Computational microbial genomics

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Chapter 1

Escherichia Coli general informations

1.1 *E. Coli* genomics

Escherichia Coli is a Gram-negative, facultative anaerobic, rodshaped, coliform bacterium, it pertains to the phylum of proteobacteria and to the family of Enterobacteriaceae. It can be grown easily and inexpensively. It has got genome with a length between 4.5 - 4.7 M bases, including about 4000-5000 genes, and about seven ribosomal RNA operons. Only the 38% of the genes of K-12 (one of the most studied bacterial strains of *E. coli*) were experimentally identified, overall 40-50% of the genes are to date without a known function. The original *E. coli* strain K-12 was obtained from a stool sample of a diphtheria patient in Palo Alto, CA in 1922.

1.1.1 *E. coli* long-term evolution experiment

The *E. coli* long-term evolution experiment led by Richard Lenski is one of the longest evolutionary experiments ever made (search "*The Longest-Running Evolution Experiment*"). The experiment started on 24th February 1988, and since that moment 12 populations of *E. coli* have been cultivated in the same environment. After each day (corresponding to the time of development of approximately 7 generations), a portion of bacteria from each flask was introduced in a new one, and let proliferating in it. Every 500 generation, it has been saved a sample of the bacteria of each flask, in order to track the evolutionary changes made. Today the experiment is on-going, and researchers reached approximately the 66000th generation. The study suggests a series of conclusions, to cyte "long-term adaptation to a fixed environment can be characterized by a rich and dynamic set of population genetic processes, in stark contrast to the evolutionary desert expected near a fitness optimum" (Good et al 2017). In fact, despite of the fixed environment, some bacteria developed the capacity to aerobically grow on citrate, which is unusual in *E. coli* (around generation 31,000) and developed complex mutation patterns.

1.1.2 *E. coli* strains

E. coli could be found as commensal strains, pathogenic strains, or environmental strains. The pathogenic strains could pertein to these categories (which are not exclusive): enteropathogenic

(EPEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), diffusely adherent (DAEC), adherent invasive (AIEC), shiga-toxin producing (STEC), enteroaggregative(EAEC), extraintestinal pathogenic (ExPEC). Resistances to antibiotics make even more difficult the process of categorization of *E. coli*. In 2011 in Germany, an outbreak of Stx-EAEC was responsible of the death of some people. An efficient counter-measure was found by sequencing the genome of those bacteria.

Shigella is *E. coli* with shiga toxin. It had been an issue for toxonomists.

Most of the genes are on plasmids, circular, additional to chromosome, and can be moved easily horizontally. Plasmids between different strains can be moved in enterobacteriacae, this doesn't happen normally in other families. Some *E. coli* strains are even capable of causing tumors in humans: for example, colibactin-positive E. coli can cause colon and rectal cancer, by creating mutations which are responsable of the of the cancer onset.

several antigens can be used by taxonomists to cathegorize *E. coli* strains. In particular there are the O, H, K antigens, respectively related to the somatic, the flagella and the capsule. O antigens are 171, Ks are 80 and Hs are 56.

1.1.3 PanPhlAn - strain detection and characterization

Pangenome-based Phylogenomic Analysis (PanPhlAn) is a strain-level metagenomic profiling tool for identifying the gene composition and in-vivo transcriptional activity of individual strains in metagenomic samples. PanPhlAn's ability for strain-tracking and functional analysis of unknown pathogens makes it an efficient tool for culture-free infectious outbreak epidemiology and microbial population studies (PanPhlAn reference). This tool was for example used to study the strain responsible of an outbreak in Germany in 2011. This strain was a shiga-toxigenic Escherichia coli (STEC), and the study was conducted by Loman and collegues in 2013. This method outlasted the traditional one.

sequencing means generally to sequence everything, it's normally difficult where to find it, although in E. coli is quite easy to understand the provenience. from all the world, populating diversity of E. coli, every time we sample we find points which are different from the reference genomes. points overlapping are people living together and share bacterias

1.1.4 Genomics of *E. coli*

In the genome of *E. coli* strains, it is possible to distinguish:

- **Core genome**: the set of all genes shared by all membrers of a bacterial species, it includes 1000 up to 3000 genes.
- **Accessory or dispensable genome**: the set of genes present in some but not all genomes within the same bacterial species. found on a single strain or in a subset of strains.
- **Pangenome**: core genome + accessory genome. set of all the genes foundable in the species strains. It is said to be ***"closed"*** when pangenome size tends to a maximum as number of genomes increases, instead it is ***"open"*** when pangenome keeps increasing as you add new genomes

Sequencing more organism of the same species means to lower the amount of genes in the core genome and augment the numer of those in the pan-genome (figure **??**). Because of technical problems the probability of getting a gene and not forget it is different from 0, so why probably the sequencing of other genomes would lead technically to a plummet to 0 of the pangenome. With some mathematical formulations we can predict a more probable plateau (Rasko David 2008).

