Analysis of RNA-Seq data at the Flemish Super Computer

Introduction to RNA-Seq Pipelines and Computational Analysis Mini Workshop

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NGS Bioinformatics

NGS data means in general big data

Raw data (uncompressed) per sample:

- Whole Human Genome:
  - 300Gb
- Exome data:
  - 6GB
- RNA-Seq
  - 1 GB

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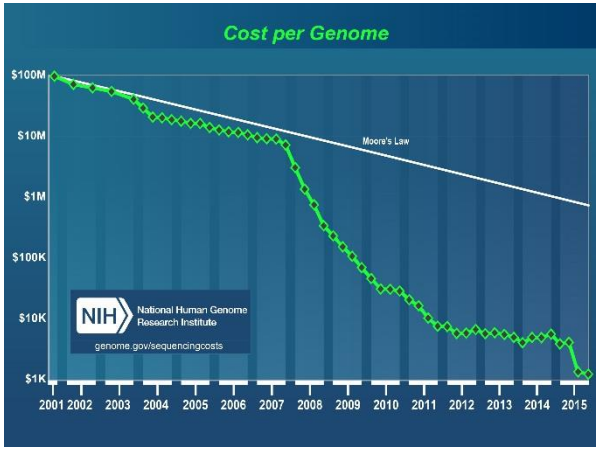
NGS Data Analysis

Sequencing technology advances, analysis remains challenging



$1.000 sequencing and $10.000 bioinformatics costs?

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NGS Data Analysis

NGS data means big data...means big computing power

NGS data is usually analyzed on a supercomputer or cluster:

- UZ Leuven: Avalok/Hydra

- KU Leuven: VSC Flemish Super Computer

- Google genomics, etc.

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High Performance Computing (HPC)

- A computer cluster consists of multiple CPUs arranged in compute nodes
  - A typical compute node consists of 10 CPUs or cores and 128GB RAM
  - A cluster consists of multiple compute nodes
    - 208 nodes with two 10-core "Ivy Bridge" Xeon E5-2680v2 CPUs

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High Performance Computing: Taking Advantage

- To take full advantage of a computer cluster for genomics, computation preferably must be

  distributed across multiple cores and nodes

  - Otherwise, not necessarily more convenient than execution in desktop computer

- Many NGS tools currently support multi-threading and/or multi-node computation: STAR, GATK 4.

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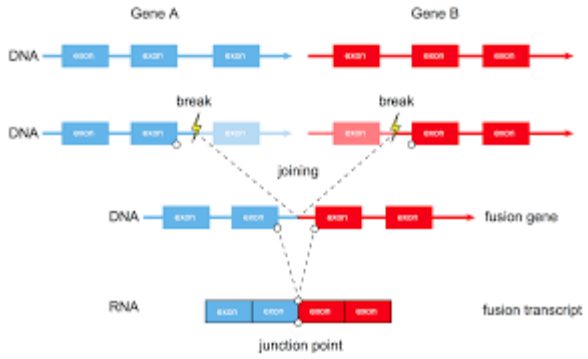
RNA-Seq and High Performance Computing

Is it needed?

- For differential expression, at the Genomics Core the analysis service is included for K.U. Leuven, UZ Leuven members
- Other pipelines: STAR, DeSeq2, Hisat2
- Analysis of public data
- Re-analysis with different experimental designs
- Downstream analysis: pathway analysis, gene ontology
- Other computational tasks:
  - Transcriptome assembly
  - Gene fusion analysis
  - SNP calling
  - Transcript discovery
  - Analysis non-model organisms



