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Identification of Critical Genes In Feline Sarcomatoid Renal Cell Carcinoma

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Short Report

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Abstract

Objective

In this study, we sequenced Feline Sarcomatoid Renal Carcinoma (OBKI) cells that would shine a spotlight on our current knowledge of feline kidney oncogenome and may serve as a foundation for much-needed research.

Method

Whole genome sequencing of OBKI cells was performed.

Results

Using loss-of-function (LOF) prediction analysis, we identified five genes associated with feline kidney cancer. TSC2 gene has highest LOF value of 1, indicating it has a high-impact effect resulting in loss of gene function in OBKI cell line.

Conclusions and relevance

Cancer represents a significant cause of morbidity and mortality in domestic cats. In humans, detailed understanding of the oncogenome across cancer types has been pivotal to modern oncology, shaping diagnostic and prognostic strategies and enabling the use of targeted therapeutic approaches. Research into the genetic basis of feline cancers initially focused on cytogenetic approaches and subsequently progressed to gene-specific analyses aimed at identifying mutations and expression-level alterations in genes frequently mutated in human malignancies.

1. Introduction

Oncology is a rapidly evolving field in small-animal medicine, highlighting the need for innovative treatment strategies. Cancer remains a major cause of illness and death in domestic cats, with numerous studies indicating that a high proportion of tumors in these animals are malignant[1–4]. Unfortunately, feline cancers, especially renal neoplasia, have not been studied as extensively as human or canine cancers.

Feline kidney cancer is a rare but clinically significant disease, characterized by its aggressive behavior and limited treatment options. The kidneys are vital organs responsible for filtering waste from the bloodstream, regulating electrolyte and fluid balance, producing hormones that stimulate red blood cell production, and maintaining blood pressure. When cancer affects the kidneys, these essential functions may be compromised. There is limited knowledge in understanding the complexities of feline kidney

cancer, including its types, clinical signs, diagnostic approaches, treatment options, and overall prognosis.

Feline renal neoplasms are uncommon but clinically significant malignancies. **Renal lymphoma** is the predominant tumor type accounting for 45% of the cases[1], typically presenting bilaterally and frequently associated with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections[2]. **Primary renal carcinomas**, while less common, demonstrate aggressive local invasion and a high propensity for metastasis. It accounts for approximately 2.5% of all feline neoplasms[3]. Rare tumor types include **transitional cell carcinoma** of the renal pelvis and nephroblastoma, the latter primarily affecting juvenile cats and offering insights into renal oncogenesis. **Mesenchymal tumors** (5% cases in cats) such as hemangiosarcoma (HSA), fibrosarcoma (FSA), chondrosarcoma (CSA), and leiomyosarcoma are exceedingly rare but highly malignant.

The kidney also serves as a common site for metastatic involvement, particularly from mammary carcinoma and systemic lymphoma. Despite their clinical impact, feline renal tumors remain poorly characterized, underscoring the need for focused studies to improve diagnostic accuracy, therapeutic strategies, and comparative oncologic understanding.

Renal cell carcinoma (RCC) is the most common tumor of renal tissue origin in cats[4]. Cats are often 8–11 y old at diagnosis, with male cats slightly over-represented[4]. RCC typically affects only one kidney, but bilateral involvement does occur[5]. Histologic subtypes include tubular, papillary, solid, and cystic morphologies. These morphologic subtypes are subclassified based on cellular appearance as clear cell, chromophobic, or oncocytic/eosinophilic[5]. Regions of different morphologic and cellular appearances may occur within a single tumor[5]. Although not considered a primary histologic RCC subtype, renal cell carcinomas containing areas of neoplastic cells with spindle-cell morphology are termed sarcomatoid renal cell carcinomas (sRCCs). Rarely, sRCCs may be composed of only spindle-shaped cells [6, 7]. In the feline population, cases of sRCC are rare but often misdiagnosed, leading to delayed treatment leading to aggressive biological behavior and lack of therapeutic benefit.

