# PRILOG 2- TEHNIČKE SPECIFIKACIJE

 **Technical specification for small RNA sequencing service:**

**General specification:**

Samples must be processed according to miND pipeline published in Khamina et al. Int. J. Mol. Sci. (2022): A MicroRNA Next-Generation-Sequencing Discovery Assay (miND) for Genome-Scale Analysis and Absolute Quantitation of Circulating MicroRNA Biomarkers.

*Tehnička specifikacija za uslugu sekvenciranja male RNA:*

***Opće specifikacije:***

*Uzorci se moraju obraditi u skladu s miND protokolom objavljenim u “Khamina et al. Int. J. Mol. Sci. (2022.): A MicroRNA Next-Generation-Sequencing Discovery Assay (miND) for Genome-Scale Analysis and Absolute Quantitation of Circulating MicroRNA Biomarkers.”*

1. **Specification for plasma samples:**

**Service must include minimally:**

* 159x total RNA processed using Maxwell RNA Purification Kit.
* 159x small RNAseq library prep (RealSeq – Biofluids Single Indexing Library Preparation Kit).
* 4x pooling and size purification (BluePippin) and DNA library QC (Bioanalyzer DNA 1000 Chip).
* 2x Illumina NovaSeq SP Flowcell 100 cycles: estimated total of 1.500-1.600 M reads.
* Demultiplexing and quality filtering.

**Data analysis service must include minimally:**

* Trimming.
* Alignment to genome reference, miRbase, and RNACentral.
* Exploratory data analysis: unsupervised clustering by PCA and hierarchical clustering.
* Differential expression analysis (EdgeR/DeSeq2).
* Reporting and interpretation support.

**Minimum service deliverables:**

* Full data report and raw data.
* Results discussion via teleconference.
1. ***Specifikacije za uzorke plazme:***

***Usluga mora uključivati ​​minimalno:***

* *159x ukupna RNA obrađena korištenjem Maxwell RNA seta reagensa za pročišćavanje*
* *159x priprema biblioteke male RNAseq (RealSeq – Biofluids Single Indexing Library Preparation Kit).*
* *4x objedinjavanje i pročišćavanje veličine (BluePippin) i DNA biblioteka QC (Bioanalyzer DNA 1000 Chip).*
* *2x Illumina NovaSeq SP Flowcell 100 ciklusa: procijenjeno ukupno 1500-1600 M očitavanja.*
* *Demultipleksiranje i filtriranje kvalitete*

***Usluga analize podataka mora uključivati ​​minimalno:***

* *Podrezivanje.*
* *Usklađivanje s referencom genoma, miRbase i RNACentral.*
* *Istraživačka analiza podataka: nenadzirano klasteriranje pomoću PCA i hijerarhijsko klasteriranje.*
* *Analiza diferencijalne ekspresije (EdgeR/DeSeq2).*
* *Podrška u izvješćivanju i tumačenju.*

***Minimalni rezultati usluge:***

* *Potpuni izvještaj s podacima uz isporuku neobrađeni podaci.*
* *Rasprava o rezultatima putem telekonferencije.*
1. **Specification for synovial fluid samples**

**Wet-lab service must include minimally:**

* 48/77x total RNA processed using Maxwell RNA Purification Kit.
* 48/77x small RNAseq library prep (RealSeq - Biofluids Single Indexing Library Preparation Kit).
* 1/2x pooling and size purification (BluePippin) and DNA library QC (Bioanalyzer DNA 1000 Chip).
* 0.75/1x Illumina NovaSeq SP Flowcell 100 cycles: estimated total of 650-800 M reads
* Demultiplexing and quality filtering.

**Data analysis service “miND” must include minimally:**

* Trimming.
* Alignment to genome reference, miRbase and RNACentral.
* Exploratory data analysis: unsupervised clustering by PCA and hierarchical clustering.
* Differential expression analysis (EdgeR/DeSeq2).
* Reporting and interpretation support.

**Minimum service deliverables:**

* Full data report and raw data.
* Results discussion via teleconference.

