Bi5444 Analysis of sequencing data

Lesson 2 - General information and introduction

General introduction

Teachers



- Dr. Eva Budinska – guarantor, teacher – RECETOX, MU
- Assoc. prof. Marek Mraz, MD – teacher - Univ. hosp. Brno & Fac. of Medicine & CEITEC MU
- Ing. Vojtěch Bartoň – teacher, RECETOX MU

Each will take care of different part of the course and at the end everything will be “merged” together

Lets get to know each other…

- Are you **familiar** with the field?
- Do you **work** with the **data** somehow?
- Do you **receive** any **outputs** from the analysis?
- Do you **plan** to **work** with it?
- Do you want to **understand** the **outputs**?

The course itself

Main objective:

- The course is **addressed** to everyone who **already works** with or **plans to work** with the NGS data, wants to learn something new from the field or wants to understand the data/outputs
- The **lectures will explain** you **the basics** and show you **examples**
- You will need to **study/exercise some extra** to get **better understanding** of the process
- During **the course,** there will be possibility to **you can discuss your own data analysis** (if any)

After the course you should…

- **Know the latest NGS methods** (next and third generation sequencing), their use and the type of data they produce
- **Be able to distinguish the type** of method based on the data
- Know the **basic scheme** of data analysis
- Able to work with **Linux**, **Bash** and **R** at a level sufficient for analysis of NGS data – partially
- Know how to **select tools** for data processing and apply them to real data
- **Be able to analyze NGS data** starting from quality control over alignment to the detection of deferentially expressed genes (in RNA-Seq), variants (CNV with SNP), genome assembly, etc.

Content of the course

- We will **not cover all topics** in the NGS field – simply there is **not enough time**
- We will provide you with **solid basics of NGS data analysis** that will allow you to **easily extend** to almost **any** NGS **application** and data types and to **work** with the **data**
- We will give you **hints** where to **look**, what to **look for**, what to **study** and how to **think** about the data
- At the beginning we will cover the **biology background** and also do the **revision of your knowledge** in the **biology/molecular biology** field – necessary for correct understanding of the NGS

Course content

- 1. Introduction to NGS technologies: a brief introduction to biology, sequencing, history, NGS technologies and their applications, sample extraction, library preparation, basic glossary. Course requirements and schedule.
- 2. Pitfalls of NGS and the consequences for data analysis.
- 3. Data sources. The basic scheme of data analysis: how the data look like, definition of general steps in NGS data analysis, basic differences in dependence on the application (eg. variant calling vs RNA-Seq …).
- 4. Introduction to software for data analysis: a brief introduction to work with Linux, Bash and R, data formats and the differences between them.
- 5. Data preprocessing and quality control: tools for quality control, Phred score, examples on sample data.
- 6. Alignment and post-processing: reference genome databases, annotations, the differences between them and application, explanations of alignment algorithms, differences between spliced/non-spliced tools and their application, alignment quality control, alignment visualization.
- 7. Analysis of RNAseq data - differentially expressed genes
- 8. Variant calling – targeted sequencing, methods for calling, specific QC steps
- 9. Metagenomics (16S, ITS, WMGS) / algorithms for taxonomy and functional assignment
- 10. Statistics and visualisation
- 11 and 12. Project defense

Let’s check the prerequisites

- Knowledge of **molecular biology**
- At least a **basic knowledge** of work with **Linux** system
- **Basic** knowledge of **R and statistics** is an **advantage**
- **Basic programming** knowledge is an **advantage**

Study materials

- There are plenty of **study materials available online**
- But the whole **field changes** very **quickly** – try to **look** for the **latest information**
- **Few years old** materials are most likely **not very useful** any more or the are already surpassed
- **Presentations** from the lectures **will be** available **online**
- There will be always a **link** to some interesting **papers** during the **course** where possible
- **It is never a bad idea to ask!**

Other recommended courses

- **C2110 UNIX and programming**
- **Bi7560 Introduction to R**
- **Bi7420 Modern methods for genome analysis**
- **Bi7528 Analysis of genomic and proteomic data**
- **Bi7527 Data Analysis in R**
- **Bi7492 DNA Sequence Analysis**
- **Bi5010 Detection of biomarkers from omics experiments**

