



100 Enterprise Way, Suite A101 Scotts Valley, CA 95066 * orders@dovetail-genomics.com * (831) 713-4465

Statement of Work

Quote # Q-03970

Czechoslovak Academy of Sciences

Karel Janko

janko@iapg.cas.cz

Spined Loach

PacBio de novo Assembly + Proximity Ligation + RNA
Seq Library + HiRise Scaffolding + Basic Annotation
Project

Scope of Work

DNA

If a tissue, blood, or cell culture Sample is provided, Dovetail will extract HMW DNA (average fragment size ~50-100kb) from a portion of the Sample, as appropriate for the Sample type. The DNA will then be checked for quality (QC'd) as described below.

If a DNA Sample is provided, Dovetail will QC the Sample by measuring the concentration, 260/280 & 260/230 ratios, and average fragment size using pulsed-field gel electrophoresis (PFGE). If the DNA passes QC, Dovetail will proceed with the library prep.

Proximity Ligation Library Preparation

Dovetail will construct one Omni-C library per 3 gigabases of the organism's genome, using the provided tissue, blood, or cell culture sample.

Dovetail will sequence ~2M PE75bp reads and map the data back to the draft assembly to assess library quality.

RNA Sequencing Library Preparation

RNA will be extracted and a standard RNA library will be prepared with rRNA-depletion. 2x150bp reads will be sequenced on an Illumina platform, and raw data from ~20M read pairs per sample will be delivered.

PacBio Library Preparation

Based on the organism's genome size and the level of PacBio sequence coverage being collected, the HMW DNA will be used to construct CLR libraries, using Blue Pippin Size selection with up to a 20Kb cutoff.

PacBio Sequencing

Sequencing of the PacBio CLR libraries will be performed on the PacBio Sequel II in an effort to collect >50Gb of raw data per 8M SMRT cell. We recommend collecting a minimum of 50X coverage for de novo assembly.

PacBio de novo Assembly

Dovetail will aim to generate 50-100X raw data coverage and assemble to generate a *de novo* assembly.

Assembly Informatics Package

Dovetail will scaffold the draft assembly (minimum N50 of 100kb is required) through our HiRise software pipeline using the proximity ligation data.

If applicable, and depending upon the metrics and complexity of the genome and HiRise output, Dovetail will perform a TAD analysis on the genome, generated from Dovetail proximity ligation libraries. The following are the input constraints for TAD analysis:

- 40% of read pairs should map at a distance > 1Kbp
- Genome size < 10Gb
- Assembly scaffold size < 400Mbp
- >40% of genome uniquely mappable
- Coverage: 100 M read pairs per 1Gb

Gene Annotation

Whole genome annotation of a genome assembly using RNA-seq data. Assembly must have >70% BUSCO single copy or duplicated score. The Basic service includes the following:

- Repeat masking
- *ab initio* gene prediction using related species
- RNAseq mapping to enhance annotation
- Manual curation of 5 genes
- Assignment of functional tags to genes

Deliverables

Dovetail will deliver the results files to the Customer electronically via secure FTP. The Results shall consist of:

- HiRise Assembly deliverables include:
 - The HiRise assembly in FASTA format
 - A report summarizing key assembly statistics, features of the proximity ligation library, and a linkage density plot of the proximity ligation library data
 - A table detailing the breaks made to the input scaffolds
 - A table describing the position of the input assembly scaffolds within the final HiRise scaffolds
 - BAM file(s) containing alignments of the proximity ligation library read pairs mapped to the draft assembly
- TAD Analysis deliverables, if applicable, include:
 - TAD calls at 10kbp, 25kbp, and 50kbp
 - CTCF binding sites
 - A/B compartmental organization
 - Isochore locations
 - Guide to loading and viewing data in HiGlass
 - Summary report
- RNA Sequencing Library deliverables includes six (6) FASTQ files, each containing ~20M read pairs.
- Genome Annotation deliverables consists of a comprehensive report including:
 - Genome annotation file in GFF3 format
 - Predicted gene CDS sequences in FASTA format
 - Predicted gene peptide sequences in FASTA format
 - Alignment files from RNA-seq and Iso-Seq data in BAM format (if evidence data is provided)
 - Repeat annotation file in GFF3 format
 - Visualization of structures of manually curated genes

The PacBio assembly outputs in FASTA format, including:

- A report summarizing key data and assembly statistics
- PacBio reads in fastq format

Target Timeline/Milestones

At typical project loads, the total run time for this project will be approximately 28 weeks from the receipt of draft assembly, sample, and raw read data when required. This assumes sequencing (when required) will be facilitated by Dovetail.

Dovetail Genomics

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Scotts Valley, CA 95066

USA

Phone: (831) 713-4465

Email: orders@dovetail-genomics.com



*Quote #:	Q-03970
Date Issued:	December 2, 2020
Expiration Date:	December 21, 2020
Payment Terms:	Net 30

To:
Czechoslovak Academy of Sciences
Karel Janko

*The above Quote # MUST appear on all purchase orders.

Invoicing Schedule

A 50% non-refundable deposit will be invoiced after receipt of Purchase Order and is due within 30 days of the invoice date (Net 30).

