WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 14, Issue 21, 558-574. Review Article ISSN 2277–7105

HOUSEKEEPING GENE AND ITS INTERNAL CONTROL

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Article Received on 02 Oct. 2025,
Article Revised on 23 Oct. 2025,
Article Published on 01 Nov. 2025,

https://doi.org/10.5281/zenodo.17474289

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How to cite this Article: Kanika Adhikari*, Harshita Singh, Devanshu Sharma, Tusharika Arora and Vinod Kumar Gupta. (2025). Housekeeping Gene and Its Internal Control. World Journal of Pharmaceutical Research, 14(21), 558–574.

ABSTRACT

Housekeeping genes are essential for maintaining the fundamental cellular processes required for cell survival and homeostasis. These genes are constitutively expressed and support core functions such as energy production, protein synthesis, structural maintenance, and DNA replication. They encode vital proteins like ribosomal components, actin, and chaperones involved in glycolysis, oxidative phosphorylation, and the citric acid cycle. In gene expression studies, housekeeping genes serve as internal controls in techniques like RT-PCR, enabling normalization of target gene expression while accounting for variations in sample quality, size, and experimental conditions. By minimizing technical variability, they ensure accurate comparisons across samples and verify that observed changes are not due to artifacts. Common examples include *GAPDH*, *ACTB*, and *β2-microglobulin*, which generally show stable expression across different cell types and conditions. However, the selection of suitable housekeeping genes requires careful consideration. Ideal reference genes should demonstrate consistent expression across tissues, remain unaffected by experimental treatments, and exhibit stability in the specific species or cell type under investigation. It's often recommended to use multiple housekeeping genes for accurate normalization and to avoid selecting genes involved in the pathway under study. Despite their widespread use, housekeeping genes can be influenced by the same variables that affect target genes, including disease states and treatment interventions. Experimental factors like RNA integrity and reverse transcription efficiency can also impact their quantification.

Therefore, the assumption of uniform expression may not always hold true, highlighting the importance of validating housekeeping genes for each specific experimental context.

KEYWORDS: Housekeeping, DNA Replication, GAPDH, RT-PCR, ACTB.

INTRODUCTION

Housekeeping genes are essential for basic cell upkeep, their expression levels should remain constant across all cells and environments. Finding these genes improves knowledge of different structural genomic traits and makes the underlying cellular architecture easier to see. Furthermore, housekeeping genes are essential for calibration in a variety of genomic research and biotechnological applications. The number of housekeeping genes that have been discovered has gradually increased as a result of improvements in our capacity to monitor RNA expression. In this article, we discuss housekeeping gene discovery in the age of RNA-Sequencing and huge parallel sequencing. We highlight the significance of consistent expression and present a list of 3804 human genes that exhibit consistent expression throughout a tissue panel**.** A collection of 575 human genes that are expressed under every test circumstance in a microarray results database that is openly accessible. Given this frequent occurrence, it is anticipated that the set will contain a large number of "housekeeping" genes that exhibit constitutive expression throughout all tissue.[1] quantitative PCR & RT-PCR has been widely used to analyse the expression of candidate genes. In recent years the utilization of the technique has grown. RT-PCR is popular mainly due to its ability to efficiently amplify small quantities of RNA in a relatively short period of time.[2] The stable expression of a housekeeping gene in any RT-PCR reaction is mandatory since it plays a critical role in the interpretation of the final result.[3] It has been suggested that housekeeping genes are conserved, necessary, and a part of cellular maintenance mechanisms. But there are four very different characteristics of a gene: expression stability (similar expression across cell types and conditions), function, essentiality (loss-of-function is lethal), and conservation (in this case, stably expressed and essential across taxa). The connections or possible connections among these four attributes have not yet been thoroughly examined in any research.[4-7] We showed that normalizing between states to ACTB instead of UBC introduced an approximately three-fold magnitude of error, and that ACTB, which was found to be one of the most stably expressed genes within states, was one of the most variable between states. UBC was the most stable housekeeping gene between states (compared to normal). Genes that encode structural proteins, metabolic enzymes, and