- You can also see the study catalogues of Mathematical Biology and Biomedicine (direction Biomedical Bioinformatics) or Chemoinformatics and bioinformatics degrees for the recommended courses

Online courses - examples

- Linux/Unix
- http://www.ee.surrey.ac.uk/Teaching/Unix/, …
- BioLinux
- http://nebc.nerc.ac.uk/nebc_website_frozen/nebc.nerc.ac.uk//support/training/course-notes/past-notes/intro-bl7, …
- R
- http://www.r-tutor.com/r-introduction, http://ww2.coastal.edu/kingw/statistics/R-tutorials/text/quick&dirty_R.txt, …
- Other interesting courses
- https://www.coursera.org/, http://online.stanford.edu/courses, https://www.edx.org/, http://www.codecademy.com/, http://ocw.mit.edu/index.htm, http://www.rna-seqblog.com/, …
- Questions & Answers
- http://seqanswers.com/, https://www.biostars.org/, http://stackoverflow.com/, …
- Blogs & Other
- www.linkedin.com, www.researchgate.net, http://core-genomics.blogspot.cz/, http://nextgenseek.com/, https://twitter.com/, …
- Introduction to Next Generation Sequencing
- https://www.ebi.ac.uk/training/course/introduction-next-generation-sequencing, …

Work with the computer

- We will try to cover the basics of work with Linux, bash, R, … BUT the course is **not** directly meant to be focused on programming and/or work with Linux system, bash, R, etc.
- It will be very helpful (for you) to look into some basics on your own
- There are numerous tutorial available online for everything (uncle Google can help you very well)
- There are also several very helpful online courses organized by top universities all over the world

Evaluation and grading

Group project

- During the semester - Group project (2-3 persons) – max 20 points – finished **before the start of the exam period**
- **Aim**: Analysis of NGS data from raw data to interpretation
- **Data**: Downloaded from databases or your own
- The projects will be **defended last two weeks of the semester**
- The project has to score **minimum 10 points**
- **PROJECTS MUST BE SELECTED before 13.10.2023**

Project

- To successfully finish the project you have to:
  - Prepare and handout a **document** with description of the project:
    - Title, background and hypothesis
    - Data description (number of samples, platform, sequencing details)
    - Methods description
    - Results
  - Handout a commented and organized **code**

Project

- **Type of samples:**
  - Bacteria/fungi/mouse/human/plant/meta genome
- **Type of sequencing:**
  - WGS, WMGS, RNAseq, variant calling, ITS/16S
- **Possible aims and numbers of samples:**
  - identification of strains (5-10) by WGS – taxonomy/phylogeny
  - differentially expressed genes (min 10 per group) by RNAseq / functions/pathways
  - identification of mutations by targeted sequencing of mutations (min 10 per group)
  - identification of taxonomical composition /functional annotation (min 10 per group)

Evaluation and grading

PROJECT

- During the semester - Group project (2-3 persons) – max 20 points – handout **before the start of the exam period** (22.12.2023)

- Project handout is **compulsory before entering the exam**
- To successfully pass you need at least 10 points from the project

EXAM

- Written test – 10 questions, 20 points

- To pass the exam you need minimally 20 points, of which 10 of the project

Attendance

- The course is structured as 2+1 (lecture + exercise)
- The presence on the “exercise” part is compulsory, 1 non-excused absence is allowed
- However, due to practical reasons, exercise and presentations are organized on the “as needed basis”
- Attendance is compulsory for the defense of the project (13.12 or 20.12)
- Every member of the project group has to present results

Computational resources

Access to the computers/resources

- Access to the resources –C4/1.18
- You need to get access to **WOLF** computers (here)
- http://wolf.ncbr.muni.cz/
- Apply for the account –now:

- https://einfra.ncbr.muni.cz/whitezone/root/index.php?lang=en&action=ncbr&show=wolf

- To the description what you want to do please put: “*Student of E5444 – fall semester 2023*”

Access to the resources - metacentrum

- MetaCentrum resources
- https://metavo.metacentrum.cz/en/index.html

- Apply for the account online “Getting an account -> Registration form”
- Login with MU identification number & secondary password and ask for account creation
- To the description what you want to do please put: “*Analysis of the sequencing data*” or similar
- You can work with you laptop but you would have to install all required tools on your own –contact us if it is your case

