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Whole-genome Sequencing Reveals Functional Genomic Variation in the Traditional Rice Landrace Iluppai Poo Samba (*Oryza sativa* L.)

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ABSTRACT

Background: Traditional rice landraces represent valuable reservoirs of genetic diversity associated with agronomically important traits, stress adaptation, nutritional quality and regional adaptation. Iluppai Poo Samba (*Oryza sativa* L.) is a traditional South Indian rice landrace cultivated in Tamil Nadu, India, valued for its characteristic aroma, grain quality and adaptation to local agroecological conditions. However, genomic information for this cultivar remains limited. The present study aimed to characterize genome-wide sequence variation in Iluppai Poo Samba through whole-genome sequencing (WGS).

Methods: High-quality paired-end sequencing libraries were prepared using the NEXTflex Rapid DNA Sequencing platform and sequenced using Illumina chemistry. Sequence reads were subjected to quality assessment, genome alignment, variant calling, genome-wide SNP density analysis and functional annotation of genomic variants.

Result: Sequencing quality assessment demonstrated high-quality reads with Q20 values exceeding 97% and Q30 values exceeding 94%, with an average GC content of approximately 45%. A total of 1,410,690 SNPs and 138,431 InDels were observed across the genome. Genome-wide SNP analysis revealed extensive chromosomal variation, heterogeneous SNP distribution patterns and distinct polymorphic hotspot regions. Functional annotation detected widespread intergenic, intronic and coding-region polymorphisms, including 44,070 synonymous and 51,380 non-synonymous SNPs. Transition/transversion (Ts/Tv) ratios ranging from 2.24 to 2.50 supported the reliability of variant identification. Variants associated with stress-responsive transporters, kinase signalling proteins, transcription factors and regulatory genes were also detected, indicating potentially important adaptive genomic signatures within the Iluppai Poo Samba genome.

Key words: Functional annotation, Genetic diversity, Genomic variation, Iluppai poo samba, *Oryza sativa*, Single nucleotide polymorphism (SNP), Traditional rice landrace.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops globally and serves as a primary dietary energy source for a large proportion of the world's population (Khush, 2005). Beyond modern high-yielding cultivars, traditional rice landraces represent valuable reservoirs of genetic diversity that contribute to crop adaptation, resilience, grain quality and sustainable agricultural production (McNally *et al*., 2009). Indigenous rice cultivars cultivated across diverse agroecological regions often harbor unique allelic combinations associated with tolerance to biotic and abiotic stresses, nutritional quality, aroma and environmental adaptation (Zhao *et al.,* 2011). India is one of the major centers of rice diversity and possesses a rich collection of traditional rice germplasm maintained through centuries of farmer-led selection and cultivation practices (Roy *et al*., 2015; Singh *et al*., 2025). Among these, the traditional Tamil Nadu landrace Iluppai Poo Samba is recognized for its characteristic aroma, grain quality and adaptation to regional agroecological conditions. Such traditional landraces constitute valuable genetic resources for rice improvement because they harbor allelic variation associated with stress resilience, grain quality and environmental adaptation (Bradbury *et al.,* 2005;Burlando and Cornara, 2014; Varshney *et al*., 2018).

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Despite their agricultural and cultural significance, many traditional rice landraces remain genomically underexplored. Advances in next-generation sequencing technologies have enabled comprehensive characterization of genome-wide variation through whole-genome sequencing (WGS), facilitating the identification of single nucleotide polymorphisms (SNPs), insertions/deletions (InDels) and other genomic variants (Doyle, 1991; Priyadharshini *et al*., 2019). More recently, advances in high-throughput sequencing, pangenome analysis and long-read genome

assembly have facilitated the discovery of functional genomic variation in rice, providing valuable insights into genetic diversity, adaptation and trait-associated alleles in traditional germplasm (Guo *et al*., 2025; Shang *et al*., 2022; Yang *et al*., 2024). Genome-wide variant analysis combined with functional annotation enables the identification of polymorphisms associated with stress-responsive pathways, signaling mechanisms and agronomically important traits in rice (McCouch *et al*., 2010; Cingolani *et al*., 2012; Huang *et al*., 2010; Trung *et al*., 2017). Despite its importance, comprehensive genomic information for Iluppai Poo Samba remains unavailable. Therefore, the present study aimed to generate a whole-genome sequencing resource for the traditional rice landrace Iluppai Poo Samba and characterize genome-wide sequence variation across its genome. High-throughput Illumina sequencing generated paired-end genomic reads, followed by SNP density analysis, chromosome-wise variation profiling and functional annotation of genomic variants. The genomic resource generated in this study provides foundational information for future functional genomics, molecular breeding, comparative genomics and conservation studies involving traditional rice germplasm.

