The following Questions and Answers are regarding RFSO# 01R11-22-S009 for High Throughput Sequencing Services, Lacombe, AB

Question 1:

Our prices for sample preparation and sequencing services are usually adjusted once (sometimes twice) a year based on quotes we get from our reagents suppliers. The Financial Proposal form asks for prices for prices for a total of 4 years. We don't know what the costs will be beyond March 2022. How will changes in prices be considered if the contract is extended beyond its first year? Can we be consider for this tender if we only provide costs for year 1 and if the contract is extended, can we revise the prices at that time?

Answer 1:

You must take into account any factors that could influence your prices, and provide prices you will be able to honour. Once pricing has been submitted (and if accepted for award of a contract), it cannot be changed.

Question 2:

The tender specifies that data are to be stored for "no less than 3 months upon completion of analysis" but there is no specification of how long we should keep the data for (our policy is 3 months). Can you please clarify what is the maximum amount of time we are expected to store the data?

Answer 2:

Three months is sufficient.

Question 3:

The names of NovaSeq flowcells are given as SP, SP2, SP3 and SP4 in the Financial Proposal, which I believe correspond to Illumina's official names of SP, S1, S2 and S4, respectively. Can you please confirm?

Answer 3:

Yes, it should be SP, S1, S2 and S4. See attached amended Financial Proposal (Attachment #2 to Appendix D)

Question 4:

For projects 2 (metagenomics shotgun sequencing), 3 (bacterial whole genome sequencing) and 4 (Bacterial RNA-Seq), some of the sequencing options requested will generate considerably more data than specified in Appendix B. Should we just indicate the price for the sequencing option that is the closest match to the required amount of data described in Appendix B? Following the same idea, NovaSeq flowcells contain either 2 or 4 lanes, and shouldn't the price be stated for the number of lanes that each project require if the project doesn't require a full flowcell?

Answer 4:

Yes, indicate the price for the sequencing option that is the closest match to the required amount of data described in Appendix B.

Question 5:

Which variable regions (V1, V2, V3, V4, etc) of the 16S rRNA gene is required for this project?

Answer 5:

The V4 hypervariable region of the 16S rRNA gene will be targeted.

Question 6:

For the RNA-Seq (project 4): "contractor will deliver sequence data with a minimum of 15 million **forward reads**." This is inconsistent with the sequencing options indicated as S1, S2, or S4 flowcells as 2x150bp in Appendix B. Can you clarify that sequencing is supposed to be paired end sequencing (2x150-bp) and that they need a minimum of 15 million paired reads, regardless if it is **forward** or **reverse** reads?

Answer 6:

15 million forward reads is what is required.

Question 7:

Since these are microbiome samples that may contain DNA or RNA from various sources present in the environment, will the laboratory submitting sample ensure that their samples were properly checked for the presence of species they may be interested in sequencing and have minimized samples that may contain environmental DNA/RNA that is not of interest to them?

Answer 7:

Yes.

Question 8:

Can you clarify what is expected from the analysis reporting in item 3.2 (more specifically Analysis Methods used and Analysis Results)? I am assuming this is not related to data analysis, which is not indicated anywhere in the tender.

Answer 8:

Correct, this is not related to data analysis. The contractor is only responsible for providing the output files from the sequencing, e.g. fastq files.

Question 9:

Do DNA and RNA samples to be submitted fall under the Canadian Biosafety Standard containment level CL2 or lower?

Answer 9:

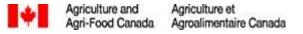
Yes.

Question 10:

Which DNA and RNA extraction methods will be used? What range of DNA and RNA amounts they expect will be available for sample preparation? Which quality assessment methods will be applied to DNA and RNA samples prior to shipping samples to us?

Answer 10:

Extraction methods will typically involve the use of commercial extraction kits with modifications based on in-house optimization. DNA and RNA amounts vary by sample type (e.g. feces vs. milk). The amount of DNA or RNA sent will depend on the contractor's requirement for each of the sequencing types. DNA and RNA will be assessed using fluorometric methods (e.g. Qubit) as well as on a Bioanalyzer and/or TapeStation system if required.



Attachment #2 to Appendix D for the Financial Proposal Amendment 001 – July 13, 2021

AAFC is not prepared to accept separate prices for any other costs. <u>All costs associated</u> with the work must be included in the Firm Unit Price (excluding applicable taxes).

