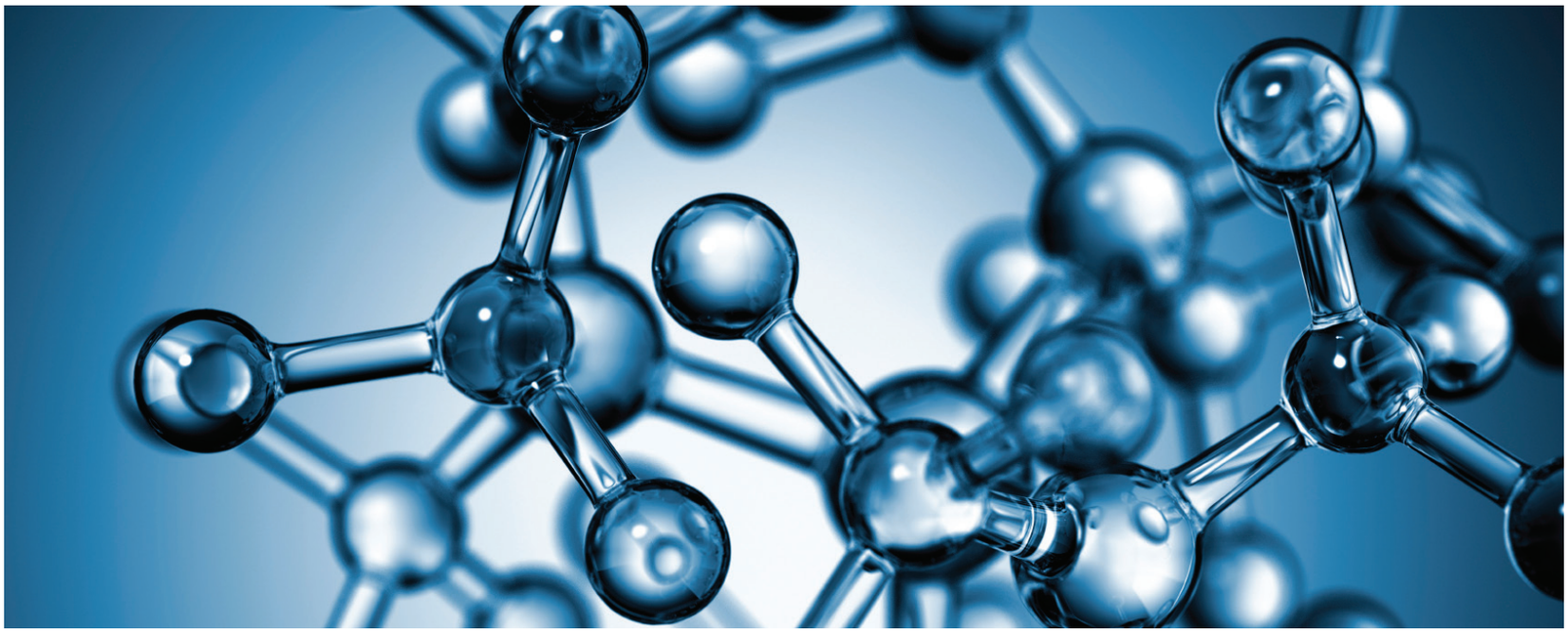
eurofins
mwg|operon



Next Generation Sequencing

High throughput sequencing services for 13 years

Custom Solutions

1. Exome Capture and Sequencing
2. De Novo Genome Sequencing
3. Re-Sequencing and Comparative Genomics
4. Transcriptome Analysis
5. Ultra-Deep Amplicon Sequencing
6. Re-Sequencing of Genomic Fragments
7. Customized Bioinformatics Service
8. NGS Library Generation Services

Latest Technologies

Illumina MiSeq

Illumina HiSeq 2500

Roche GS FLX/FLX+

Roche GS Junior

PACBIO RS

In combination with classical

Sanger technology on ABI 3730 XL

Quality Management System ISO 9001 2008 CERTIFIED

Quality Management System ISO 13485 2003 CERTIFIED

operon.com

The Best Solution for Your Application

Powered by the Illumina HiSeq 2500, Illumina MiSeq, Genome Sequencer FLX/FLX+, GS Junior, and PACBIO RS, Eurofins MWG Operon offers all next generation sequencing (NGS) applications on most of the available NGS platforms.