MATERIALS AND METHODS

Plant material and genomic DNA isolation

Seeds of the traditional rice landrace Iluppai Poo Samba (*Oryza sativa* L.) were obtained from farmers in Tamil Nadu, India. Seedlings were grown under greenhouse conditions at VIT School of Agricultural Innovations and Advanced Learning (VAIAL), Vellore Institute of Technology, Tamil Nadu, India (Lat. 12°58′05.56″ N, Lon. 079°09′39.96″ E). Young healthy leaves collected from greenhouse-grown seedlings were used for genomic DNA isolation. Genomic DNA was extracted from young leaf tissues using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle, 1991; Priyadharshini *et al*., 2019). DNA quality and concentration were assessed using NanoDrop spectrophotometry, Qubit fluorometric quantification and agarose gel electrophoresis prior to library preparation. High-quality genomic DNA samples exhibiting optimal purity ratios with 260/280 values ranging from 1.97 to 2.01 and 260/230 values of approximately 2.05 were selected for downstream sequencing analysis. Qubit-based DNA concentrations ranged from 176 to 178 ng μL^{-1} prior to library preparation.

Whole-genome library preparation and sequencing

Whole-genome sequencing libraries were prepared using the NEXTflex Rapid DNA Sequencing Bundle (BIOO Scientific, USA) following the manufacturer's instructions for Illumina-compatible paired-end sequencing. Approximately 500 ng of genomic DNA was fragmented using a Covaris S220 sonicator to obtain DNA fragments within the target size range of 200-350 bp. Fragmented DNA was purified using JetSeq magnetic beads followed by end repair, adenylation and ligation with indexed Illumina adapters. Adapter-ligated fragments were PCR amplified and purified prior to library quality assessment.

Library quality and fragment size distribution were evaluated using the Agilent TapeStation platform. Average library fragment sizes ranged from 433 to 446 bp, indicating successful library preparation and appropriate fragment distribution for Illumina sequencing. Paired-end sequencing was performed using Illumina sequencing chemistry with a read length of 150 bp. Sequencing reads were generated in FASTQ format for downstream bioinformatic analysis.

Sequencing quality assessment

Raw sequencing quality was evaluated based on total reads, total bases, GC content and base quality score distribution. Sequencing quality metrics including Q20 and Q30 values were calculated to assess overall sequencing performance and read reliability (Bolger *et al.,* 2014). Read quality distribution across sequencing cycles was further evaluated using quality profiling analysis to ensure consistency of base-calling performance across generated datasets.

Genome alignment and variant calling

Raw paired-end sequencing reads generated from Iluppai Poo Samba samples were subjected to adapter trimming and quality filtering using Trim Galore to obtain high-quality adapter-free reads. The filtered reads were aligned against the reference genome *Oryza sativa japonica* (NCBI assembly accession: GCA_034140825.1) using the Burrows-Wheeler Aligner (BWA) algorithm (Li and Durbin, 2009). Following alignment, duplicate reads were characterised and filtered using SAMtools fixmate and markdup utilities to improve alignment accuracy and reduce PCR-derived redundancy (Li *et al.,* 2009).

Variant calling was performed using bcftools mpileup to identify genome-wide single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) (Danecek *et al.,* 2021). The resulting variants were filtered based on quality parameters following previously reported criteria (Bindusree *et al.,* 2017). Variants exhibiting low mapping quality, insufficient read support and ambiguous base calls were excluded to retain high-confidence SNPs and InDels for downstream analyses.