Column B (Unit Price Offered) and Column C (Extended Cost) must be completed with a dollar value for all line items or your Offer may be considered non-compliant.

The estimates provided in Column A will be used for cost evaluation purposes only and do not constitute a guarantee or commitment of work on behalf of Canada. Actual usage may vary from amounts shown below for each year.

1. 16S rRNA GENE SEQUENCING				
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRA	RY PREPARATION			
.1	PCR (per sample)	388	\$/ Sample	
.2	Amplicon barcoding (per sample)	388	\$/ Sample	
.3	Amplicon normalization (per sample)	388	\$/ Sample	
.4	Amplicon library quality control (per library)	1	\$/ Library	
SEQUE	INCING OPTIONS			
.5	Illumina MiSeq PE 300 bp sequencing run	1	\$/ Run	
.6	Illumina MiSeq PE 250 bp sequencing run	1	\$/ Run	
			Total	T1

PRICING FOR INITIAL STANDING OFFER PERIOD (1 YEAR)



2. SHOTGUN METAGENOMIC SEQUENCING				
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRA	RY PREPARATION			
.1	Shotgun metagenomic DNA library preparation (per sample)	32	\$/ Sample	
.2	Library Quality Control (per sample)	32	\$/ Sample	
SEQUE	INCING OPTIONS			
.3	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell	
.4	NovaSeq 6000 <mark>SP1</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell	
.5	NovaSeq 6000 <mark>SP2</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell	
.6	NovaSeq 6000 SP4 (1 x 150 bp) flow cell	1	\$/ Flow cell	
			Total	Τ2

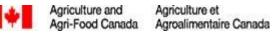
3A. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – SHORT READ				
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRA	RY PREPARATION			
.1	Library preparation	24	\$/ Sample	
.2	Library Quality Control (per sample)	24	\$/ Sample	
SEQUENCING OPTIONS				
.3	Illumina MiSeq PE 300 bp sequencing run	1	\$ <u>/</u> Lane	



.4	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell	
			Total	ТЗА

3B. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – LONG READ					
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRA	LIBRARY PREPARATION				
.1	Library preparation	8	\$/ Sample		
.2	Library Quality Control (per sample)	8	\$/ Sample		
SEQUE	SEQUENCING OPTIONS				
.3	PacBio Sequel sequencing SMRT cell	1	\$/ Cell		
			Total	T3B	

4. RNA	4. RNA SEQUENCING OF BACTERIAL TRANSCRIPTOMES				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRARY PREPARATION					
.1	Bioanalyzer for RNA samples (per sample)	24	\$/ Sample		
.2	Bacterial RNA-seq library preparation (per sample)	24	\$/ Sample		
.3	Library Quality Control (per sample)	24	\$ <u>/</u> Sample		
SEQUE	SEQUENCING OPTIONS				
.4	NovaSeq 6000 <mark>S1</mark> PE150 flow cell	1	\$/ Flow cell		



.5	NovaSeq 6000 <mark>S2</mark> PE150 flow cell	1	\$/ Flow cell	
.6	NovaSeq 6000 S4 PE150 flow cell	1	\$/ Flow cell	
			Total	Τ4

5. DISPOSAL FEE'S (if applicable) Bidders must enter a value here. If no charge for these than enter \$0.00.	\$/ Sample	
	Total	Т5

Total Cost for Initial Standing Offer Period (T1+T2+T3A+T3B+T4+T5) = _____

PRICING FOR OPTION PERIOD ONE (1)

1. 16	1. 16S rRNA GENE SEQUENCING			
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRAI	RY PREPARATION			
.1	PCR (per sample)	388	\$/ Sample	
.2	Amplicon barcoding (per sample)	388	\$/ Sample	
.3	Amplicon normalization (per sample)	388	\$/ Sample	
.4	Amplicon library quality control (per library)	1	\$/ Library	
SEQUE	ENCING OPTIONS			
.5	Illumina MiSeq PE 300 bp sequencing run	1	\$/ Run	
.6	Illumina MiSeq PE 250 bp sequencing run	1	\$/ Run	
			Total	T1



2. SHC	2. SHOTGUN METAGENOMIC SEQUENCING				
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRAI	RY PREPARATION				
.1	Shotgun metagenomic DNA library preparation (per sample)	32	\$/ Sample		
.2	Library Quality Control (per sample)	32	\$/ Sample		
SEQUE	INCING OPTIONS				
.3	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell		
.4	NovaSeq 6000 <mark>SP1</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell		
.5	NovaSeq 6000 <mark>SP2</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell		
.6	NovaSeq 6000 SP4 (1 x 150 bp) flow cell	1	\$/ Flow cell		
			Total	T2	

