KNIME and Next Generation Sequencing: From data cleansing to systems biology

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NGS at PF2

Technological approaches for NGS:

RNA seq

- Gene expression profiling (mRNAs,miRNAs, small RNAs...)

- Transcriptome annotation (TSS mapping, isoforms...)

ChiPSeq

- DNA-protein interactions (histone modifications, transcription factor binding sites...)

NGS at PF2

Many different organisms under study:

Viruses: **Rift valley fever, Measles**

Bacteria: ***Listeria, Streptococcus, Enterococcus, Legionella, Clostridium, Thiomonas, Helicobacter***

Yeasts: ***Candida, Yarrowia, Aspergillus, Saccharomyces, Trichoderma***

Protozoans: ***Plasmodium, Entamoeba***

Insects: ***Drosophila***

Mammals: **Mouse, Human**

NGS at PF2

Biological questions under study:

Developmental biology

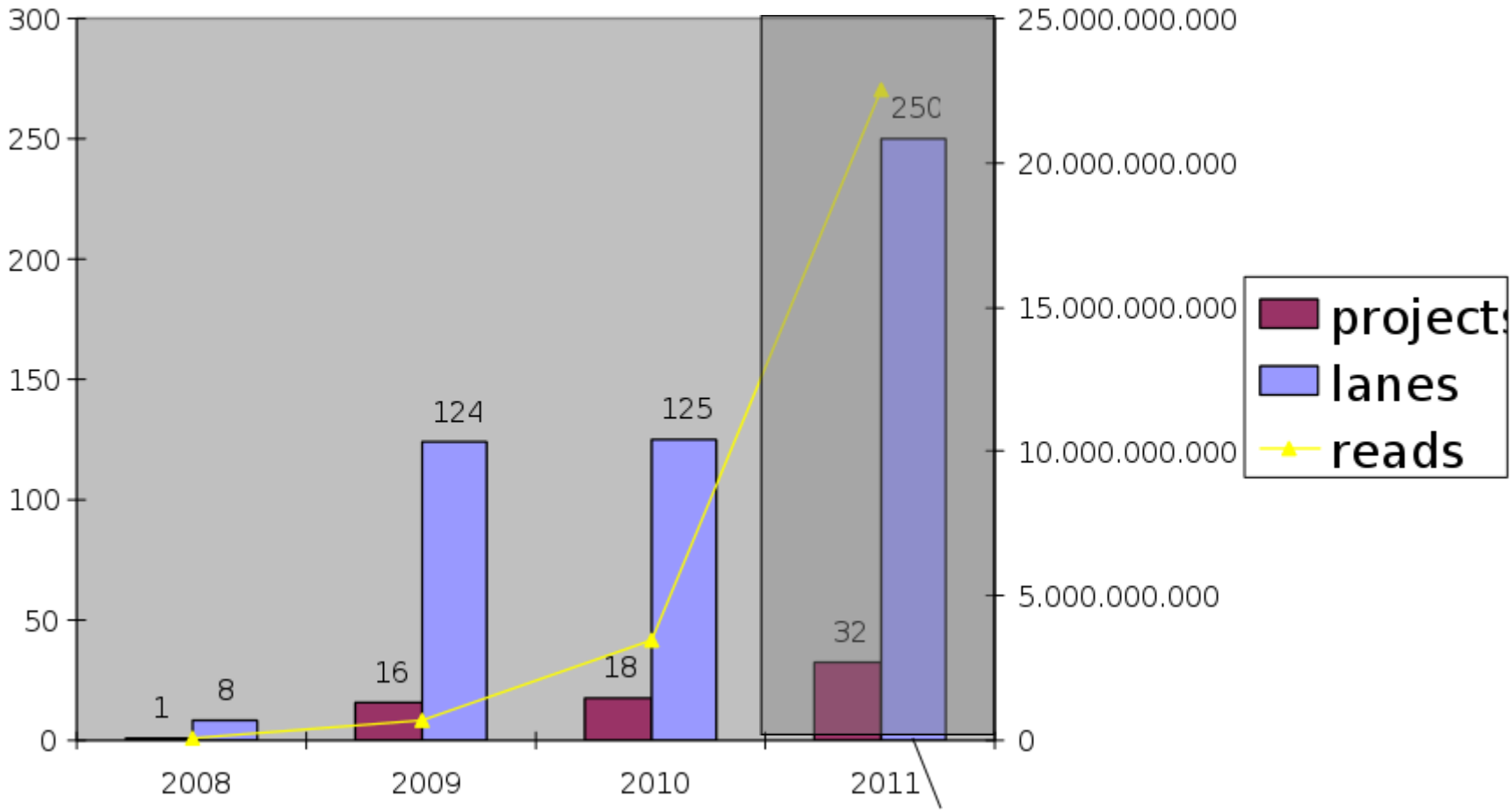
Infectious diseases (host-pathogen interactions, virulence factors...)