elements of the transcription and translation machinery are examples of common housekeeping genes. In gene expression investigations, for example, genes like ACTB (β-actin), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and HPRT (hypoxanthine-guanine phosphoribosyltransferase) are commonly employed as internal controls.[8] The term "housekeeping gene" has no known origin. The word was first used In literature in 1976 to refer exclusively to tRNA and rRNA.[9] Housekeeping genes are frequently described as being conserved, important, part of cellular maintenance pathways, and expressed consistently in all cells and circumstances. Both theoretical and applied biology, particularly the study of evolution, have benefited from the idea of housekeeping genes.[10] The bare minimum of genes needed to support life is known as housekeeping genes. At the practical level, they can be described as markers of an organism's healthy biological state or as genes that are consistently expressed in all of its cells, regardless of the tissue type, developmental stage, cell cycle status, or external signal. They might enable us to identify the genetic traits and gene functions unique to species and higher taxa at the evolutionary level, which could promote conservation or change.[11]

ROLE OF HOUSEKEEPING GENES

These genes encode for proteins that are involved in fundamental cellular processes, such as cell cycle regulation, DNA replication, and metabolism. Housekeeping genes are widely used as internal controls for experimental studies.[12] Internal controls are used as indicator of perfect nucleic acid extraction, quality of samples, quality of PCR.[13]

Reverse transcription quantitative PCR (RT-PCR) and other gene expression investigations use housekeeping genes as reference or internal controls. Data is normalized using their stable expression, guaranteeing precise comparisons between various samples or experimental settings. The stability of these genes under certain experimental conditions must be confirmed, though, because their expression can change depending on the circumstances.[14]

The transcriptional regulation of housekeeping genes is influenced by their distinct regulatory architectures, which include high GC content and CpG islands in their promoter regions. Knowledge of these regulatory mechanisms is crucial for understanding how fundamental cellular functions are preserved in various cell types.[15]

They guarantee the validity of experimental results by ensuring their dependability and reproducibility.[12]

Fig-1: Role of Housekeeping gene in Research.

IMPORTANCE OF HOUSEKEEPING GENE IN RESEARCH

In research, they act as internal controls to normalize data, which guarantees accurate and trustworthy results, especially in gene expression investigations. When using methods such as reverse transcription quantitative PCR (RT-PCR), precise normalization is fundamental. Since their steady expression helps explain differences in sample quantity and quality, housekeeping genes are frequently used as reference points. Variability can result in incorrect interpretations, so it's crucial to confirm that the selected housekeeping gene exhibits constant expression under particular experimental circumstances.

It's not always accurate to assume that some genes are constant under all circumstances. As a result, choosing appropriate housekeeping genes necessitates thorough validation specific to each experimental configuration. To help researchers find the right reference genes for their research, resources like as the Housekeeping and Reference Transcript Atlas (HRT Atlas) offer up-to-date listings of human and mouse housekeeping genes.[16]

Housekeeping genes are frequently employed as reference genes in gene expression studies and are crucial for preserving fundamental biological functioning. They are useful for normalizing data in quantitative PCR (qPCR) and other gene expression analyses because their expression levels are often constant across different tissues and experimental settings.[17]

Recent studies have shown, however, that the stability of housekeeping gene expression might change according on the particular experimental setting. One study, for example, looked at the expression stability of common housekeeping genes in relation to cancer and bowel inflammation. It was published in the journal Inflammatory Bowel Diseases. The significance of choosing appropriate normalizers for inflammatory bowel disease research is highlighted by the researchers' discovery that intestinal inflammation and cancer had distinct effects on the expression stability of these genes.[18]

Some common housekeeping genes are 18S rRNA, β-actin, and GAPDH. But no single housekeeping gene is consistently stable under every circumstance. For example, research has demonstrated that these genes' expression might vary depending on the density of cells or the type of treatment.[16] GAPDH is commonly used as a housekeeping gene in gene expression research, acting as a reference for data standardization because of its steady expression in a wide variety of tissues.[19] Nonetheless, studies reveal that the expression of GAPDH can range among various tissues and experimental settings. An analysis of GAPDH mRNA expression in 72 human tissues, for example, revealed notable variability, with the highest and lowest expressing tissues differing by 15 times.[20]