NGS introduction

Next-generation sequencing introduction

- Deciphering DNA sequence is essential for all the branches of “biological” research
- It has become widely adopted in numerous laboratories all over the world
- **Next-generation sequencing (NGS)** is a new (almost) technology in the sequencing
- It helps to overcome the limitations of older techniques such as speed, scalability, throughput and resolution
- In this course you will get familiar with NGS as itself, its use and basic data processing

Before we proceed any further

- **Bioinformatics** (and especially the sequencing bioinformatics) is a **very new field**
- No good books, no standards, nothing lasts forever, … **almost everything** is old and **outdated**!
- **Bioinformaticians** have to be **always** looking for **new methods**, tools, algorithms, … it's the same when wet-lab people must search for novel methods which for decrease bias, are faster, require less input material, …
- The good thing is that there is **still a space for improvement** – for you!
- However, the data **analysis is never trivial**
- **Garbage in –garbage out**
- If you **do not understand** the whole process you **don't know** what the **results** mean

Very short history

- **Maxam–Gilbert** sequencing 1977 –complex, very radioactive, …
- **Sanger** sequencing 1977 –widely used, dideoxy method, “golden standard” (??), slow, low throughput, …
- **Next-generation sequencing** since 2001
- Started with pyrosequencing (1999 in Sweden) – later “rented” by 454 -> Roche, now discontinued
- Big leap forward thanks to the **Human Genome Project**
- HGP was launched in 1990 and finished in 2003 by publishing first complete human genome (**$2.7 billion**) – classic Sanger
- They had competition – Celera genomics founded in 1998 and finished in 2003 ($300 million) –shotgun sequencing
- But Celera cheated a bit

Year 2010


华大基因
BGI
Complete genomics
illumina
Roche
life technologies
PACIFIC BIOSCIENCES
Helicos
BioSciences Corporation
ion torrent
Oxford NANOPORE Technologies
RainDance Technologies
VISIGEN BIOTECHNOLOGIES, INC