Due to the lack of molecular characterization of feline sRCC, there are currently no effective diagnostic biomarkers or targeted treatment options available for affected cats. Most information about them comes from human studies. sRCC accounts for only 8% of human RCCs[7]. sRCC is an aggressive form of kidney cancer with a poor prognosis, having a median overall survival of approximately 8 months. The presence of distant metastases commonly in the lungs, bones, liver, and brain significantly worsens outcomes, increasing the risk of mortality. However, treatment with nephrectomy and systemic therapies has been shown to improve survival, reducing the mortality risk for affected patients.

Our previous study of feline sRCC[8] is similarly invasive with near effacement of the left kidney, areas of necrosis, and extension beyond the renal capsule. Although peritoneal metastasis has been reported in feline sRCC elsewhere[9, 10], we observed significant peritoneal effusion that has not been described previously. Immunohistochemical (IHC) evaluation of the renal mass showed positive results for screening panels like pancytokeratin, vimentin, cytokeratin 18, and smooth muscle actin (SMA, alpha sm-

1) immunolabeling[8]. sRCC was confirmed with positive staining of immunoreactivity for PAX8 [8]. Then the spindle-shaped epithelial cells were immortalized commercially. These cells are characterized by a doubling time of 36–48 hours and are utilized in cancer research, particularly for studying feline renal tumors. Thus, this study reports on the genetic profile of sRCC cells and identified critical oncogenic genes.

2. Materials and Methods

2.1 Cell Culture

OBKI cells were established from neoplastic tissue obtained at autopsy from the primary renal mass of a 9-year-old castrated male domestic medium-hair cat. These cells were cultured in RPMI-1640 supplemented with 10% FBS (Cat. no. A5256701, ThermoFisher Scientific, USA). OBKI cells are commercially available through Applied Biological Materials (Abm) Inc (Cat. No. T8093).

2.2 Whole-Genome Sequencing

2.2.1. DNA Isolation

Primary cells were cultured using the RPMI, and we used passage 9 to isolate DNA using QIAamp DNA Micro Kit (Cat. No. 56304, QIAGEN, Hilden, Germany). The quality and quantity of DNA were analyzed using an Invitrogen™ Nanodrop™ One C (Thermo Fisher Scientific, Waltham, MA, USA) and Invitrogen™ Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.2.2. Library Preparation and Sequencing

gDNA was used to make a 150-base-pair paired-end library (NextSeq DNA PCR kits, Illumina, San Diego, CA, USA) and sequenced on a NextSeq1000, with a quality score of greater than 30 achieved. The raw reads were filtered to remove low-quality reads and adaptor sequences. These filtered reads were then analyzed using the fastqc program. After analyzing the quality of the reads, the reference genome was indexed using Bowtie2 and subsequently mapped to the NIH reference genome, Felis catus 9.0, using Bowtie2. After alignment to the reference genome, the resulting output was converted to BAM format using the Samtools function. The BAM files were then sorted using Samtools. Variant detection was performed using the Sneff. Initial variant calling was conducted using the Felis catus 9.0 reference genome and high-quality BAM files, followed by variant annotation using a genomic feature file (GFF). Several filtering steps were applied to enhance data quality, including filtering based on variant quality scores and read depth thresholds. Summary statistics were then generated to provide an overview of the identified and annotated variants.

3. Results

Quality Control Summary:

The initial quantity 75.8 ng/µl of DNA was used for library preparation. A quality control showed 6.46 ng/µl of DNA using Qubit 4.0. As shown in Figure 1, DNA fragments of size 575 bp on Agilent Tapestation 4200 was loaded on the P2 600 cycles cartridge for whole genome sequencing.

Quality control analysis of the raw sequencing data was performed using FastQC version 0.12.0. The dataset contained 797,553,317 reads, comprising a total of 58.8 Gb of sequence data. The per-base sequence quality remained high across all reads in Figure 2, with the majority of bases scoring Q33, indicating excellent read accuracy. Reads were generated using Illumina sequencing with Sanger/Illumina 1.9 (Phred+33) encoding. Read lengths ranged from 35 to 151 bp, indicating variable read length consistent with quality and/or adapter trimming as shown in Figure 3. No sequences were flagged as poor quality, demonstrating excellent overall read quality. The GC content was 41%, which falls within the expected range for mammalian genomes and indicates no obvious GC bias.

