



NEXT GENERATION SEQUENCING

Powered by the
Genome Sequencer™ FLX
in Combination with
Titanium-Series Chemistry
and Classical
Sanger Technology

De Novo Sequencing of Genomes

Transcriptome Analysis

Ultra Deep Amplicon Sequencing

Re-Sequencing of Genomic Fragments

Re-Sequencing of Genomes and
Comparative Genomics

Customised Bioinformatic Services

Library Generation

The Best Solution for Your Application

Powered by the Genome Sequencer™ FLX operated with Standard- or Titanium-series chemistry, a multitude of well known and new applications are provided. Optimum results are achieved in rapid time by combining next generation sequencing technology with classical Sanger sequencing.

De Novo Sequencing of Genomes

Whole Genome Shotgun Sequencing of Prokaryotic and Eukaryotic Genomes

- Fragmentation by nebulisation of the genomic DNA
- Preparation of a non cloned single stranded shotgun library (sstDNA) and clonal amplification by emulsion PCR (emPCR)
- Ultra high throughput sequencing with GS FLX Titanium-series chemistry up to 20 fold coverage
- Assembly with appropriate assembly software
- Generation and sequencing of non cloned paired end libraries (Fig. 1)

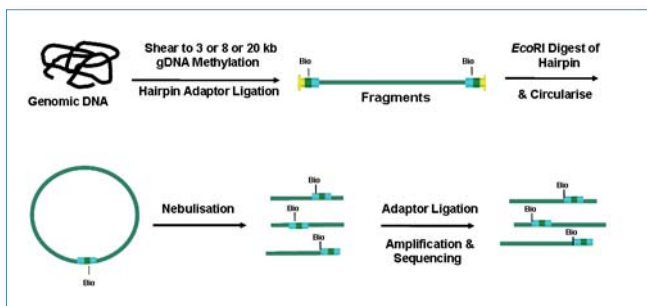


Fig. 1: Generation of long tag paired end libraries

- Assembly of the paired ends and scaffolding the contigs (Fig. 2, 3)
- Closing of gaps by designing primer pairs on adjacent contig ends and sequencing PCR amplicons of the genomic region of interest
- Bioinformatic analysis such as ORF calling and annotation
- Subsequent gene synthesis services can be performed to generate clones for specific genes of interest

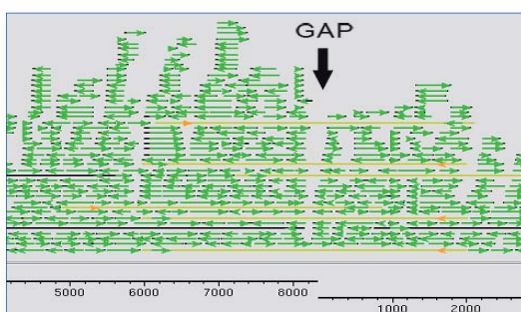


Fig. 2: Shotgun reads displayed in green

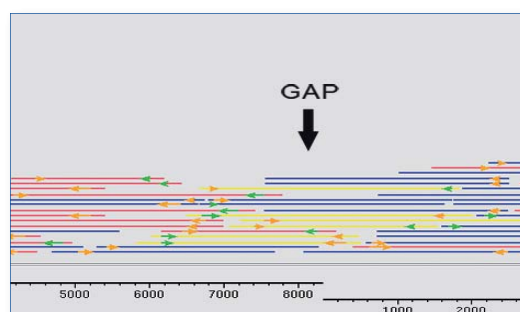


Fig. 3: Scaffolding of contigs with longtag paired end libraries

BAC Sequencing Approaches for Eukaryotic Genomes

- Generation of BAC libraries (on demand)
- Sequencing or multiplex sequencing of selected BAC clones
- Fragmentation by nebulisation of the BAC DNA
- Preparation of the non cloned single stranded shotgun library (sstDNA) and clonal amplification by emulsion PCR (emPCR)
- Ultra high throughput sequencing with GS FLX Titanium-series chemistry up to 20 fold coverage
- Assembly by using optimised assembly software
- Closing of gaps by designing primer pairs on the contig ends and primer walking on the respective BAC clone
- Bioinformatic analysis such as ORF calling and annotation