Year 2023


10x GENOMICS
华大基因 BGI
Complete genomics
illumina
Roche
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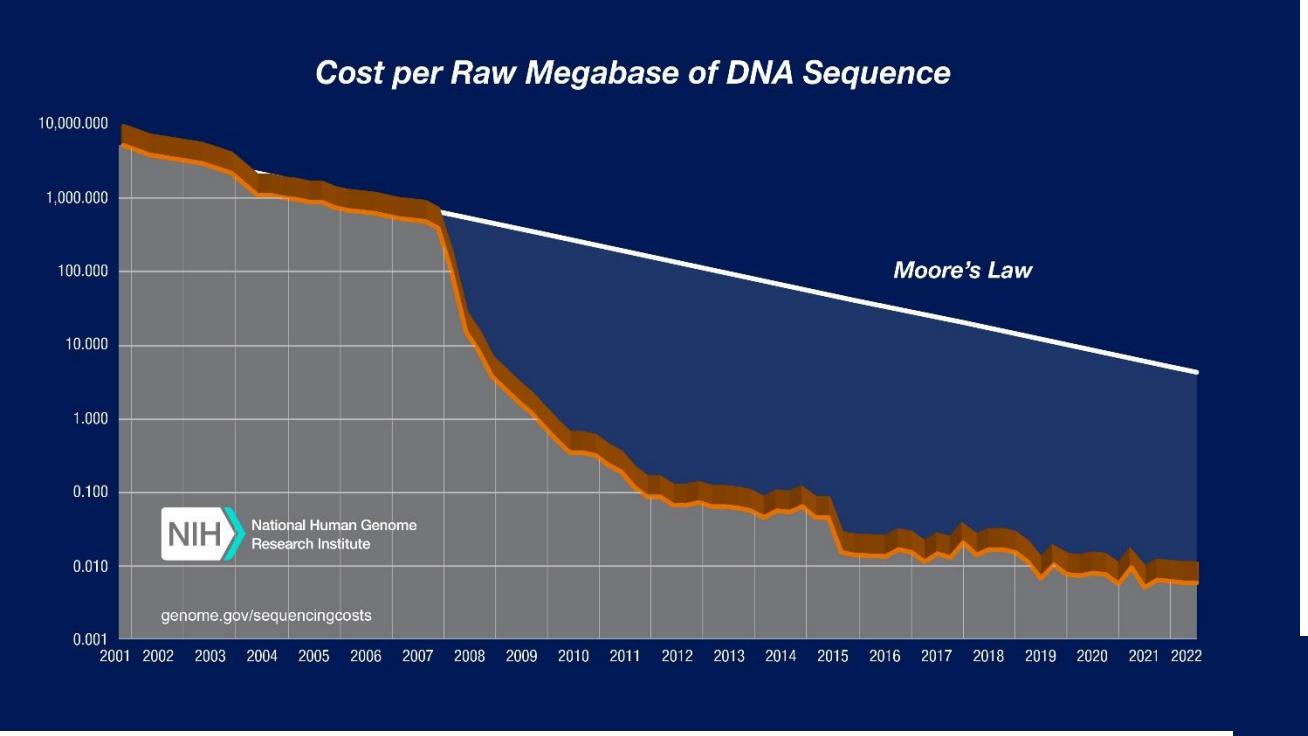
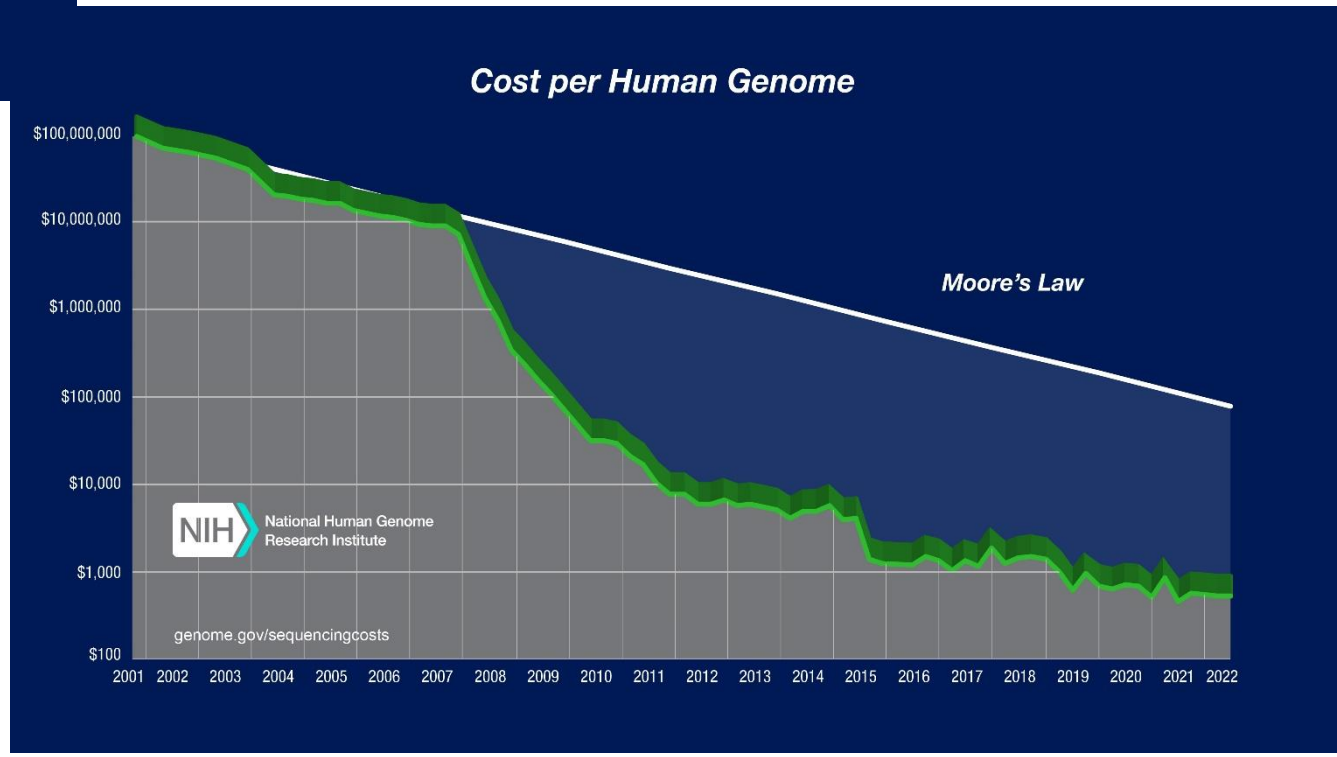
PACIFIC BIOSCIENCES
Helicos BioSciences Corporation
ion torrent
Oxford NANOPORE Technologies
RainDance Technologies
ThermoFisher SCIENTIFIC
VISIGEN BIOTECHNOLOGIES, INC