Alignment and Duplicate Summary:

Sequencing yielded 797,553,317 reads, of which 93.6% (746,490,972 reads) aligned to the reference genome. Of the 791,403,706 paired-end reads, 91.6% (724,852,608 reads) were properly paired, indicating high alignment concordance. Only 675,641 reads (0.09%) were classified as singletons. A small proportion of reads (9,858,002; ~1.2%) had mates mapping to different chromosomes, including 5,683,483 reads with mapping quality ≥5. Supplementary alignments accounted for 6,149,611 reads, consistent with split or chimeric mappings.

Duplicate read analysis identified an overall duplication rate of 18.1%, comprising 66,973,285 duplicated read pairs, 4,633,468 optical duplicates, and 406,235 duplicated unpaired reads. The estimated library size was 9.44×10^8 reads, indicating high library complexity despite deep sequencing coverage.

Whole genome sequencing of OBKI cells achieved an average coverage of 30x with alignment to reference feline genome (Cinnamon), confirming a feline genome and ruling out any other contamination. We filtered feline cancer genes and matched ~100 genes listed in Table 1. This study further explored kidney cancer genes in OBKI cells listed in Table 2. Impact LOF prediction refers to the assessment of whether a genetic variant is likely to cause a loss of function (LOF) in a gene and how severe that effect is. Based on loss of function (LOF) prediction we found 5 genes related to feline kidney cancer.

Table 1: List of Cancer Genes in OBKI cells

Table 2: List of Kidney Cancer Genes in OBKI cells

BAP1 encodes a ubiquitously expressed de-ubiquitinating enzyme that has important roles in a range of cellular processes, including contributions to transcriptional regulation, cell cycle and growth, response to DNA damage and chromatin dynamics[11]. *BAP1* have been associated with Küry–Isidor syndrome (Online Mendelian Inheritance in Man, 619762), a neurodevelopmental disorder[12]. It remains unclear why BAP1 loss is associated with uveal melanoma, cutaneous melanoma, mesothelioma,

cholangiocarcinoma, renal cancer and meningioma, which are proportionally uncommon malignancies[11].

MET also known as KIT, is routinely assessed by IHC as a diagnostic biomarker to distinguish between different cancer types. The proto-oncogene KIT, is a receptor tyrosine kinase (RTK) encoded by the KIT gene[13]. Signal transduction pathways downstream of activated KIT include the mitogen-activated protein kinase/EC signal-regulated kinase (MAPK/ERK), phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), Janus kinase/signal transducer and activator of transcription (JAK/STAT), and phospholipase C-γ (PLCγ) pathways [14]. Dysregulated KIT function promotes tumorigenesis and progression of various cancer types through its activation or inappropriate expression. As mentioned elsewhere [15], KIT mutations have been reported in multiple feline cancer studies.

Interestingly, we found a high impact for TSC2 gene. In human, SEGA is most often present in the context of tuberous sclerosis, an autosomal dominant suppressor gene syndrome linked to two genetic loci: TSC1 and TSC2 [16, 17]. SEGA is also reported in a 6-year old female domestic short hair cat [18]. Tuberous sclerosis is characterized clinically by seizures, mental retardation, and autism and histopathologically by hamartomas and benign tumors arising in the brain, skin, kidney, and retina [19-21]. The TSC2 gene locus codes for the protein tuberin, which has GTPase activating protein (GAP) activity for Rap1 and Rab5 [16, 22, 23]. TSC2 gene has highest LOF value of 1, indicating it has a high-impact effect resulting in loss of gene function in OBKI cell line.

The endothelial Per-Arnt-Sim (PAS) domain protein 1 (EPAS1) gene encodes hypoxia-inducible-factor-2 alpha (HIF2a, a subunit of heterodimeric of transcription complex HIF-2) is a transcription factor that is involved in the response to hypoxia[24]. HIFs regulate numerous responses to hypoxia through transcription activation of multiple genes that either increase oxygen delivery or decrease oxygen consumption. It also regulates gene for vascular endothelial growth factor (VEGF) which promotes angiogenesis and increase vascular permeability in cancer [25, 26].

