TECHNICAL DOSSIER OF THE SERVICE

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1. INTRODUCTION TO THE PROJECT

The requested services are integral to the research project titled ***"Comprehensive Analysis of Clinical and Transcriptomic Data (Liquid Biopsy) to Identify Biomarkers in Patients with GNAO1-Related Disorders."*** This project, funded by the Famiglie GNAO1 Research Grant 2024 and the Torrons Vicens-Rac1 2024, aims to advance the understanding of GNAO1-related disorders (GNAO1-RD) by identifying potential biomarkers in cell-free RNA (cfRNA) obtained through liquid biopsies. GNAO1-RD is a rare neurogenetic disorder characterized by movement abnormalities, epilepsy, developmental delay, and significant morbidity.

The project’s overarching objective is to discover and validate a cfRNA-based biomarker signature specific to GNAO1-RD. This will be achieved by analyzing cfRNA from plasma samples of 20 patients with GNAO1-RD and 20 age- and sex-matched healthy controls, followed by validation in an additional 20 GNAO1-RD patients from international cohorts and 20 age- and sex-matched healthy controls. The analysis will include differential gene expression, gene ontology analysis, and machine learning techniques to correlate identified biomarkers with clinical features. The results are anticipated to contribute to both diagnostic and prognostic advancements in GNAO1-RD, as well as to facilitate more personalized therapeutic strategies.

The study will be conducted at Hospital Sant Joan de Déu, Barcelona, in collaboration with the GNAO1 Spain patient association and other international partners. The findings will be disseminated across the scientific, clinical, and patient communities, with the potential to inform future clinical trials and therapeutic options, thereby improving quality of life for affected patients and families.

2. CONTRACTING SERVICES

**2.1. REQUIRED SERVICES**

The proposed project necessitates the engagement of an external organization to carry out high-quality cfRNA sequencing and analysis services for both the discovery and validation phases of the study. The primary tasks to be outsourced include RNA extraction, quality control, sequencing, and bioinformatics analysis. Specifically, the contractor must:

1. **RNA Extraction and Quality Control**:
   * Extract cfRNA from plasma samples provided by Hospital Sant Joan de Déu.
   * Conduct rigorous quality control to ensure RNA purity and integrity, employing standard protocols and methods suitable for liquid biopsy-derived RNA.
2. **Sequencing**:
   * Perform high-throughput RNA sequencing, ensuring comprehensive transcriptomic coverage and appropriate depth to detect differentially expressed genes.
   * Use advanced RNA-sequencing platforms (such as Illumina or equivalent) with a minimum of 30 million reads per sample to ensure robust data for subsequent analysis.
3. **Bioinformatics and Data Analysis**:
   * Provide pre-processing, alignment, and quantification of RNA-seq data, using tools such as FastQC, STAR, and Salmon.
   * Perform differential gene expression analysis using EdgeR or similar packages, ensuring correction for batch effects and applying a significance threshold of |log2 fold-change (FC)| ≥ 1 and false discovery rate (FDR) ≤ 0.1.
   * Conduct gene ontology analysis and machine learning-based classification, employing methods such as LASSO, Random Forest, or SVM, as required to develop cfRNA signatures for distinguishing GNAO1-RD patients and assessing disease severity.
4. **Tissue-Specific Analysis and Pathway Enrichment**:
   * Determine the tissue specificity of identified biomarkers using databases like the Human Tissue Atlas of miRNAs.
   * Conduct pathway enrichment analysis of miRNA targets with software such as miRPath v4.0, using TarBase v8.0 as a reference, and identify pathways enriched in miRNA target genes with an FDR <0.05.

3. REQUIREMENTS

The selected contractor must fulfill the following requirements:

* **Technical Expertise in RNA Analysis**: Demonstrated experience in cfRNA extraction, RNA sequencing, and bioinformatics analysis, particularly in projects related to neurogenetic disorders or rare diseases.
* **Laboratory Accreditation**: The laboratory must be certified (e.g., ISO 17025 or equivalent) to ensure high standards in cfRNA processing and sequencing.
* **Data Security and Confidentiality**: The contractor must implement strict data security protocols to protect the confidentiality of patient samples and related data, adhering to relevant data protection regulations (e.g., GDPR).
* **Scalability**: Ability to process additional samples in case the study expands in scope or includes further validation cohorts.
* **Timeliness**: Commitment to the project timeline and flexibility to adjust as per project needs while ensuring high-quality data and results.
* **Ethical Standards**: Compliance with ethical standards in biomedical research, including the management of human-derived materials and adherence to the highest standards of scientific integrity.

