By Speed Post

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F.No. : 3-15/S&P/2022-23 **Dt. : 05-09-2022**

प्रति,

विषयः– **Transcriptome sequencing** कराने के संबंध में।

महोदय,

निदेशक, खरपतवार अनुसंधान निदेशालय की ओर से **Transcriptome sequencing** कराने हेतु सील बंद निविदा/ऑफर पोस्ट के द्वारा आमंत्रित है। विवरण निम्नानुसार हैः–

Sr. No.	Description	Qty.
1.	❖ **Transcriptome sequencing of *Parthenium hysterophorus*** **RNA extraction and quality control** • Extraction of high quality total RNA from the Parthenium sample followed by quality control sing bioanalyzer and QC reports RIN number should be provided before library preparation. • Library type: Ribodepletion for all the samples has to be done. • Preparation of paired end RNA sequencing library using illumina adapters. • Sequencing reads of 2X150 base pairs using Novaseq 6000 sequencing platform should be generated, minimum no of good quality reads should be 60 million (PE 150) for each sample. **Transcriptome assembly and data analysis** • Transcriptome data should be De novo assembled. Combined read assembly to be constructed. Reference based shall be done for rest of the samples using Draft assembly as reference. • Comparative transcriptome analysis and prediction of differentially expressed genes and their expression levels should be done. Differential gene expression in terms of TPM (Transcripts per million), RPKM (Reads per Kilobase of Exon per Million Fragments) and differential Gene Expression using *commander Ver.1.77* tool or any equivalent tools has to be generated and Reports using DeSeq Tool (**DESeq** is an R package to analyse count data from high-throughput sequencing assays	18

such as RNA-Seq and test for differential expression.)

- Identification of differentially expressed genes and their GO enrichment among the samples and isoform specific differential expression.
- Finding homologous genes and transcriptional factors, functional annotation (GO and KEGG analysis) non-redundant protein database (NR, NCBI), gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The output of these analyses should be given in all presentable formats-suitable graphical charts.
- Gene prediction, molecular pathway analysis, annotation of contigs for putative genes following by discovery of SSRs, SNPs, long non-coding RNAs, COGs, transcription factors and miRNA information along with methodology and QC reports should be done.
- Advanced analysis-Digital gene expression, GC content identification and functional annotation to mRNA and proteins of related species. Submission of raw sequence data in FASTQ, annotated contigs, SSRs and SNPs information along with methodology and QC reports.
- Functional classification of pathway terms for differentially expressed genes. The pathway annotations must be acquired with KAS using an e-value threshold of $\leq 10^{-10}$. Total differentially expressed genes must be classified into pathway categories. The data for pathway categories that represented less than 1% of the differentially expressed genes should be included in other pathway categories.
- Alignment of reads across splice junctions to identify isoforms, novel transcripts, gene dosage and gene fusions.
- Identify and quantify both rare and common transcripts among the samples.
- Prediction of different classes of proteins (membrane proteins, secreted proteins, and other cellular localization) based on signal peptides using SignalP, TargetP, WolfPSORT, etc.
- Mappling of reads or overall transcript synteny with *Oryza sativa* and *Zea mays* genes, if required.
- Reference based assembly: high quality reads has to mapped on the parthenium reference genome; mitochondrial and chloroplast genome for control and treated samples. Unmapped references should to be given separately. Predicted transcripts have to be delivered based on their position in Parthenium reference genome.
- Statistical validation of the gene expression data using standard methodologies.
- Prediction of significant changes in the metabolic and hormonal pathways among the sample and annotation using KEGG.
- Global analysis of transcriptome datasets of biological replicates. (a) Bar plot describing the number of expressed transcripts after filtering. (b) principal component analysis (PCA) of transcriptome data with principal component value.
- Prediction of candidate secreted effector proteins (CSEPs) and

with their separate comparative expression data

- Should provide the Venn diagramme with the number of overlapping genes between and among the samples. Venn diagrammes must represent the overlaps of induced and repressed genes across comparisions. Induced and repressed genes for each pairwise comparison.Heatmap generation using RScript Tool (Rscript is the **R Interpreter** which helps in the sequential execution of R commands in the script file).
- Hierarchically clustered heat map of differentially expressed genes/gene family as groups.

Additional post sequencing free support

- Lab and bioinformatics training should be provided for at least two persons.
- The service provider should provide publication quality data and support for publishing the NGS data.
- Turnaround time should be 8 to 12 weeks from samples pass to the QC.