Recent research, however, has shown that changing experimental setups, therapeutic approaches, or disease states might affect how housekeeping genes, like as β-actin, are expressed. For example, studies have revealed that tissue fibrosis and other variables might affect β-actin expression, making it an unreliable reference in some situations.[21] Furthermore, β-actin expression can change among cell types and experimental treatments, which could cause confusing results if utilized carelessly as a control.[22]

Before utilizing β-actin as a reference gene, researchers must confirm that its expression is stable in their particular experimental setups. Accurate normalization and interpretation of gene expression data are guaranteed by this validation. To obtain more dependable results when β-actin expression is unpredictable, numerous reference genes or alternative housekeeping genes should be taken into consideration**.** In order to normalize gene

expression data and guarantee the precision and dependability of experimental outcomes, housekeeping genes are essential in research. In experimental design, careful gene selection and validation are crucial processes.[14]

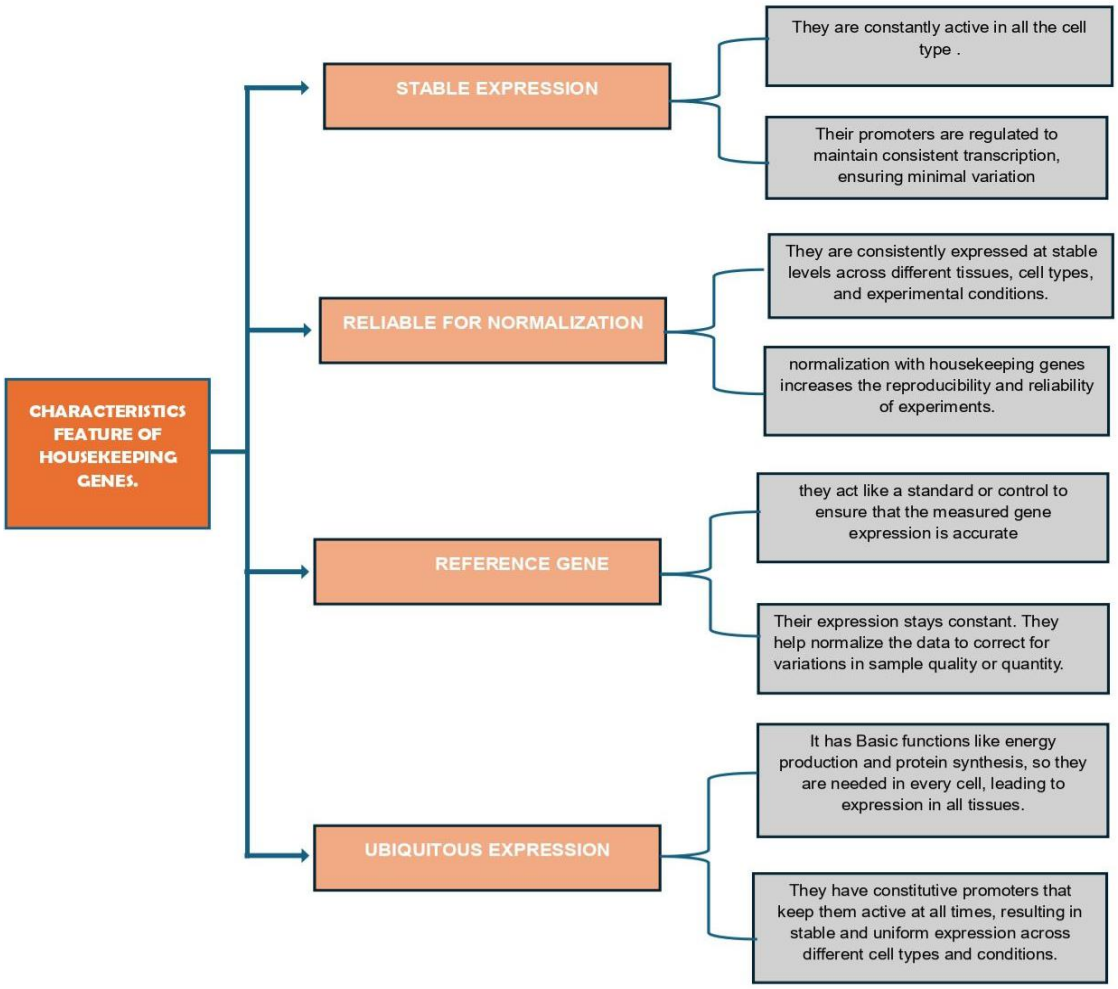

Fig. 2: Characteristic Feature of Housekeeping Gene.

REGULATION OF HOUSEKEEPING GENES

Housekeeping genes are typically controlled by clustering of promoters and frequently do not interact with distal enhancers, in contrast to genes essential for cell identity, which are controlled by cell-type-specific enhancers. Numerous housekeeping and metabolic genes are regulated by this promoter clustering, guaranteeing their steady expression, which is essential for regular cell growth and metabolism.[23]

Housekeeping genes are regulated by certain transcription factors and complexes. For example, it has been demonstrated that the NSL complex controls housekeeping genes in Drosophila, which helps to minimize transcriptional noise and preserve transcription

fidelity.[24] Unique epigenetic landscapes, such as particular histone modifications and chromatin architecture that support their open and accessible state and encourage consistent transcription across various cell types, are characteristics of housekeeping gene promoters.[25]

Homeostasis and cell identity depend on the proper control of gene expression. Dynamic interactions between DNA, protein, and RNA molecules are involved in this control, which affects the local genome architecture. DNA components known as promoters and enhancers are essential for controlling genes. Genes' transcriptional start sites and binding sites for elements typically engaged in early transcriptional activities are found in promoters. Enhancers are components unique to a certain cell type that bind transcription factors (TFs) and raise the expression of the genes they bind to. The capacity to bind transcription factors, recruit transcription machinery, and start transcription is shared by promoter and enhancer elements.[26]