Functional annotation of variants

Functional annotation of identified variants was performed using SnpEff to classify variants based on their genomic location and predicted functional impact (Cingolani *et al.,* 2012). Functional annotation databases implemented within SnpEff were used to predict variant effects relative to annotated rice gene models. Variants were categorized into intergenic, intronic, upstream, downstream, synonymous, non-synonymous, splice-region, stop-gain and stop-loss variants. Transition and transversion mutations were further quantified to evaluate mutation patterns and variant calling reliability. Candidate genes were identified from SnpEff-annotated variants based on

their predicted functional effects and previously reported biological roles in stress adaptation, signaling pathways, transport mechanisms and developmental regulation in rice.

Genome-wide SNP density analysis

Genome-wide SNP density analysis was performed using non-overlapping 100 kb genomic windows across all rice chromosomes. SNP density distributions were visualized to identify chromosomal regions exhibiting elevated polymorphism frequencies and heterogeneous variation patterns throughout the genome. Chromosome-wise SNP distribution analysis was further performed to examine genomic regions exhibiting variable SNP abundance across the Iluppai Poo Samba genome.

Data visualization and statistical analysis

Genome-wide SNP density plots, chromosome-specific variation profiles and functional variant classification plots were generated using R software. Variant summary statistics, including transition/transversion ratios and functional annotation categories, were calculated from filtered high-confidence variant datasets. Quality assessment and downstream analyses were performed using standard bioinformatics workflows implemented in Linux and R environments.

RESULTS AND DISCUSSION

Sequencing quality and library assessment

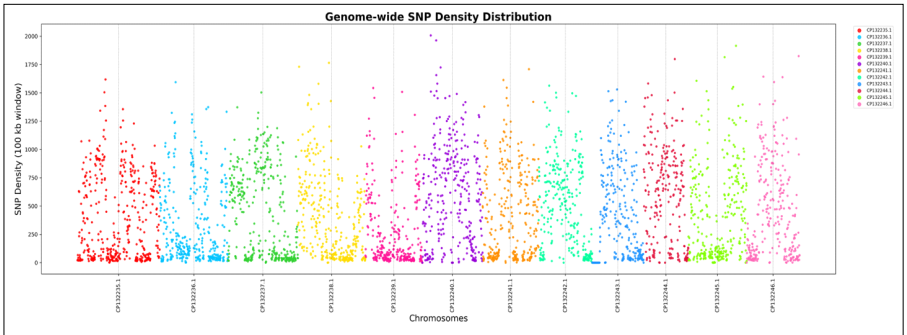
Whole-genome sequencing of the traditional rice landrace Iluppai Poo Samba generated high-quality paired-end sequencing data suitable for downstream analysis. Sequencing quality assessment demonstrated consistently high base-calling accuracy across both technical replicates. Quality metrics indicated that more than 97% of bases achieved Q20 scores, while over 94% of bases exceeded Q30 quality thresholds, confirming the generation of high-confidence sequencing reads. The average GC content of the sequencing libraries was approximately 45%, which is consistent with the expected genomic GC composition of *Oryza sativa*. Quality score distribution profiles further demonstrated stable sequencing performance across read lengths, indicating minimal decline in base quality across sequencing cycles.

Library quality assessment using the Agilent TapeStation platform confirmed successful library preparation and appropriate fragment size distribution for Illumina sequencing. Average library fragment sizes ranged from approximately 433 to 446 bp, indicating uniform fragmentation and efficient adapter ligation during library preparation. The high sequencing quality and optimal library profiles collectively supported the reliability of downstream genome alignment and variant detection analyses.

Genome-wide SNP distribution and genomic variation patterns

Genome-wide variant analysis revealed extensive sequence variation across all rice chromosomes in the Iluppai Poo Samba genome. SNP density analysis using non-overlapping 100 kb genomic windows demonstrated heterogeneous distribution of polymorphisms throughout the genome, with several chromosomal intervals exhibiting elevated SNP density relative to the reference genome (Fig 1). Distinct SNP-enriched hotspot regions and low-variation intervals were observed across multiple chromosomes, indicating uneven genome-wide polymorphism patterns.

The observed variation in SNP density suggests differential accumulation of sequence variation across chromosomal regions, potentially reflecting differences in evolutionary conservation, recombination frequency, selection pressure and genomic organization (Huang *et al.,* 2011). Similar heterogeneous SNP distribution patterns have been reported in other rice genomic studies and are often associated with adaptive divergence and cultivar-specific variation (Trung *et al.*, 2017; Wang *et al.*, 2009).



