Next generation sequencing: principles and approaches in PGx

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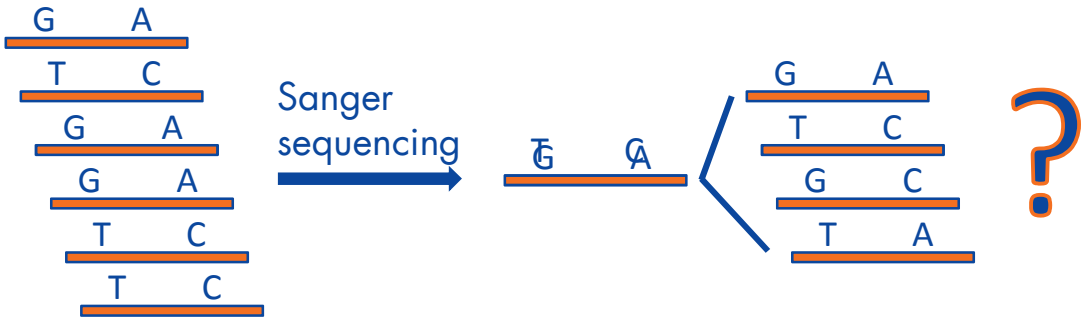
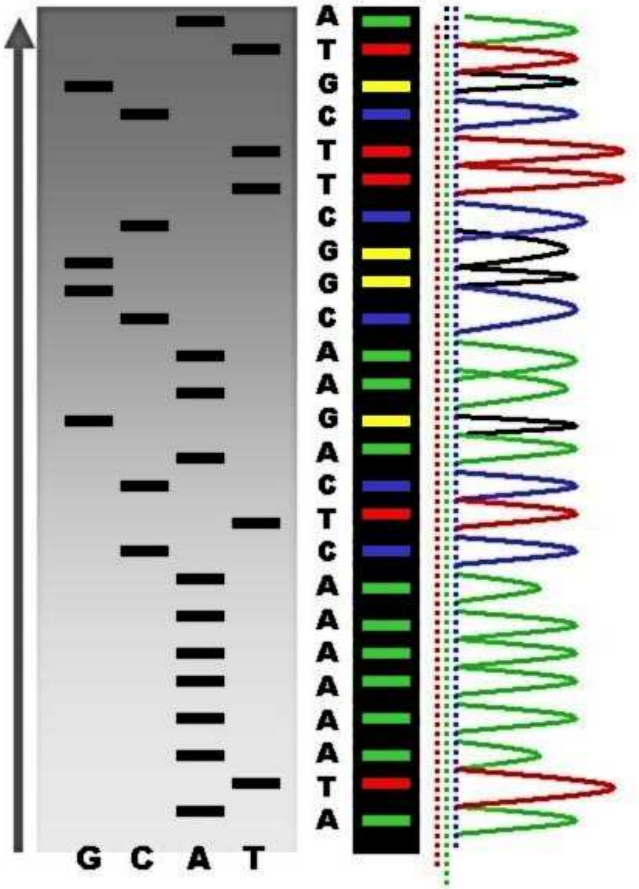
IMGM Laboratories GmbH

your outsourcing partner for
genomic services

What was before „next-generation“ sequencing?

- Sanger sequencing (chain termination)
- Since its development in1977 the standard and most widely used method
- Long read length (up to 1000 bp)
- Low throughput (1-96 reads/run)
- Mixture sequence = sequence mixture

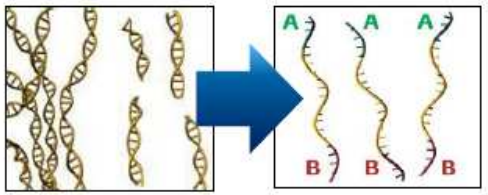




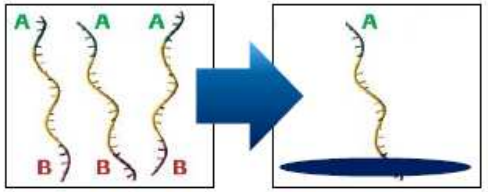
http://en.wikipedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg

How does NGS work?