Transcriptome Analysis

The transcriptome analysis service covers the construction and sequencing of standard, normalised or 3'-fragment cDNA libraries and miRNA or snRNA (small non coding RNA) libraries. New and unknown ESTs (expressed sequence tags) for expression profiling or new rare transcripts can be identified and differential gene expression studies can be performed. Depending on the application, different project strategies are carried out.

- Generation of standard or normalised (Fig. 4, 5) cDNA libraries
- Sequencing of EST or SAGE clones by Sanger technology
 - DNA preparation and purification
 - Sanger sequencing from the 5'-end or both ends (read lengths up to 1,100, > 750 Q20 bases)
 - Clones for further experiments are available
- Generation of 3' or 5' fragment and non coding RNA cDNA libraries. Libraries are non cloned since the protocol is specially developed for the GS FLX technology. No nebulisation step is needed.

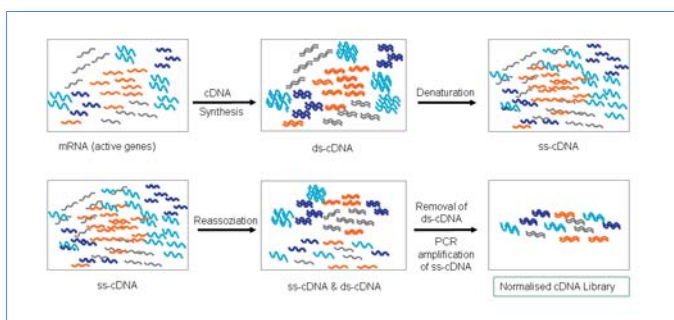


Fig. 4: Generation of a normalised cDNA library

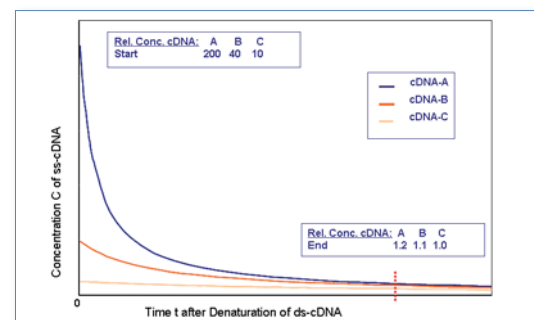


Fig. 5: Kinetics of Normalisation

- Ultra parallel sequencing of non cloned cDNA libraries with GS FLX Standard- or Titanium-series chemistry
 - Preparation of the non cloned single stranded shotgun library (sstDNA) and clonal amplification by emulsion PCR (emPCR)
 - Ultra high throughput sequencing with the GS FLX
- State-of-the-art clustering and assembly service (Fig. 6)
 - Overall clustering and assembly statistics including the number of contigs and singlets, number of reads per cluster, information on contig lengths and allocation of reads to contigs/clusters, etc.
 - FASTA files of all singlets and contigs
 - BLASTn and BLASTx analysis and filtered output files in standard GFF format

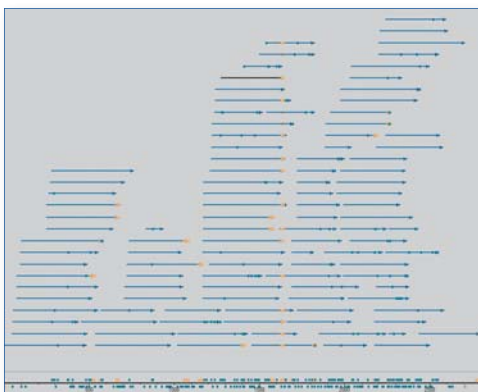


Fig. 6: Example of a de novo cDNA assembly of GS FLX Titanium reads

Amplicon Sequencing

Amplicon sequencing by GS FLX technology refers to ultra deep sequencing of exons or other PCR products for analysing genetic variations in eukaryotic or prokaryotic populations, e.g. for SNP or mutation detection or for identifying and qualifying methylation patterns.

