



# GenXPro

*genes never seen before!*

**Transcriptomics**

**Genomics**

**Epigenomics**

**Bioinformatics**

**GenXPro GmbH**

**Altenhöferallee 3, 60438 Frankfurt am Main, Germany**

**Phone: +49 (0)69- 95 73 97 10**

**[www.genxpro.de](http://www.genxpro.de) | [info@genxpro.de](mailto:info@genxpro.de)**



## GenXPro Service Portfolio

By applying intelligent and most sensitive sample preparation techniques in combination with the latest next generation sequencing procedures, GenXPro offers a wide spectrum of possibilities to analyse genomes, transcriptomes and epigenomes. Intensive customer consultation and guidance prior to a project and expert mentoring for handling vast amounts of data is part of our service. Data is delivered in convenient-to-analyze and ready-to-use format and includes quality information, quantification, annotation, mapping, Gene Ontology analysis, plots and other graphics.

Our team of skilled bioinformaticians applies the most advanced software for standard applications, and designs individual customer’s solutions to handle the data, using our powerful CPU clusters.

### Keywords of our portfolio are:

*RNAseq, 3’ DGE, SUpersAGE, MACE, PARE, Transcriptomics, De novo Sequencing, Assembly, SNPs, Copy Number Variations (CNVs) , RADseq, Reduced Representation Libraries, Normalisation of cDNA and DNA, CpG Islands, Methylation, Digital Karyotyping, Exome Sequencing, Target enrichment, qPCR, ChiP Seq and barcoded Sequencing.*

### Transcriptomics: Some Facts...

#### **Some transcripts in many copies, many transcripts in few copies.**

The typical relation between different transcript species and their abundance in a transcription profile (here from healthy pancreatic tissue) is shown below. The outer circle shows the abundance of different transcript species (in % of total transcripts), the inner circle details how many transcripts they represent in total. Although most transcript species are expressed at only low levels (5-9 copies per

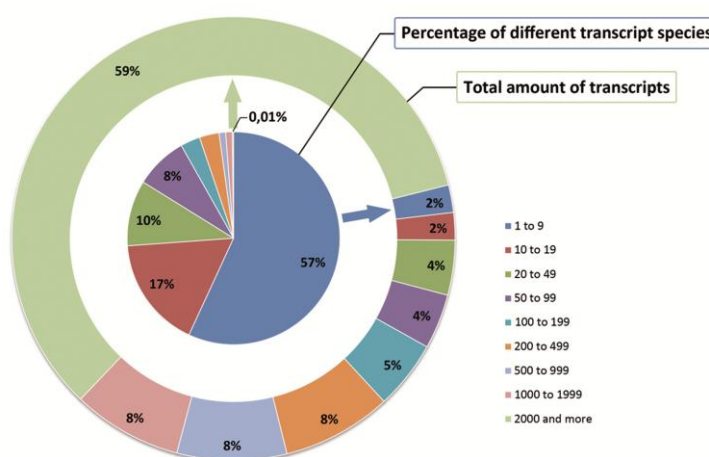


Fig. 1 Transcript-Species and Abundance

million), they merely represent 2% of the entire transcript population. On the other hand, a few transcript species (here less than 0.01% of the total) are present in so many copies, that they add up to ~ 60% of the entire transcript population (Fig. 1).

## GenXPro's Next Generation Transcriptome Services

### RNA-Seq: we maintain strand-specificity and include the polyA(-) transcripts!

RNA-Seq is a Next Generation Sequencing (NGS) approach for the qualitative and quantitative analysis of entire transcriptomes. Fragmented cDNA is sequenced by NGS. The resulting sequences can either be assembled for non-model organisms, or mapped to known