TP53 is a tumor suppressor gene frequently mutated in human cancers [27] and genetic aberrations in this gene have been observed in feline lymphoma cell lines [28] as well as in cat tumor tissues [29]. TP53 is a key player in cell cycle regulation and DNA damage response and its loss results in uncontrolled proliferation of damaged cells[30].

4. Discussion

The investigation of OBKI cells holds significant promise in advancing our understanding of cellular biology and their potential applications in medical research. Understanding the genetic architecture of feline sRCC will not only benefit veterinary medicine by enabling earlier diagnosis and more effective treatment strategies but also contribute to the growing field of comparative oncology, where naturally occurring cancers in companion animals serve as powerful models for human disease. Given the anatomical, physiological, and genomic similarities between cats and humans, studying cancer genomics in cats may uncover conserved oncogenic mechanisms relevant to both species. This study

addressed a critical knowledge gap by generating a comprehensive genomic and transcriptomic profile of feline sRCC and identifying novel molecular signatures associated with tumorigenesis. Future studies include the alteration of these genes, mode of action in tumorigenesis, and targeted drugs of these pathways.

Declarations

Ethical Approval:

This work did not involve the use of animals and therefore ethical approval was not specifically required for publication in *JFMS*.

Informed Consent:

This work did not involve the use of animals (including cadavers) and therefore informed consent was not required. No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

Funding Declaration

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Author Contribution

S.K. carried out experimental designs, performed data analysis, and drafted the manuscript.T.A.N. generated cell line, led the conceptualization of the project, provided technical and financial support, and finalized the manuscript.

Data Availability

The whole genome sequencing data of feline sarcomatoid renal cell will be available via the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject accession.

References

1. *Veterinary Society of Surgical Oncology-Renal Lymphosarcoma*. [cited 2025 9/11]; Available from: https://vsso.org/renal-tumors-feline
2. *Kidney (Renal) Cancer in Cats*. 9/11/2025; Available from: https://petcureoncology.com/kidney-renal-cancer-in-cats/
3. Crow S. *Urinary tract neoplasms in dogs and cats.* 1985.

4. Henry C, et al. Primary renal tumours in cats: 19 cases (1992–1998). J Feline Med Surg. 1999;1(3):165–70.
5. Meuten DJ, Meuten TL. Tumors of the urinary system. Tumors Domest Anim, 2016: pp. 632–88.
6. Gulbahar M, et al. Sarcomatoid renal cell carcinoma with scant epithelial components in an Angora cat. N Z Vet J. 2013;61(6):362–6.
7. Delahunt B. Sarcomatoid renal carcinoma: the final common dedifferentiation pathway of renal epithelial malignancies. Pathology. 1999;31(3):185–90.
8. Wu B, et al. Feline sarcomatoid renal cell carcinoma with peritoneal carcinomatosis and effusion. J Vet Diagn Invest. 2022;34(1):153–9.
9. Britt J, Ryan C, Howard E. Sarcomatoid renal adenocarcinoma in a cat. Vet Pathol. 1985;22(5):514–5.
10. Matsumoto I, et al. Histopathologic and immunohistochemistry findings in feline renal cell carcinoma. Vet Pathol. 2018;55(5):663–72.
11. Carbone M, et al. BAP1 and cancer. Nat Rev Cancer. 2013;13(3):153–9.
12. Küry S, et al. Rare germline heterozygous missense variants in BRCA1-associated protein 1, BAP1, cause a syndromic neurodevelopmental disorder. Am J Hum Genet. 2022;109(2):361–72.
13. Sabattini S, et al. Prognostic significance of Kit receptor tyrosine kinase dysregulations in feline cutaneous mast cell tumors. Vet Pathol. 2013;50(5):797–805.
14. Liang J, et al. The C-kit receptor-mediated signal transduction and tumor-related diseases. Int J Biol Sci. 2013;9(5):435.
15. Aupperle-Lellbach H, Kehl A, de Brot S, van der Weyden L. Clinical use of molecular biomarkers in canine and feline oncology: Current and future. Veterinary Sci. 2024;11(5):199.
16. Plank TL, Yeung RS, Henske EP. Hamartin, the product of the tuberous sclerosis 1 (TSC1) gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. Cancer Res. 1998;58(21):4766–70.
17. Yeung RS, Katsetos CD, Klein-Szanto A. Subependymal astrocytic hamartomas in the Eker rat model of tuberous sclerosis. Am J Pathol. 1997;151(5):1477.
18. Duniho S, et al. A subependymal giant cell astrocytoma in a cat. Vet Pathol. 2000;37(3):275–8.
19. Boesel CP, Paulson GW, Kosnik EJ, Earle KM. Brain hamartomas and tumors associated with tuberous sclerosis. Neurosurgery. 1979;4(5):410–7.
20. Nakamura Y, Becker L. Subependymal giant-cell tumor: astrocytic or neuronal? Acta Neuropathol. 1983;60(3):271–7.
21. Shepherd CW, et al. Subependymal giant cell astrocytoma: a clinical, pathological, and flow cytometric study. Neurosurgery. 1991;28(6):864–8.
22. Jin F et al. *Suppression of tumorigenicity by the wild-type tuberous sclerosis 2 (Tsc2) gene and its C-terminal region.* Proceedings of the National Academy of Sciences, 1996. 93(17): pp. 9154–9159.