- Starting material can be provided as amplicons with specific adaptors (A and B fusion primers) or alternatively as PCR products without adaptors
- Clonal amplification by emulsion PCR (emPCR)
- Sample pooling (Fig. 7) using adaptors and specific sequence tags (barcodes)
- Ultra deep sequencing with the GS FLX system
- Clustering of the sequence reads
- Further bioinformatic analysis, if required

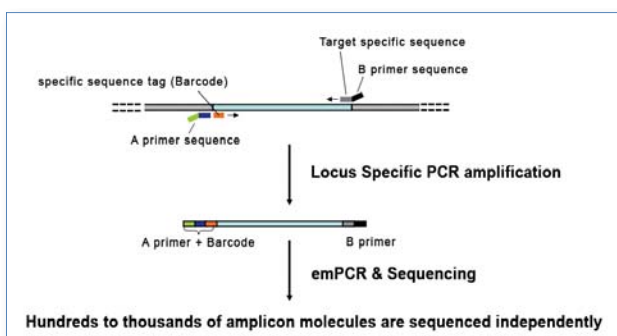


Fig. 7: Scheme of the generation of pooled amplicons

Re-Sequencing of Genomic Fragments

Re-sequencing of genomic fragments is the sequencing of presequenced and preselected genomic regions such as exons in order to find a correlation between sequence deviations (e.g. SNPs or indel mutations) and phenotypes. Based on Sanger technology, this service is performed in three steps:

Phase I: Establishment of the PCR

- Primer design and synthesis for all exon regions
- Establishment of PCR amplification on a minimum of two DNA samples
- Alternatively, establishment of PCR amplification on a standard DNA
- Quality check by double stranded sequencing of the test samples

Phase II: PCR and Sequencing

- High throughput PCR of all DNA samples
- High throughput purification and Sanger sequencing of PCR products

Phase III: Bioinformatic Analysis

- Identification of all differences between reference and samples
- Homozygous and heterozygous substitutions
- Detection of insertions/deletions

Re-Sequencing of Genomes and Comparative Genomics

Genome comparison or re-sequencing of genomes using the next generation Genome Sequencer™ FLX (GS FLX) is a convenient and economic way to identify genetic variations including single base mutations, inserted and deleted genes or heterozygous SNPs, e.g. for production strain optimisation, metabolic engineering or mutation analysis.

- Fragmentation by nebulisation of the genomic DNA
- Preparation of a non cloned single stranded shotgun library (sstDNA) and clonal amplification by emulsion PCR (emPCR)
- Shotgun sequencing by GS FLX Titanium-series chemistry up to a 20 fold sequence coverage
- Assembly of the sequence data with appropriate assembly software using a reference sequence as backbone
- Sequence reads which are not present on the reference genome (e.g. due to phage insertions or plasmids) are automatically assembled de novo
- Closing of gaps by designing primer pairs on adjacent contig ends and sequencing PCR amplicons of the genomic region of interest (optional)
- Genome comparison by a matched assembly against the reference genome (Fig. 8)
- Additional bioinformatic analysis e.g. annotation

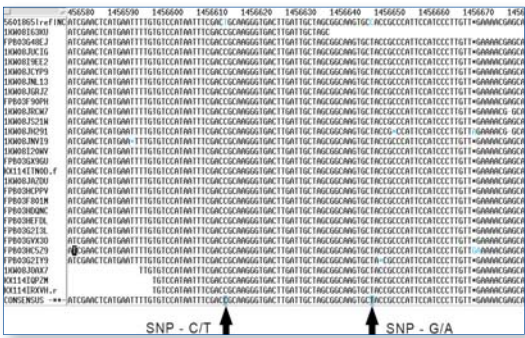


Fig. 8: Screenshot of a matched assembly; example of SNP identification

With the proprietary AnnotationViewer (Fig. 2), a software designed and built by Eurofins MWG Operon, the sequence and all of its annotated features can be displayed.



