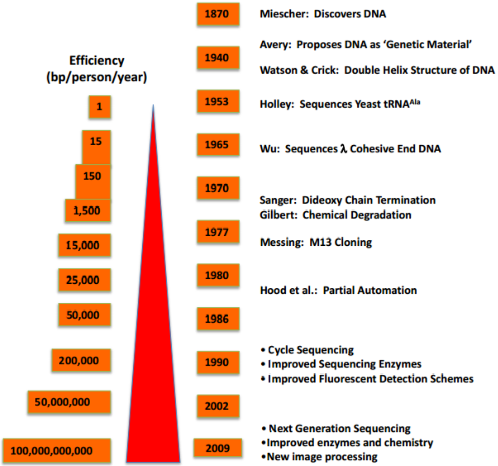
Gene expression affected by this type of SNP is referred to as an **eSNP** (*expression SNP*).

How to found SNPs: DNA sequencing

DNA sequencing

"DNA Sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases — adenine, guanine, cytosine, and thymine — in a strand of DNA."

History of DNA Sequencing



Efficiency (bp/person/year)

1

15

150

1,500

15,000

25,000

50,000

200,000

50,000,000

100,000,000,000

1870 Miescher: Discovers DNA

1940 Avery: Proposes DNA as ‘Genetic Material’

Watson & Crick: Double Helix Structure of DNA

1953 Holley: Sequences Yeast tRNA^{Ala}

1965 Wu: Sequences λ Cohesive End DNA

1970 Sanger: Dideoxy Chain Termination

Gilbert: Chemical Degradation

1977 Messing: M13 Cloning

1980 Hood et al.: Partial Automation

1986

1990

- Cycle Sequencing
- Improved Sequencing Enzymes
- Improved Fluorescent Detection Schemes

2002

2009

- Next Generation Sequencing
- Improved enzymes and chemistry
- New image processing

Figure 4: History of DNA Sequencing

Sequencing methods

Over the years, many methods have been developed for sequencing the DNA:

1. **basic methods**, such as the *Maxam-Gilbert sequencing* and *Chain-termination methods*
2. **advanced methods**, such as the *Shotgun sequencing* or *PCR Bridge*
3. **next-generation methods**, such as *Massively Parallel Signature se-quencing (MPSS), Polony sequencing, 454 pyrosequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Single Molecule Real Time (SMRT) sequencing, ...*

Next-Generation Sequencing

Nowadays, thanks to technological progress we pushed further forward. It is possible to sequence **more than 100 million base pairs in about a week** (generating a very high amount of data).
This is called the **Next-Generation Sequencing**.

However, the higher the speed of sequencing, the more there is a problem: **interpretation**. It often represents a real bottleneck; a single computer is not able to interpret a sequencing at the same speed of which it is presented to him.

Solution: *cloud computing*.