23. Wienecke R, et al. *Reduced TSC2 RNA and protein in sporadic astrocytomas and ependymomas.* Annals of Neurology. Official J Am Neurol Association Child Neurol Soc. 1997;42(2):230–5.
24. Semenza GL. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. Annu Rev Pathol. 2014;9(1):47–71.
25. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell. 2012;148(3):399–408.
26. Sutter CH, Laughner E, Semenza GL. *Hypoxia-inducible factor 1α protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations.* Proceedings of the National Academy of Sciences, 2000. 97(9): pp. 4748–4753.
27. Kim S. New and emerging factors in tumorigenesis: an overview. Cancer Manage Res, 2015: pp. 225–39.
28. Mayr B, et al. Presence of p53 mutations in feline neoplasms. Res Vet Sci. 2000;68(1):63–9.
29. Miki R, et al. Centrosome amplification and chromosomal instability in feline lymphoma cell lines. J Vet Med Sci. 2004;66(7):797–805.
30. Marei HE, et al. p53 signaling in cancer progression and therapy. Cancer Cell Int. 2021;21(1):703.

Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

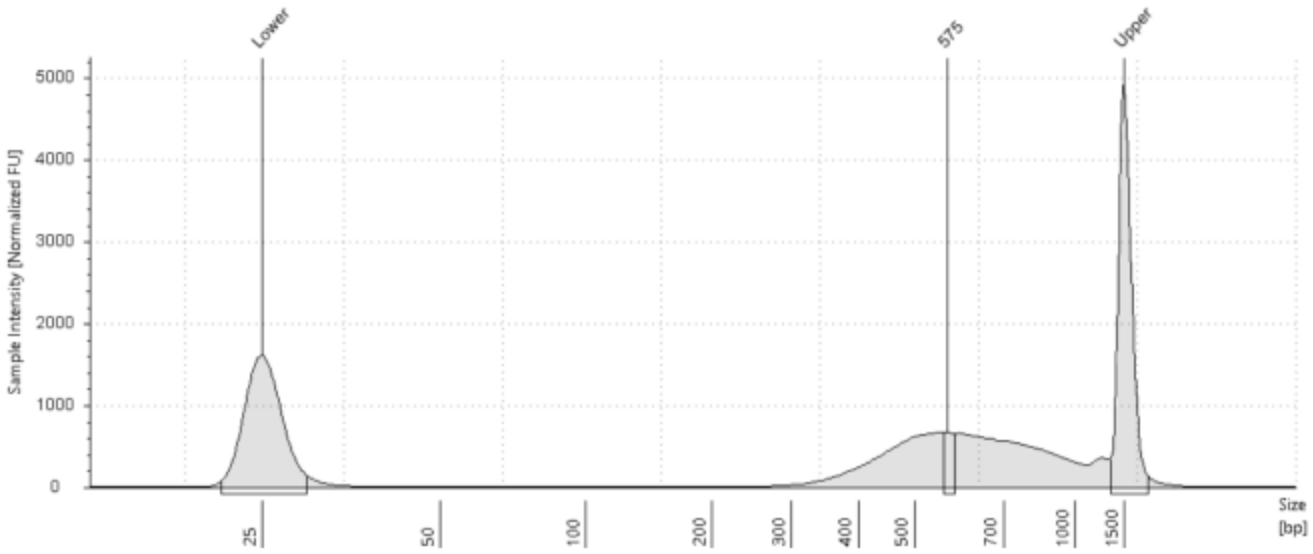


Figure 1

Quality control of DNA fragments on Tapestation. The figure shows DNA fragments of 300-575 bp after library preparation stage.