SNP density was calculated using non-overlapping 100 kb genomic windows relative to the *Oryza sativa japonica* reference genome. Distinct chromosomal variation patterns and polymorphism hotspot regions were observed across multiple chromosomes, indicating heterogeneous genome-wide sequence variation within the Iluppai Poo Samba genome.

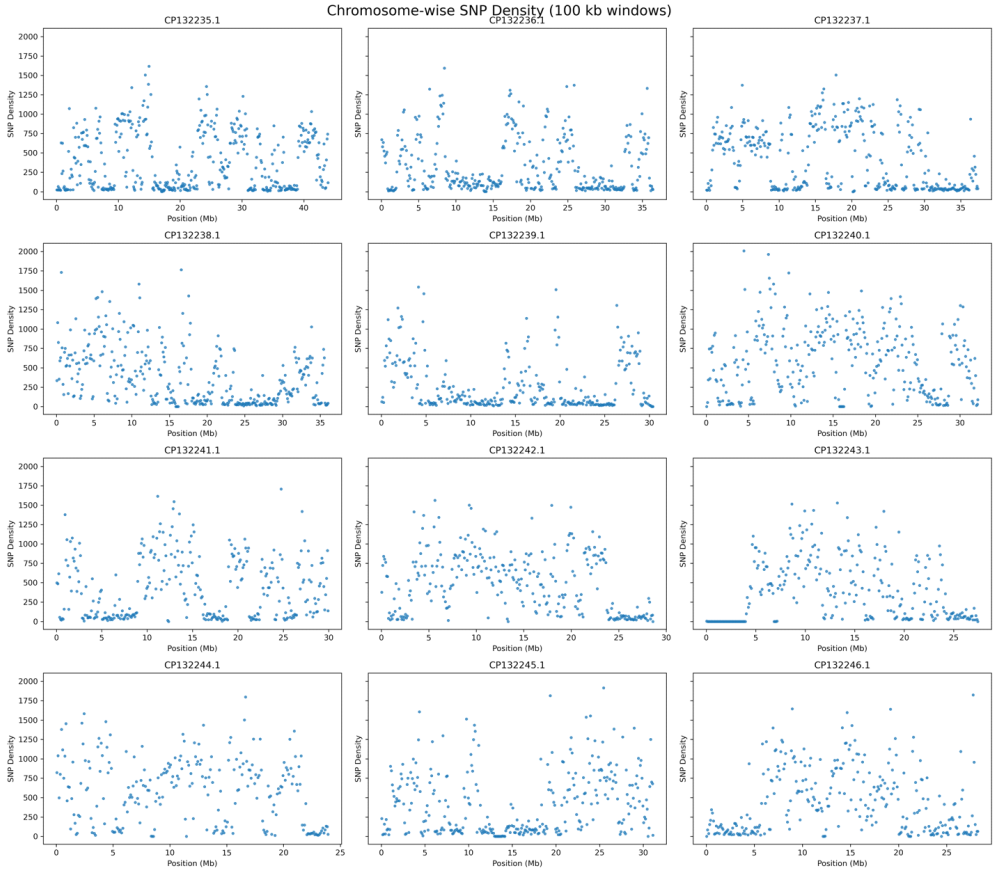
Fig 1: Genome-wide SNP density distribution across the Iluppai Poo Samba genome.

The widespread polymorphism detected in Iluppai Poo Samba indicates substantial genomic differentiation from the *Oryza sativa* japonica reference genome and highlights the unique genetic diversity retained within this traditional landrace. The heterogeneous distribution of SNPs across the genome suggest that different chromosomal regions have experienced varying levels of genetic diversification and selective pressure during the evolution of Iluppai Poo Samba. Polymorphism-rich genomic regions may harbor genes associated with environmental adaptation, stress tolerance and other agronomically important traits, whereas relatively conserved regions are likely enriched for genes under stronger functional constraint. These genomic variation patterns provide valuable targets for future trait mapping, functional validation and marker-assisted breeding in traditional rice germplasm (Huang *et al.*, 2011; Wang *et al.*, 2009).

