नेपाल सरकार
कृषि तथा पशुपंछी विकास मन्त्रालय
कृषि विभाग
राष्ट्रिय फलफूल विकास केन्द्र, कीर्तिपुर

शिलबन्दी दरभाउपत्र आह्वान सम्बन्धी सूचना

प्रथम पटक सूचना प्रकाशित मितिः- २०८२/०९/१७ गते

यस केन्द्र अन्तर्गत तपसिलमा उल्लेखित कार्यक्रम संचालन गर्न सिलबन्दी दरभाउपत्र प्रस्ताव आह्वान गरिएको हुदा सम्वन्धित सबैको जानकारीका लागि यो सूचना प्रकाशित गरिएको छ।

शर्तहरूः

१. यो सिलबन्दी दरभाउपत्र सूचना प्रकाशित भएको मितिले १५ औं दिनसम्म कार्यालय समय भित्र कार्यालयको कोड नं.३१२०१३५०२, मा रू.१०००/- (एक हजार) (फिर्ता नहुनेगरी) राजश्व शिर्षक नं. १४२२९, राजश्व खाता नं.१०००१००२०००१०००० मा दाखिला गरेको सक्कल बैंक भौचर पेश गरी यस केन्द्रबाट खरिद गर्न सकिनेछ।

२. सिलबन्दी दरभाउपत्र खरिद गर्दा नविकरण भएको इजाजतपत्र, आयकर दर्ता प्रमाणपत्र, भ्याट दर्ता भएको प्रमाण पत्र, कर चुक्ता प्रमाणपत्रको प्रतिलिपि पेश गर्नुपर्नेछ।

३. यो सूचना प्रकाशित भएको मितिले १६ औं दिन दिनको १२;०० बजे सम्ममा सिलबन्दी दरभाउपत्र पेश गरिसक्नुपर्नेछ र दिनको १:०० बजे खोलिनेछ।

४. सिलबन्दी दरभाउपत्र बुझाउने अन्तिम दिन विदा पर्न गएमा सो पछि कार्यालय खुल्ने दिनमा तोकिएको समयमा बुझाउन सकिनेछ।

५. हुलाक, कुरियर र इन्टरनेटबाट पठाईएको सिलबन्दी दरभाउपत्रलाई मान्यता दिइने छैन।

६. सिलबन्दी दरभाउपत्रसाथ माग गरिएको विड सेक्युरिटी को.ले.नि.का. त्रिपुरेश्वरको नाममा रहेको रा.बा.बैंक त्रिपुरेश्वर, काठमाण्डौको धरौटी खाता नं.१७००१००१०२०३०००० , कार्यालयको कोड नं.३१२०१३५०२ मा नगद जम्मा गरेको सक्कल भौचर वा मान्यता प्राप्त क वर्गको वाणिज्य बैंकबाट (अन्तिम दर्ता मितिले ७५ दिन म्याद भएको) यस कार्यालयको नाममा खिचिएको बैंक जमानत अनिवार्य रूपले रहेको हुनुपर्नेछ।

७. अन्य थप जानकारीको लागि यस केन्द्रमा कार्यालय समयभित्र फोन नं.०१-५९०५७४२, ५९०५०५३ मा सम्पर्क गर्न सकिनेछ।

S.N.	Contract No.	Description of work	Estimate Amount Including VAT (NRS.)	Bid Security (NRS.)
1	NCFD- 2082/83-01	DNA fingerprinting; SSR Sequencing of Mandarin and Molecular Characterization	994383.33	17600.00

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1. Introduction

Mandarin (*Citrus reticulata*), the national fruit of Nepal, is a high-value horticultural crop of cultural, nutritional, and economic importance. Despite its widespread cultivation across diverse agro-ecological regions, the genetic diversity, population structure, and varietal distinctiveness of Nepalese mandarin germplasm remain inadequately characterized. Observed phenotypic variation across regions reflects both underlying genetic differences and environmental influences, underscoring the need for comprehensive molecular characterization.

This assignment aims to characterize mandarin germplasm using DNA-based markers to identify distinct genotypes, assess population structure, and generate standardized DNA fingerprints. The outputs will support varietal identification, breeding programs, conservation planning, and sustainable utilization of mandarin genetic resources in Nepal.

2. Objectives of the Assignment

The outsourced laboratory/service provider shall:

1. Perform high-quality DNA extraction from fresh mandarin leaf tissues provided by the client.
2. Conduct molecular genotyping using one of the following approaches:
    - **SSR genotyping** using 10–20 high-PIC loci, **or**
    - **SNP genotyping** (GBS/RADseq/array-based or targeted amplicon sequencing).
3. Generate and deliver raw and analyzed genomic data, including genetic diversity indices, population structure, clustering, and phylogenetic relationships.
4. Develop a standardized minimal marker panel for routine DNA fingerprinting of mandarin genotypes.
5. Submit comprehensive technical reports and datasets in agreed formats.