Figure 1.1: Core- and pan- genome of *E. coli*

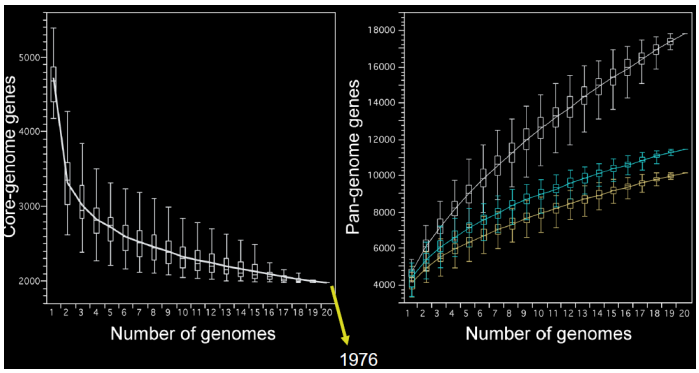
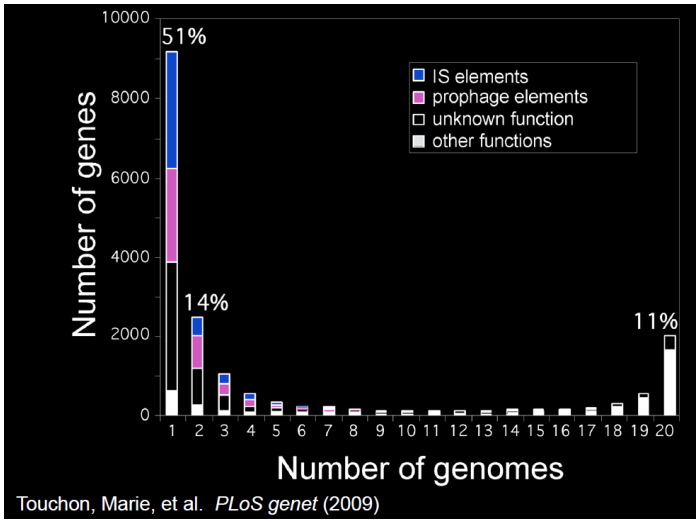


Figure 1.2: It can be seen that 51% of the genes are strain specific, and the other are shared between 2 to 20. of *E. coli*



each *E. Coli* genome contains in a balanced way genes of the core genome and of the pan genome, for a total amount of genes correspondant to about 4700 genes (figure **??**). Core genomes' genes are responsible of some basic cellular functionalities and utilities to survive environent, while instead elements of the pangenome are quite usually specific to the single strains, they are not always functionally well known.

ratios of the pan-genome and the core-genome are not equal in other organisms behave differently

Figure 1.3: A different definition of orthologous genes can modify the number of pan genes of a species.

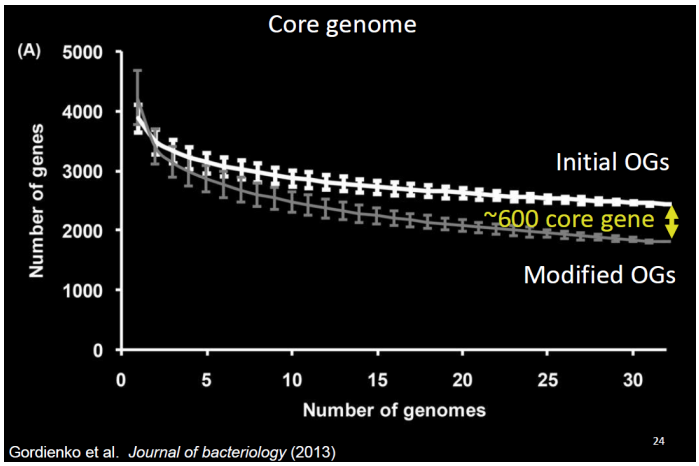


Figure 1.4: we predict a core genome size of 3079 genes for extraintestinal pathogenic *E. coli*