A 50% final payment will be invoiced upon delivery of the Results and is due within 30 days of the invoice date (Net 30).

ACTIVITY	QTY	DISC (%)	UNIT PRICE	EXTENDED
PacBio de novo assembly	1.00		\$4,800.00	\$4,800.00
Sequel II SMRT Cell	2.00		\$3,000.00	\$6,000.00
Proximity Ligation Library Prep and Sequencing for 1-3Gb	1.00		\$5,959.86	\$5,959.86
RNA Library Prep and Sequencing	1.00		\$2,883.80	\$2,883.80
Assembly Informatics Package	1.00		\$3,364.44	\$3,364.44
Genome Annotation, Basic	1.00		\$1,441.90	\$1,441.90

***Quote Total:**
\$24,450.00

*Payable in US Dollars, inclusive of wire and bank fees. Price does not include any applicable international duties or taxes, or applicable US sales tax; any such taxes or fees will be added to the invoice.

Purchase Order Requirements: All orders must include: Quotation Number, and **Bill to** contact details: name, phone, **email address**. **The above Quotation number must appear on all purchase orders**, which signifies the parties agree to the Project details described in this Statement of Work (SOW). Acceptance of this SOW/Quote is also acknowledgment and acceptance of our Standard Terms and Conditions. All deposits are non-refundable. Work will only commence upon receipt of initial deposit.

Dovetail Genomics, LLC Service Terms and Conditions

Thank you for your interest in purchasing the Dovetail Genomics genome assembly service. Our goal is to make your experience a smooth one. Below are our Service Terms and Conditions. **We understand that certain state and federal institutions are subject to specific jurisdictional laws, which may be in conflict with these terms and conditions. In the event of such a conflict these terms and conditions are hereby modified to the extent necessary to conform to those laws.** We may change our terms and conditions or the pricing of our products from time to time so the below terms and attached quotation are intended for a single order.