3. Scope of Work

3.1 Sample

- **Number of samples for amplification** :40
- **Number of samples for sequencing**: 25
- **Handling requirements:** Maintenance of DNA quality under cold-chain conditions, where required

3.2 DNA Extraction

- **Method:** CTAB-based protocol or commercial kits optimized for citrus polysaccharide- and polyphenol-rich tissues
- **Quality control:** Agarose gel images and DNA quantification using Nanodrop and/or Qubit fluorometry to be shared with the client

3.3 Molecular Marker Analysis

A. SSR Genotyping

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प्रमुख

- Number of loci: 12–20 SSR markers
- Marker selection criteria:
    - High polymorphism (PIC ≥ 0.5 preferred)
    - Genome-wide distribution
    - Prior validation in citrus/mandarin

B. SNP Genotyping

- Approach: Targeted amplicon sequencing or other suitable SNP genotyping platforms
- Minimum sequencing depth: ~100× per locus

4. Bioinformatics and Data Analysis

4.1 Data Processing

- Raw data quality assessment (Fast QC or equivalent)
- Adapter trimming
- Alignment to a reference citrus genome
- SNP/SSR calling and filtering

4.2 Genetic Diversity Statistics

- Number of alleles (Na)
- Effective number of alleles (Ne) and allelic richness
- Major allele frequency
- Observed heterozygosity (Ho)
- Expected heterozygosity (He)
- Polymorphism information content (PIC)

4.3 Population Genetics and Structure Analysis

- Genetic distance matrix
- Principal Component Analysis (PCA) / Principal Coordinate Analysis (PCoA)
- Analysis of Molecular Variance (AMOVA)
- Pairwise FST estimation
- UPGMA and/or Neighbor-Joining phylogenetic tree
- STRUCTURE or ADMIXTURE analysis with K-value determination

4.4 Fingerprinting Panel Development

- Identification of a minimal informative marker set (8–12 SSR loci or SNPs) capable of uniquely distinguishing all genotypes

5. Deliverables

5.1 Technical Deliverables

- DNA quality control report (Nanodrop/Qubit results and gel images)
- Raw sequencing data (FASTQ files)
- Cleaned reads and alignment files (BAM)
- SNP/SSR genotype matrices (VCF and Excel formats)
- Genetic diversity analysis outputs, including:
    - Dendrograms and clustering outputs

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प्रमुख

- PCA/PCoA plots
- STRUCTURE/ADMIXTURE plots
- Diversity statistics tables
- Pairwise FST matrix
- Heatmaps

5.2 Final Report

- Detailed methodology
- Marker and fingerprinting panel specifications
- Summary of genetic diversity and population structure results
- Biological interpretation of findings
- Recommendations for marker deployment and future work

6. Timeline

- DNA extraction and QC: 2–4 weeks
- SSR genotyping or sequencing: 2–4 weeks
- Bioinformatics analysis and reporting: 4–6 weeks
- **Total duration:** Approximately 3–3.5 months from receipt of samples

7. Required Qualifications of the Service Provider

- Dedicated bioinformatics team with demonstrated experience in SSR/SNP data analysis
- Past work experience of the laboratory in the molecular research in agriculture or allied biological sciences
- Valid license from the competent authority to conduct molecular tests for at least the past two years
- Availability of next-generation sequencing platforms will be given priority

8. Confidentiality and Data Ownership

- All raw and analyzed data generated under this assignment shall remain the sole property of the client
- No publication, dissemination, or third-party sharing of data is permitted without prior written consent of the client

9. Proposal Submission Requirements

- Technical proposal
- Laboratory biosafety and QA/QC procedures
- Detailed project timeline
- CVs of key technical and bioinformatics personnel
- Cost breakdown (DNA extraction, genotyping/sequencing, bioinformatics analysis)

10. Laboratory Infrastructure, Quality Assurance, and Compliance Requirements

10.1 Laboratory Infrastructure

- Separate pre-PCR, PCR, and post-PCR areas
- Dedicated DNA/RNA extraction facility with contamination control
- Functional clean benches/laminar flow hoods, biosafety cabinets, and fume hoods
- Power backup systems (UPS/inverter)
- Autoclave facilities

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प्रमुख

10.2 Equipment and Instrumentation

- Tissue homogenization system (e.g., TissueLyser or LN-based facility)
- Calibrated PCR thermal cyclers and real-time PCR systems
- Gel electrophoresis and documentation systems
- Refrigerators (4°C) and freezers (−20°C/−80°C) with temperature logs
- Microcentrifuges (refrigerated and non-refrigerated)
- DNA quantification instruments (Nanodrop/Qubit/fluorometer)
- Pipettes (0.1-1000 µL), pipette tips
- Vortex mixer
- Magnetic stirrer (optional?)
- Water bath/dry heat block
- Analytical balance
- Water deionizer/mol. grade water unit
- Autoclave

10.3 Quality Assurance and SOPs

- Documented SOPs for molecular workflows, sequencing, genotyping, and data analysis
- Equipment calibration certificates and maintenance records
- Negative controls included in all PCR assays

10.4 Data Management and Biosafety

- Secure data storage with regular backups
- Traceable documentation from sample receipt to final analysis
- Compliance with national biosafety, ethical, and regulatory requirements

11. Audits and Performance Indicators

- Reproducibility and failure/contamination rates of molecular assays
- Turnaround time for workflows
- Validation of new assays and pipelines
- Periodic performance and compliance audits

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प्रमुख