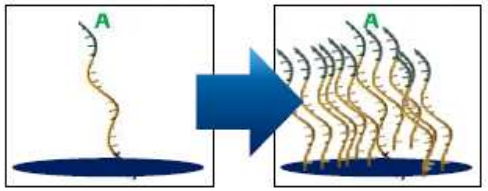
1. Production of handy, manageable DNA fragments
   - gDNA fragmentation
   - Enrichment by hybridization
   - Target enrichment by amplification



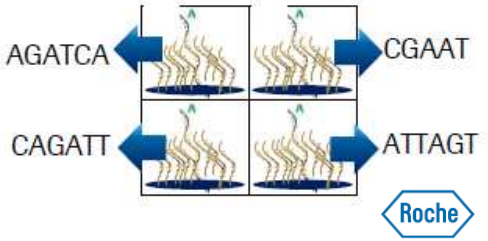
2. Separate and fix DNA fragments within compartments
   - Beads
   - Glass slide



3. Amplification of fixed DNA fragments
   - Clonal through PCR



4. Massive parallel sequencing of DNA fragments over all compartments
   - Sequencing by synthesis



→ High-throughput

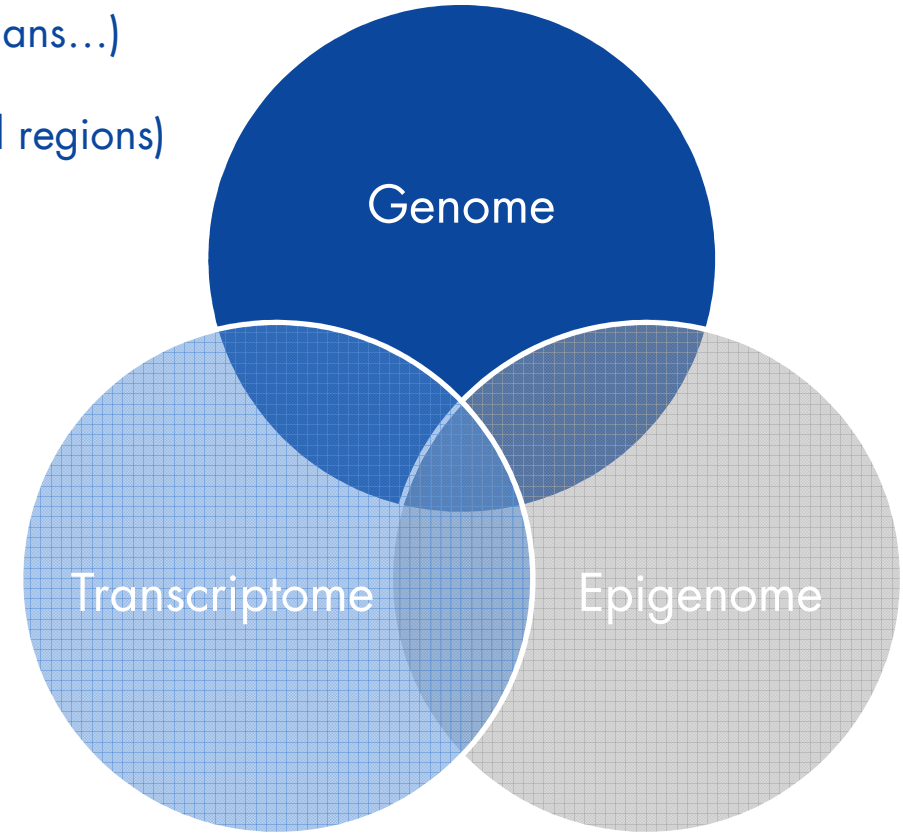
→ Resolution of sequence mixture

NGS Applications

- De novo sequencing
  (viruses, bacteria, fungi, plants, mammalians…)
- Targeted resequencing
  (single genes, gene panels, chromosomal regions)
- Whole exome resequencing
- Whole genome resequencing
- Metagenome