Our NGS experts will help to guide the optimum selection of technologies and platforms to yield the best data for your experiments. Shortened and more flexible service times are available including optional express delivery.

1. Exome Capture and Sequencing

- Guaranteed coverage
- 3-6 weeks turnaround time
- Sequencing files, alignment files and variance reports for each sample
- Expert consulting and project customization

Eurofins MWG Operon provides fast, reliable and consistent results for human exome sequencing services using Illumina Nextera and Agilent SureSelect capture and enrichment protocols in combination with Illumina HiSeq2000/2500 sequencing using 100 base PE runs. Services for non-human samples are available by request.

Just send your genomic DNA samples. Our highly-experienced technical staff perform library construction and enrichment capture followed by quantified massive parallel Illumina sequencing. Consult with one of our specialists today to discuss the optimal enrichment/capture method and coverage for your experimental needs!

	NGS Coverage	Price/sample*
Illumina Nextera™ Rapid Exomes 37 Mb target capture of coding exons	30x	$920
	50x	$1050
	100x	$1470
Illumina Nextera™ Rapid Exomes Extended 62 Mb target capture of exons and UTRs	30x	$1350
	50x	$1550
	100x	$1970
Agilent SureSelectTM All Exon V4 50 Mb target capture of coding exons	30x	$1030
	50x	$1290
	100x	$2360
Agilent SureSelectTM All Exon V5 50 Mb target capture of coding exons	30x	$1250
	50x	$1350
	100x	$2420

*Minimum number of samples applies depending on selected services. All prices in US dollars.

2. De Novo Genome Sequencing

The most effective methods for NGS sequencing of unknown DNA viruses, prokaryotic, or eukaryotic genomes are determined by balancing the size and complexity of the genome and the research requirements.

Our experts may recommend construction of specialized longer interval libraries along with shotgun libraries to create reliable backbone sequence (especially for eukaryotes) and collection of massive numbers of sequence reads on Illumina sequencers. At times, long reads are essential for creation of reliable backbone sequence and Roche technologies may be recommended.

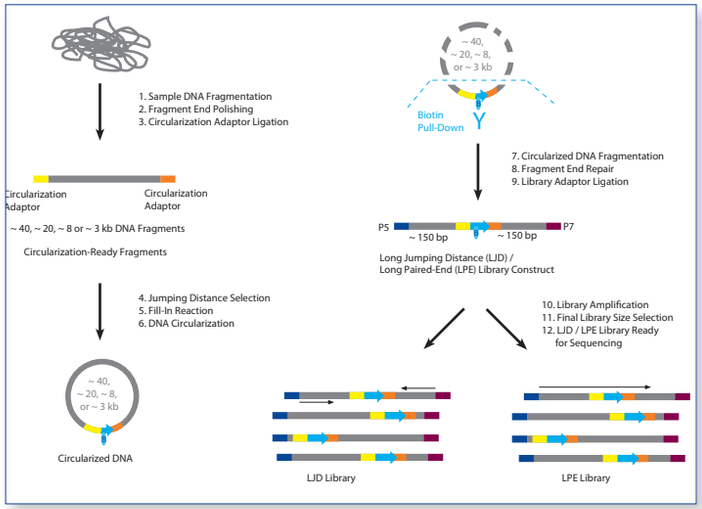


Fig. 1: Generation of a long jumping distance or a long paired-end library

Whole genome sequencing of viral, prokaryotic and eukaryotic genomes

- Generation of standard and long jumping distance (LJD) or long paired-end (LPE) libraries (Fig. 1)
- Sequencing of all libraries on Illumina HiSeq 2500/MiSeq or LPE libraries on Roche GS FLX/FLX+
- Data assembly with appropriate assembly software (gsMapper, MIRA or Celera)
- Mapping of LJD or LPE read pairs and scaffolding of the contigs
- Closing of gaps by semi-automated primer design on adjacent contig ends
  - Sequencing PCR amplicons of the genomic region of interest
  - Sequencing of long range PCR by primer walking
- Analyses such as reference-guided ORF finding, annotation or sample comparison (synteny plot)

3. Re-Sequencing and Comparative Genomics

Genome comparison or re-sequencing of genomes using NGS technologies is the most reliable method to identify genetic variations including single base mutations (SNPs), inserted and deleted genes or heterozygous SNPs. Re-sequencing is often used for production strain optimization, metabolic engineering, and mutation analysis.