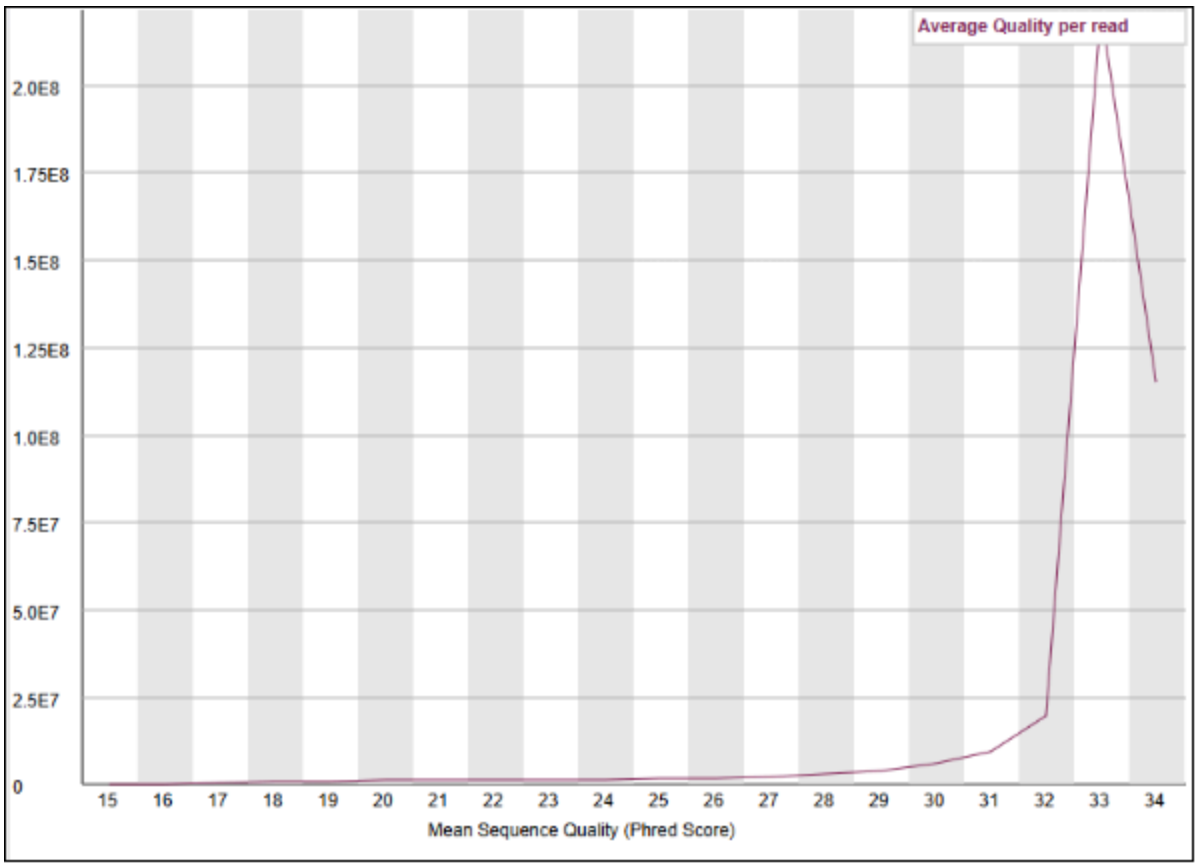


Figure 2

Quality score distribution across all sequences. The plot shows the mean sequence quality (Phred score) distribution, with most reads having an average quality score around Q33.

