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GATC Biotech AG - Jakob-Stadler-Platz 7 - D-78467 Konstanz

Czech University of Life Sciences Prague
Mr. Ales Bucek
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Your Customer No.
6063-75976

Illumina Sequencing

Offer No. V3_6535764 14.12.2016

This offer replaces offer no. V2_6535764

Dear Mr. Bucek and colleagues,

We thank you very much for your interest in GATC Biotech as exclusive commercial service provider for the IOCB for the herewith described services. GATC is pleased to offer this frame contract for a period of 6 month. You are interested in Illumina sequencing of different samples. As starting material you will provide already isolated genomic DNA (Part I), total RNA (Part II) or PCR products (Part III). The samples will be delivered in several batches.

Complying with your request we are happy to offer you the following services:

Art. no.	Service	Qty	Unit	Unit price Euro net	Total price Euro net
Library Preparation - Option I:					
10140200	Standard genomic library DNA fragmentation Adapter ligation Size selection Amplification Delivery time: Approx. 10 working days for up to 12 samples	1	library	110,00	110,00
Library Preparation - Option II:					
10140204	Random primed cDNA library Purification of poly-A containing mRNA molecules mRNA fragmentation Random primed cDNA synthesis Adapter ligation and adapter specific PCR amplification Delivery time: Approx. 12 working days for up to 12 samples	1	library	115,00	115,00

Now featuring an improved library preparation protocol for an even more accurate and balanced amplification across the wide range of transcriptomic GC content.

Library Preparation Option III:

10140222	Adapter ligation Adapter ligation Size selection Amplification	1	library	230,00	230,00

Delivery time: Approx. 10 working days for up to 12 samples

For samples with low diversity or unbalanced base composition (e.g., amplicons or bisulfite converted samples) a spike-in of 20% PhiX will be used to increase the diversity and to improve the sequence quality. Therefore 20% of the above mentioned reads will be represented by the PhiX control library (for further information please refer to "Requirements and Conditions").

Sequencing Option I:

10140525	Data package: 5 million reads (1 x 50 bp) Specifications per package: - Technology: Illumina - Run type: Single read - Read length: 1 x 50 bp - Guaranteed 5 million reads per package (+/- 3%) - Guaranteed 250 Mb raw data per package (+/- 3%) Deliverables: - FastQ Files (sequences and quality scores)'	1	Package	35,00	35,00

Delivery time: Approx. 10 working days for up to 384 packages

Sequencing Option II:

10140526	Data package 5 million read pairs (2 x 50 bp) Specifications per package: - Technology: Illumina - Run type: Paired end - Read length: 2 x 50 bp - Guaranteed 5 million read pairs (10 million reads) per package (+/- 3%) - Guaranteed 500 Mb raw data per package (+/- 3%) Deliverables - FastQ Files (sequences and quality scores)	1	Package	65,00	65,00

Delivery time: Approx. 12 working days for up to 384 packages

Sequencing Option III:

10140527	Data package: 5 million read pairs (2 x 125 bp) Specifications per package: - Technology: Illumina - Run type: Paired end - Read length: 2 x 125 bp - Guaranteed 5 million read pairs (10 million reads) per package (+/- 3%) - Guaranteed 1.25 Gb raw data per package (+/- 3%) Deliverables - FastQ Files (sequences and quality scores) Delivery time: Approx. 14 working days for up to 384 packages	1	Package	85,00	85,00

Bioinformatic Option I:

10140901	Detection and annotation of SNPs and InDels Semiautomatic mapping against one reference Detection of SNPs and InDels Annotation of detected SNPs and InDels (using dbSNP) Allocation of effects on protein level (using Ensembl) Deliverables: - Alignment file (bam) - SNP and InDel tables including annotated variants and effects (vcf, tsv, bed) - Comprehensive Data Analysis Report (pdf) incl. description of delivered data, overview of results, information on tools and references used, etc. Delivery time: Approx. 6 working days for up to 12 samples	1	sample	110,00	110,00

Bioinformatic Option II:

10140946	Transcriptome Analysis	1	sample	170,00	170,00

Mapping against one reference
Identification and quantification of transcripts
Pairwise comparison (samples or groups of samples) of expression levels and determination of significant fold differences
Alternative splicing analysis based on known/provided gene models for eukaryotes
Detection and annotation of SNPs and InDels
Deliverables:
- Alignment file (bam)
- Gene expression table including FPKM value (tsv)
- Table of pair-wise differential expression including fold change and p-value (tsv)
- Combined gene expression table of all samples (tsv)
- Table of top genes expressed (tsv)
- Tables of alternative splicing events (tsv)
- SNP and InDel tables including annotated variants and effects (vcf, tsv, bed)
- Comprehensive Data Analysis Report (pdf)

Delivery time: Approx. 6 working days for up to 12 samples

90910015	UPS-Label (EU) For sample shipment to GATC Biotech - not available for dry ice shipment (RNA samples) -	25	piece	25,00	625,00

Delivery time

Delivery time (mentioned above) only applies after receipt of written order confirmation and arrival of samples at the lab of GATC Biotech in Constance, Germany.

If samples do not pass the quality check the above mentioned delivery time will be delayed until new material is provided or until we receive customer's consent to continue the project with the existing material.

The sequencing service will be carried out under S1 safety conditions.

Requirements and Conditions

Starting material:

Library Option I: Standard genomic library

Type: high molecular and RNA-free DNA
Quantity: 1 µg per sample
Concentration: > 10 ng/µl
Purity: OD 260/280 ≥ 1.8; OD 260/230 ≥ 1.9
Dissolved in: RNase-, DNase- and protease-free Tris-HCl buffer (pH 8.0 - 8.5)

Library Option II: Random primed cDNA library

Type: total RNA
Quantity: 1 µg per sample
Concentration: > 20 ng/µl
Purity: OD 260/280: 1.8 - 2.2; RIN value ≥ 8
Dissolved in: RNase-, DNase- and protease-free molecular grade water or Tris buffer

RNA quality and degradation have influence on the analysis results of gene expression profiles. For degraded samples an overrepresentation of 3' ends has to be expected which increases proportional to any RNA degradation.

Library Option III: Adapter ligation

Type: purified PCR products / amplicons
Quantity: 500 ng per sample
Concentration: > 10 ng/µl
Dissolved in: RNase-, DNase- and protease-free Tris-HCl buffer (pH 8.0 - 8.5)

Information on PCR cycle conditions applied, sequence of your PCR product and primer sequences should be provided upfront. The size of the amplicons should ideally range from 150bp to 250bp (absolute minimum 100bp, absolute maximum 450bp). In samples containing a mixture of different amplicons, the amplicons should be designed to be close in length (within approximately 100bp of each other). Shorter fragments will be preferentially amplified over the longer ones. This potentially lowers the sequencing run yield and an over representation of the shorter fragments in the sequencing results has to be expected.

Illumina cluster detection algorithms are optimized around a balanced representation of A, T, G, and C nucleotides. Any divergence from equal base distribution will negatively influence the amount and quality of sequencing data produced. To increase the library nucleotide balance a spike-in of 20% PhiX will be used for samples with low diversity or unbalanced base composition (e.g., amplicons, bisulfite converted samples). This shall improve cross-talk and phasing and create a more diverse set of clusters. The extent to which the negative impact of the unbalanced base composition will be reduced by spiking the PhiX control depends on individual sample and sequence characteristics. For more information please refer to the Illumina website.

Any biological samples that are submitted to GATC Biotech must comply with biosafety S1/L1 classification according to the German "Technical Rules for Biological Agents" (TRBA). If biosafety S2 and/or L2 biomaterials shall be analysed, we would kindly ask our customers to contact GATC Biotech regarding our approval prior to sample submission.

Bioinformatic Analyses:

Bioinformatic Analyses Option I (valid for library preparation I or III):

Reference sequence has to be clearly defined and provided in Fasta (.fas, .fa, .fasta) including annotation (in .gff format) or GenBank (.gbk, .gb) format. Alternatively, provide the GenBank GI or accession number of the sequence(s).
The NCBI record should contain the sequence and annotation information.

SNP and InDel discovery is performed by mapping all sequencing reads against a reference sequence with subsequent variant calling using programs and parameters optimized for the sequencing technology, read length and type of raw data. Sequencing coverage and reference sequence similarity play an essential role in the accuracy, confidence and number of discovered variants. The percentage of mappable reads and average coverage achieved is directly correlated with the similarity between sequenced organism and reference sequence used. To facilitate high quality analyses please take care that the reference sequence to be used for the analyses is closely related to your organism. The discovered variants are not further validated or verified.

