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Genome Sequencer FLX System

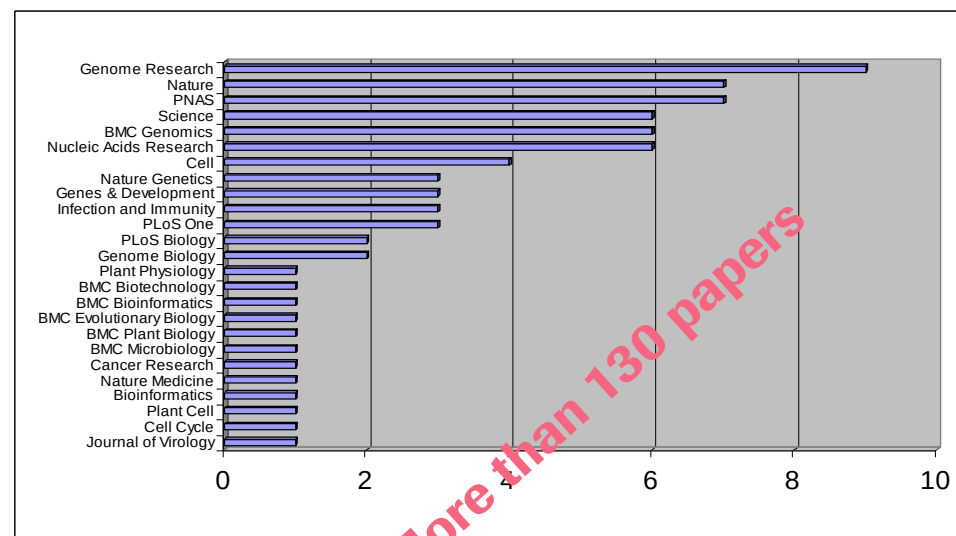
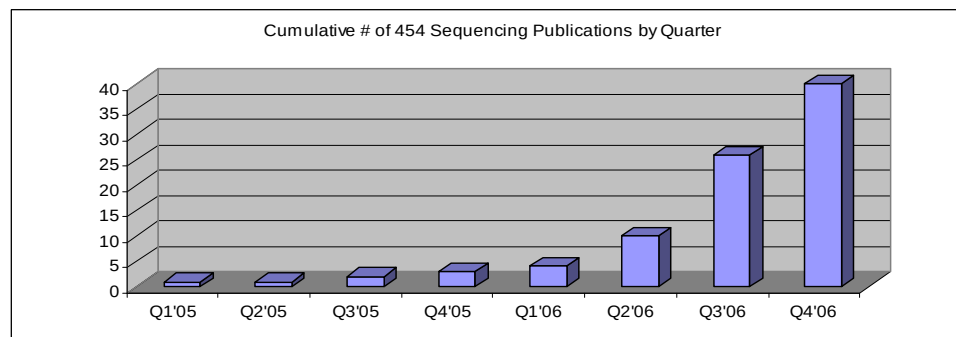
Longer reads mean

