

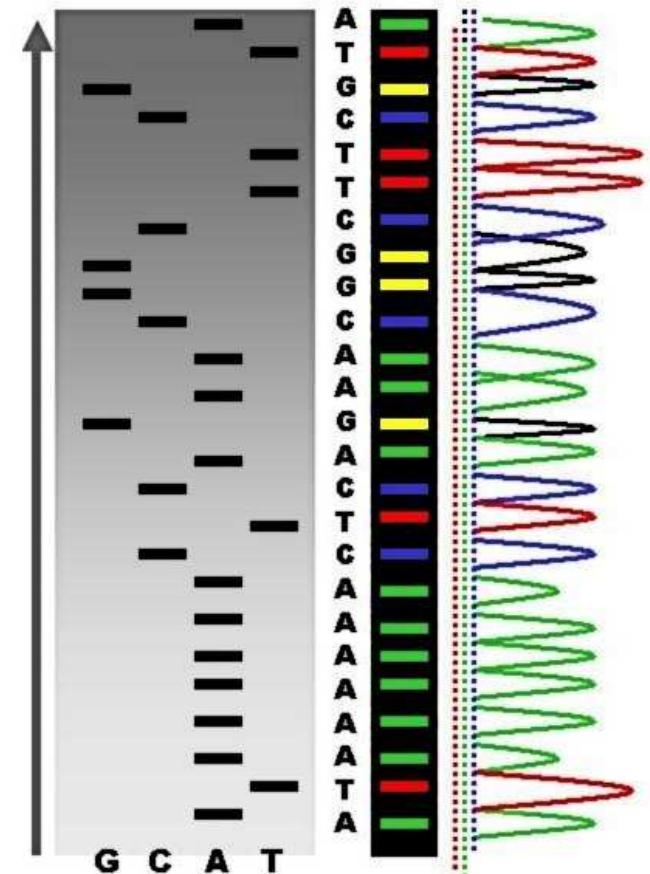
Next generation sequencing: principles and approaches in PGx

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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 in 1977 the standard and most widely used method
- Long read length (up to 1000 bp)
- Low throughput (1-96 reads/run)
- Mixture sequence = sequence mixture



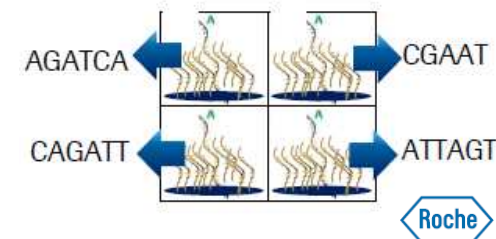
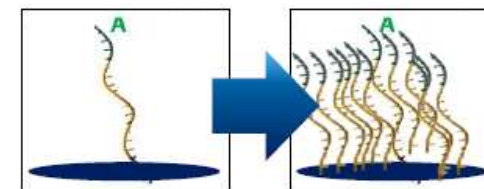
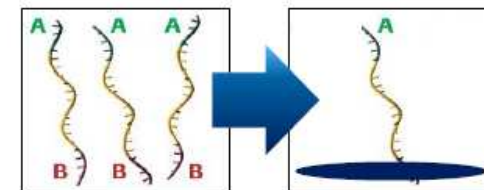
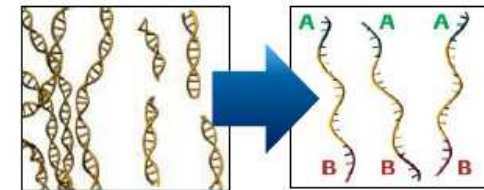
http://en.wikipedia.org/wiki/File:Radioactive_Fluorescent_Seq.jpg