3A. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – SHORT READ					
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRA	LIBRARY PREPARATION				
.1	Library preparation	24	\$/ Sample		
.2	Library Quality Control (per sample)	24	\$/ Sample		
SEQUENCING OPTIONS					
.3	Illumina MiSeq PE 300 bp sequencing run	1	\$/ Lane		



.4	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell	
			Total	ТЗА

3B. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – LONG READ				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRAI	RY PREPARATION			
.1	Library preparation	8	\$/ Sample	
.2	Library Quality Control (per sample)	8	\$/ Sample	
SEQUENCING OPTIONS				
.3	PacBio Sequel sequencing SMRT cell	1	\$/ Cell	
			Total	T3B

4. RNA SEQUENCING OF BACTERIAL TRANSCRIPTOMES							
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)			
LIBRAI	RY PREPARATION						
.1	Bioanalyzer for RNA samples (per sample)	24	\$/ Sample				
.2	Bacterial RNA-seq library preparation (per sample)	24	\$/ Sample				
.3	.3 Library Quality Control (per sample) 24 \$/ Sample						
SEQUE							



.4	NovaSeq 6000 <mark>S1</mark> PE150 flow cell	1	\$/ Flow cell	
.5	NovaSeq 6000 <mark>S2</mark> PE150 flow cell	1	\$/ Flow cell	
.6	NovaSeq 6000 S4 PE150 flow cell	1	\$/ Flow cell	
			Total	Τ4

5. DISPOSAL FEE'S (if applicable) Bidders must enter a value here. If no charge for these than enter \$0.00.	\$/ Sample	
	Total	Τ5

Total Cost for Option Period One (T1+T2+T3A+T3B+T4+T5) = _____

PRICING FOR OPTION PERIOD TWO (2)

1. 16	1. 16S rRNA GENE SEQUENCING				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRAI	RY PREPARATION				
.1	PCR (per sample)	388	\$/ Sample		
.2	Amplicon barcoding (per sample)	388	\$/ Sample		
.3	Amplicon normalization (per sample)	388	\$/ Sample		
.4	Amplicon library quality control (per library)	1	\$/ Library		
SEQUE	ENCING OPTIONS				
.5	Illumina MiSeq PE 300 bp sequencing run	1	\$ <u>/</u> Run		
.6	Illumina MiSeq PE 250 bp sequencing run	1	\$/ Run		
			Total	T1	



2. SHOTGUN METAGENOMIC SEQUENCING				
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRAI	RY PREPARATION			
.1	Shotgun metagenomic DNA library preparation (per sample)	32	\$/ Sample	
.2	Library Quality Control (per sample)	32	\$/ Sample	
SEQUE	NCING OPTIONS			
.3	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell	
.4	NovaSeq 6000 <mark>SP1</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell	
.5	NovaSeq 6000 <mark>SP2</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell	
.6	NovaSeq 6000 SP4 (1 x 150 bp) flow cell	1	\$/ Flow cell	
			Total	T2

3A. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – SHORT READ				
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRA	RY PREPARATION			
.1	Library preparation	24	\$/ Sample	
.2	Library Quality Control (per sample)	24	\$/ Sample	
SEQUENCING OPTIONS				
.3	Illumina MiSeq PE 300 bp sequencing run	1	\$/ Lane	



.4	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell	
			Total	ТЗА

3B. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – LONG READ				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)
LIBRAI	RY PREPARATION			
.1	Library preparation	8	\$/ Sample	
.2	Library Quality Control (per sample)	8	\$/ Sample	
SEQUENCING OPTIONS				
.3	PacBio Sequel sequencing SMRT cell	1	\$/ Cell	
			Total	T3B

4. RNA SEQUENCING OF BACTERIAL TRANSCRIPTOMES							
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)			
LIBRAI	RY PREPARATION						
.1	Bioanalyzer for RNA samples (per sample)	24	\$/ Sample				
.2	Bacterial RNA-seq library preparation (per sample)	24	\$/ Sample				
.3	.3 Library Quality Control (per sample) 24 Sample						
SEQUE							