Microbiology of model organisms

This represents 35 different projects in the last 2 years

Most of them require specific bioinformatics developments

Data throughput

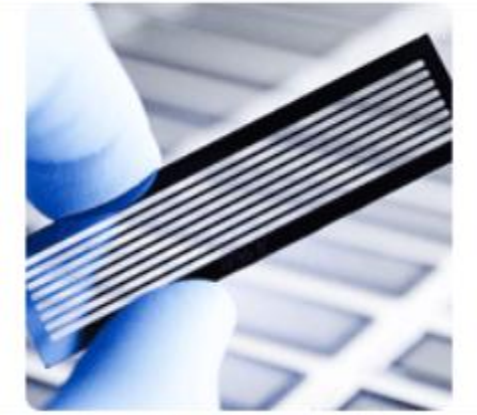


GAII: average number of reads: 25M / lane
HiSeq: first run: 85M / lane

Projected throughput with HiSeq

Equipment

Flow cell

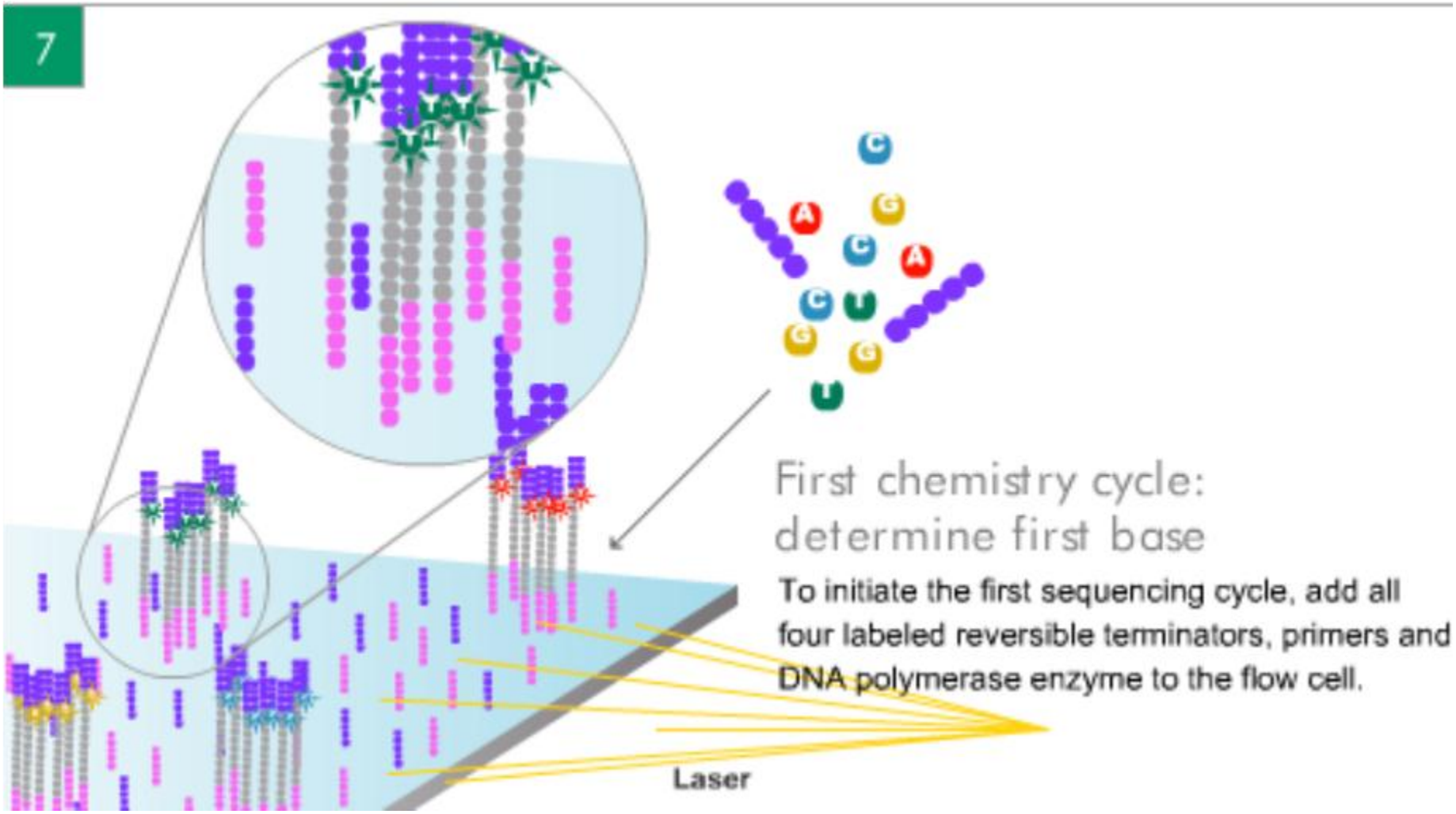


Cluster Station

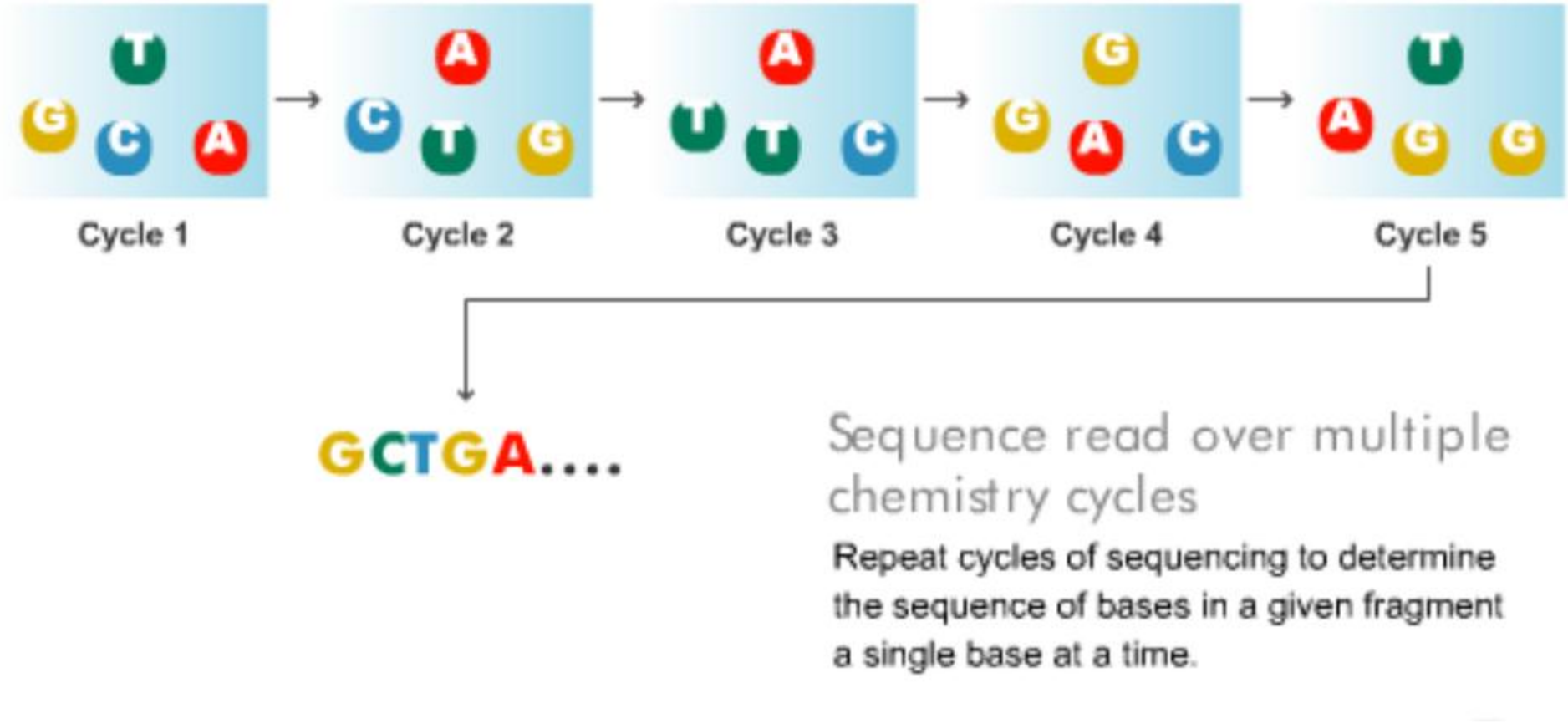
Genome Analyser

Paired-end module

Principle: sequencing by synthesis

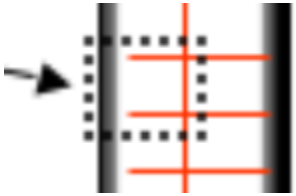


Sequence by synthesis



1 Cycle

« tile »