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

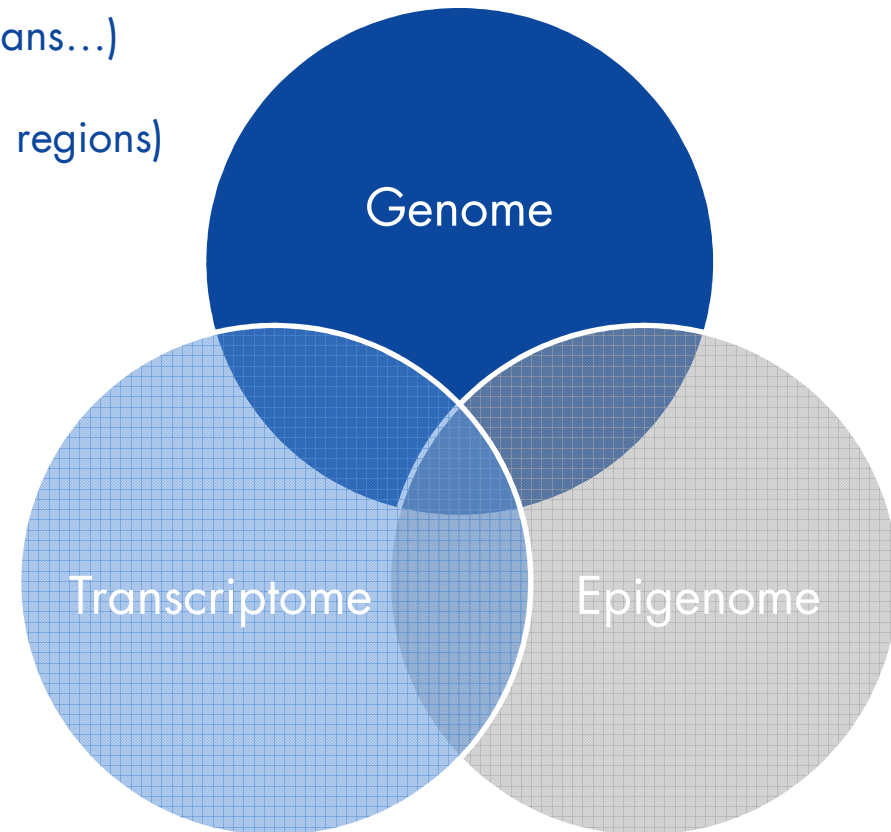


NGS Applications

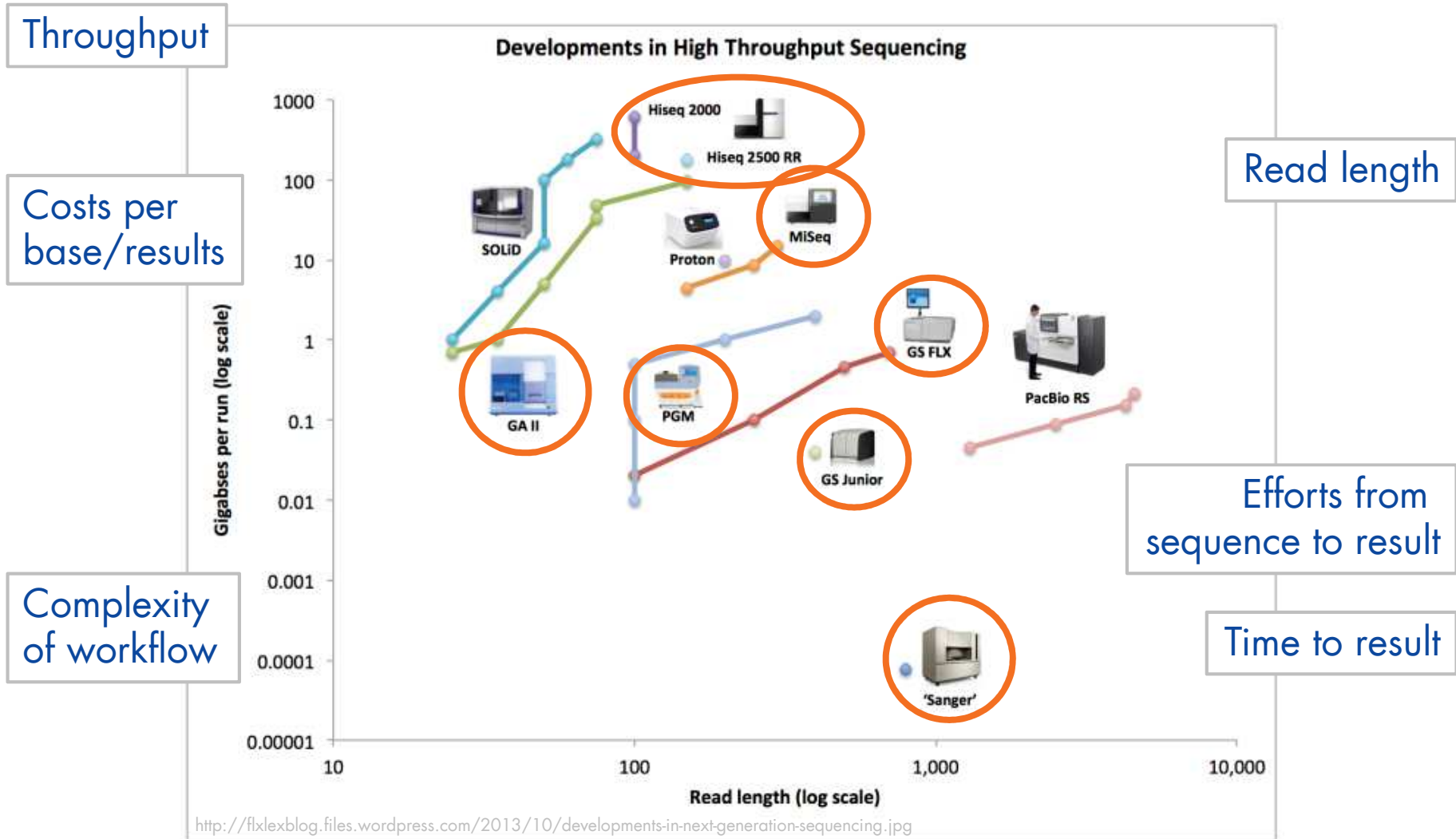
- De novo sequencing
(viruses, bacteria, fungi, plants, mammals...)
- Targeted resequencing
(single genes, gene panels, chromosomal regions)
- Whole exome resequencing
- Whole genome resequencing
- Metagenome