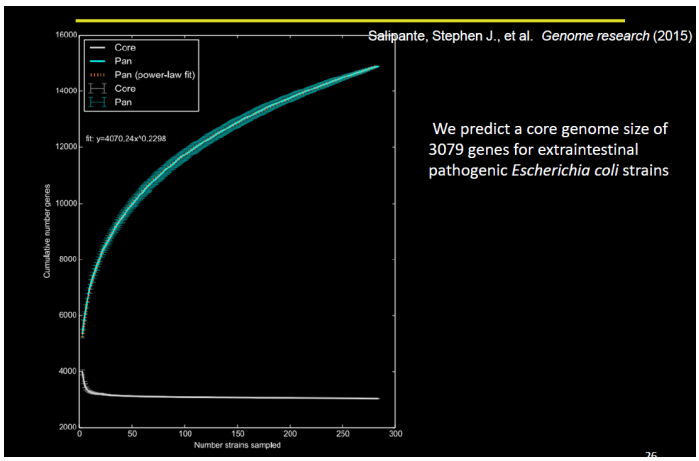
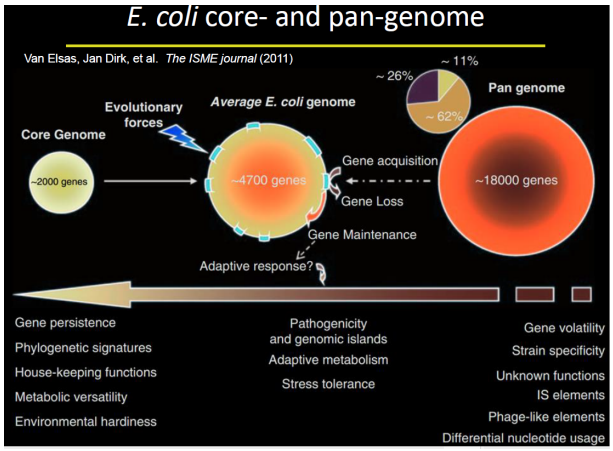


Figure 1.5: Balance between genes of the core- and of the pan-genome



Chapter 2

Sequencing Data

hard drive with the sequencing data

2.0.0.0.1 Given a biological problem, which sequencing machine is optimal?

- (no problem with) repeats
- read line
- troughput ($\frac{Millionsofreads}{run}$)
- library preparation compatibility
- coverage
- cost: of the sequencing of the machine itself. $\frac{Reagentcost}{run}$, $\frac{cost}{Mb}$, $\frac{cost}{run}$, service contract
- accuracy: multipe types of errors: Indel, Substitution, CG deletions (deletions o full CG), A-T bias. The error rate takes in account all the primary errors of the specific instrument.
- speed / run-time
- contamination

GA II was the first version of illumina. SOLID was a sequencing machine which is not anymore used. Rouche454 responsible of revolution in 2007, not used anymore. MiSeq is also used today. Long read sequencing MinION and pacBio are probably next generation. pacBio has very log reads and close to single modlecule sequencing. Ion-Torrent is not anymore used. MinIon and PacBio gives real time sequencing. 454 quite hifh number of reads but the trouput low.

Going towards high throughput.

Illumina NovaSeq is the biggest in the market. very high throughput need.

ILLUMINA has very small machines, not expensive

MinION is portable, wet-lab free, real time sequencing. It was used in Ebola pandemic- Used for sequencing DNA, RNA, proteins. The read length follows a Gaussian curve, as there can be also really long reads.

Afer base calling, which is the process through machine converts signals in digits representing the bases. It is made a FASTQ format file. other machines generate other files, but all can at least

Figure 2.1: Machines for sequencing

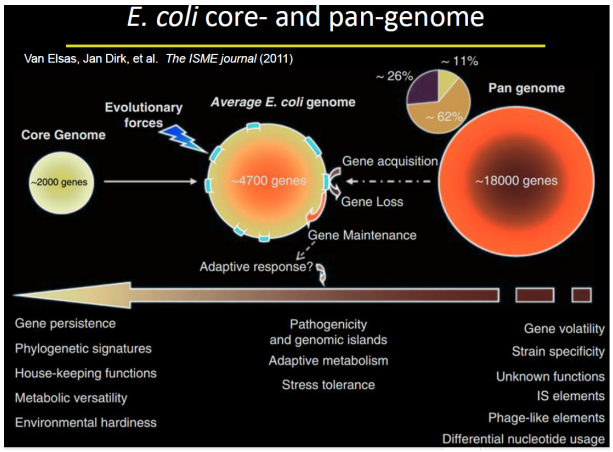
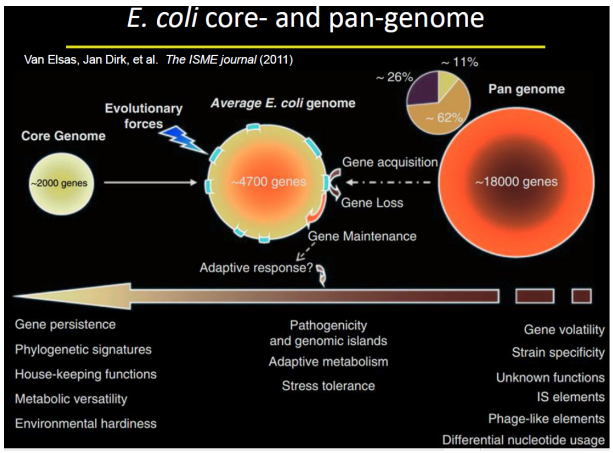


Figure 2.2: Machines for sequencing



convert their output in FASTQ. FASTQ contains the sequencing reads and the quality of each base. FASTQ needs to be stored compressed.