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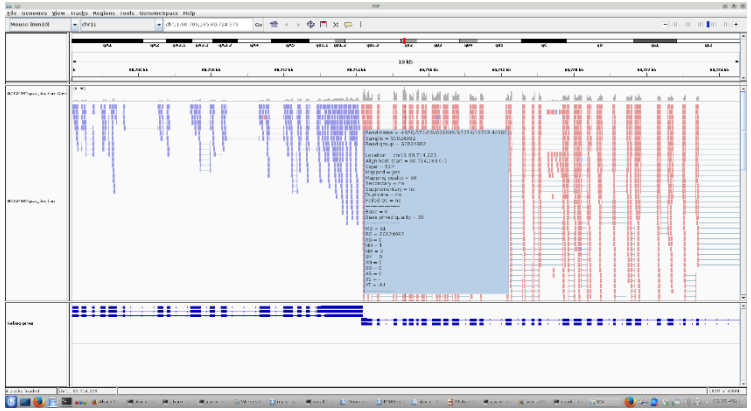
RNA-Seq and High Performance Computing

Differential Expression Analysis:

- Computationally intense for:
  - mapping,
  - counting,
  - and differential expression of genes



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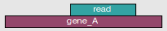
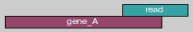
	gene_A	gene_A	gene_A
	gene_A	no_feature	gene_A
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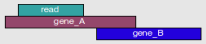
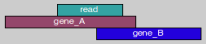
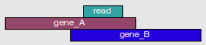
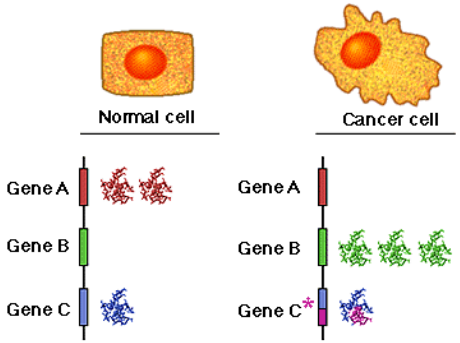
	gene_A	gene_A	gene_A
	ambiguous	gene_A	gene_A
	ambiguous	ambiguous	ambiguous



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RNA-Seq at VSC

To star working at the VSC:

- A VSC account and compute credits:

  https://www.vscentrum.be/

- Raw or preprocessed RNA-Seq data:

  Genomics Core transfers fastq files or preprocessed data (i.e. unnormalized counts) directly into a specified location at the VSC.

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RNA-Seq at VSC

Any analysis at the VSC:

- Number of nodes
- Number of cores per node
- Memory
- Maximum time the analysis will need the resources: walltime
  - Estimation?
- Project to charge the computation
- An email and a job name

PBS Script

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Portable Batch System (PBS)

Portable Batch System (or simply PBS) is the name of computer software that performs job scheduling. Its primary task is to allocate computational tasks, i.e., batch jobs, among the available computing resources. It is often used in conjunction with UNIX cluster environments. PBS is supported as a job scheduler mechanism by several meta schedulers ...

-- by Wikipedia

```
#!/bin/bash -l
#PBS -l walltime=48:00:00
#PBS -l mem=100gb
#PBS -l nodes=15:ppn=20
#PBS -M alvaro.cortes@uzleuven.be
#PBS -m aeb
#PBS -N star_mapping_population
#PBS -A lp_biogenomics
```

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RNA-Seq and PBS

- One PBS script per task:
  - Quality checking
  - Adaptor trimming
  - Mapping
  - Counting
  - Differential Expression analysis
    - EdgeR
    - DeSeq2
    - Bayseq
  - Summary of results and report

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RNA-Seq and more on PBS

- The input data
  - SAMPLE_DIR="$PROJECT_DIR/htseq";
- Location output
  - OUTPUT_DIR="$PROJECT_DIR/deseq2_16_10_2017_all"
- Software package to perform the actual analysis
  - Name
  - Version
    - module load R
- Input parameters software package:

```
Rscript $R_SCRIPT_DIR/deseq2.2conditions.R $CONDITION1
$CONDITION2 > $COMP_NAME".rscript.log" 2> $COMP\
_NAME".rscript.log";
```

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Submitting PBS Jobs

- Thinking

  $ qsub step8_deseq2.pbs

- Cerebro

  $ module load cerebro/2014a

  $ qsub step8_deseq2.pbs

- When a job is submitted, the job id is returned.