Essential terms and conditions

- The company should have good experience/ record in conducting such experiments.
- Participating companies (the service provider) should have their own Novaseq 6000 sequencing platform (should enclose a certificate for it).
- Proof of lab facility: Illumina installation certificate in India should be provided, partner/collaborator company installation certificate located abroad will not be considered.
- The bidder should provide proof of in-house NGS facility and bioinformatics analysis in India and price quoted must be valid for two years and DSIR certificate should be enclosed for the same.
- Service provider should be certified as per ISO standards.
- Sample purification and QC check-up by Agillent bioanalyzer should be performed by the service provider.
- Data generated from RNA quality check passed the samples should be comparable to other samples; firm must generate the comparable data (without any additional charges). If the generated data is found to be deviating significantly from other samples, necessary actions should be taken to address them. Also, if there is any error in sequencing or bioinformatics analysis, it should be rectified without any additional charges.
- Price should be quoted as per the sample cost (price per transcriptome sample).
- Samples or data should not be outsourced outside India at any stage.
- Should maintain the confidentiality of the project
- The service provider should have well supported bioinformatics expertise to execute the given specifications.

	• Validity of the quotation should be for at least 90Days. • Assistance should be provided for submission of transcriptome datasets to NCBI. • Data analysis training for two persons must be provided at their site of analysis and participation of the trainings at any point of the study is required. • Further assistance on the part of data analysis, as and when required (at least for the period of 24 months from the date of handing over the analyzed data). • The details of progress of experiment will be intimated to the customer/scientist and further processing in case of any issues will be based on the instructions of the customer only. Experiment should be carried out in the presence of principle scientist. • Failure to submit quotation as per the technical specification may deprive the concerned firm from consideration. • Quotation should be on firm's letter head with GST Number, PAN Number and CST Number mentioned clearly. • Data should be delivered through secured server & HDD only. To maintain data confidentiality, firm should not write data in CDs/DVDs. • Time lines to complete the project with complete analysis should be written clearly and should not exceed more than 4-6 weeks.	

नियम एवं शर्तें :–

1. दरों का उल्लेख वस्तु को इस निदेशालय परिसर, महाराजपुर, जबलपुर पहुंचाने तक का होना चाहिये।
2. टैक्स का स्पष्ट रूप से उल्लेख होना चाहिये यदि टेक्स का उल्लेख नहीं रहता है, तो यह माना जावेगा कि दर्शायी गई दरों में टेक्स सम्मिलित है। इस निदेशालय से कोई ''सी'' अथवा ''डी'' फॉर्म जारी नहीं किया जाता है।
3. बिलों से टी.डी.एस. यदि लागू हो तो कटौती नियमानुसार की जावेगी।
4. दर्शायी गई दरों की वैधता निविदा खोलने की तिथि से कम से कम 90 दिनों तक की होनी चाहिये, दरों में पैकिंग forwarding एवं ट्रांसपोर्ट्रेशन चार्ज स्पष्ट रूप से उल्लेख होना चाहिये।
5. सम्पूर्ण रूप से पूर्ण सीलबंद निविदा/कोटेशन निदेशालय में रखी निविदा पेटी या पोस्ट के द्वारा दिनांक 21/09/2022 दोपहर 2.30 बजे तक स्वीकार की जावेगी। फर्म इस पर ध्यान दें, कि किसी भी प्रकार अस्पष्ट, मिटी हुई, सफेदी लगी लिखाई नहीं होनी चाहिये, यदि कोई कटिंग अन्य ओवर राईटिंग होती है, तो उसे सत्यापित (उस स्थान पर हस्ताक्षर होना चाहिये) अन्यथा ऐसी दशा में कोटेशन पर कोई विचार नहीं किया जावेगा।
6. सभी मामलों में निदेशक, ख.अनु. निदेशालय का निर्णय अंतिम होगा तथा निविदाकारों को मान्य होगा ।
7. बिल का भुगतान 30 दिनों में किया जावेगा। चूंकि भुगतान ई–पेमेंट के द्वारा किया जाता है, इसलिए फर्म का जी.एस.टी. नं., बैक खाता नं. पता एवं आई.एफ.एस.सी. कोड बिल में स्पष्ट रूप से उल्लेखित किया जाना चाहिये।

8. निविदा/कोटेशन में रू. 5,000/– की राशि बतौर धरोहर राशि (ई.एम.डी.) के रूप में एकाउंट पेई डिमांड ड्राफ्ट/पे. आर्डर **ICAR UNIT DWR, JABALPUR** के पक्ष में देय हो आवश्यक रूप से निविदा/कोटेशन के साथ संलग्न होना चाहिये। अन्यथा निविदा पर विचार न करते हुये रद्द कर दिया जावेगा।

9. धरोहर राशि (ईएम.डी) की राशि लघु उद्योग **(MSME)** के लिए छूट है। इस हेतु निविदा के साथ वांछित प्रमाण पत्र को संलग्न करना अनिवार्य है ।