- Gene expression profiling
- Small RNA analysis
- Whole transcriptome analysis

- Methylation analysis
- ChIP-Seq

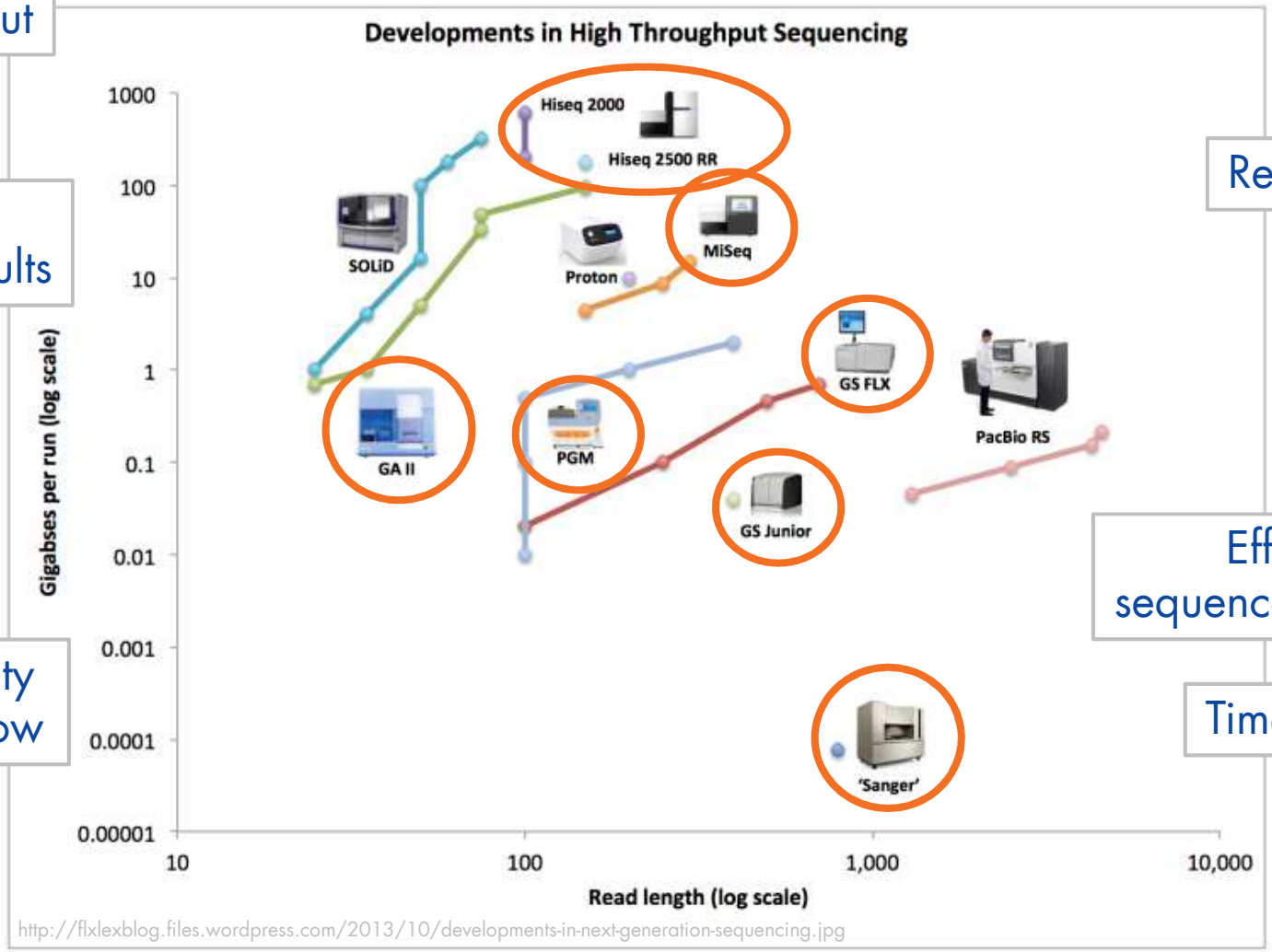


NGS Platforms

Throughput

Costs per base/results

Complexity of workflow



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Read length

Efforts from sequence to result

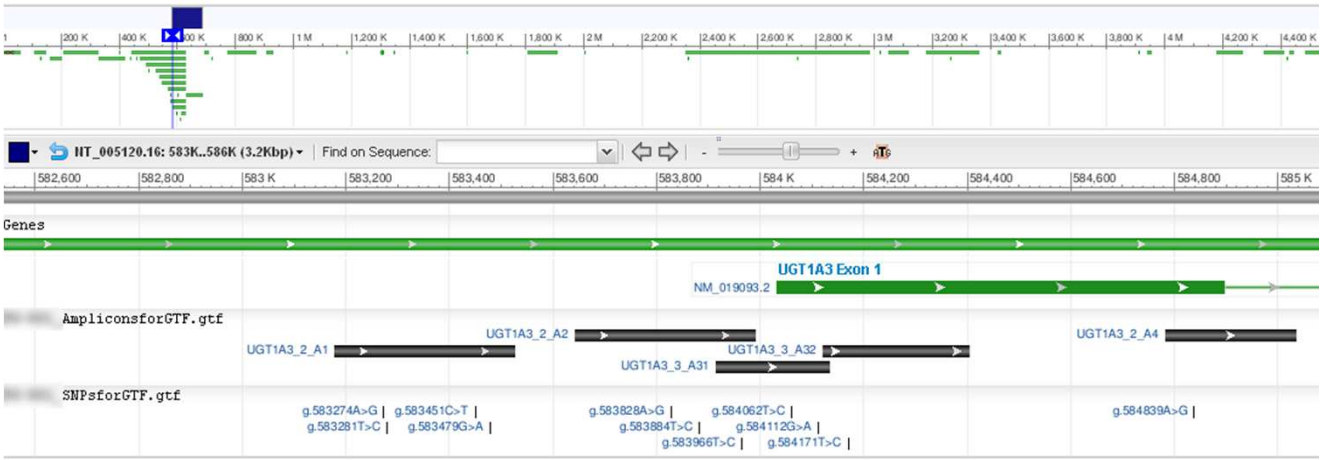
Time to result

NGS in PGx
Custom assays

IMGM® LABORATORIES

Custom assays for non-standard targets

- Assay design (PCR, hybridization)
- Assay validation
- Analysis of study samples
- Data analysis
- Reporting of genotypes, haplotypes and if available phenotypes



→ High-throughput analysis of many samples and few targets

→ Post-hoc studies

NGS in PGx
HLA typing

Performed in cooperation with the „Center of human genetics and laboratory diagnostics (MVZ), Dr. Klein, Dr. Rost and Colleagues“

- Accreditation according to ISO 15189