- jobIDs are unique.

  Thinking jobs starts with 2

  Cerebro jobs starts with 3

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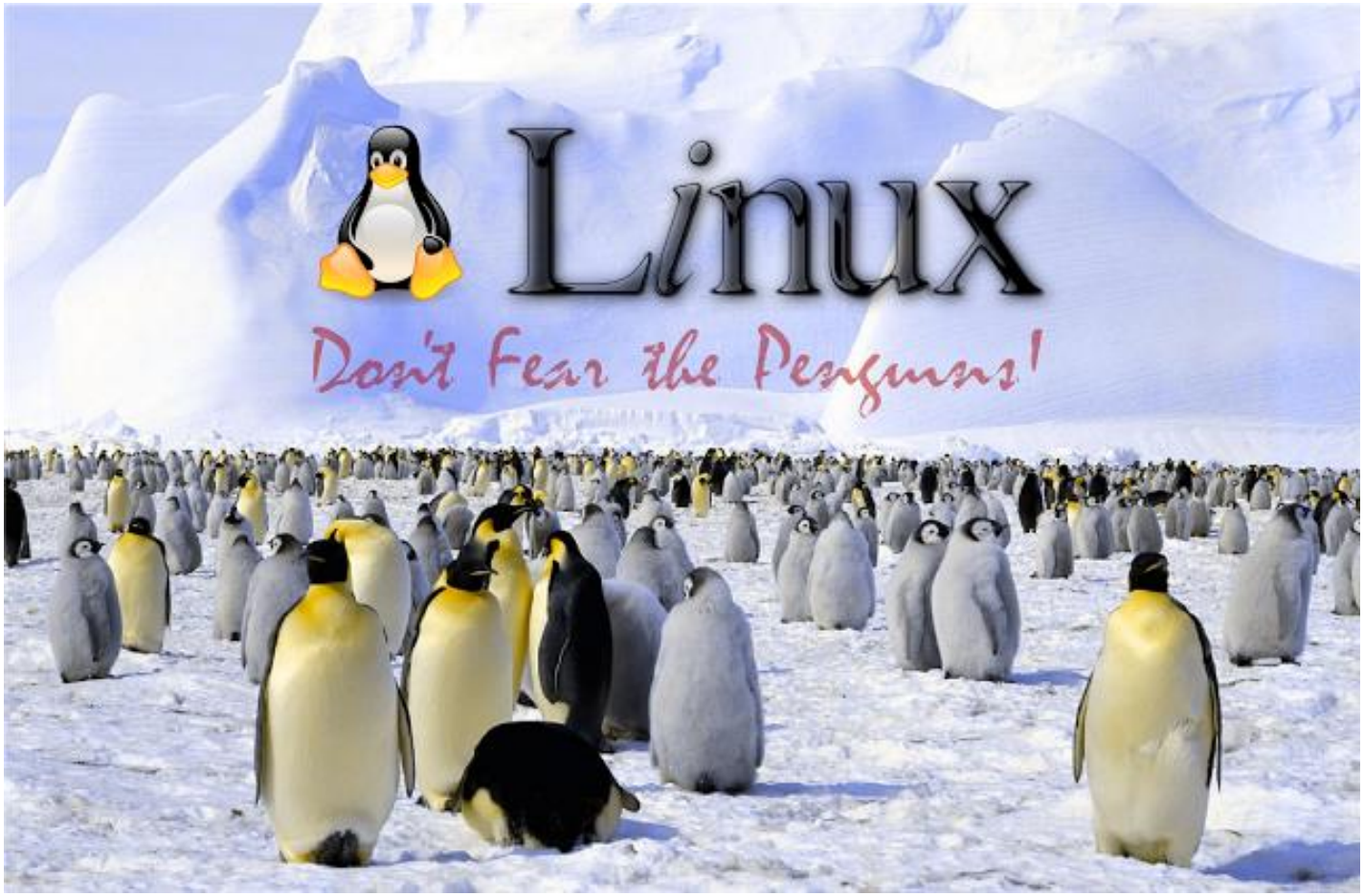
Hands-on Project