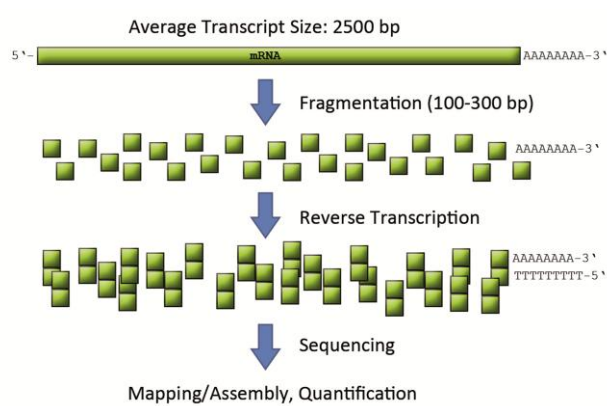


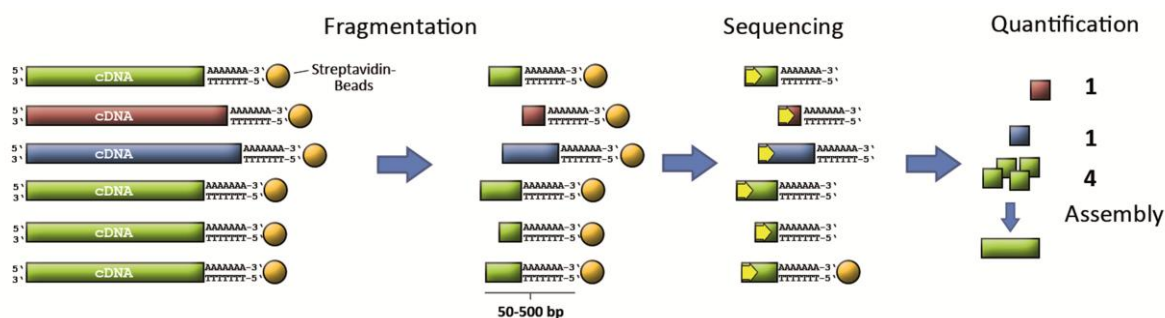
Fig. 2: Scheme of RNA-Seq

genomes. Quantification of the RNAs or contigs is achieved by counting the corresponding fragments. As full-length transcripts can be analyzed, the technique allows identifying splice-variants and SNPs. GenXPro's protocols for RNA seq include the information about

strand specificity and effectively exclude artifact during cDNA production. We can therefore distinguish between sense- and antisense transcripts. Moreover, we offer to deplete ribosomal RNA and sequence the remaining RNA to include the non-polyadenylated transcripts.

### SuperSAGE and MACE: Ultra Deep Digital Gene Expression

SuperSAGE is an improved version of the SAGE technique and MACE stands for "Massive analysis of cDNA Ends". Both techniques are based on the analysis of one single cDNA fragment ("tag") per transcript-molecule. Because of this, SuperSAGE and MACE capture millions of transcript-molecules and even very low-level expressed transcripts such as for receptors, transcription factors or naturally occurring antisense transcripts are included. These low-level transcripts are invisible on microarrays. Since up to 80% of a cell's transcript species are expressed at low levels (1-20 copies per Million - see above) their analysis is crucial to understand the cells function. Our tag-based analyses display these low level transcripts for only a fraction of the costs of RNA-Seq.