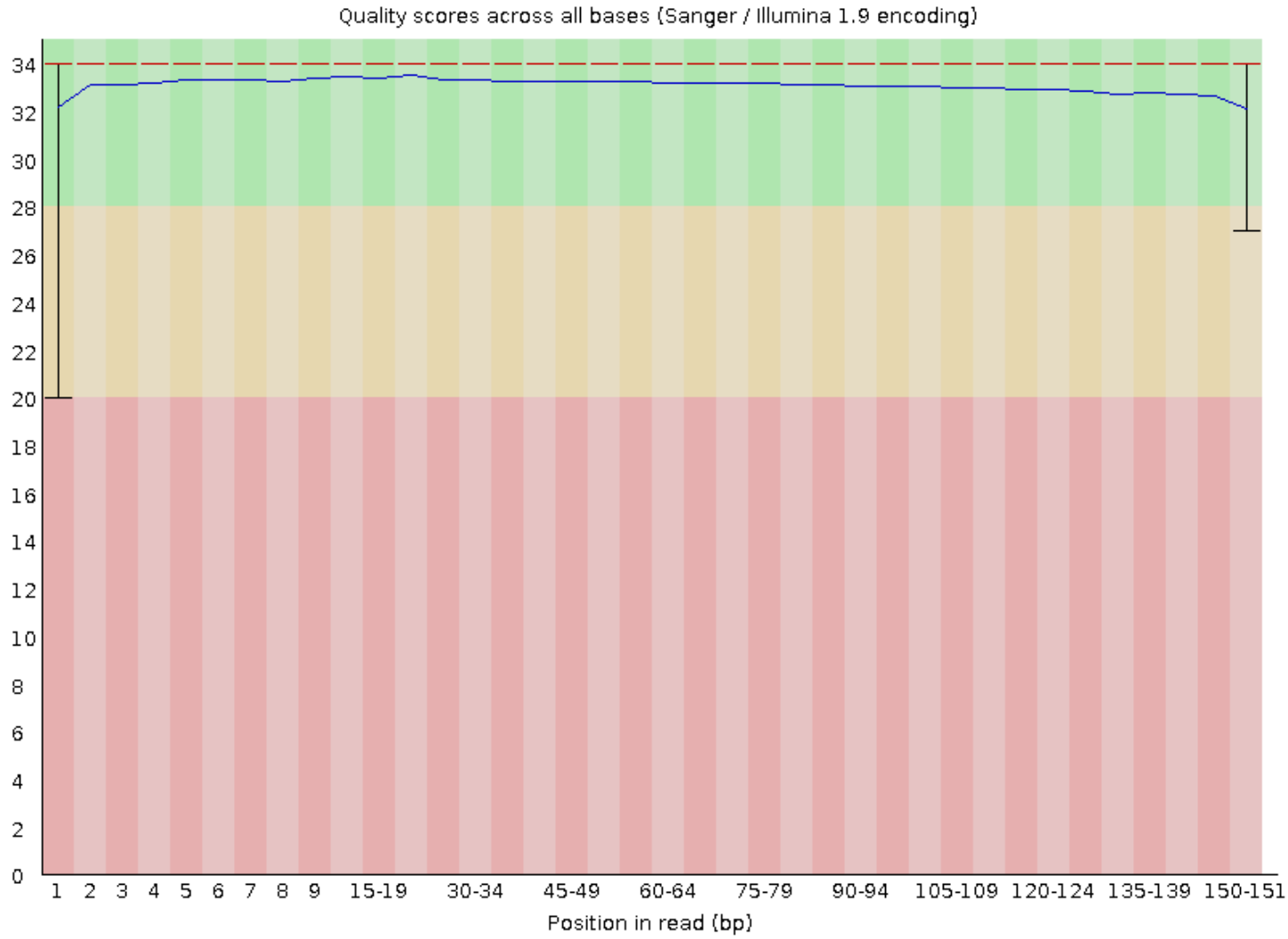


Figure 3

Quality scores (Phred score) across all bases in 151 bp reads. The plot illustrates the per base quality scores for each position in the read, with the majority of bases maintaining a high Phred score above Q30 throughout the length of the read. The green, yellow, and red shaded areas indicate high, moderate, and low-quality ranges, respectively.

Supplementary Files

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- Table12.docx