For sequence projects where a well-known reference is available, sequencing on Illumina HiSeq 2500 or Illumina MiSeq is most often the most efficient methodology. For example, up to 20 *E.coli*-like bacteria can be sequenced in one run using the 2x 250 bp paired-end read module of our MiSeq instruments.

- Library preparation(s), optimally using varying fragment size shotgun libraries or LJD libraries
- Shotgun sequencing on HiSeq 2500/MiSeq to 30- to 50-fold coverage
- Reference guided assembly to map the new sequence data
- Sequence reads not present on the reference genome (e.g. due to phage insertions or plasmids) are automatically sorted and delivered with your data
- Optional, closing of gaps by designing primer pairs for adjacent contig ends and sequencing PCR amplicons in the region of interest
- Genome comparison using SNP analysis (Fig. 2)
- Annotation of contigs and sequencing of PCR amplicons of the genomic region of interest

Fig. 2: Screenshot of Illumina read mapping on a reference; example of a SNP identification

4. Transcriptome Analysis

Our transcriptome analysis service covers the construction and sequencing of random primed, 3-fragment and/or 5-fragment cDNA libraries as is, or with optional normalization. Libraries prepared and sequencing may be transcriptome libraries, miRNA, sncRNA (small non-coding RNA), or other libraries.

Results often identify unknown expressed sequence tags (ESTs) or rare transcripts. Expression profiling and differential gene expression analyses may also performed using these sequencing methods. Depending on the research goals, different sequencing platforms and/or strategies are recommended.

- Generation of random primed cDNA libraries (no nebulization step recommended)
- Generation of 3- or 5-fragment and miRNA cDNA libraries (no nebulization step recommended)
- Generation of normalized cDNA libraries (see Fig. 3)
- Optional protocol for strand-specific sequencing using MiSeq libraries and technology
- Ultra high throughput sequencing of cDNA libraries with Illumina HiSeq 2500, MiSeq, or GS FLX/FLX+

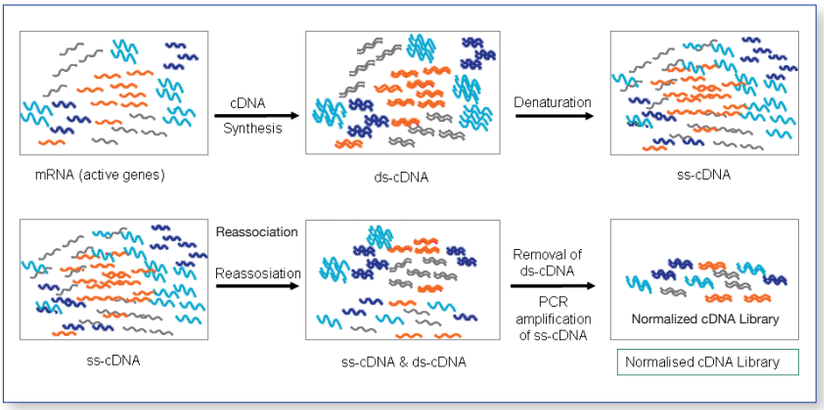


Fig. 3: Generation of a normalized cDNA library

- State-of-the-art clustering and assembly service (Fig. 4)
  - 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/FASTQ files of all contigs and singlets; BLASTn and BLASTx analysis with optional HTML output