Chromosome-wise variation profiling

Chromosome-wise SNP distribution analysis revealed considerable variation in polymorphism abundance across individual rice chromosomes. Several chromosomes exhibited broader high-density polymorphic regions, whereas others contained localized SNP-enriched intervals interspersed with relatively conserved regions (Fig 2). In particular, chromosomes CP132240.1, CP132244.1, CP132245.1 and CP132246.1 displayed extensive high-density polymorphic regions, while chromosomes CP132239.1 and CP132243.1 exhibited more localized SNP enrichment patterns.

The uneven distribution of SNPs across chromosomes may reflect differences in recombination dynamics, mutation rates, selective constraints and historical evolutionary processes. Regions exhibiting elevated SNP abundance may represent genomic intervals associated with increased genetic diversity and adaptive variation, whereas regions with lower SNP density may correspond to more conserved genomic segments. These chromosome-specific variation landscapes provide useful targets for future investigations of agronomically important traits, including stress adaptation,



SNP density profiles were generated using non-overlapping 100 kb genomic windows across individual rice chromosomes. Distinct chromosome-specific polymorphism landscapes and SNP-enriched hotspot regions were observed across multiple chromosomal intervals, indicating heterogeneous genomic diversification within the Iluppai Poo Samba genome.

Fig 2: Chromosome-wise SNP density distribution across the Iluppai Poo Samba genome.

grain quality and aroma-associated characteristics in traditional rice germplasm. Chromosome-specific differences in SNP abundance may also reflect historical recombination events and cultivar-specific evolutionary processes that have shaped the genetic architecture of Iluppai Poo Samba. The identification of polymorphism-rich chromosomal intervals provides a valuable framework for future quantitative trait locus (QTL) mapping and genome-wide association studies aimed at identifying loci controlling stress tolerance, grain quality and other economically important traits (Huang *et al.,* 2011; 2010).

Functional classification of genomic variants

Functional annotation of genome-wide variants revealed extensive sequence variation distributed across coding and non-coding genomic regions of the Iluppai Poo Samba genome. A substantial proportion of identified variants were localized within intergenic and intronic regions, indicating widespread regulatory and non-coding genomic variation across the genome. In addition, large numbers of synonymous and non-synonymous SNPs were identified across all chromosomes, suggesting substantial functional variation within protein-coding genes. High-impact variants including stop-gain and stop-loss mutations were also detected across multiple chromosomal regions, potentially contributing to altered gene function and phenotypic diversification. The presence of extensive coding and regulatory-region polymorphisms indicates that genomic variation within Iluppai Poo Samba extends beyond neutral variation and may influence multiple biological pathways associated with adaptation and agronomic performance.

Transition mutations were consistently more abundant than transversion mutations across all chromosomes, with Ts/Tv ratios ranging from 2.24 to 2.50. These values are consistent with mutation patterns commonly observed in plant genomes and support the reliability and biological consistency of the identified variants. Furthermore, the substantial number of non-synonymous variants identified in coding regions suggests the presence of functional allelic diversity that may influence protein function and contribute to phenotypic variation associated with stress responses, development and environmental adaptation. Although experimental validation is required to confirm their biological effects, these variants represent promising candidate loci for future functional genomics, marker development and rice improvement programs (Depristo *et al.*, 2011; Wakeley, 1996). Detailed summary statistics of genomic variants identified throughout the Iluppai Poo Samba genome are presented in Table 1.

Candidate genes harboring functional variants

Functional annotation identified variants in biologically important genes involved in stress adaptation, ion transport, signaling pathways and transcriptional regulation. Variants were identified in HKT transporter family genes including HKT1;1, HKT1;3, HKT2;1 and HKT2;4, which are known to

Table 1: Summary statistics of genomic variants identified across the Iluppai Poo Samba genome.