More applications, More breakthroughs, More publications

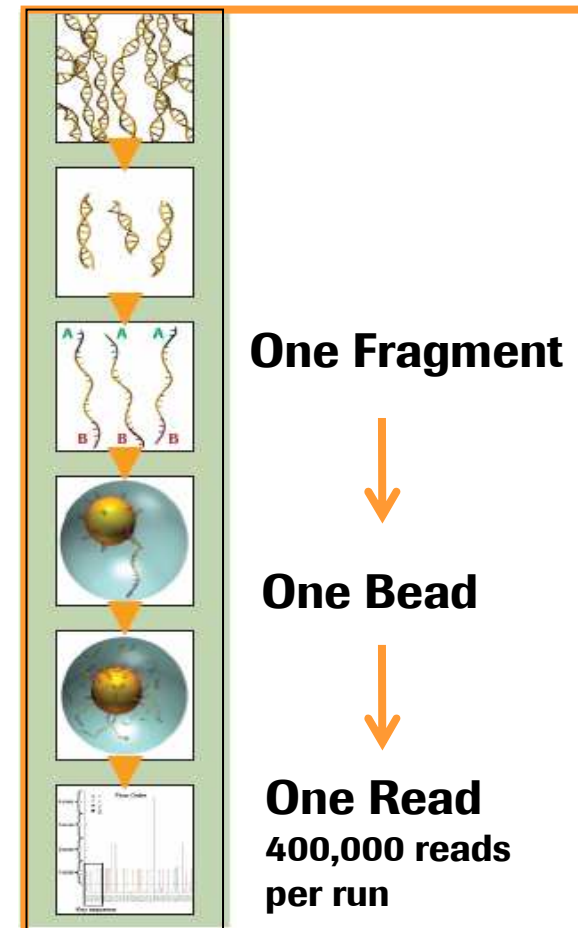
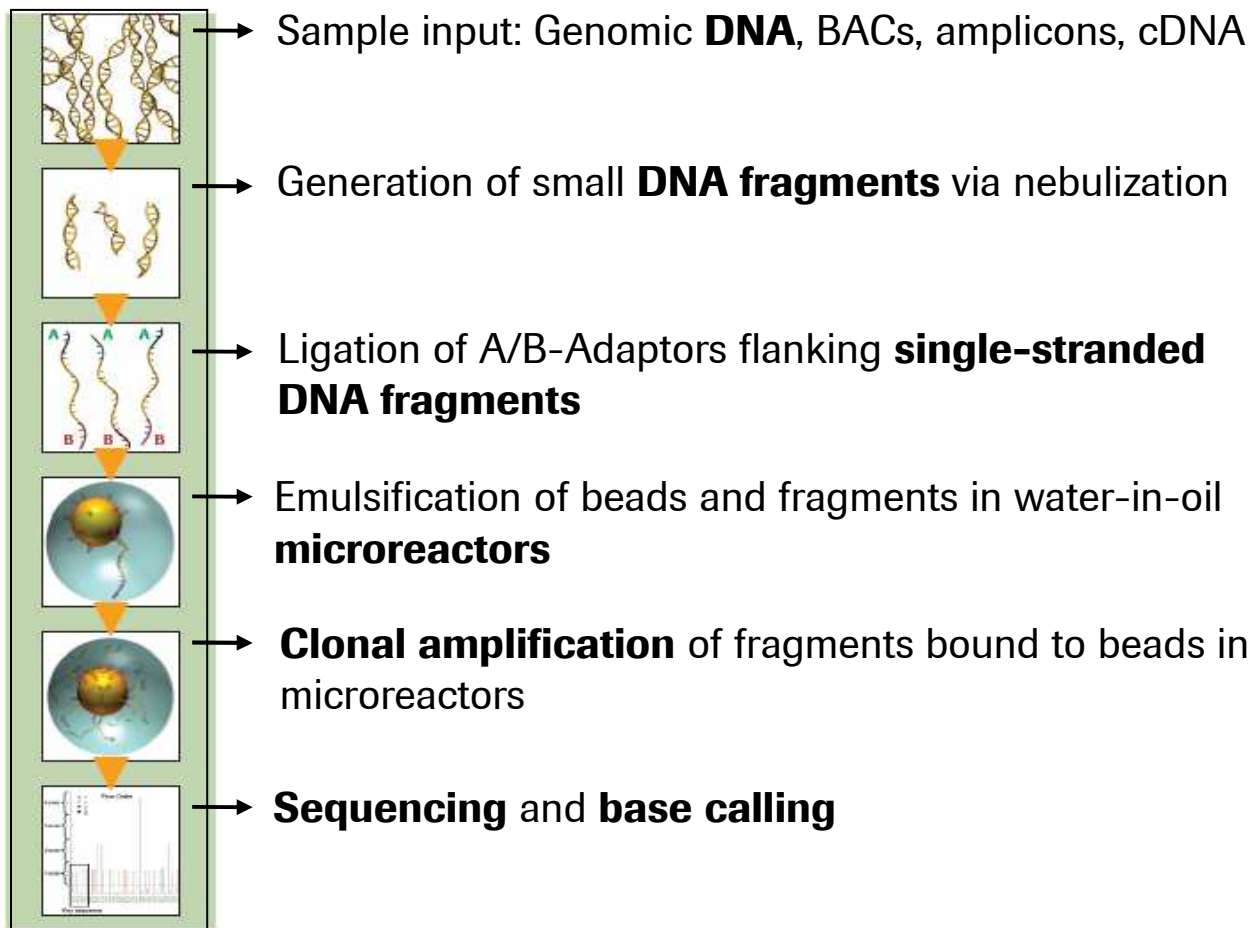
Katia Accorsi Roche-Applied-Science