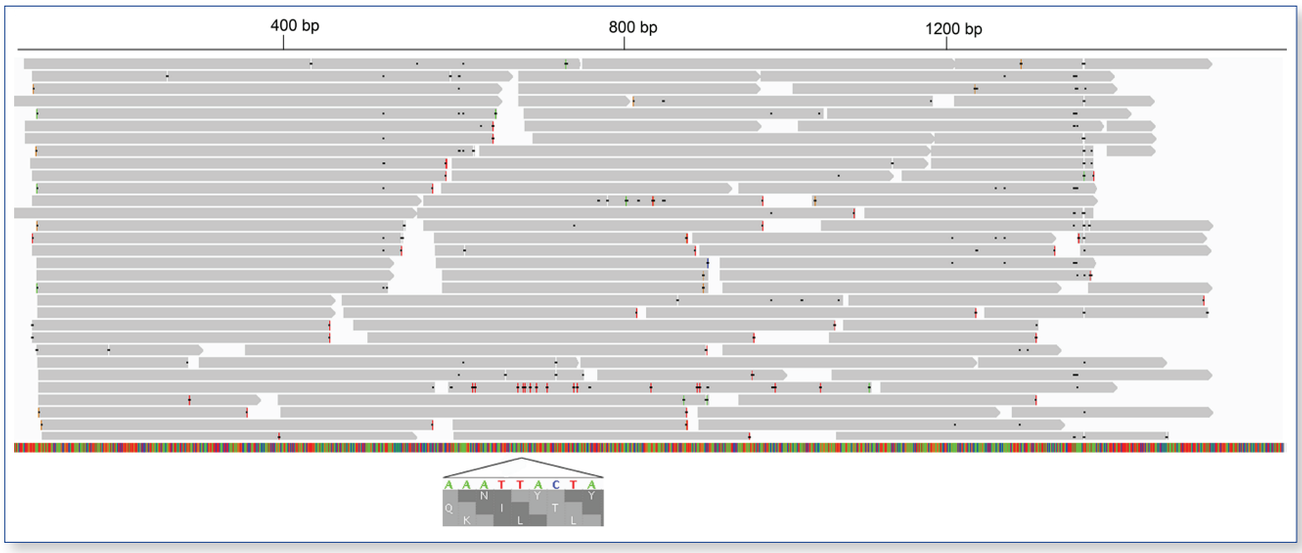


Fig. 4: Sector of a de novo assembly of cDNA reads derived from sequencing a random primed, normalized cDNA library with GS FLX+

5. Ultra-Deep Amplicon Sequencing

New advances in amplicon sequencing using the MiSeq sequencer and chemistry has brought the cost of amplicon sequencing projects to a level that is more accessible than ever. Amplicon sequencing provides ultra-deep sequencing of PCR products, exons or multiplexed samples. Analysis of genetic variations, SNP or mutation detection, variance analysis of the data set, and identification and qualification methylation patterns are within the scope of an amplicon project.

- Illumina MiSeq sequencing of amplicons which include PCR incorporated specific adaptor
- PCR products can also be sequenced with GS FLX+ enabling modal read lengths up to 800 bp
- Clonal amplification by emulsion PCR (emPCR)
- Sequencing of samples and optional pooling with specific sequence tags
- Clustering of the sequence reads, sophisticated variance analysis

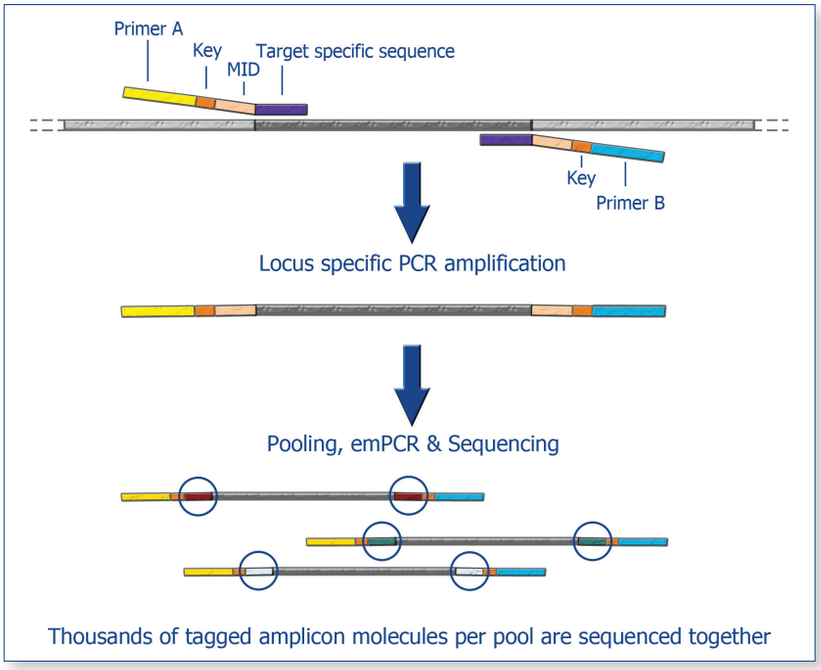


Fig. 5: Generalized scheme for the generation of ready-to-sequence bar coded amplicons