Chromosome	SNPs	InDels	Intergenic	Intronic	Coding variants	Synonymous	Non-synonymous	Stop gain	Stop loss	Ts/Tv ratio
Chr01	156,213	18,527	124,713	28,342	12,101	5,338	6,638	86	36	2.50
Chr02	107,443	12,426	83,640	21,148	8,422	3,893	4,440	60	19	2.47
Chr03	48,567	6,102	37,346	10,665	3,404	1,661	1,703	28	8	2.45
Chr04	126,531	11,035	103,059	20,674	8,420	3,773	4,546	80	26	2.33
Chr05	73,671	8,713	60,225	12,302	4,981	2,320	2,605	45	11	2.39
Chr06	174,405	16,915	141,050	28,972	10,972	5,255	5,634	61	28	2.40
Chr07	118,602	11,535	96,208	19,057	8,159	3,656	4,431	63	20	2.40
Chr08	134,004	12,688	111,008	20,220	7,845	3,576	4,166	77	19	2.48
Chr09	99,964	8,831	82,541	15,350	5,681	2,639	2,979	38	19	2.40
Chr10	131,750	11,370	107,849	20,339	7,639	3,616	3,962	44	22	2.47
Chr11	122,106	10,882	89,806	23,340	11,951	5,274	6,566	100	38	2.24
Chr12	117,434	9,407	99,456	15,202	6,864	3,069	3,710	62	28	2.28
Total	1,410,690	138,431	1,136,901	235,611	96,439	44,070	51,380	744	274	2.40

Variant annotation included coding and non-coding polymorphisms identified following genome-wide SNP and InDel analysis relative to the *Oryza sativa japonica* reference genome. Transition/transversion (Ts/Tv) ratios indicated high-confidence genome-wide variant identification across all rice chromosomes.

regulate sodium transport and ion homeostasis during salinity stress in rice. Genetic variation within these transporter-associated loci may influence salinity tolerance and environmental adaptation by altering ion transport efficiency. Although functional validation is required, these variants represent promising candidate alleles for future studies aimed at improving stress resilience in rice cultivars (Liu *et al.,* 2022).

In addition, variants were detected in kinase-related genes including CPK7, CPK13 and CPK19, which function as key regulators of cellular signaling pathways involved in plant responses to abiotic and biotic stresses. Variants were also identified in KIN17, a conserved DNA/RNA-binding protein implicated in genome maintenance and cellular homeostasis, suggesting additional functional diversity within genes associated with cellular regulation. Additionally, variants were identified in transcription factor and regulatory genes including HSFA2D, GATA15 and BZIP39. These transcription factors regulate stress-responsive gene expression, developmental processes and metabolic pathways, suggesting that functional polymorphisms within these genes may contribute to cultivar-specific adaptation and phenotypic diversity.

Furthermore, variants were detected in genes associated with developmental regulation and cell wall biosynthesis, including IRX9L and IRX14, indicating additional sources of functional genomic diversity that may influence plant growth and structural development. Collectively, these findings suggest that Iluppai Poo Samba harbors a diverse repertoire of functional genetic variation extending beyond neutral sequence polymorphisms. The identified candidate genes provide promising targets for future functional validation, marker-assisted breeding and the genetic improvement of stress resilience and grain quality in rice.

CONCLUSION AND FUTURE PERSPECTIVES

The present study provides the first comprehensive whole-genome and functional variant characterization of the traditional rice landrace Iluppai Poo Samba (*Oryza sativa* L.) and establishes a foundational genomic resource for this indigenous cultivar. High-quality Illumina sequencing generated reliable genomic datasets, while genome-wide SNP profiling revealed heterogeneous patterns of polymorphism distribution and distinct chromosomal hotspot regions across the rice genome.

Functional annotation identified extensive variation across coding and non-coding genomic regions, including synonymous, non-synonymous and high-impact variants. The observed transition/transversion ratios supported the reliability of variant identification. Variants detected in stress-responsive transporters, kinase signalling proteins, transcription factors and developmental regulatory genes further indicated the presence of potentially important adaptive genomic signatures within the Iluppai Poo Samba genome.

The genomic resource generated in this study provides a valuable foundation for the conservation, characterization and utilization of traditional rice germplasm and will facilitate future molecular breeding, marker-assisted selection and comparative genomics studies. Future integration of transcriptomics, functional genomics and trait-associated marker validation will further improve understanding of the genetic basis of agronomically important traits in Iluppai Poo Samba and related traditional rice landraces.

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Data availability statement

The raw whole-genome sequencing data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject accession number PRJNA1473127. The associated BioSample accession number is SAMN60541900 and the raw sequencing reads are available under SRA accession numbers SRR38927863 and SRR38927864.

Disclaimers

The views and conclusions expressed in this article are those of the authors and do not necessarily reflect the views of their affiliated institutions.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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