Comparison of NGS

Method	Read length	Accuracy (single read not consensus)	Reads per run	Time per run	Cost per 1 million bases (in US$)	Advantages	Disadvantages
Single-molecule real-time sequencing (Pacific Biosciences)	30,000 bp (N50); maximum read length >100,000 bases[66][67][68]	87% raw-read accuracy[69]	500,000 per Sequel SMRT cell, 10–20 gigabases[66][70][71]	30 minutes to 20 hours[66][72]	$0.05–$0.08	Fast. Detects 4mC, 5mC, 6mA.[73]	Moderate throughput. Equipment can be very expensive.
Ion semiconductor (Ion Torrent sequencing)	up to 600 bp[74]	99.6%[75]	up to 80 million	2 hours	$1	Less expensive equipment. Fast.	Homopolymer errors.
Pyrosequencing (454)	700 bp	99.9%	1 million	24 hours	$10	Long read size. Fast.	Runs are expensive. Homopolymer errors.
Sequencing by synthesis (Illumina)	MiniSeq, NextSeq: 75-300 bp; MiSeq: 50-600 bp; HiSeq 2500: 50-500 bp; HiSeq 3/4000: 50-300 bp; HiSeq X: 300 bp	99.9% (Phred30)	MiniSeq/MiSeq: 1-25 Million; NextSeq: 130-00 Million, HiSeq 2500: 300 million - 2 billion, HiSeq 3/4000 2.5 billion, HiSeq X: 3 billion	1 to 11 days, depending upon sequencer and specified read length[76]	$0.05 to $0.15	Potential for high sequence yield, depending upon sequencer model and desired application.	Equipment can be very expensive. Requires high concentrations of DNA.
Combinatorial probe anchor synthesis (cPAS- BGI/MGI)	BGISEQ-50: 35-50bp, MGISEQ 200: 50-200bp, BGISEQ-500, MGISEQ-2000: 50-300bp[77]	99.9% (Phred30)	BGISEQ-50: 160M, MGISEQ 200: 300M, BGISEQ-500: 1300M per flow cell, MGISEQ-2000: 375M FCS flow cell, 1500M FCL flow cell per flow cell.	1 to 9 days depending on instrument, read length and number of flow cells run at a time.	$0.035- $0.12		
Sequencing by ligation (SOLiD sequencing)	50+35 or 50+50 bp	99.9%	1.2 to 1.4 billion	1 to 2 weeks	$0.13	Low cost per base.	Slower than other methods. Has issues sequencing palindromic sequences.[78]
Nanopore Sequencing	Dependent on library prep, not the device, so user chooses read length. (up to 500 kb reported)	~92–97% single read	dependent on read length selected by user	data streamed in real time. Choose 1 min to 48 hrs	$500–999 per Flow Cell, base cost dependent on expt	Longest individual reads. Accessible user community. Portable (Palm sized).	Lower throughput than other machines, Single read accuracy in 90s.
Chain termination (Sanger sequencing)	400 to 900 bp	99.9%	N/A	20 minutes to 3 hours	$2400	Useful for many applications.	More expensive and impractical for larger sequencing projects. This method also requires the time





[DNA Sequencing Costs: Data (genome.gov)]

*Seq things

- NGS sequencing has a **wide range of use**
- One of many nice list give you an example of all possible applications
- http://enseqlopedia.com/enseqlopedia/

- Approximately (on this list) ~**200 different** techniques…
- Another (simple) list of NGS based techniques
- https://liorpachter.wordpress.com/seq/

BGI

See you next week, same place, same time

PacBio Sequel – Pacific Biosciences Technologies (SMRT)

MinION - Oxford Nanopore

Extra

SmidgION: Oxford Nanopore, iPhone-powered sequencing