genome analyser

x 100

x 8

7 Mb +	7 Mb +	7 Mb +	7 Mb = 28 Mb	x 100	2,8 Gb	x 8
1 +	1 +	1 +	1 = 4 files		400 files	3200 files

22,4 Gb

* Each file contains 50 000 - 150 000 lines (clusters)

HiSeq 2000 - throughput

HiSeq 2000 (Paired-end run)
Gb per run 150-200
Gb per day 20-25
Cluster density in KClusters/mm²** 260-350
Read length 2 x100
Available surface area (mm²)* 2880

1 run

1 cycle 22.4 Gb – 3200 files

36 cycles 806 Gb – 115.200 files

72 cycles 1.6 Tb – 230.400 files

72 cycles 3.2 Tb – 460.800 files
Paired end

Illumina Centers

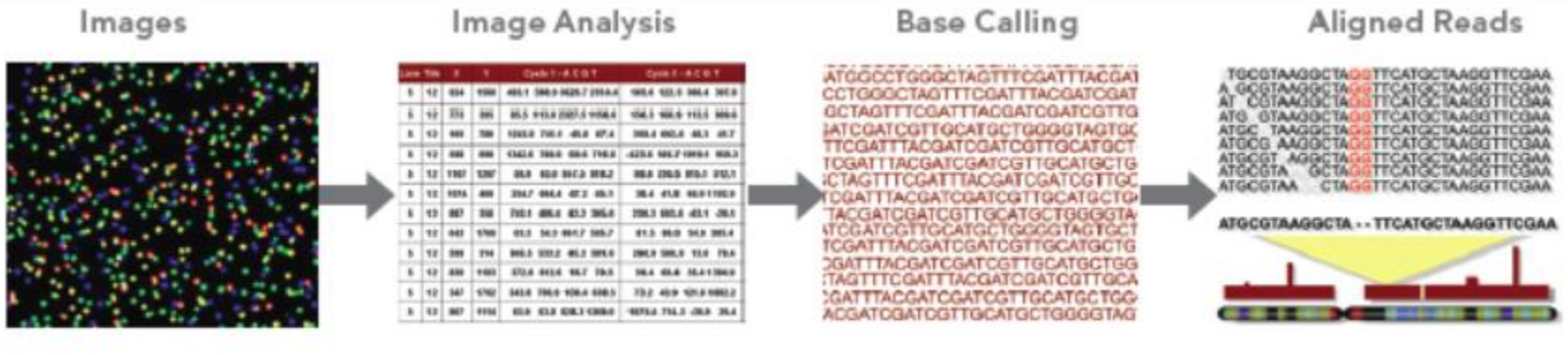
BGI (Beijing Genomics Inst., China)
    128 HiSeq 2000
Broad (Cambrige, MA, USA)
    51 HiSeq 2000
Sanger Center (UK)
    ~ 37 Solexa machines

=> just these 3 centers can produce almost
  1 PB per month

NGS data processing

- ***NGS Data primary data analysis***
  - ***types and size; workflow overview; Image analysis; Base calling; Alignment; Quality control***
- ***NGS Data secondary data analysis***
  - ***Functional annotation; Visualization; Classification; Quantitative/Qualitative analysis***

NGS – primary data analysis



Images	Image Analysis	Base Calling	Aligned Reads
• Transfert of image files • Identification of clusters	• Cluster position • Clusters intensities • Signal to noise analysis => One file per cycle	• Intensity correction • Quality assesment • Calculate sequence for each cluster	• Align sequences to reference genome • Sequence files • Statistics • Graphics
Number of files			
360.000+	5.000	10.000	36.000
Disc space used			
TBs	~100 GB	~ 80 GB	~ 25 GB