.4	NovaSeq 6000 <mark>S1</mark> PE150 flow cell	1	\$/ Flow cell	
.5	NovaSeq 6000 <mark>S2</mark> PE150 flow cell	1	\$/ Flow cell	
.6	NovaSeq 6000 S4 PE150 flow cell	1	\$/ Flow cell	
			Total	Τ4

5. DISPOSAL FEE'S (if applicable) Bidders must enter a value here. If no charge for these than enter \$0.00.	\$/ Sample	
	Total	Τ5

Total Cost for Option Period Two (T1+T2+T3A+T3B+T4+T5) = _____

PRICING FOR OPTION PERIOD THREE (3)

1. 16	1. 16S rRNA GENE SEQUENCING				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRA	RY PREPARATION				
.1	PCR (per sample)	388	\$/ Sample		
.2	Amplicon barcoding (per sample)	388	\$/ Sample		
.3	Amplicon normalization (per sample)	388	\$/ Sample		
.4	Amplicon library quality control (per library)	1	\$/ Library		
SEQUE	SEQUENCING OPTIONS				
.5	Illumina MiSeq PE 300 bp sequencing run	1	\$ <u>/</u> Run		
.6	Illumina MiSeq PE 250 bp sequencing run	1	\$/ Run		
Total				T1	



2. SHC	2. SHOTGUN METAGENOMIC SEQUENCING				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRAI	LIBRARY PREPARATION				
.1	Shotgun metagenomic DNA library preparation (per sample)	32	\$/ Sample		
.2	Library Quality Control (per sample)	32	\$/ Sample		
SEQUE	SEQUENCING OPTIONS				
.3	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell		
.4	NovaSeq 6000 <mark>SP1</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell		
.5	NovaSeq 6000 <mark>SP2</mark> (1 x 150 bp) flow cell	1	\$/ Flow cell		
.6	NovaSeq 6000 SP4 (1 x 150 bp) flow cell	1	\$/ Flow cell		
Total				T2	

3A. W	3A. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – SHORT READ				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRA	LIBRARY PREPARATION				
.1	Library preparation	24	\$/ Sample		
.2	Library Quality Control (per sample)	24	\$/ Sample		
SEQUE	SEQUENCING OPTIONS				
.3	Illumina MiSeq PE 300 bp sequencing run	1	\$/ Lane		



.4	NovaSeq 6000 SP (2 x 250 bp) flow cell	1	\$/ Flow cell	
			Total	ТЗА

3B. WI	3B. WHOLE GENOME SEQUENCING OF BACTERIAL GENOMES – LONG READ				
Item	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)	
LIBRAI	LIBRARY PREPARATION				
.1	Library preparation	8	\$/ Sample		
.2	Library Quality Control (per sample)	8	\$/ Sample		
SEQUENCING OPTIONS					
.3	PacBio Sequel sequencing SMRT cell	1	\$/ Cell		
			Total	T3B	

4. RNA	4. RNA SEQUENCING OF BACTERIAL TRANSCRIPTOMES					
ltem	Description	Estimated # of Units (A)	Firm Unit Price Offered (CDN\$) (B)	Extended Cost (C) = (A x B)		
LIBRAI	LIBRARY PREPARATION					
.1	Bioanalyzer for RNA samples (per sample)	24	\$ <u>/</u> Sample			
.2	Bacterial RNA-seq library preparation (per sample)	24	\$/ Sample			
.3 Library Quality Control (per sample) 24 \$/ Sample						
SEQUE	SEQUENCING OPTIONS					



.4	NovaSeq 6000 <mark>S1</mark> PE150 flow cell	1	\$/ Flow cell	
.5	NovaSeq 6000 <mark>S2</mark> PE150 flow cell	1	\$/ Flow cell	
.6	NovaSeq 6000 S4 PE150 flow cell	1	\$/ Flow cell	
			Total	Τ4

5. DISPOSAL FEE'S (if applicable) Bidders must enter a value here. If no charge for these than enter \$0.00.	\$/ Sample	
	Total	Τ5

Total Cost for Option Period Three (T1+T2+T3A+T3B+T4+T5) = _____

Total Cost for Initial Standing Offer Period

Total Cost for Option Period One (1) +

Total Cost for Option Period Two (2) +_____ Total Cost for Option Period Three (3) +_____

TOTAL COST for all periods = _____

Supplier to indicate:	
Vendor / Company Name:	
Signature :	
Date:	