4. WORKING METHODOLOGY

The contractor is expected to adhere to the following working methodology:

1. **Sample Reception and Processing**:
   * Receive and log plasma samples from Hospital Sant Joan de Déu, ensuring secure storage and handling protocols.
   * Extract cfRNA from plasma samples within two weeks of receipt to minimize degradation risks.
2. **Quality Control and Sequencing**:
   * Conduct initial RNA quality assessment and send a preliminary report to the research team.
   * Sequence cfRNA samples using high-throughput next-generation sequencing (NGS) with a minimum read depth of 30 million reads per sample, ensuring comprehensive transcriptome coverage.
3. **Data Analysis and Reporting**:
   * Carry out data preprocessing, alignment, and quantification using industry-standard bioinformatics tools.
   * Perform differential gene expression analysis, pathway enrichment analysis, and machine learning to identify disease-specific cfRNA signatures.
   * Conduct regular progress meetings with the research team to discuss interim results and any methodological adjustments.
4. **Model Development and Validation**:
   * Develop a machine learning model to classify patients and healthy controls based on cfRNA expression patterns.
   * Test the predictive model's performance using cross-validation and provide an evaluative report on its robustness and accuracy.
5. **Documentation and Communication**:
   * Maintain clear and detailed documentation of procedures, methodologies, and results.
   * Communicate any delays, issues, or changes in methodology to the research team promptly.
   * Submit comprehensive final reports covering the analysis workflow, findings, and any recommended future directions.

5. BUDGET

The proposed budget should cover the following components:

1. **RNA Extraction and Quality Control**: Include costs for cfRNA extraction from 40 discovery samples (20 GNAO1-RD patients and 20 age-matched controls) and 40 validation samples (20 GNAO1-RD patients and 20 age-matched controls), along with quality assessment using industry-standard methods.
2. **Sequencing Costs**: Provide itemized costs for RNA sequencing of the discovery and validation cohorts, specifying costs per sample and any discounts for larger sample batches if applicable.
3. **Bioinformatics Analysis**: Outline costs associated with data preprocessing, differential expression analysis, gene ontology and pathway enrichment, and machine learning for biomarker discovery.
4. **Model Development and Validation**: Specify costs related to the development, testing, and validation of machine learning models for cfRNA signature identification.
5. **Reporting and Documentation**: Include any additional costs for generating comprehensive reports, data visualization, and documentation as specified in the deliverables.
6. **Contingency Funds**: Allocate contingency funds for unforeseen expenses, such as additional samples or repeated analyses due to quality control issues.

The total budget should provide a breakdown of each cost category, along with a detailed justification. Flexibility in the budget to accommodate possible project expansions is preferred.

6. SUBMISSION EVALUATION

**Submission Guidelines**:

Interested contractors should submit a comprehensive proposal by **29th November, 2024** that includes:

* Company profile, highlighting relevant expertise and previous work on cfRNA or neurogenetic disorders.
* Detailed methodology for cfRNA extraction, sequencing, and analysis, specifying platforms, protocols, and bioinformatics tools to be used.
* Itemized budget as outlined in Section 5.
* Timeline of deliverables aligned with the project duration.
* References from previous clients or collaborators in similar projects.

**Evaluation Criteria**:

Proposals will be evaluated based on the following criteria:

1. **Technical Capability (40%)**: Demonstrated expertise in cfRNA extraction, sequencing, and analysis, including prior experience in similar research projects.
2. **Quality Assurance (20%)**: Standards and protocols for ensuring high data quality and reproducibility.
3. **Budget and Cost-Effectiveness (20%)**: Competitive pricing for the scope of services offered, with detailed justifications for each cost component.
4. **Timeline Adherence (10%)**: Ability to meet project deadlines and provide prompt communication on progress.
5. **Reputation and References (10%)**: Positive references from previous clients or collaborators, especially in related fields.

Shortlisted contractors may be invited for an interview to discuss their methodology, experience, and commitment to the project timeline.

7. CONTRACT DURATION

The contract duration will be for a period of 12 months from the start date of the project. Key milestones and deliverables are expected as follows:

* **Month 1-3**: Sample processing, cfRNA extraction, and initial quality control.
* **Month 3-6**: RNA sequencing and preliminary data analysis.
* **Month 6-9**: Bioinformatics analysis and development of predictive models.
* **Month 9-12**: Final report submission, including the cfRNA signatures and biomarker validation results.

The contract may be extended if additional samples are required or if unforeseen delays occur. Any such changes will be discussed and agreed upon by both parties.

8. ADDITIONAL INFORMATION

All data generated through this project remains the property of Hospital Sant Joan de Déu and must be handled in strict compliance with applicable data protection and privacy regulations (GDPR). The contractor agrees to maintain the confidentiality of all data and results, and to share data only with the designated members of the research team.

Upon project completion, the contractor is expected to provide all raw and processed data, scripts, and documentation used in the analysis, enabling replication and further research by Hospital Sant Joan de Déu or authorized collaborators.

For any additional queries, prospective contractors are encouraged to contact [juandario.ortigoza@sjd.es](mailto:juandario.ortigoza@sjd.es) before the submission deadline.