Extent and outcome of the performed analyses depend on the quality and completeness of the provided reference. Annotation of SNPs and InDels can only be performed if the selected reference includes gene annotations.

Bioinformatic Analyses Option II (valid for library preparation II):

Clearly defined Ensembl genome assembly name (e.g. GRCh37 or Rnor_5.0) for the annotated genomic reference sequence has to be provided prior to project start. Alternatively the genome sequence can be provided in Fasta (along with the respective annotation in Gene transfer format (gtf)) or in GenBank format (including annotation).

If no genomic reference sequence is available transcriptomic reference sequences can be used for the analyses. Clearly defined reference sequences of all genes that shall be used for the analyses have to be provided as multiple sequences in one FASTA file (multiFASTA format). If available please also provide the corresponding annotation in gene transfer format (gtf).
Gene expression analyses is performed by mapping all sequencing reads against a reference sequence with subsequent analyses using programs and parameters optimized for the sequencing technology, read length and type of raw data.
Sequencing coverage and reference sequence similarity play an essential role in the accuracy, confidence and number of discovered variants. Extent and outcome of the performed analyses depend on the quality and completeness of the provided reference. Identification and annotation of expressed genes can only be performed if the selected reference includes gene annotations.
To facilitate high quality analyses please take care that the reference sequence to be used for the analyses is closely related to your organism. The results are not further validated or interpretated.
For splice variant analysis we highly recommend paired-end sequencing and long read lengths.

Disclaimer

Specific costs might occur for additional pre-processing steps and expenses due to insufficient quality of the provided starting material. Please refer to section "Technical Appendix" below for further information about technical requirements / specifications and potential costs for additional services.

Prices only valid if all samples are provided in sufficient quality and quantity within 12 months after GATC Biotech's receipt of the purchase order. If total number of samples is not delivered within that timeframe we reserve the right to withdraw from the contract and will additionally charge 10% of the outstanding purchase order amount.

If there are more than 12 weeks between offer acceptance and project start (delivery of suitable starting material) it might no longer be possible to fulfill the stated technical specifications. If changes in services occur due to technological product and process innovation, we will reserve the right to present a proposal for substantially similar services with comparable outcomes.

Liability
As far as it does not concern direct damages to property and person, the Seller can only be made liable up to the order sum or in accordance with the Seller´s liability insurance. Seller is not responsible for loss of profit, missed savings or indirect and/or consequential damages.

All analyses results are for research use only.

Terms of payment and delivery

Offer validity:	14.06.17
Delivery:	Delivery of all raw data as well as the analysed data online via your secured myGATC-account (free of charge). On request data delivery on hard drive can be offered at an extra charge.
Delivery time:	Delivery time does not include eventually necessary consultations with the customer e.g., for approval in case of insufficient starting material, additionally required sample pre-processing steps or outstanding prepayment. No reimbursement can be made for delays due to any cause beyond GATC Biotech's control, including e.g., supply shortage of third-party reagents or technical failures of the machines. Bank holidays in Konstanz/Germany are no working days.
Terms of payment:	Payment 14 days net We reserve the right to invoice for individual sample processing steps and partial data delivery.

For our customers who are members of the European Union applies the following:
According to the European Union VAT Directive, the beneficiary is engaged to send us - when placing an order at the latest - a document which is established by the beneficiary's local tax and revenue authority and which is showing the customer's tax ID number. If we don't receive such a document, we are obliged to invoice the german VAT which is currently 19 %.

Our General Business Conditions apply.

Material and data storage

We will keep your starting material as well as all materials and sequence data pertaining to the project for a maximum time period of 3 months after sequence data have been uploaded. You can place a re-order within this time frame if needed. After 3 months, all project materials will be disposed of and data will be deleted. If required, GATC offers prolonged data and sample storage at an extra charge.

Confidentiality and property rights

GATC guarantees the strict confidentiality of all data and information resulting from this project. All data, results and materials remain the sole property of the customer and GATC will not acquire any intellectual rights resulting from information or materials generated as a result of this project.

Sincerely yours

GATC Biotech AG

Your Customer Service Team

Acceptance	offer No. V3_6535764

I herewith confirm that I read and understood above described services and conditions and order the described services.