The Genome Sequencer FLX System enables applications which were previously unfeasible

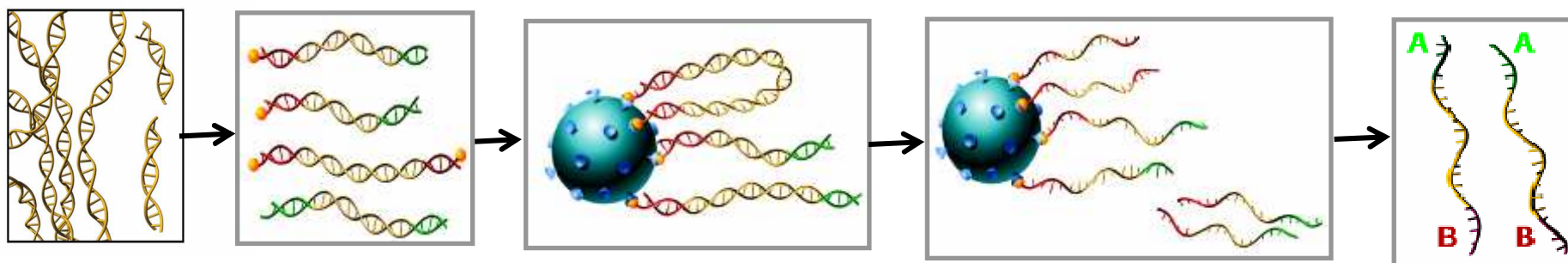


Sequencing Workflow Overview



Sequencing Workflow

Library Preparation



- Genome fragmented by nebulization
- sstDNA library created with adaptors
- A/B fragments selected using streptavidin-biotin purification