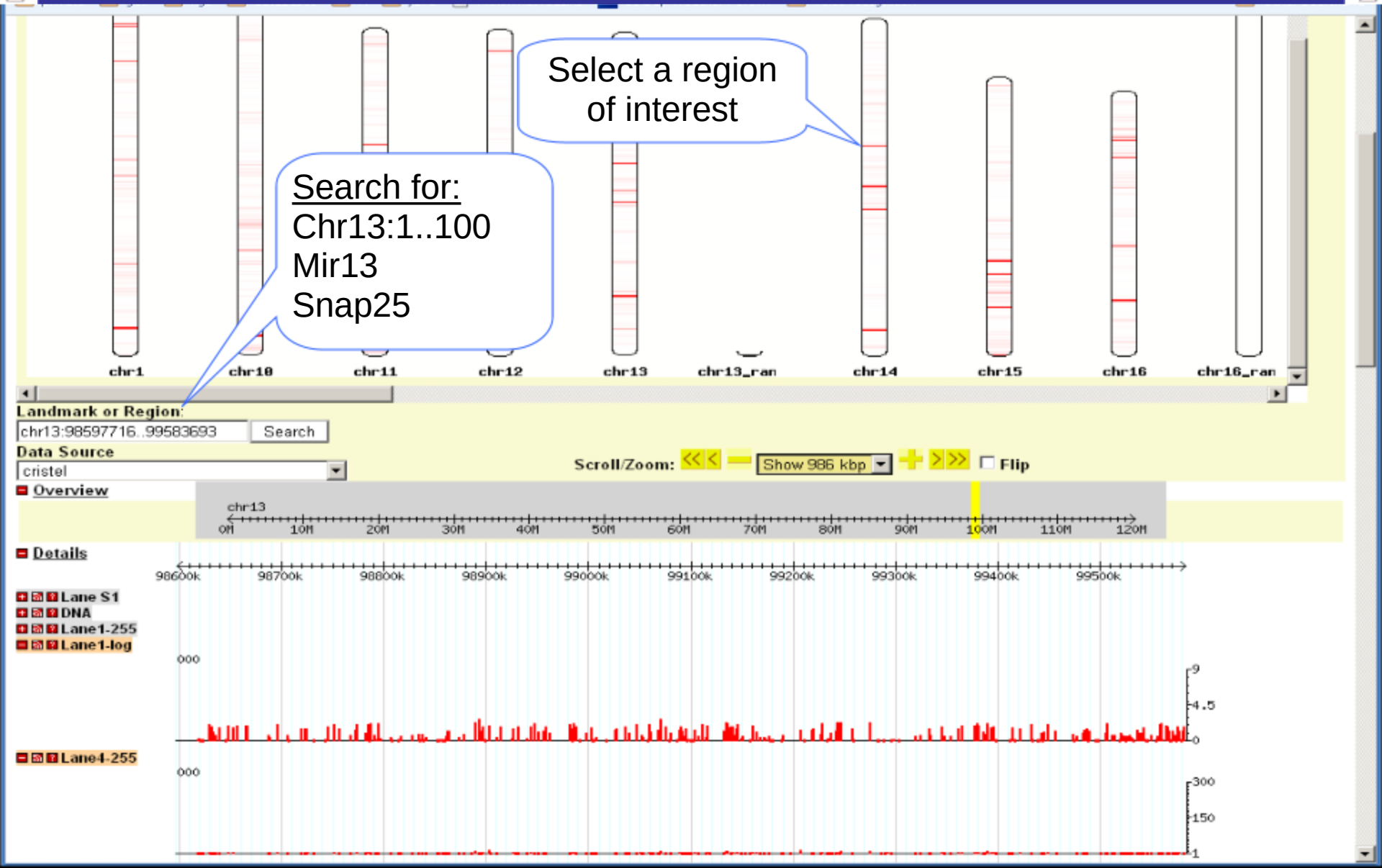
Bioinformatics tasks

- Quality control
- Descriptive statistics
- Mapping to reference genomes
- De-novo alignment
- Intersection with annotation
- Preparation for statistical analysis

NGS – secondary data analysis

- Making sense of the alignments
  - Visualization (Genome Browser)
  - Statistical interpretation

Visualization: GBrowse



Nodes implemented

- **FastQReader** Reads in FastQ file into table. One FASTQ entry (i.e. 4 lines) are translated into one row. This node is using BioJava
- **FastQWriter** Writes out FastQ file into a file. This node is using BioJava.
- **BEDGraphWriter** Writes out BED files.
- **SAMReader** Reads Sam or Bam files.