1. **General.** These Dovetail Genomics, LLC ("Dovetail") Service Terms and Conditions, any attached Addenda, and the accompanying Genome Assembly Statement of Work ("SOW") and formal price quotation ("Quote"), (collectively, the "Agreement") shall exclusively govern Dovetail's provision of services ("Services"), to the purchaser ("Customer"). If a purchase order referencing the applicable Quote by quotation number is provided to Dovetail by Customer, then the customer's purchase order shall become an additional attached Addenda to the "Agreement".
2. **Price.** The price(s) for the Services will be those listed on the accompanying Quote and expire on the date specified on the Quote. In the event the Customer terminates the Agreement prior to the delivery of all the Results, the Customer may be charged a cancellation fee equal to the costs incurred by Dovetail up to that point and for which payment has not been received.
3. **Acceptance.** Dovetail will use commercially reasonable efforts to deliver the results as specified in the SOW ("Results"). All Services shall be deemed accepted by Customer upon delivery. Customer will notify Dovetail in writing of any nonconformity promptly after delivery, describing the nonconformity in detail.
4. **Payment.** Customer will be invoiced as set forth in the Quote. Payments for Results will be due as described therein. Payments are not subject to Customer's inspection or acceptance of the Services. Late payments shall incur a charge at the rate of one and one-half percent (1.5%) per month, or the maximum allowed by law, whichever is less.
5. **Unforeseen Events.** Dovetail shall not be liable for delay or failure in performance of any obligations if performance is rendered impracticable by any condition beyond Dovetail's reasonable control, including global health emergencies and pandemics. In such a case, Dovetail shall have additional necessary time to perform its obligations and shall have the right to apportion the Services then available for delivery among its various customers in such manner as Dovetail considers appropriate.
6. **Customer Information and Samples.** Customer shall provide all relevant information as described in the SOW (e.g. sequencing data, draft assemblies, etc.) and biological sample(s) (e.g., cell, tissue or DNA samples) ("Sample") in a timely manner to Dovetail. Customer represents and warrants that it owns or otherwise controls the Samples and that it has the right to provide the Samples to Dovetail for the purpose described herein. Customer acknowledges that any failure to provide the Samples, information, and any other materials in accordance with Dovetail requirements may result in delay or cancellation of the Services. Dovetail shall use the Samples only for the purpose of providing the Services. Upon completion or termination of Services, Dovetail shall destroy any remaining Samples according to its internal procedures. Through the time of destruction, Customer shall at all times retain all right, title, and interest in the Samples provided hereunder. Unless explicitly set forth in a written Addendum, Samples and Results are not considered to be confidential information.
7. **Compliance with Law.** Customer agrees that it will comply with all applicable United States laws and the laws of any other jurisdiction, including all laws related to the export or re-export of any Samples or the Results or accompanying documentation. Customer agrees that a false representation and warranty regarding Customer's ownership or control of Samples is willful misconduct and a material breach of the Agreement.
8. **Disclaimer.** The Services are for RESEARCH USE ONLY. The Services do not have FDA or other regulatory approval. Customer agrees not to use the Services, Results or any accompanying documentation in any setting requiring FDA or similar regulatory approval or exploit the Services in a clinical or veterinary diagnostic or therapeutic setting.
9. **No Warranty. THE SERVICES, RESULTS AND/OR RAW DATA AND ANY ACCOMPANYING DOCUMENTATION ARE PROVIDED "AS IS." PROVIDER MAKES NO WARRANTIES, EXPRESS, IMPLIED, STATUTORY OR OTHERWISE WITH RESPECT TO THE SERVICES AND EACH PARTY EXPRESSLY DISCLAIMS ALL REPRESENTATIONS AND WARRANTIES THAT ARE NOT SET FORTH IN THIS AGREEMENT INCLUDING IMPLIED WARRANTIES, THE WARRANTIES OF MERCHANTABILITY, NONINFRINGEMENT AND FITNESS FOR A PARTICULAR PURPOSE.**
10. **Liability Limitation.** EXCEPT TO THE EXTENT (i) CAUSED BY DOVETAIL'S GROSS NEGLIGENCE OR WILLFUL MISCONDUCT, OR (ii) REQUIRED BY APPLICABLE LAW, DOVETAIL AND ITS REPRESENTATIVES SHALL HAVE NO LIABILITY FOR (A) ANY LOSS OF USE, PROFITS, REVENUE, GOODWILL, BUSINESS, OR OTHER FINANCIAL LOSS, (B) COSTS OF SUBSTITUTE GOODS OR SERVICES, OR (C) ANY LOST PROFITS, INDIRECT, CONSEQUENTIAL, INCIDENTAL, OR SPECIAL DAMAGES OF ANY KIND, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF DOVETAIL OR ITS REPRESENTATIVE HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. IN ADDITION, DOVETAIL'S LIABILITY SHALL NOT EXCEED THE AMOUNT PAID BY BUYER TO DOVETAIL FOR THE SERVICES. BUYER UNDERSTANDS THAT THE RISKS OF LOSS HEREUNDER ARE REFLECTED IN THE PRICE OF THE SERVICES AND THAT THESE TERMS WOULD HAVE BEEN DIFFERENT IF THERE HAD BEEN A DIFFERENT ALLOCATION OF RISK.
11. **Ownership of Results.** Customer shall own the exclusive rights to all Results and raw data. After Delivery of Results and raw data to Customer, Dovetail is not responsible for maintaining backup or archive copies of Results or raw data Backup or archive copies of Results or raw data (if any) are subject to destruction or deletion after six (6) months following delivery to Customer.
12. **Indemnification.** Customer shall indemnify, defend, and hold Dovetail harmless from and against any and all losses, damages and expenses (including reasonable attorneys' fees and other costs of defending any action) that Dovetail may incur as a result of Customer's use or resale or other transfer (authorized or unauthorized) of Services or Results or by reason of Customer's breach of or failure to perform any of its obligations hereunder. Customer shall fully cooperate with Dovetail in any investigation relating to any such claims and, at no charge to Dovetail, make available to Dovetail all related statements, reports and tests available to Customer.
13. **Arbitration.** Dovetail and Customer agree that any dispute or controversy arising out of or in connection with this Agreement shall be finally settled by binding arbitration under the extant rules of the American Arbitration Association. The parties agree that, any provision of applicable law notwithstanding, they will not request, and the arbitrator shall have no authority to award, punitive or exemplary damages against any party.
14. **Miscellaneous.** This Agreement constitutes the entire agreement between Customer and Dovetail for the Services and is the final, complete, and exclusive statement of the terms of the Agreement, superseding all prior written and oral agreements, understandings and undertakings. This Agreement shall exclusively govern the ordering, purchase, and supply of the Services, and shall override any conflicting, amending, and/or additional terms contained in any purchase orders, invoices, or similar documents, which are hereby rejected and shall be null and void. Dovetail's failure to object to any such terms shall not constitute a waiver by Dovetail, nor constitute acceptance by Dovetail of such terms and conditions. Modifications may be made only in writing and signed by an authorized corporate officer of Dovetail. The waiver of any term or condition or any breach thereof shall not affect any other term or condition of this Agreement. This Agreement shall be governed by and construed according to the laws of California. Customer may not assign this Agreement, and any change of control of Customer shall be deemed to be an assignment. In any legal action commenced to enforce or interpret this Agreement, the prevailing party shall be entitled to reasonable attorneys' fees and expenses. Subject to filling any orders that have been accepted by Dovetail, Dovetail may terminate this Agreement without cause upon thirty (30) days written notice. Dovetail may terminate this Agreement prior to Customer providing the Samples and/or Customer information or if these do not conform to Dovetail requirements or quality control standards. Sections 7 through 14 and all attached Addenda shall survive termination. Time is not of the essence for Dovetail's obligations herein. In the event that any provision of this Agreement or portion thereof is found to be illegal or unenforceable, the Agreement shall be construed without the unenforceable provision or portion thereof. Services may be covered by and/or sold under one or more U.S. or other patents licensed from third parties. "DOVETAIL GENOMICS," "HIRISE," "OMNI-C," and "CHICAGO" and related logos are trademarks or registered trademarks of Dovetail in the U.S. and/or other countries.

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