- Accreditation by European Federation of Immunogenetics
- Operates according to GMP guidelines

1. Exon-based typing

2. cDNA-based typing

3. Typing of whole genes (shot gun)

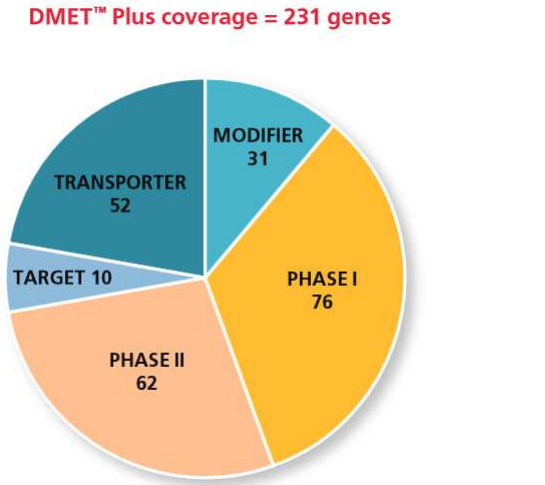
→ Less ambiguities

→ Detection of minorities, chimeras

→ High-throughput analysis of many samples and few targets

NGS in PGx
NGS panels

- DMET™ Plus (Affymetrix):
  - 231 genes
  - 1,936 SNP, copy number and indel markers
- NGS Panel: customize targets
  - Transporter
  - Targets
  - Receptors
  - Enzymes
- IMGM: Assay design using Illumina DesignStudio
  - TruSeq Custom Amplicon (> 50 amplicons)
  - Nextera Custom Enrichment (> 0.5 Mb)



http://media.affymetrix.com/support/technical/brochures/dmet_drug_metabolism_brochure.pdf

→ Screening analysis of many targets of interest

→ Screening analysis of non-standard PGx targets

→ Does one size fit all?