Phred- the base calling program, are based on algorithms, to check statistically a sequence. The phasing noise $\phi, the intensity scew the intensity of the signal of the closest nucleotide.$

siignal decay δ

on ILLUMINA there can be mixed clusters.

Boundary effects: image if zoomed in it is not really possible to give perimeters to the images of the signals. Overclusteringm akes it very difficult to separate different nucleotide produced signals. Underclustering and Overclustering, optimal clustering in between.

Base-caller has to be performing. By sequencing a known sequence it is possible to evaluate the base caller.

Figure 2.3: Machines for sequencing

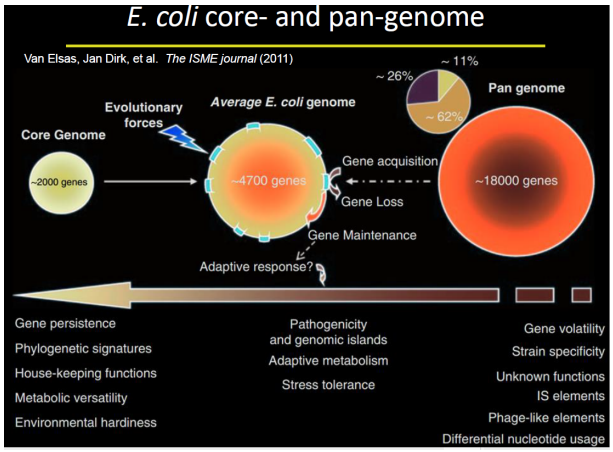
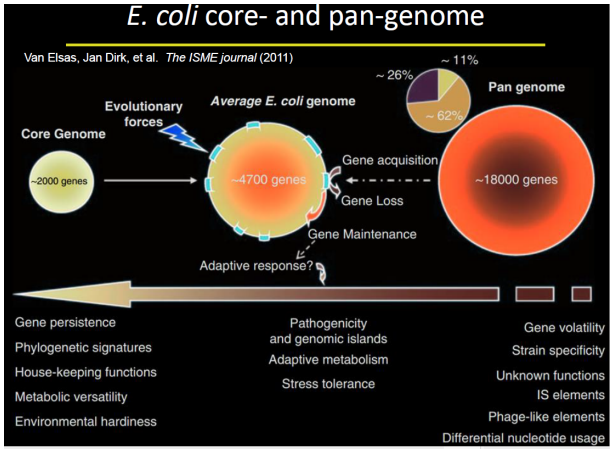


Figure 2.4: Factors to take into account by the base caller



2.0.1 FASTQ format

1. "@" followed bt a sequence identifier
2. the sequence
3. "+", optionally followd by a sequence identifier
4. The quality scores: several symbols.

Base quality:

$$Q = -10\dot{\log}_1 0p$$

p = probability of error

Phred+33 transforms integers with characters. Each number can be told in an integer.

FASTA format

Figure 2.5: Machines for sequencing

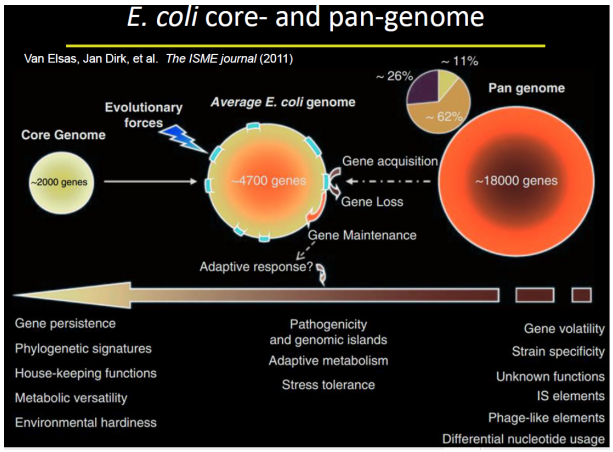


Figure 2.6: Base quality. Going under 0.01% is quite impossible. 3 to 40

1. ">" followed by a sequence identifier
2. The sequence

2.0.1.0.1 Transform FASTQ in FASTA format file

Figure 2.7: Base quality. Going under 0.01% is quite impossible. 3 to 40