Linux - Don't Fear the Penguins by Rob A. Shinn

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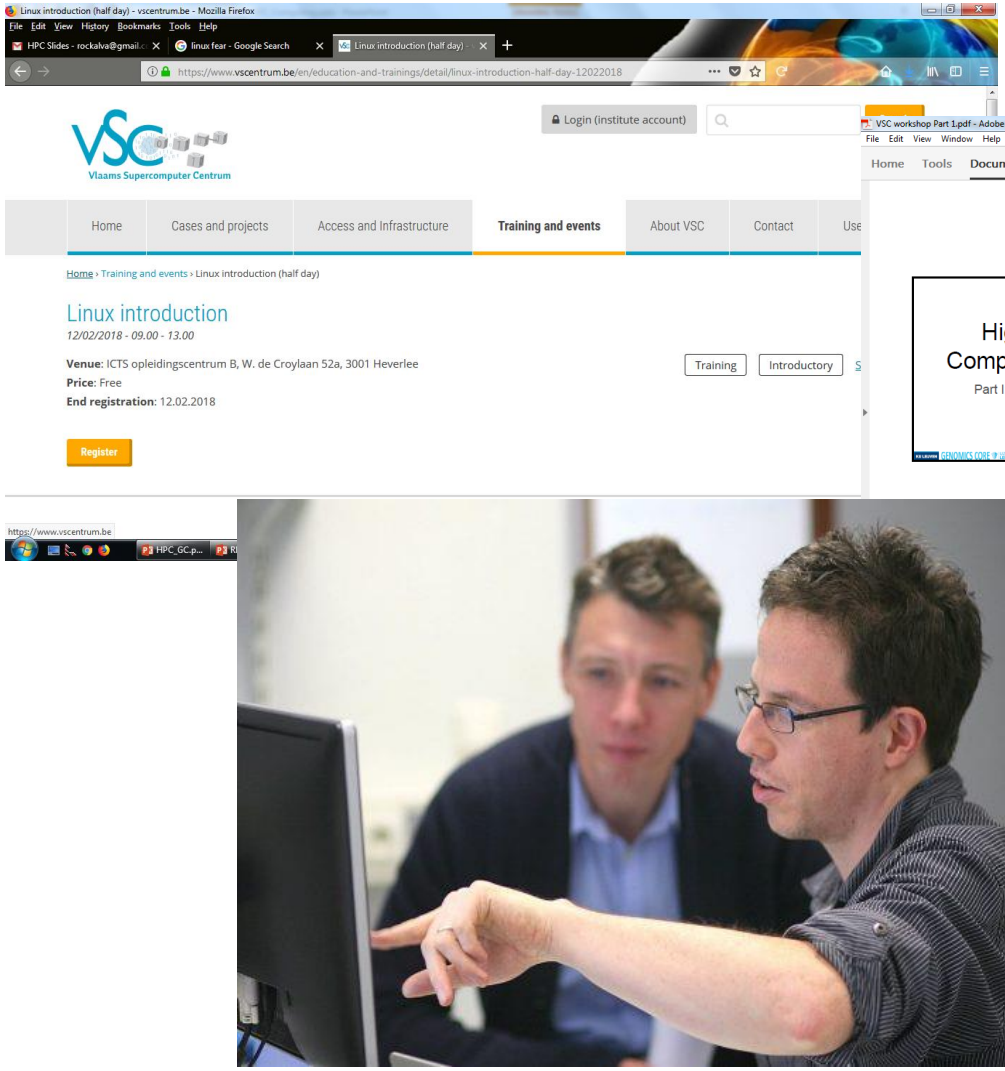
An Ecosystem of Support



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LIVE Demo

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Thanks!

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