9. निदेशक, ख.अनु. निदेशालय के पास यह अधिकार सुरक्षित है, कि कोई भी निविदा/कोटेशन को स्वीकार अथवा अस्वीकार बिना किसी कारण के किया जा सकेगा।

10. किसी भी प्रकार के पत्राचार अधोहस्ताक्षरकर्ता के पद नाम से ही किया जावे न कि उसके नाम से।

11. सभी संलग्न **Annexure (I, II and III)** भरना अनिवार्य है।

12. किसी भी प्रकार का विवाद जबलपुर न्यायालय (jurisdicsion) के अधीन रहेगा।

13. आपूर्ति की गई सामग्री की गुणवत्ता की गारन्टी का संबंधित फर्म द्वारा रूपष्ट रूप से उल्लेख होना

14. उपकरण के कय हेतु 2 बिड प्रणाली की प्रकिया अर्जित है, अतः

- **ANNEXURE I and II,** को एक लफाफे में तथा
- **फाइनेंशियल बिड ANNEXURE- III,** को अलग लिफाफे में प्रस्तुत किया जाना होगा।

प्रभारी कय एवं भण्डार अनुभाग

फर्म का नाम एवम पता : .....

(फर्म का लेटर हेड का उपयोग करे)

ANNEXURE – 1

Essential Basic Information

1	Name of the Firm/Agency	
2	Full address with Tele./Mob. No., Fax No., e-mail	
3	Details of Tender Fee & EMD (DD/No., date, Name of the Bank/Branch)	
4	Registration Certificate of the Firm/Company	
5	GST Registration no.	
6	I.T. PAN No./ TIN No.	
7	Proprietary Certificate /Authorized dealer certificate	
8	**Bank Details**	
	Name of the Account Holder /firm/ Company (Payee's Account Name)	
	Nature of Account (saving/current)	
	Name of the Bank	
	Bank Account No.	
	Branch Address	
	IFSC Code of Bank/Branch	
9	Check List	

Date

Place

Name of the Authorized Signatory

Stamp & Signature

फर्म का नाम एवम पता : .....

(फर्म का लेटर हेड का उपयोग करे)

ANNEXURE – II

TECHNICAL BID

Tender Ref : ..... **Date : .....**

Sr. No.	Description	Qty.	Ready to provide the analysis **Yes/No**
1	❖ **Transcriptome sequencing of *Parthenium hysterophorus*** **RNA extraction and quality control**	18	
	• Extraction of high quality total RNA from the Parthenium sample followed by quality control sing bioanalyzer and QC reports RIN number should be provided before library preparation.		
	• Library type: Ribodepletion for all the samples has to be done.		
	• Preparation of paired end RNA sequencing library using illumina adapters.		
	• Sequencing reads of 2X150 base pairs using Novaseq 6000 sequencing platform should be generated, minimum no of good quality reads should be 60 million (PE 150) for each sample.		
	Transcriptome assembly and data analysis		
	• Transcriptome data should be De novo assembled. Combined read assembly to be constructed. Reference based shall be done for rest of the samples using Draft assembly as reference.		
	• Comparative transcriptome analysis and prediction of differentially expressed genes and their expression levels should be done. Differential gene expression in terms of TPM (Transcripts per million), RPKM (Reads per Kilobase of Exon per Million Fragments) and differential Gene Expression using *commander Ver.1.77* tool or any equivalent tools has to be generated and Reports using DeSeq Tool (**DESeq** is an R package to analyse count data from high-throughput sequencing assays such as RNA-Seq and test for differential expression.)		
	• Identification of differentially expressed genes and their GO enrichment among the samples and isoform specific differential expression.		
	• Finding homologous genes and transcriptional factors, functional annotation (GO and KEGG analysis) non-		