Fig. 9: Example of a mutation displayed in the AnnotationViewer

Customised Bioinformatic Services

Analysis of data from a sequencing project requires industrial scale bioinformatics. We offer standard BLAST analysis, annotation or clustering and assembly services and in addition we can support specific requests with customised bioinformatic solutions.

Library Generation

- BAC libraries, arraying into plates and spotting onto nylon filters
- Cosmid and Fosmid library generation services
- Standard, normalised, 3'- and 5'-fragment cDNA libraries are available for transcriptome analysis
- Generation of shotgun libraries (mechanically sheared shotgun fragments cloned into plasmids) of large constructs or bacterial genomes

Advantages of Two Technologies

Utilising the respective advantages of each technology – pyrosequencing with GS FLX and Sanger sequencing with ABI 3730 XL – adds strength and a higher degree of flexibility than any technology would contribute on its own.

Genome Sequencer™ FLX operated with Standard- and Titanium-Series Chemistry

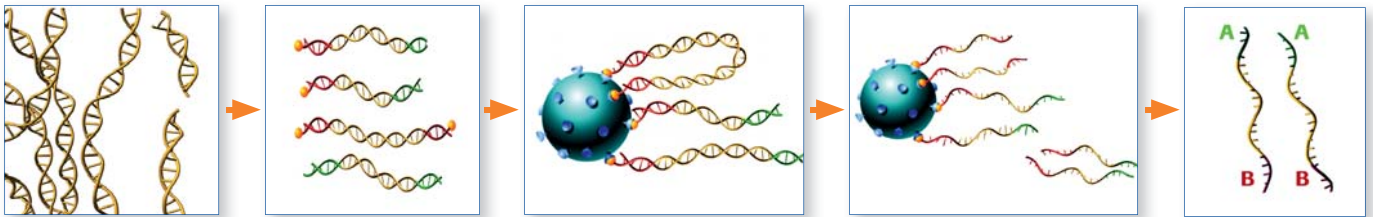
- Cloning is not necessary – due to molecular titration and amplification of purified DNA
 - Good representation of AT-rich areas – no cloning bias in library generation
 - Biosafety is guaranteed – easier handling of pathogens
- Throughput with Titanium-series chemistry:
 - Generation of 400-500 MB per run or 1 GB per day
 - 1,2 million reads with an average length of 350-450 base pairs
- Throughput with Standard-series chemistry:
 - Generation of over 100 million bases within an 8-hour run
 - 400,000 reads with an average read length of 220-270 bases per run
- Trouble-free sequencing of regions with high GC content – no “full stops”
- Reliable sequence quality
 - Consensus accuracy > 99.994 % at a 20 fold sequence coverage (Q45)
 - Single read accuracy > 99.5 %

Sanger Technology

- System of choice for finishing projects
 - Gap closure with primer walking strategy
 - Sequencing of amplified PCR products of interest
- Read lengths of up to 1,100 bases
- Gold standard for single read approaches
 - Re-sequencing of selected PCR products or plasmid clones
 - Sequencing of specific regions of large constructs like BAC, PAC, Cosmid or Fosmid clones