SOME COMMON TYPE OF HOUSEKEEPING GENES

GAPDH, a vital enzyme in glycolysis and a reference gene frequently employed in expression investigations plays role in apoptosis, oxidative stress response and gene transcription. It is located on chromosome 12(12p13), GAPDH Enzyme consists of about 335 amino acids having molecular weight 36kDa.GAPDH functions as **tetramer (**have four identical subunit), and **ACTB** (beta-actin), which is essential for cytoskeleton formation. Protein synthesis depends on ribosomal RNA genes like **18S rRNA** and **28S rRNA**, while **B2M** (beta-2-microglobulin) plays a role in the immunological response. Typical housekeeping genes also include structural proteins like **TUBB** (beta-tubulin) and enzymes like **HPRT1** (involved in purine metabolism). Additional examples include **TBP** for transcription initiation, **SDHA** from the mitochondrial respiratory chain, **UBC**, which is essential for protein degradation, and **PPIA** (Cyclophilin A) for protein folding. These genes are often employed as controls in gene expression investigations due to their stable expression across varied situations.

GAPDH, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the most prominent housekeeping proteins and is often employed as an internal control in various semi-quantitative tests. In addition to glycolysis, GAPDH is involved in various cancer-related biological processes and has been documented to be routinely dysregulated in multiple cancer types. Therefore, it is crucially necessary to clarify its role in the physiological process of cancer. According to pan-cancer studies, GAPDH is widely

expressed at high levels in the majority of cancer types, and patients with high tumor tissue GAPDH expression have a bad prognosis.[27]

ACTB, A common reference gene/protein for determining the expression levels in malignancies, beta-actin (ACTB) has long been thought of as an endogenous housekeeping gene. But ACTB is closely linked to many malignancies, and there is growing evidence that ACTB is dysregulated in cancers of the liver, melanoma, kidneys, colon, stomach, pancreas, oesophagus, lung, breast, prostate, ovary, leukemia, and lymphoma. Most tumor cells and tissues have been shown to have up-regulated ACTB. It is discovered that the invasiveness and metastasis of malignancies are linked to aberrant ACTB expression and polymerization as well as the alterations to the cytoskeleton that emerge from these processes.[28]