	redundant protein database (NR, NCBI), gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The output of these analyses should be given in all presentable formats-suitable graphical charts.		
•	Gene prediction, molecular pathway analysis, annotation of contigs for putative genes following by discovery of SSRs, SNPs, long non-coding RNAs, COGs, transcription factors and miRNA information along with methodology and QC reports should be done.		
•	Advanced analysis-Digital gene expression, GC content identification and functional annotation to mRNA and proteins of related species. Submission of raw sequence data in FASTQ, annotated contigs, SSRs and SNPs information along with methodology and QC reports.		
•	Functional classification of pathway terms for differentially expressed genes. The pathway annotations must be acquired with KAS using an e-value threshold of $\leq 10^{-10}$. Total differentially expressed genes must be classified into pathway categories. The data for pathway categories that represented less than 1% of the differentially expressed genes should be included in other pathway categories.		
•	Alignment of reads across splice junctions to identify isoforms, novel transcripts, gene dosage and gene fusions.		
•	Identify and quantify both rare and common transcripts among the samples.		
•	Prediction of different classes of proteins (membrane proteins, secreted proteins, and other cellular localization) based on signal peptides using SignalP, TargetP, WolfPSORT, etc.		
•	Mappling of reads or overall transcript synteny with *Oryza sativa* and *Zea mays* genes, if required.		
•	Reference based assembly: high quality reads has to mapped on the parthenium reference genome; mitochondrial and chloroplast genome for control and treated samples. Unmapped references should to be given separately. Predicted transcripts have to be delivered based on their position in Parthenium reference genome.		
•	Statistical validation of the gene expression data using standard methodologies.		
•	Prediction of significant changes in the metabolic and hormonal pathways among the sample and annotation using KEGG.		
•	Global analysis of transcriptome datasets of biological		

replicates. (a) Bar plot describing the number of expressed transcripts after filtering. (b) principal component analysis (PCA) of transcriptome data with principal component value.		
• Prediction of candidate secreted effector proteins (CSEPs) and with their separate comparative expression data		
• Should provide the Venn diagramme with the number of overlapping genes between and among the samples. Venn diagrammes must represent the overlaps of induced and repressed genes across comparisions. Induced and repressed genes for each pairwise comparison.Heatmap generation using RScript Tool (Rscript is the **R Interpreter** which helps in the sequential execution of R commands in the script file). • Hierarchically clustered heat map of differentially expressed genes/gene family as groups.		
Additional post sequencing free support		
• Lab and bioinformatics training should be provided for at least two persons.		
• The service provider should provide publication quality data and support for publishing the NGS data.		
• Turnaround time should be 8 to 12 weeks from samples pass to the QC.		
Essential terms and conditions		
• The company should have good experience/ record in conducting such experiments.		
• Participating companies (the service provider) should have their own Novaseq 6000 sequencing platform (should enclose a certificate for it).		
• Proof of lab facility: Illumina installation certificate in India should be provided, partner/collaborator company installation certificate located abroad will not be considered.		
• The bidder should provide proof of in-house NGS facility and bioinformatics analysis in India and price quoted must be valid for two years and DSIR certificate should be enclosed for the same.		
• Service provider should be certified as per ISO standards.		
• Sample purification and QC check-up by Agillent bioanalyzer should be performed by the service provider.		
• Data generated from RNA quality check passed the samples should be comparable to other samples; firm must generate the comparable data (without any additional charges). If the generated data is found to be deviating significantly from other samples, necessary		

	actions should be taken to address them. Also, if there is any error in sequencing or bioinformatics analysis, it should be rectified without any additional charges.		
	• Price should be quoted as per the sample cost (price per transcriptome sample).		
	• Samples or data should not be outsourced outside India at any stage		
	• Should maintain the confidentiality of the project		
	• The service provider should have well supported bioinformatics expertise to execute the given specifications.		
	• Validity of the quotation should be for at least 90Days.		
	• Assistance should be provided for submission of transcriptome datasets to NCBI.		
	• Data analysis training for two persons must be provided at their site of analysis and participation of the trainings at any point of the study is required.		
	• Further assistance on the part of data analysis, as and when required (at least for the period of 24 months from the date of handing over the analyzed data).		
	• The details of progress of experiment will be intimated to the customer/scientist and further processing in case of any issues will be based on the instructions of the customer only. Experiment should be carried out in the presence of principle scientist.		
	• Failure to submit quotation as per the technical specification may deprive the concerned firm from consideration**.**		
	• Quotation should be on firm's letter head with GST Number, PAN Number and CST Number mentioned clearly.		
	• Data should be delivered through secured server & HDD only. To maintain data confidentiality, firm should not write data in CDs/DVDs.		
	• Time lines to complete the project with complete analysis should be written clearly and should not exceed more than 4-6 weeks.		

Date Name of the Authorized Signatory

Place Stamp & Signature

फर्म का नाम एवम पता : .....

(फर्म का लेटर हेड का उपयोग करे)

ANNEXURE – III

FINANCIAL BID

Tender Ref : …….. **Date : ……..**

Sr. No.	Description	Qty.	Total Amount Rs.
1	❖ **Transcriptome sequencing of *Parthenium hysterophorus***	18	Rs..... **In word** (Rupees .. ..)

Terms and condition

1. **Tax/GST :** Inclusive or Exclusive
2. **Validity :** …….. days
3. **Payment condition :** No advance payment will be given
4. **Delivery :** ……..

Date — Name of the Authorized Signatory

Place — Stamp & Signature