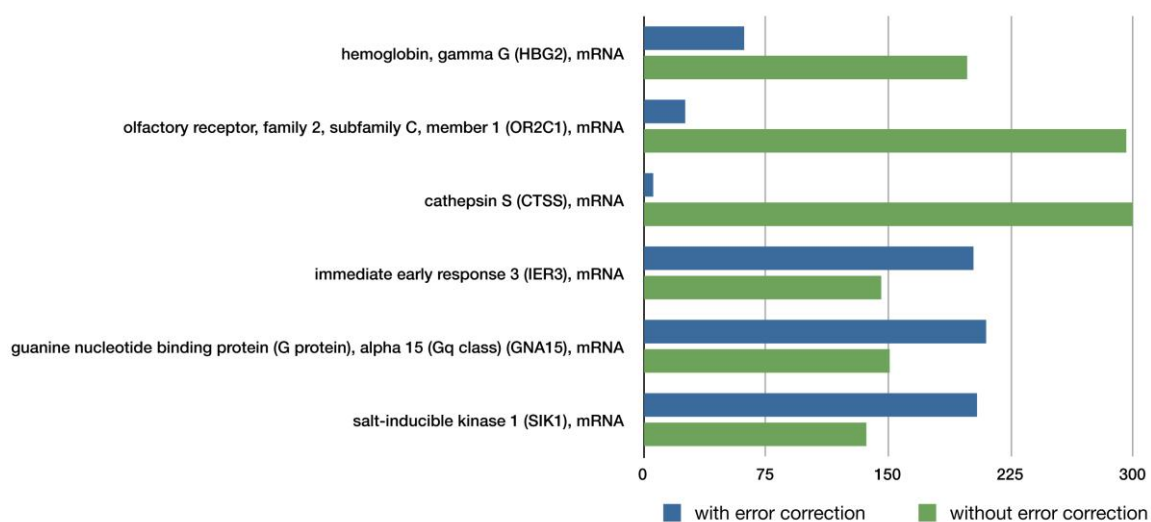


**Fig. 3: Scheme of MACE**

A population of cDNAs is first bound to a streptavidin matrix via 3'-biotin. The cDNAs are then sheared to 50-500 bp fragments, and unbound fragments discarded. The bound cDNA ends are sequenced by NGS, starting at the fragmentation site, generating 50-500 bp "tags", depending on the NGS-platform. Frequent tags can be assembled into contigs, all tags can be annotated to database entries and counted.

### PCR-Bias Free Data!

**Quantification:** All second-generation sequencing-based data are prone to PCR-bias, because different DNA fragments are amplified with different amplification efficiency. GenXPro has developed a method to eliminate such bias by its "PCR-Copy-Elimination" technique.



Negative common logarithm of the p-value for differential expression of gene expression comparisons (Audic & Claverie; 1997). If not corrected for PCR bias, the p-value changes dramatically for many transcript as a consequence of the biased data.

### Normalization of cDNA, Sequencing and Assembly

To obtain a wider spectrum of different transcripts, frequent transcripts are depleted prior to sequencing. GenXPro offers a full service from RNA isolation, cDNA

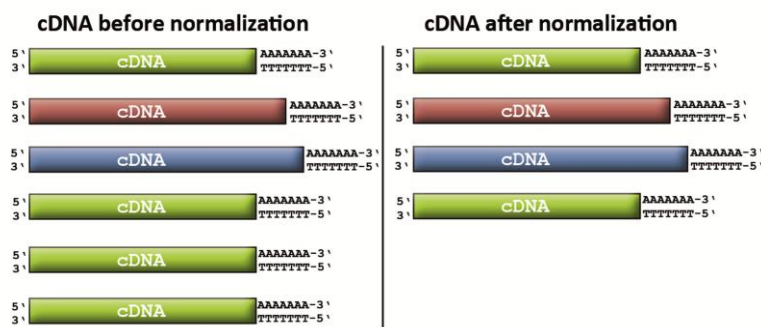


Fig. 4 cDNA normalization

preparation, normalization, sequencing by whatever NGS technology to assembly and mapping of cDNAs. Our optimized methods for the preparation of normalized cDNA libraries reduce 3' and 5' end biases and eliminate frequent transcripts as much as is possible, without losing rare transcripts. Our bioinformatics service includes also mapping to reference genomes and SNP detection (Fig. 4).

### MicroRNA Analysis Service

Quantify known and discover new microRNAs and small RNAs from any biological sample by ultra-deep miRNA and small RNA sequencing. GenXPro delivers miRNA expression profiles, annotation to existing databases and reliable miRNA quantification by applying our proprietary, PCR bias-free quantification.

### Genomic DNA analysis

#### Restriction Site Anchored Sequencing (RAD-Seq)

RAD-Seq is the ideal technique for genotyping, comparative genomics, analysis of gene copy number variations (CNVs), and detection of chromosomal changes and of viral or xeno DNA of any organism.

DNA is fragmented with one or several restriction enzymes. The fragment-ends are then sequenced in their millions and counted. In different genotypes, sequence differences reveal polymorphisms (e.g. SNPs) in the analyzed fragments. The method is therefore ideally suited for genetic marker discovery, genotyping and phylogenetic studies. Differences in the abundance of specific fragments reveal copy number variations (Fig. 5).