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

- Methylation analysis
- ChIP-Seq



NGS Platforms

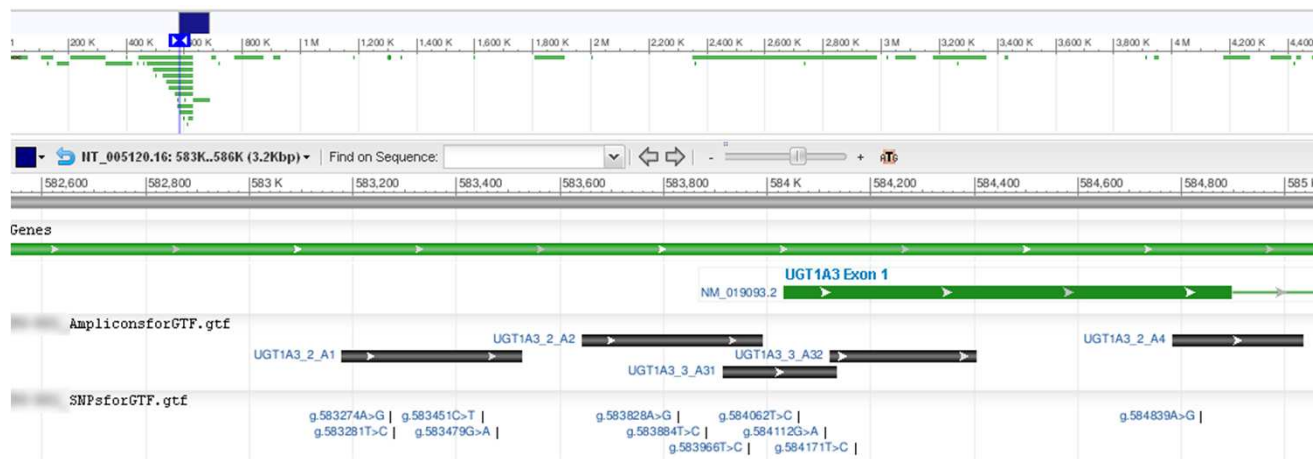


NGS in PGx

Custom assays

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

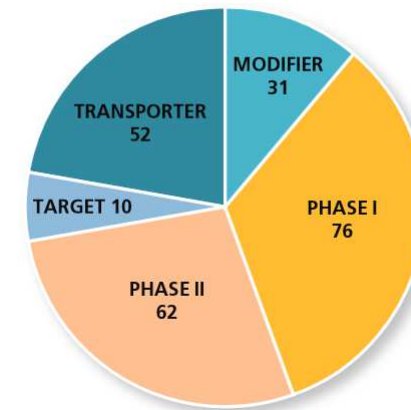
→ High-throughput analysis of many samples and few targets

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
- IMG: Assay design using Illumina DesignStudio
 - TruSeq Custom Amplicon (> 50 amplicons)
 - Nextera Custom Enrichment (> 0.5 Mb)

DMET™ Plus coverage = 231 genes



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

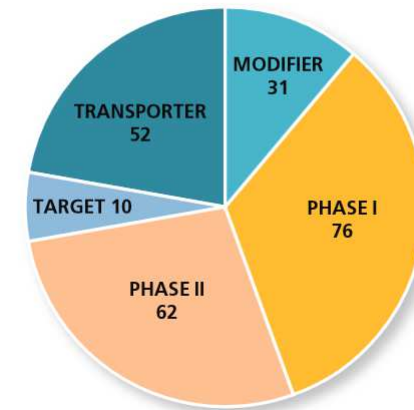
- Screening analysis of many targets of interest
- Screening analysis of non-standard PGx targets
- Does one size fit all?

NGS in PGx

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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

- 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



<http://image.slidesharecdn.com/nextgenerationsequencinginpharmacogenomics-120804080844-phpapp02/95/slide-12-1024.jpg?1344353852>

IMG M'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 IMG M
- 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