DNA library preparation and titration
4.5 h and 10.5 h

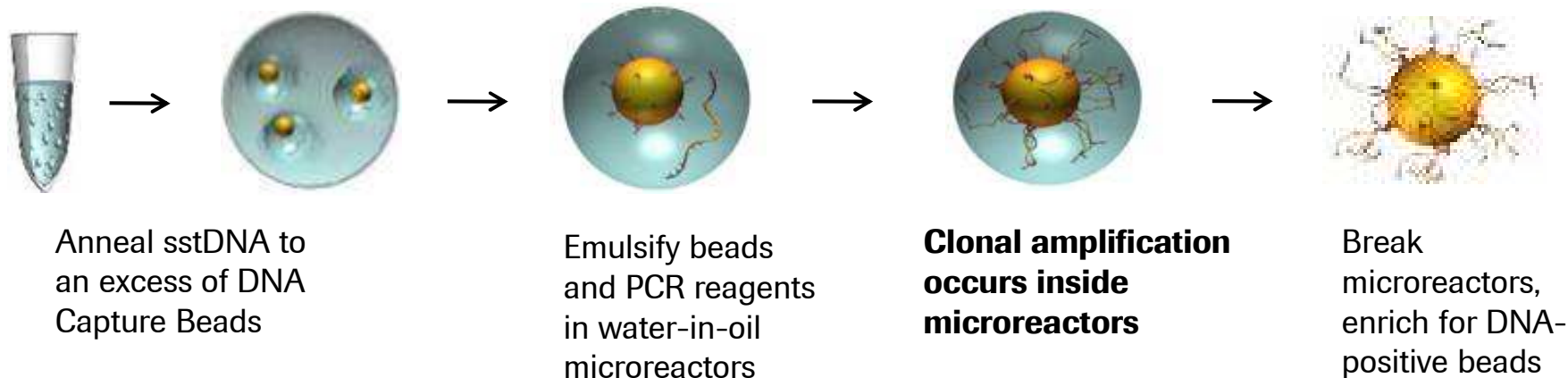
emPCR
8.0 h

Sequencing
7.5 h

Sequencing Workflow

Emulsion PCR

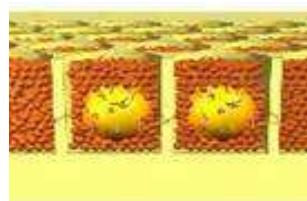
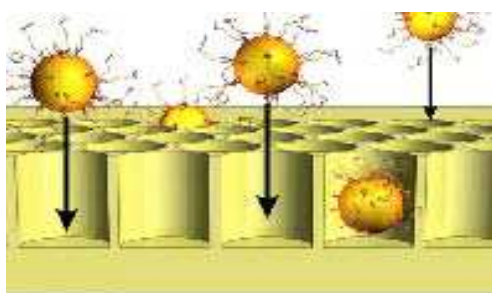
Emulsion-based clonal amplification



Sequencing Workflow

Loading of PicoTiterPlate Device

Depositing DNA beads into the PicoTiterPlate device

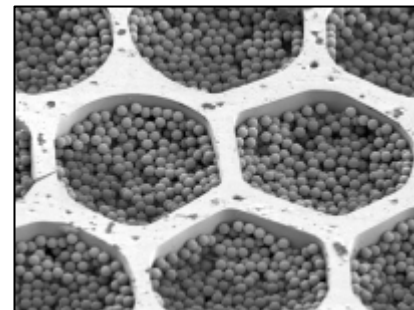
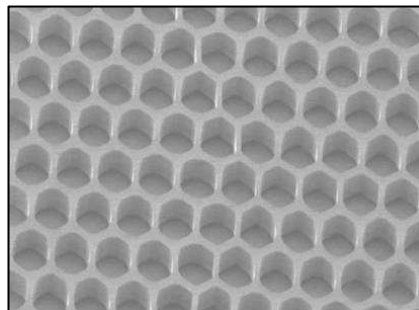


- Well diameter: average of 44 μm
- >400,000 reads obtained in parallel
- A single clonally amplified sstDNA bead is deposited per well