- **AdapterRemoval** Node to remove adapter sequences.

Nodes implemented

- **Bash** Executes commands in bash or cmd.exe
- **CmdwInput** Similar to the bash node only that it takes the input table and executes strings within that table.
- **JoinSorted** Creates a full outer join of two sorted tables.
- **CountSorted** Counts occurrences within a sorted column. It is faster than the ValueCounter and useful for counting reads from a FASTQ file as they are already sorted. It also uses minimum amount of memory.

Nodes implemented

- **GetRegions** Identifies regions of interest (ROI). A ROI is defined as a chromosomal region that has no gaps. This node also produces a string of concatenated values (i.e. counts)
- **PositionStr2Position** Takes a string (chr1_123) and translates it into two columns (“chr1”, 123)
- **RegionOverlap** Identifies regions that overlap. This node is usually used within a sub-workflow that divides the data set per chromosome. The first input node is being retained.
- **Seq2PosIncidents** This node splits a sequence into one nucleotide per row.

Nodes implemented

- **OneString** This creates a single cell of type String, Integer, or Double. It is use full when executing workflows from the command line.
- **Wait** Does nothing other than synchronizing executions. This can also be done using the Variable Ports of existing nodes

KNIME workflows implemented

- RNA-Seq
  - Gene expression profiling
  - TSS mapping
  - Small RNA characterization
- ChiP-Seq
  - Histone modifications
  - DNA/protein interactions
- Implemented workflows are routinely being executed by Caroline Proux and Odile Sismeiro

KNIME workflows implemented

- Adapter removal
  - Multiple copies of adapter with variations
  - Length selection
  - Quality score selection
  - Complexity filter

KNIME workflows implemented

- Mapping to reference genome
  - Using bowtie
  - Creating SAM/BAM files
  - Creates Pileup (counts per position)

Node in Alpha

- **FASTAReader** reads fasta, genbank, uniprot, embl, INSDseq files and creates a new sequence object including annotations
- **GetSequenceName** extracts the sequence name from a sequence object
- **SubSequence** extracts a sequence from a sequence object
- **IGVView** open IGV at a particular chromosomal location
- **GBrowseViewer** opens GBrowse at a particular chromosomal position
- **Upload2Gbrowse** uploads data to a GBrowse instance
- **DASClient** retrieve annotation from a DAS server
- **MobyleWebServiceClient** launches a program through Mobyle
- **AdapterRemovalAdv** more advance read cleansing.

KNIME workflows in development

- Region of interest (ROI) identification
  - Select mapped reads
  - Distinguish between forward/reverse strand
  - Distinguish between unique/non-unique/all aligned reads
  - Generate ROIs of ungapped genomic regions
  - Identify possible Mutations/SNPs
  - Generate BED files and table for further analysis
- KNIME integration of Statistical analysis tools for normalization / differential analysis
- Integration of Cytoscape / GOSeq,
- Specific developments according to user's needs

Conclusions

- 2nd stage data analysis for NGS is
  complex
  time-consuming
  individual
  rewarding
- Completed (KNIME) workflows available for
  - Data cleansing
  - 1st stage data analysis (mapping)
  - Intersection with annotation
  - Most of the individual tasks are available
    (TSS, strand specificity, intersection with annotation, Pivoting, prototypes for integration with: DAS , UCSC & GBrowse, IGV, BioJava, Mobyle, SAMtools, ROI tools)

Whish list

- Random access tables (see samtools, bzip)
- Visualization of arbitrary large data sets (pre-rendered graphs?)
- Vector/matrix operations for LARGE data
- Parallelization
- Faster chunk-loops (no rereading of the input table)
- Partial results should be available after cancel or failure…
- Workflows with the same name that are located in different wkfl groups cannot be distinguished
- Templates for java/python/perl snippets (see R Plot from community nodes)
- node to save workflow

Thanks

PF2

- Jean-Yves Coppee
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- Caroline Proux
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KNIME

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- Michael Berthold
- Torsten Meinl
- Martin Horn
- ….

Collaborators

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- PF1 (Christiane Bouchier, Sophie Creno)
- PF8 (Ghislaine Guigon)
- ENS (Laurent Jourdren, S Le Crom)
- Mobyle (Hervé Ménager, Bertrand Néron)
- LBD (Nicolas Joly, Bernard Caudron, Louis Jones)
- SR (Youssef Ghorbal, Jerome Sobecki)
- NGS users