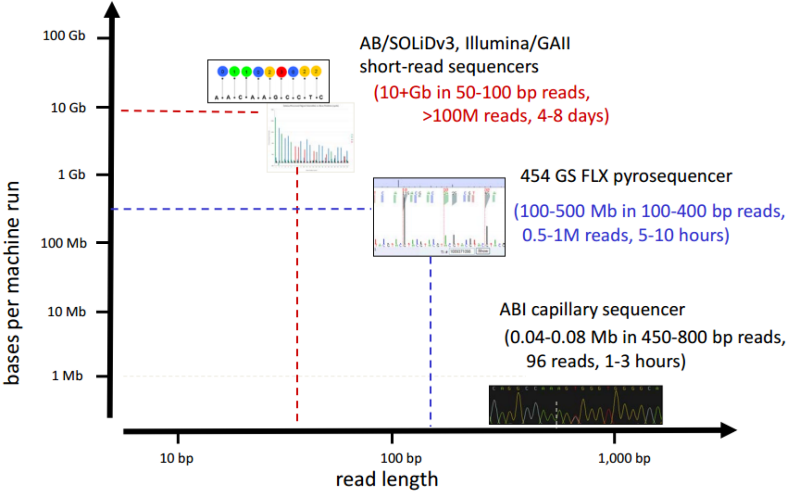
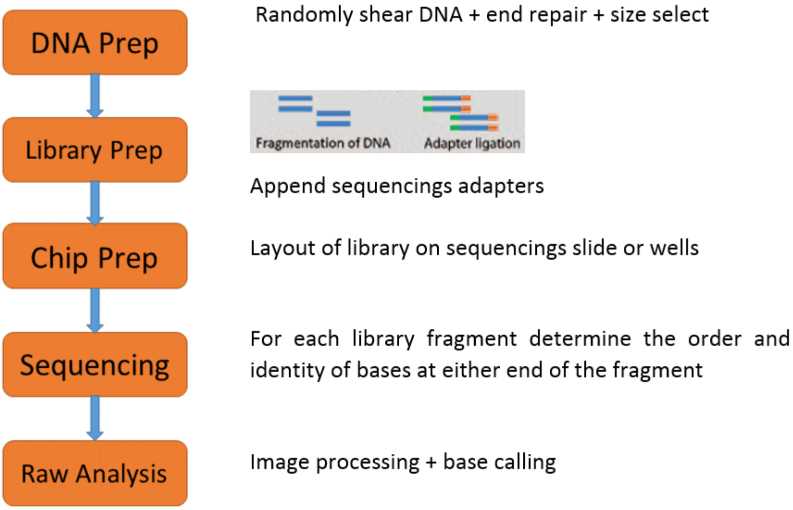


Figure 5: Nowadays Sequencing



Randomly shear DNA + end repair + size select

Append sequencings adapters

Layout of library on sequencings slide or wells

For each library fragment determine the order and identity of bases at either end of the fragment

Image processing + base calling

Figure 6: NGS work-flow

Whole Exome Sequencing

- The type of sequencing used to obtain the data that our web-app manages.
- The **Whole Exome Sequencing** test is a highly complex test that is newly developed
- In contrast to “common” sequencing tests that analyze one gene or small groups of related genes at a time, the WES test analyze the *exons or coding regions of thousands of genes simultaneously* using next-generation sequencing techniques.

Exome: portion of the human genome that contains functionally important sequences of DNA that direct the body to make proteins essential for the body to function properly

Whole Exome Sequencing

- It is known that **most of the errors that occur in DNA sequences that then lead to genetic disorders are located in the exons**. Therefore, sequencing of the exome is thought to be an efficient method of analyzing a patient's DNA to discover the genetic cause of diseases or disabilities.
- WES includes a **mitochondrial genome sequencing**. (Mitochondria: structures within cells that convert the energy from food into a form that cells can use).

Data Format

CSFASTA

The sequencer that generates the data managed by our web-app provides the results of the sequencing using format **CSFASTA**

>MCHU - Calmodulin - Human, rabbit, bovine, rat, and chicken

ADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQD MINEVDADGNGTID FPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREA DIDGDGQVNYEEFVQMMTAK*

SNP Databases

Why databases?

Because SNPs are expected to facilitate large-scale association genetics studies, there has recently been great interest in SNP discovery and detection. For this reason databases can to serve as a central repository. Once discovered, polymorphisms could be used by additional laboratories, using the sequence information around the polymorphism and the specific experimental conditions.

Most important DBs

1. **dbSNP**, a SNP database from the *National Center for Biotechnology Information (NCBI)*
2. **SNPedia**, a wiki-style database supporting personal genome annotation, interpretation and analysis

Support DBs

Furthermore, there are various support database that allow, for example, to bind a SNP to the disease that causes:

1. **OMIM** database describes the association between polymorphisms and diseases (e.g., gives diseases in text form)
2. **Human Gene Mutation Database** provides gene mutations causing or associated with human inherited diseases and functional SNPs
3. **GWAS Central** allows users to visually interrogate the actual summary-level association data in one or more genome-wide association studies
4. . . .