6. Re-Sequencing of Genomic Fragments Using Sanger Technology

Sequencing of specific genomic regions such as exons or genes of interest is often done to find a correlation between sequence deviations (e.g. SNPs or in/del mutations) and phenotypes. Using Sanger technology, this service is performed in three steps:

Phase I: PCR Optimization

- Primer design and synthesis for all exon regions
- Establishment of PCR amplification
- 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 each PCR product

Phase III: Bioinformatics Analyses

- Identification of differences between reference and samples
- Discovery of homozygous and heterozygous substitutions
- Detection of in/dels

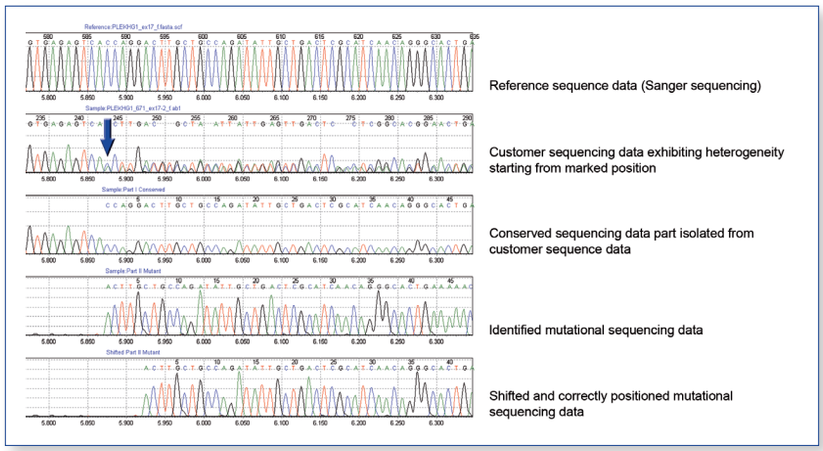


Fig. 6: Mutation Surveyor™ (screenshot from sequencing data analysis)

7. Customized Bioinformatics Services

Eurofins MWG Operon offers -

- Sequence clustering and assembly services for HiSeq 2500/MiSeq or GS FLX/FLX+ data
- De novo assembly of genome data. A wide variety of assembly programs and options are available
- Genome annotation services and protein function prediction
- BLAST analysis for singlet reads and assembled contigs, genomic and cDNA sequencing data
- Mapping and cross-mapping of sequencing data on reference sequences
- Expression profiling for transcriptome analysis data
- SNP and in/del analyses for re-sequencing projects

We offer clustering and assembly services for Illumina, Roche and Pacific Biosciences sequencing data, standard BLAST analysis, and annotation services. In addition, our team can support specific requests with customized bioinformatics solutions.