***2. Specifikacija za uzorke sinovijalne tekućine***

***Usluga mora uključivati ​​minimalno:***

* *48/77x ukupna RNA obrađena korištenjem Maxwell RNA seta reagensa za pročišćavanje*
* *48/77x priprema biblioteke male RNAseq (RealSeq – Biofluids Single Indexing Library Preparation Kit).*
* *1/2x objedinjavanje i pročišćavanje veličine (BluePippin) i DNA biblioteka QC (Bioanalyzer DNA 1000 Chip).*
* *0,75/1x Illumina NovaSeq SP Flowcell 100 ciklusa: procijenjeno ukupno 650-800 M očitavanja*
* *Demultipleksiranje i filtriranje kvalitete*

***Usluga analize podataka mora uključivati ​​minimalno:***

* *Podrezivanje.*
* *Usklađivanje s referencom genoma, miRbase i RNACentral.*
* *Istraživačka analiza podataka: nenadzirano klasteriranje pomoću PCA i hijerarhijsko klasteriranje.*
* *Analiza diferencijalne ekspresije (EdgeR/DeSeq2).*
* *Podrška u izvješćivanju i tumačenju.*

***Minimalni rezultati usluge:***

* *Potpuni izvještaj s podacima uz isporuku neobrađeni podaci.*
* *Rasprava o rezultatima putem telekonferencije.*
1. **Specification for fat tissue samples**

**Wet-lab service must include minimally:**

* 36x total RNA processed using Qiagen miRNeasy Mini kit with workflow to enhance RNA yield.
* 36x small RNAseq library prep (RealSeq – Biofluids Single Indexing Library Preparation Kit).
* 1x pooling and size purification (BluePippin) and DNA library QC (Bioanalyzer DNA 1000 Chip).
* 1x Illumina NovaSeq SP Lane 100 cycles: estimated total of 350 M reads.
* Demultiplexing and quality filtering.

**Data analysis service must include minimally:**

* Trimming.
* Alignment to genome reference, miRbase, and RNACentral.
* Exploratory data analysis: unsupervised clustering by PCA and hierarchical clustering.
* Differential expression analysis (EdgeR/DeSeq2).
* Reporting and interpretation support.

**Minimum service deliverables:**

* Full data report and raw data.
* Results discussion via teleconference.

***3. Specifikacija za uzorke masnog tkiva***

***Usluga mora uključivati ​​minimalno:***

* *36x ukupna RNK obrađena pomoću Qiagen miRNeasy Mini seta reagensa uz protokol za povećanje prinosa RNK.*
* *36x priprema biblioteke male RNAseq (RealSeq – Biofluids Single Indexing Library Preparation Kit).*
* *1x objedinjavanje i pročišćavanje veličine (BluePippin) i DNA biblioteka QC (Bioanalyzer DNA 1000 Chip).*
* *1x Illumina NovaSeq SP Lane 100 ciklusa: procijenjeno ukupno 350 milijuna očitavanja.*
* *Demultipleksiranje i filtriranje kvalitete*

***Usluga analize podataka mora uključivati ​​minimalno:***

* *Podrezivanje.*
* *Usklađivanje s referencom genoma, miRbase i RNACentral.*
* *Istraživačka analiza podataka: nenadzirano klasteriranje pomoću PCA i hijerarhijsko klasteriranje.*
* *Analiza diferencijalne ekspresije (EdgeR/DeSeq2).*
* *Podrška u izvješćivanju i tumačenju.*

***Minimalni rezultati usluge:***

* *Potpuni izvještaj s podacima uz isporuku neobrađeni podaci.*
* *Rasprava o rezultatima putem telekonferencije.*

By signing this form, we confirm that we will perform the service until December 1, 2023. / *Potpisom ovog obrasca potvrđujemo da ćemo izvršiti uslugu do dana 01.12.2023. godine.*

Datum: \_\_\_\_\_\_\_\_\_\_\_\_\_2023. g.

 M.P. ZA PONUDITELJA:

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(ime, prezime i potpis ovlaštene osobe

za zastupanje gospodarskog subjekta)

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