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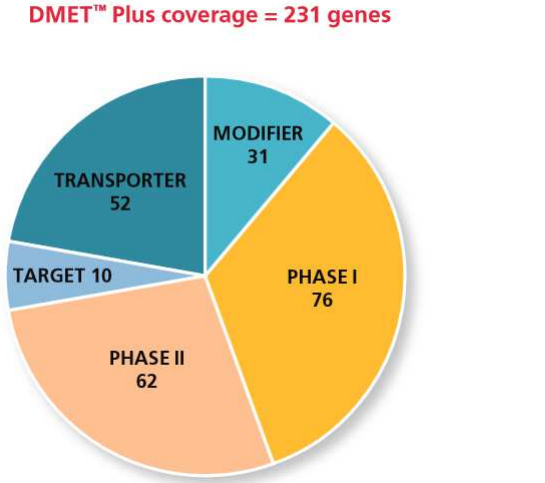

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→ Screening analysis of non-standard PGx targets

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Exome sequencing

Illumina	„Clinical“ exome (HGMD)	Whole exome
Target region size	7 Mb	62 Mb
Number of target genes	2,761	20,794
Number of target exons	~ 40,000	201,121
Probe size	80-mer	95-mer
Number of probes	> 50,000	340,427
Percent bases covered at 0.2x mean coverage	> 95%	>80%

→ Screening analysis of complete exome

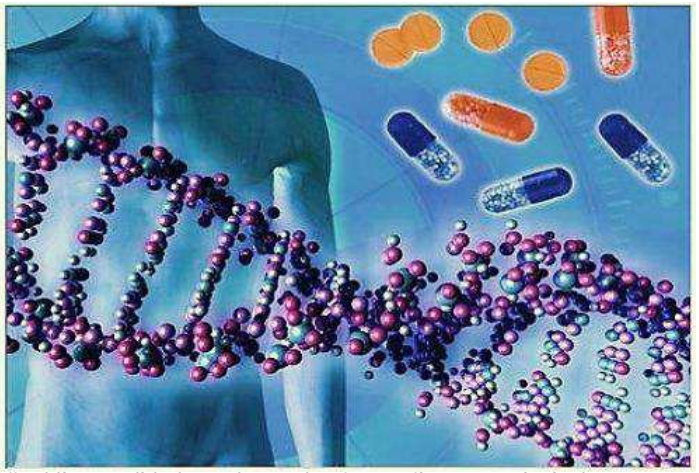
→ Screening analysis to identify new targets

Possibilities and limits of genotyping analysis using NGS

IMGM® LABORATORIES

- Errors and drop outs:
  - Gene families and pseudogenes
  - Regions of high GC content (exon 1)
  - Known structural variants
  - Repeat sequences
  - Homopolymers/InDels
- TAT
- Informed consent

+ High throughput analysis
+ Costs
+ Screening of many and/or unknown targets
+ Customizable: analysis of non-standard targets
+ Resolution of ambiguities
+ Detection of minorities, chimeras



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IMGM's Service Portfolio

Pharmacogenetics Services

- Using our whole spectrum of small- to high-throughput technologies, including Pharma-specific applications such as DMET Plus and U133 Arrays by Affymetrix
- Further parameters needed for clinical studies available through one of Europe's leading Diagnostic Centers within the same building and under common leadership with IMGM
- In-depth consultation including gene and polymorphism selection, clinical protocol, informed consent, appropriate technical approach, result interpretation, etc.
- Analytical plan and report (GCP compliant, e.g. customizable data formats)
- Rigorous QA program

IMGM's Service Portfolio

IMGM - YOUR flexible and reliable partner…

- Longstanding experience in biomarker discovery
- Expert in unique metagenomics NGS sequencing
- Applying our knowledge to pharmacogenetic services
- Find out more at www.imgm.com