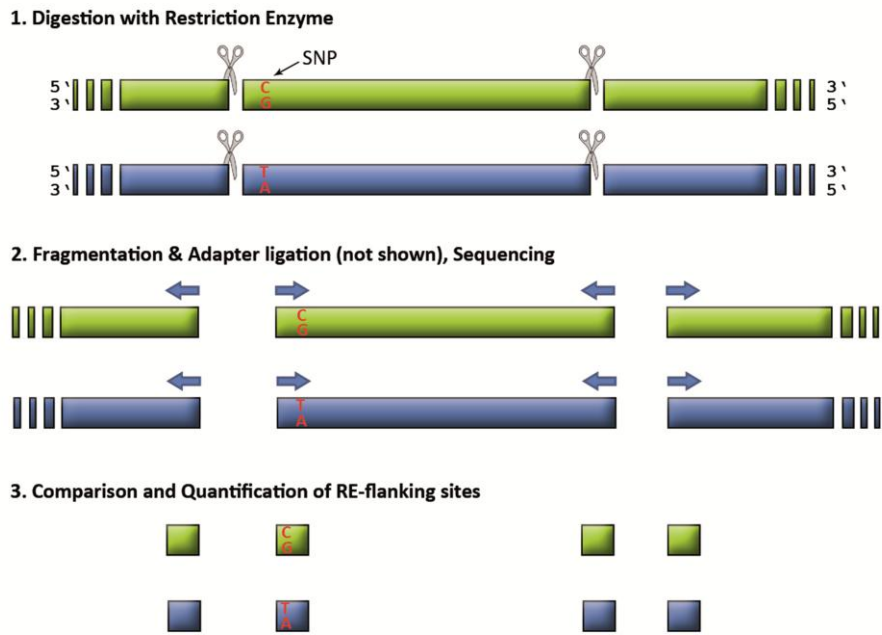


Fig. 5: Scheme of RAD-Seq

## Genome Sequencing

GenXPro uses Reduced Representation (RR) strategies for genome sequencing, by separately sequencing different size fractions of restriction enzyme digested DNA, as described by Young et al. 2010 (Genome Research). In addition, we apply techniques to deplete highly repetitive DNA, reducing the sequencing costs and computational requirements by 50% and more (Fig. 6).

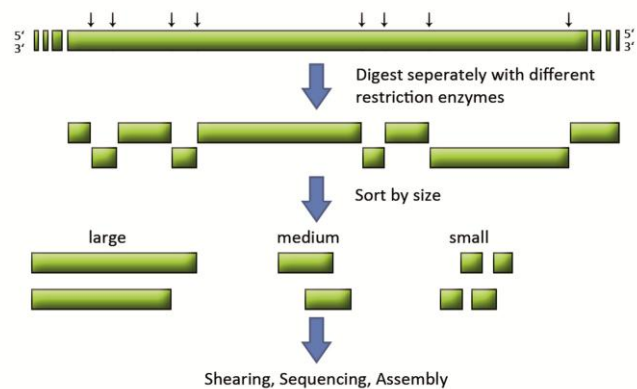


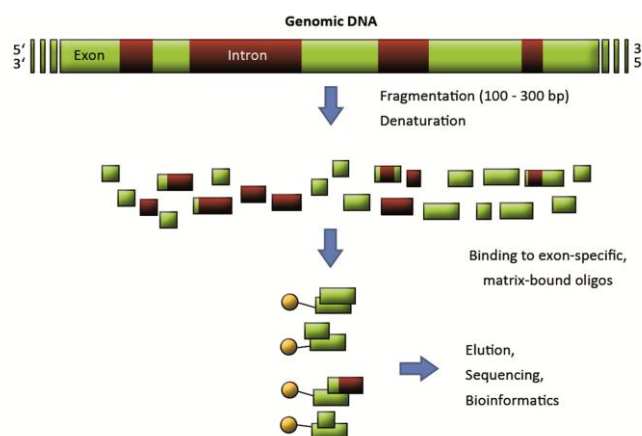
Fig. 6: Genome Sequencing

## Whole Exome Sequencing, Target Sequencing

Whole exome sequencing aims at sequencing all genomic regions which represent an exon (Fig. 7). By reducing large and complex genomes to the exomes, extensive genetic analyses can be achieved at low costs. About 85% of all genetic diseases are associated with, or caused by, changes in exonic regions. Because not RNA, but genomic DNA is the source

material, formalin-fixed paraffin-embedded material or peripheral blood samples can be used for exome sequencing.