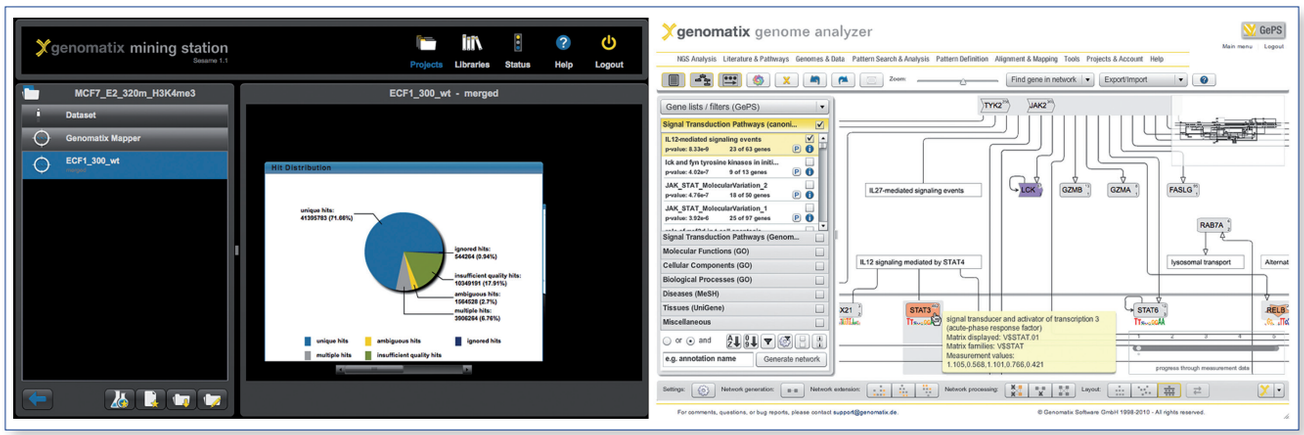


Fig. 7: Screenshot genome mining station and genome analyzer software

Eurofins offers bioinformatics services in an exclusive worldwide partnership with Genomatix Software GmbH.

Genomatix is a leading company in complex analysis and interpretation of NGS data and state-of-the-art solutions for your individual project. Genomatix 180 peer-reviewed publications, have >5,000 citations and >35,000 users.

Genomatix Mining Station (GMS)

GMS provides an integrated environment for all mapping related analyses of your NGS data.
Assembly, genome indexing, genomic positioning, SNP detection, CNV and structural variations, identification of alternative splicing and transcript variants (Fig. 7).

Genomatix Genome Analyzer (GGA)

GGA analyses address deeper biological data correlations and interpretations. More than 3 terabyte of structured, pre-analyzed background data shed light on biological context, intra- and cross species. ChIP-Seq, RNA-Seq or genotyping experiments, gene regulation analysis, epigenetics, comparative genomics, pathway and literature mining (Fig. 7).

Genomatix Software Suite (GSS)

Besides providing visualization of your comprehensive genome annotation, GSS integrates the scientific analysis of genomic data, gene regulation and expression. GSS can generate and evaluate networks and pathways of interest along with extended literature searches and sequence analyses and extraction.

8. NGS Library Generation Services

Specialized library construction techniques and the correct selection of those library types to address the experimental needs of each project are critical to Eurofins MWG Operon – and your – success. Whether your project involves sequencing on HiSeq 2500/MiSeq, GS FLX/FLX+, or the PACBIO RS system, Eurofins creates the highest quality genomic DNA, cDNA, or other ready-to-sequence libraries.

- Genomic shotgun libraries to be sequenced on GS FLX/FLX+, HiSeq 2500 or MiSeq
- Long jumping distance libraries (LJD) for sequencing on HiSeq 2500/MiSeq
- Long paired end (LPE) libraries adapted to be sequenced on GS FLX/FLX+
- Random primed, 3-fragment and 5-fragment cDNA libraries with or without normalization

Expertise and References

Eurofins MWG Operon has been providing sequencing services for NGS projects since 2005.

Brede DA, Snipen LG, Ussery DW, Nederbragt AJ, Nes IF. Complete genome sequence of the commensal *Enterococcus faecalis* 62, isolated from a healthy Norwegian infant. J Bacteriol. 2011 May;193(9):2377-8.

Buckwalter MR, Nga PT, Gouilh MA, Fiette L, Bureau JF, Laird ME, Buchrieser J, Ozden S, Cheval J, Eloit M, Manuguerra JC, Gessain A, Brey PT, Fontanet A, Albert ML. Identification of a novel neuropathogenic Theiler's murine encephalomyelitis virus. J Virol. 2011 Jul;85(14):6893-905.

Coucheron DH, Nymark M, Breines R, Karlsen BO, Andreassen M, Jørgensen TE, Moum T, Johansen SD. Characterization of mitochondrial mRNAs in codfish reveals unique features compared to mammals. Curr Genet. 2011 Jun;57(3):213-22.

De Schutter et al. Genome sequence of the recombinant protein production host *Pichia pastoris*. Nature Biotech, 2009 Jun; 27(6):561-6.

Gross JB, Furterer A, Carlson BM, Stahl BA. An integrated transcriptome-wide analysis of cave and surface dwelling *Astyanax mexicanus*. PLoS One. 2013;8(2):e55659. doi: 10.1371/journal.pone.0055659.