Technical Details - GS FLX Process

1. Preparation of a non cloned single stranded (sstDNA) Shotgun Library



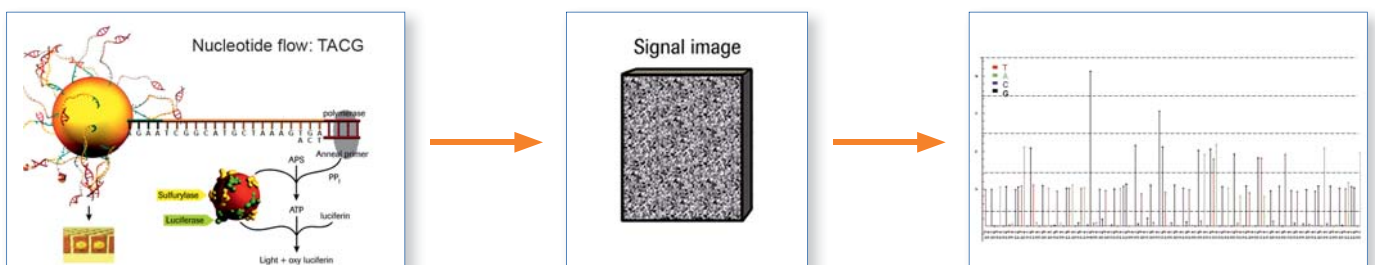
- Genomic DNA, cDNA, BAC DNA or PCR products can be used as starting material
- Samples > 700 bp are fragmented by nebulisation
- Fragments are labelled with adapters (A and B), which are ligated blunt end to each fragment
- Fragments with biotin labelled adaptors (A/B or B/B) attach to a streptavidin coated bead
- Only single stranded fragments with A/B labelled ends are selected using streptavidin-biotin purification

2. Clonal Amplification by Emulsion PCR (emPCR)



- Due to the adaptors the single stranded fragments bind to beads
- Using a water-in-oil emulsion the beads are packed into droplets (so called microreactors)
- Titration experiments are necessary to ensure that only one sstDNA molecule is bound to one bead and only one bead is packed into one emulsion oil droplet
- The fragments are amplified in parallel within the microreactors up to several million copies per bead
- After enrichment, the DNA-positive beads are ready for sequencing

3. Sequencing by Synthesis with the Genome Sequencer™ FLX Standard- or Titanium-Series Chemistry



- DNA beads are deposited onto the PicoTiterPlate device
- A single clonally amplified sstDNA bead is deposited per well
- 400,000 or 1,2 million reads are obtained in parallel with an average read length of 220–270 bases or 350–450 bases respectively
- The light signals are detected by a CCD camera and the images are translated into Flowgrams

Expertise and References

Eurofins MWG Operon has been providing sequencing services for projects of all sizes since 1996.

Expertise in de novo Sequencing and Re-Sequencing of Genomes:

We have determined over 50 microbial genomes such as several E.coli strains, strains of Streptomyces, Chlamydomonas, Lactococcus, Salmonella, Clostridium, Mycoplasma, Pseudomonas, etc. In the first year after the implementation of the GS FLX system we finalised several dozen larger projects such as fungal genomes and metagenome projects. Over 1,600 BAC sequencing projects have been performed. We have developed an advanced multiplex approach to sequence up to 96 BACs in parallel.

Expertise in Transcriptome Analysis and Amplicon Sequencing:

We have carried out more than 100 large EST projects using Sanger technology. For ultra deep transcriptome analysis we established a new type of non cloned normalised and 3'-fragment cDNA libraries and miRNA libraries. This enabled us to sequence over 80 libraries as well as over 50 amplicon projects within the first year.

Selected Publications:

De Schutter et al. Genome sequence of the recombinant protein production host *Pichia pastoris*
Nature Biotechnology, Volume 27, Number 6, June 2009

Strittmatter et al. Genome sequence of *Desulfobacterium autotrophicum* HRM2, a marine sulfate reducer oxidizing organic carbon completely to carbon dioxide
Environmental Microbiology (2008), 10, 1462-2920

Torres et al. Gene expression profiling by massively parallel sequencing.
Genome Res. 2008 18: 172-177 originally published online Nov 21, 2007

Horn et al. Illuminating the evolutionary history of *Chlamydia*.
(Sequencing of the genome of *Protochlamydia amoebiphila*). Science. 2004 Apr 30; 304 (5671): 728-30

Feel Free to Contact Us

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