Signature Ales Bucek, Ing. Jana Vohralikova or a other *signature authorized purchaser:
Name in block letters
Date:
Your purchase order number:
Estimated arrival date of starting material*:

* mandatory information, without this order cannot be handled

Additional contact

Additional contact (if any) that shall be actively informed about project details:

Name of additional contact:
E-mail address of additional contact:

As project owner you may at any time invite additional project users (must be registered at myGATC) to track the current project status in real time over your secure myGATC account.

Invoicing details

Please fill in the following details if above receiver's address is not the same as the invoice address:

Name of invoice receiver:
Institute / Department:
Street:
Postal Code, City, Country:
Signature invoice receiver:

Please complete if debiting from existing "Customer Benefit Account" is required:

Account number:
Account owner:
Signature account owner:

Differing invoice recipients or account owners require the recipient/owner to affix his signature.
Change requests in absence of the signature cannot be edited.

Shipping address

GATC Biotech
European Genome and Diagnostics Centre
Jakob-Stadler-Platz 7
78467 Konstanz
Germany

Please note, that the GATC Collection Points cannot be used for the shipment of NextGen samples.

Sample Submission Checklist

☐ Concentration has been determined for each sample

☐ Samples are labelled and prepared for shipment

☐ Samples are assigned to the corresponding barcodes in your myGATC account

☐ All information and documents (e.g., offer acceptance in written form) are available for GATC Biotech

Contact

Any queries? Please don't hesitate to contact us:

Germany: +49 - 7531 81 60 68
Great Britain: +44 - 207 691 4921
France: +33 - 491 82 84 88
Sweden: +46 - 8 655 36 09

customerservice@gatc-biotech.com

Technical Appendix

General

For each library that shall be prepared and analysed the integrity and quantity of the provided starting material (one sample per library) will be determined by appropriate methods. If the amount, concentration and / or quality of the starting material do not meet the requirements for further processing, we will contact you to discuss how to proceed. We reserve the right to invoice for any additionally services performed as e.g., further quality checks or other pre-processing steps for optimizing the sample quality.

The following prices per sample apply for additional services performed in consultation with customer:

1. RNA / DNA concentrating: € 60
2. RNA / DNA purification: € 60
3. RNase treatment: € 60
4. Sample pooling after quantification by agarose gel: € 25
5. Sample pooling after quantification by capillary electrophoresis or other method: € 40
6. Additional quality check: € 10

To help assure a fast and optimized processing please send an electronic copy of all available quality and quantity measurement results to nextgen@gatc-biotech.com prior to sending your samples or contact our experienced Project Management Team by telephone under +49 (0)7531 8160-5060 if you have any further questions regarding sample requirements.
Customer will choose the appropriate shipment method and scheduling to ensure arrival of intact samples during working hours at GATC Biotech. Please refer to above mentioned requirements for detailed information on required sample quantity, quality and concentration.

For libraries prepared from starting material which has not successfully passed our initial QC all output specifications and guarantees are invalid. If you decide to proceed with the project besides failed QC this will be done at your own risk and all services performed will be invoiced as specified in the corresponding offer.

Due to technical limitations of assessment methods (e.g., detection of single-strand breaks, presence of inhibitors) a residual risk exists that besides successful QC the average read length; read quality and number of reads achieved may vary from the specifications given in your offer. This is especially true for samples with altered DNA/RNA composition due to specific preparation protocols (like e.g. any sort of enrichment, depletion, amplification or chemical treatment) or inadequate sample handling. The performed QC conduces the assessment of sample quantity, purity and integrity and does not indicate the composition of the material (e.g. contamination through other organisms, RNA content).

Starting material that has been subjected to any specific upfront treatment or does not match the specifications mentioned in your offer can only be accepted upon consultation and will be considered as non-standard customized starting material.

Customization

Non-standard starting material or customized library preparation protocols that are experimentally not validated by GATC Biotech are considered as "proof of principle" and will be performed solely on customer risk. Final yield, characteristics and content of the resulting library depend on the nature of the starting material like e.g., length of dsDNA fragments and presence of secondary structures and may not be predictable upfront. The actual achieved sequencing output (average read length; read quality and number of reads) may therefore vary from the specifications given in your offer.

Necessary adaptions will be performed with extreme care to meet the customer requirements and expectations. Despite this care we cannot completely rule out that due to the given nature of the starting material or unforeseen difficulties the library preparation and/or sequencing fails. We therefore reserve the right to abort the further processing at any timepoint if the outcome of individual steps does not meet our requirements in terms of quality and performance or if the sample preparation fails repeatedly. All services and intermediate steps performed until then will be invoiced even if we no results have been achieved.