Moreover, a specific subset of genomic regions can be sequenced after “target enrichment” strategies, either by PCR amplification of the target sites or by capturing of the sites via biotinylated oligonucleotides. Genes of interest of hundreds of individually barcoded genotypes can hence be analyzed simultaneously.



**Fig. 7: Whole Exome Sequencing**

## Epigenetics

### **Methylation Specific Digital Karyotyping (MSDK)**

GenXPro’s genome-wide analysis of C-methylation reveals the individual methylation pattern of genomic DNA by analyzing the digestion products of methylation sensitive restriction enzymes. Genomic DNA is digested with one or several methylation-sensitive restriction enzymes (MSREs). DNA will either be digested (at the non-methylated sites) or not (at the methylated sites). Hence, different fragments will be generated from differently methylated genomic DNA. By sequencing and counting millions of these fragments, a genome-wide, quantitative analysis of the methylation-status is established (Fig. 8).

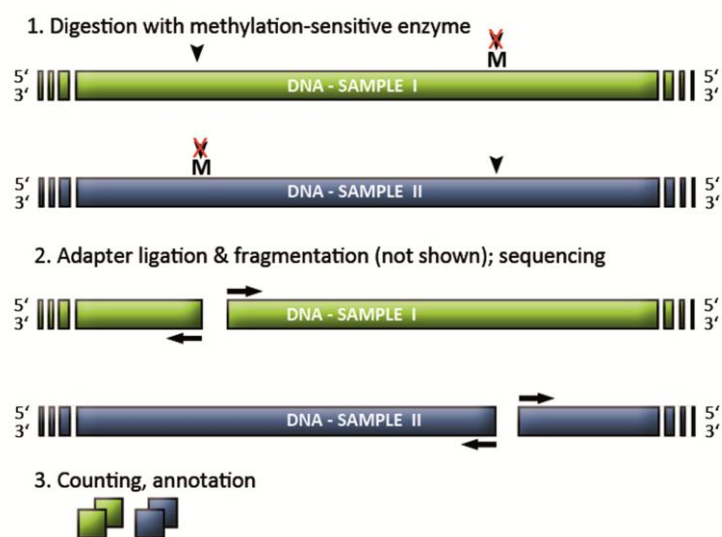


Fig. 8:Scheme of MSDK

### ***Methylation specific qPCR***

Differentially methylated DNA is first digested by methylation sensitive restriction enzymes (MSRE). Specific primers, whose binding site is located within the CpG Island of interest are used in a qPCR reaction to quantify the methylation of the site. The PCR product will only appear, if the site is methylated and cannot be digested by the MSRE.

### ***ChIP-Seq***

Identify and quantify the DNA-binding sites of proteins by sequencing immune precipitated DNA. Profit from our reliable quantification method for PCR bias-proof quantification. Analyze multiple samples simultaneously at low costs using our barcode-system.

### ***Barcoded sequencing: Amplicon Sequencing, Target Sequencing etc.***

With our 96 different barcodes, we can individually label hundreds of samples and sequence them for you- save the time and money to set up a barcoding system- we have it ready to use for all your samples!

**Profit from our vast experience in any NGS-based approach for your question of interest:**

**...ChIP-Seq ... Metagenome Analysis ... Amplicon Sequencing...**

## **qPCR Service**

### **Real Time and Quantitative PCR Service**

#### **The qPCR Service of GenXPro includes:**

- qPCR for Transcripts, Copy Number Variations
- methylation specific qPCR
- Design of gene-specific primers and TaqMan™ probes
- Definition of internal controls
- Optimization of qRT-PCR assays
- Data interpretation including standard curves and calculation of copy numbers
- All experiments according to MIQE-standards (Minimum Information about qPCR Experiments)

**Stay competitive! Exploit the turnkey solutions of**

**GenXPro**  
genes never seen before