Amplified sstDNA library beads

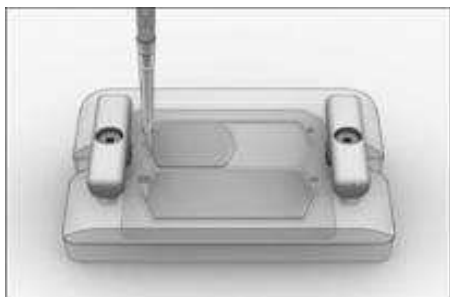


Quality filtered bases

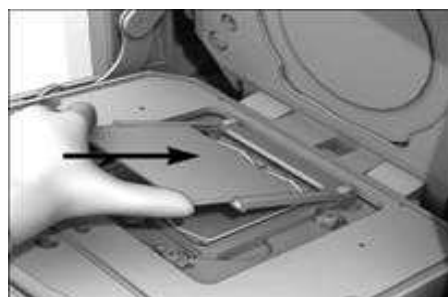


Sequencing Workflow

Instrument Loading



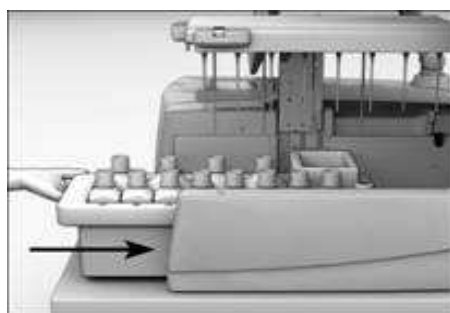
Load beads onto
PicoTiterPlate device



Load PicoTiterPlate device on
instrument



Open instrument



Load sequencing reagents

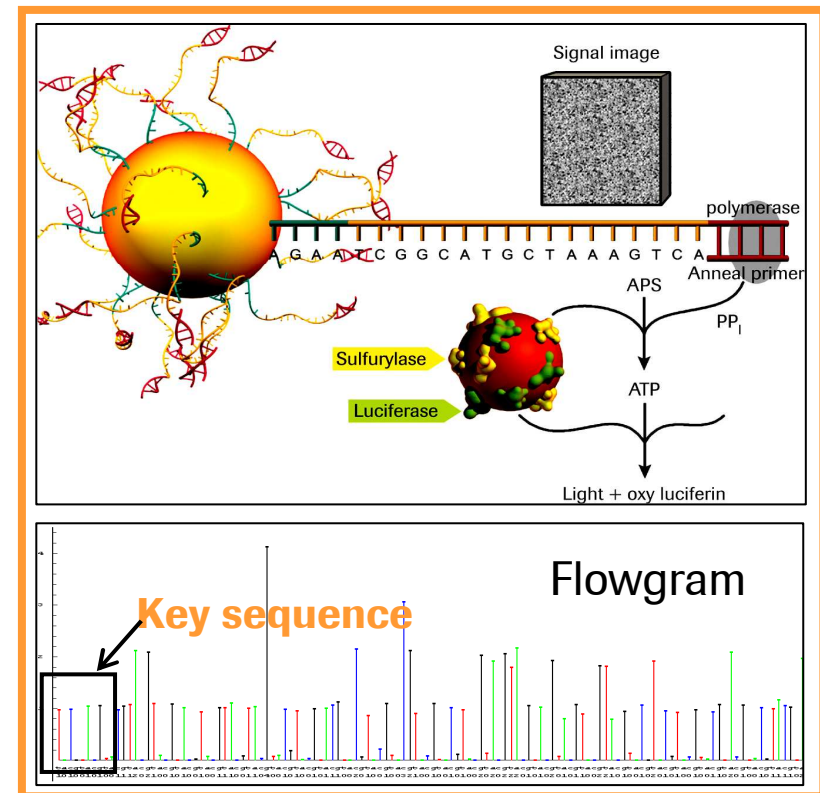


Press START for Sequencing!

Sequencing Workflow

Sequencing by Synthesis

- Bases (TACG) are flowed sequentially and always in the same order (100 times for a large GS FLX run) across the PicoTiterPlate device during a sequencing run.
- A nucleotide complementary to the template strand generates a light signal.
- The light signal is recorded by the CCD camera.
- The signal strength is proportional to the number of nucleotides incorporated.



