Question: Regarding Return of Remaining RNA Samples

- The Performance Work Statement states that remaining RNA samples are to be returned upon project completion.
- Would the Government consider secure destruction of remaining sample material, in accordance with a laboratory's documented retention and disposal procedures, acceptable in lieu of returning the remaining material?

Answer: The vendor shall return all unused samples (do not destroy them) in dry ice. USDA ARS will pay for the shipping cost with dry ice

Question: Regarding NCBI Sequence Read Archive (SRA) Submission

- Is the contractor required to perform the NCBI SRA submission directly?
- Alternatively, would it be acceptable for the contractor to provide all raw sequencing files, metadata, and supporting documentation necessary for the Government to complete the submission?

Answer: Yes. Vendor is required to submit all raw sequences to NCBI SRA under PI's instruction.
All processed data , including analysis results and QC reports, shall be emailed to PI.

Question: Regarding Sequencing Depth

- The PWS references approximately 25–40 million paired-end reads per sample.
- Is this range considered acceptable, or is there a preferred target read depth within that range?

Answer: Yes. This range is considered acceptable

Question: Regarding Sample Quality

- If certain samples received by the contractor do not meet quality standards for optimal RNA sequencing, should the contractor proceed with sequencing and provide the resulting data, or should sequencing be paused pending Government direction?

Answer: Show the QC report to PI and let him decide if proceeding or dropping off particular samples. All depend on the issues identified.

Question: Regarding Sample Batching

- May samples be submitted and processed in multiple batches throughout the period of performance, provided all required deliverables are completed within the contract period?

Answer: Up to three batches.

Question: Regarding Bioinformatics Analysis Requirements

- Please confirm whether the following analyses satisfy the Government's requirements:
  - Quality control of raw sequencing data
  - Alignment to a reference genome
  - Gene expression quantification
  - Differential expression analysis
  - Functional enrichment analysis (e.g., GO and/or KEGG)
  - Standard visualizations such as PCA plots, heatmaps, and volcano plots

Answer: These are acceptable.

Question: Regarding Data Delivery Format

- Please confirm whether delivery of raw FASTQ files, alignment files (BAM), expression count tables, differential expression results, and final analysis reports in electronic format satisfies the data delivery requirement.

Answer: PI needs to download these files.
FASTQ files will also need to deposit to NCBI SRA. The quote shall include this deposition cost.

Question: Regarding Equivalent Sequencing Platforms

- The solicitation references Illumina NovaSeq X or equal.
- Please confirm whether equivalent Illumina short-read platforms capable of achieving the required sequencing performance are acceptable.

Answer: Any instrument from the Illumina (or equivalent) platform is okay

Question: Regarding Data Retention

- Is there a minimum data-retention period required after final delivery to the Government?

Answer: Standard 3 to 4 weeks.

Question: Reference Genome and Annotation Files

- Will the Government provide all required reference genomes and annotation files necessary for alignment and downstream bioinformatics analysis, if applicable?

Answer: These files are in public domains and can be downloaded freely.

Question: Could USDA-ARS please clarify the desired RNA library preparation method for the 110 tissue samples — specifically, whether libraries should be prepared using mRNA enrichment (polyA selection) or rRNA depletion?

Answer: The rRNA depletion is desired and should be applied to all samples.
If rRNA depletion method, which carries higher cost, is not available, the mRNA enrichment method should apply to all samples. All samples should be either rRNA depletion or polyA mRNA enrichment, not both or mixed.

Question: Will polyA enrichment protocol be acceptable?

Answer: Yes

Question: Can USDA confirm the species represented in the 110 tissue samples? The Statement of Work references research involving goats, sheep, and rodents, but the species for the samples to be sequenced are not explicitly stated. If multiple species are anticipated during the contract period, please provide an estimated breakdown.

Answer: We do not know the number or ratios, because the animal experiments have not started yet. Tentatively 40 lambs (sheep) and 70 rodents.

Question: Regarding the tissue samples from goats, sheep, and rodents: Will any of these contain parasites? If so, what specific protocols have been implemented to ensure the tissues are safe for handling?

Answer: Yes. The tissues are stored in TRIzol

Question: What is the biosafety level (BSL) associated with these tissue samples?

Answer: BSL2

Question: What specific type(s) of tissue will you be sending?

Answer: Intestine

Question: What is the expected amount or volume of tissue per sample?

Answer: Sufficient for >100 ug of total RNA (or ~2 cm in length of the intestine)

Question: You mentioned "providing digital gene expression signature profiles with full data analysis." Could you clarify if standard differential gene expression analysis is sufficient, or if you require any specific downstream analysis beyond that?

Answer: Standard gene expression analysis is sufficient (must include software version and parameters used, raw count table, FPKM table, DEG results and annotation), but the raw

sequence data will need to be deposited to NCBI SRA wit PI's permission.

Question: Will your project require strand-specific information?

Answer: Yes

Question: Do you plan to send the samples altogether in one batch? If they will be split up, how many batches should we expect, and how many samples will be in each?

Answer: Up to two batches. The number of samples in each batch may vary.

Question: What type of RNA pre-processing is required for your project (e.g., poly-A selection or rRNA depletion)?

Answer: rRNA depletion is required.