Hsu YH, Cook SR, Alexander TW, Klima CL, Niu YD, Brent Selinger L, McAllister TA. Investigation of *Mannheimia haemolytica* bacteriophages relative to host diversity. J Appl Microbiol. 2013 Mar 12. doi: 10.1111/jam.12185.

Johansen SD, Karlsen BO, Furmanek T, Andreassen M, Jørgensen TE, Bizuayehu TT, Breines R, Emblem A, Kettunen P, Luukko K, Edvardsen RB, Nordeide JT, Coucheron DH, Moum T. RNA deep sequencing of the Atlantic cod transcriptome. Comp Biochem Physiol Part D Genomics Proteomics. 2011 Mar;6(1):18-22.

Lynch T, Chong P, Zhang J, Hizon R, Du T, Graham MR, Beniac DR, Booth TF, Kibsey P, Miller M, Gravel D, Mulvey MR; Canadian Nosocomial Infection Surveillance Program (CNISP). Characterization of a stable, metronidazole-resistant *Clostridium difficile* clinical isolate. PLoS One. 2013;8(1):e53757. doi: 10.1371/journal.pone.0053757.

Mallona I, Egea-Cortines M, Weiss J. Conserved and divergent rhythms of crassulacean acid metabolism-related and core clock gene expression in the cactus *Opuntia ficus-indica*. Plant Physiol. 2011 Aug;156(4):1978-89.

Monstein HJ, Ryberg A, Karlsson A. Assessment of the mosaic structure in the *Helicobacter pylori* cagA gene 3'-region using an improved polymerase chain reaction-based assay. Diagn Microbiol Infect Dis. 2012 Jul;73(3):281-3.

Mraheil MA, Billion A, Mohamed W, Mukherjee K, Kuenne C, Pischimarov J, Krawitz C, Retey J, Hartsch T, Chakraborty T, Hain T. The intracellular sRNA transcriptome of *Listeria monocytogenes* during growth in macrophages. Nucleic Acids Res. 2011 May;39(10):4235-48.

Robinson CM, Zhou X, Rajaiya J, Yousuf MA, Singh G, Deserres JJ, Walsh MP, Wong S, Seto D, Dyer DW, Chodosh J, Jones MS. Predicting the next eye pathogen: analysis of a novel adenovirus. MBio. 2013 Apr 9;4(2). pii: e00595-12. doi: 10.1128/mBio.00595-12.

Sass P, Berscheid A, Jansen A, Oedenkoven M, Szekat C, Strittmatter A, Gottschalk G, Bierbaum G. Genome sequence of *Staphylococcus aureus* VC40, a vancomycin- and daptomycin-resistant strain, to study the genetics of development of resistance to currently applied last resort antibiotics. J Bacteriol. 2012 Apr;194(8):2107-8.

Singh G, Robinson CM, Dehghan S, Schmidt T, Seto D, Jones MS, Dyer DW, Chodosh J. Overreliance on the hexon gene, leading to misclassification of human adenoviruses. J Virol. 2012 Apr;86(8):4693-5. doi: 10.1128/JVI.06969-11.

Urbarova et al. Digital marine bioprospecting: mining new neurotoxin drug candidates from the transcriptomes of cold-water sea anemones. Marine Drugs 2012; 10(10).
Visi DK, D'Souza N, Ayre BG, Webber Iii CL, Allen MS. Investigation of the bacterial retting community of kenaf (*Hibiscus cannabinus*) under different conditions using next-generation semiconductor sequencing. J Ind Microbiol Biotechnol. 2013 May;40(5):465-75. doi: 10.1007/s10295-013-1242-1.

Yu L, Nicolaisen M, Larsen J, Ravnskov S. Succession of root-associated fungi in *Pisum sativum* during a plant growth cycle as examined by 454 pyro-sequencing. Plant and Soil 2012, DOI: 10.1007/s11104-012-1188-5.

For custom pricing for any of these products, please contact:

Our Next Generation Sequencing Specialists

1-800-688-2248

NGSSupport@eurofins.com

Feel Free to Contact Us

We invite you to send us your request using our online request form at operon.com, by email or contact us directly by phone.

Quality Management System ISO 9001 2008 CERTIFIED

Cert. # FM 572466

Quality Management System ISO 13485 2003 CERTIFIED

Cert. # FM 574234

operon.com

eurofins mwg|operon