Pooling

Sample quantitation and pooling will be performed with extreme care to achieve uniform read distribution across all samples. Despite this care, the number of reads per library may significantly vary between samples / libraries due to technical limitations in equimolar pooling and individual sample characteristics (genome size, fragment length, GC content, quality of isolated DNA). By default samples are pooled equimolar. Pooling in different ratios can be performed upon consultation.

The pooling of different library types is generally not recommended. The insert size and insert size distribution of the sequencing library directly influences the number of clusters generated and as a result the number of reads achieved per library. Different medium insert sizes sequencing libraries impair the quantification and equimolar pooling. Therefore the number of reads per library might exceed resp. fall below the above mentioned deviation.

Ready-to-load libraries

Technical specifications are according to the manufacturer's specifications. The actual achieved sequencing output (read length, read quality and number of reads) is directly correlated with the quality of the sequencing library used. GATC Biotech can therefore under no circumstances assume any responsibility for sequencing data resulting from "ready-to-load" (platform specific adapters already attached) libraries prepared by the customer. Thus the entire amount for each performed service is due for payment even if no results are achieved.

To ensure optimal sample preparation, please refer to the corresponding guidelines provided by the manufacturer. The final responsibility for selection, usage and compatibility assurance of the used adapter sequences and other library characteristics remains with the customer. If unsatisfactory sequencing results are due to technical problems with the sequencing kits or machines, the sequencing will be repeated at no additional cost to the customer.

Glossary and terms

Coverage
Average coverage is calculated as the sum of the mapped bases at each reference position, divided by the total number of bases of the reference (bases on reference / total length of reference sequence). The range and uniformity of coverage varies over reference sequence.

Library insert size
Library insert size is defined as stretch of sequence between the sequencing adapters. The medium insert size of produced sequencing libraries depends on sequencing technology used and library preparation protocol. Each sequencing library will show a Gaussian distribution of DNA fragments with different length.

Paired end sequencing
During paired end sequencing the same library molecule is sequenced from both ends. The distance between the resulting read pair corresponds to the library insert size and is optimized in such a way to avoid an overlap of reads from both ends. Nevertheless a certain percentage of reads might partially overlap and cover the same bases. This artificial doubling of coverage at certain positions should be excluded further downstream analyses to assure accurate analyses.

Contractual Appendix

Between

GATC Biotech AG, Jakob-Stadler-Platz 7, 78467 Konstanz, Germany
represented by Dr. Marcus Benz, COO

and

Czech University of Life Sciences Prague, Kamycka 129 16500 Prague 6-Suchdol, Czech Republic
represented by Ing. Janou Vohralíkovou, Quaestor

The Agreement is drawn up in 4 (four) identical copies, each copy having the value of the original. Each party will receive 2 (two) identical copies of the Contract.

Any changes or amendments to this Agreement are possible only on the basis of a written agreement of the Parties. Addendums to the Agreement must be dated, numbered and signed by both Parties.

If the reason for invalidating the Agreement is based solely on specific provisions of this Agreement, only the specific provisions may be invalidated, provided that from their character, contents or circumstances, in which they were agreed upon, cannot be separated from the remaining contents of the Agreement.

The GATC Biotech AG agrees with the publication of the full text of this Contract so that this Contract can be deemed information provided pursuant to Act No. 106/1999 Coll., on Freedom of Access to Information, as amended and Act No. 340/2015 Coll., on register of contracts.

The GATC Biotech AG is aware and agrees with the fact that he is a person bound by the duty of financial control, in accordance with Art.2, Letter e) of the Act No. 320/2001 Coll., as amended. The GATC Biotech AG is obliged to fulfill all conditions which are related to his person as stipulated in the above mentioned Act.

The Parties declare that before signing hereunder they have read the Agreement and unreservedly agree to its contents. The Agreement is an expression of their true, genuine, free and serious will. The proof of the authenticity and veracity of these statements has to be provided by the authorized representatives of the Parties in the form of their signatures.

For GATC Biotech AG:

By: _____

Name: Dr. Marcus Benz

Title: COO GATC Biotech AG

Date: 14.12.2016

For Czech University of Life Sciences Prague:

By: _____

Name: Ing. Janou Vohralíkovou

Title: Quaestor Czech University of Life Sciences

Date: 15 -12- 2016

ČZU ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE (2)