DNA library preparation and titration

4.5 h

and 10.5 h

emPCR

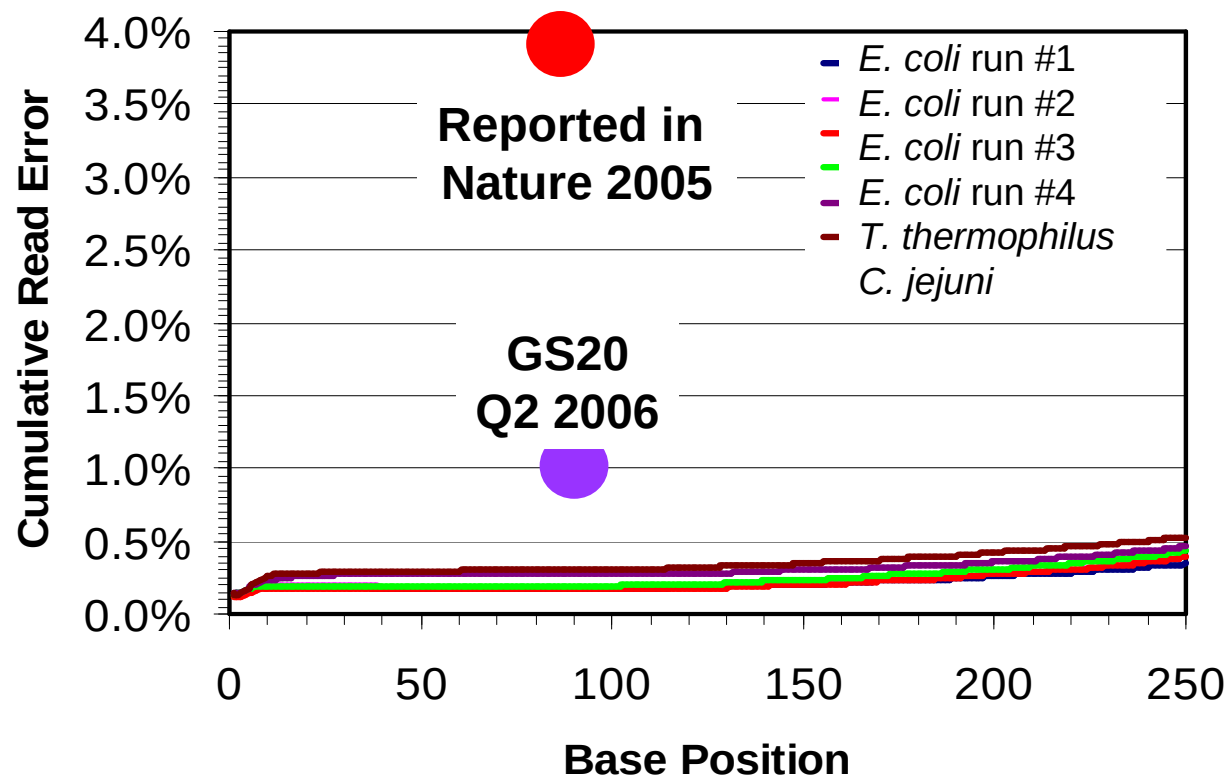
8.0 h

Sequencing

7.5 h

Superior Single Read (*De novo*) Accuracy

No filtering based on comparison with reference sequence needed



- **>99.5%** (includes all homopolymer overcalls and undercalls)
- **>99.99%** (substitution errors are exceedingly rare)

Many customers quote:

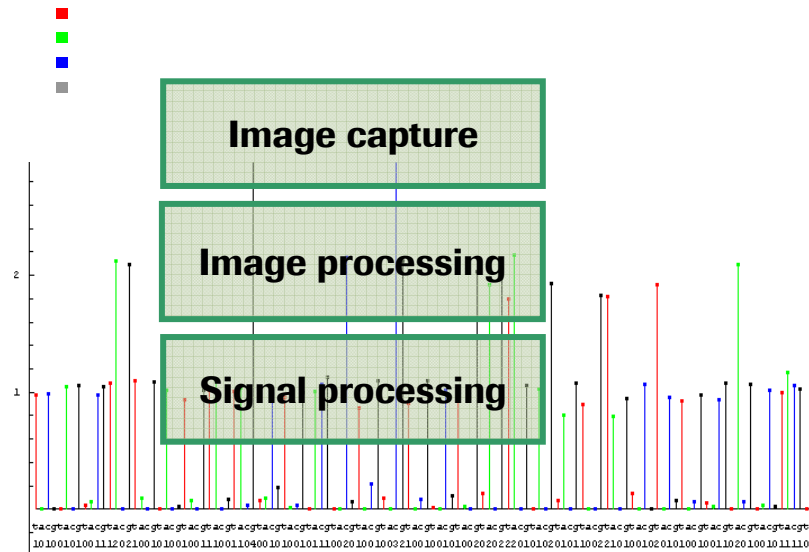
“If the GS tells that there is a substitution - you can be very sure that it is real”

GS FLX Sequencing

Bioinformatics



**De novo assembly of a 2 Mb bacterial genome
(20fold) takes 7 min.**



Basecalling and quality filtering

(no comparison with reference
sequence needed)

Bioinformatics Modules

The Genome Sequencer FLX System

Simply better quality, easier, faster and more reliable

2007

- 400,000 reads per run
- 200 - 300 nt per read
- 100 MB per run
- 2 runs per day
- 1.0 GB in 5 days



Mid 2008

- >1.250.000 reads per run
- **>400 nt per read (100 nt run available)**
- 2 runs per day
- **5.0 GB in 5 days**

All on the same instrument, no hardware upgrade

Unique combination of read length & reads per run ensure the broadest applications portfolio

De Novo Sequencing

- *Microorganisms* (genome plasticity)
- Plants, Animals
- BACs, YACs, Fosmids, Viruses etc.

Resequencing

- Whole Genomes
- Disease associated regions
- Somatic mutations (cancer research via amplicon sequencing)
- Structural variations of the human genome

Transcriptome Analysis

- Expression profiling (e.g. SAGE-like, CAGE-like, GIS-PET)
- EST-sequencing
- Full length cDNA sequencing

Gene Regulation Studies

- Identification of transcription factor binding sites (ChIP-Sequencing)
- Identification and quantification of sncRNAs sequences

Epigenetic Changes

- DNA-Methylation patterns

Metagenomes & Microbial Diversity

- Shotgun sequencing of the metagenome
- 16S amplicon sequencing

Ancient DNA

- Neanderthals, Mammoths and many more