As part of the purine salvage pathway, **HPRT** is a housekeeping enzyme that recycles guanine and inosine. HPRT has been used extensively as an endogenous control as a housekeeping gene for molecular studies assessing alterations in gene expression. Yet, recent evidence has shown that HPRT exhibits high variability within malignant samples.[29]

SELECTION CRITERIA OF HOUSEKEEPING GENES

The expression levels of the gene should be constant in many tissues and under various experimental settings.[19] Reliable normalization is ensured when there is minimal variance in expression across samples. Experimental treatments should not alter the gene's function in order to prevent biased results. In a variety of tissues and experimental settings, housekeeping genes need to exhibit constant expression levels. When it comes to gene expression analysis, this uniformity guarantees accurate normalization.[9] Since no single housekeeping gene is consistently stable under all circumstances, empirical validation is crucial. It is frequently believed that housekeeping genes express themselves consistently in all tissues and under all experimental circumstances. However, a number of variables, including tissue type, developmental stage, disease status, and environmental circumstances, might affect how they manifest. For example, it has been demonstrated that genes frequently utilized housekeeping genes, such as GAPDH and ACTB, display varying amounts of expression depending on the experimental setting.[30]

BETA – ACTIN

For many years, the highly conserved cytoskeleton structural protein beta-actin (ACTB) has been used as a reference gene and considered a common housekeeping gene.[31] Actin beta

(abbreviated ACTB/ACTB by the HGNCC) is one of six different actin isoforms that have been identified in humans. One of the two cytoskeletal actins that are absent from muscles is this one. The integrity, structure, and mobility of cells are attributed to highly conserved proteins called actins. Alpha actins are one of the primary parts of the contractile apparatus.[32]

B-actin is commonly used to normalize molecular expression studies because of its high conservation as an endogenous housekeeping gene. However, recent studies have shown that β-actin expression can change throughout growth and differentiation, in response to biochemical stimuli, and during illnesses. As expected, these occurrences pose a serious threat to the use of β-actin as an internal reference marker. Different β-actin expression under these conditions most likely indicates a change in the function of this maintenance molecule. More investigation into the role of actin in different contexts is probably going to lead to a more thorough comprehension of the roles played by this housekeeping gene. β-actin and γ-actin are found in nearly all cells, although α-actin is usually only found in smooth muscle cells. The three main isoform families of actins—α, β, and γ—are the most common proteins found in eukaryotic cells. Both cytoskeleton maintenance and cell mobility are dependent on these proteins.[33-34] B-actin is one of the most commonly utilized reference genes because its expression levels are more consistent than those of other internal controls.[35]

FUNCTIONS

By managing the cell's supply of globular actin (G-actin), β-actin is essential for controlling cell migration and proliferation. This control is necessary for functions including gene expression and cell migration. An essential part of the cytoskeleton, beta-actin supports intracellular transport and preserves the structural integrity and form of cells.[36] It alters gene transcription and expression through interactions with chromatin remodelers and transcription factors.[37]

Actin networks sustain cells mechanically and facilitate signal transduction by providing pathways for trafficking across the cytoplasm.[38]

In quantitative PCR investigations, beta-actin (ACTB) is a frequently utilized housekeeping gene to normalize gene expression data. It codes for a protein that is an essential part of the cytoskeleton and is involved in both cell motility and structure. It is frequently believed that beta-actin is expressed consistently in a variety of tissues and experimental settings because

of its vital roles. Concerns regarding the stability of beta-actin expression under various circumstances have been brought up by recent research, nevertheless. For instance, studies on the tissues of gastric cancer discovered that, in contrast to peritumoral tissues, the expression of beta-actin was markedly elevated in gastric cancer samples, although it remained constant in normal stomach epithelial cells. This implies that in some cancer research, beta-actin might not be a trustworthy reference gene.[39]

STRUCTURE OF BETA ACTIN HOUSEKEEPING GENE

One of the six human actin isoforms, beta-actin is encoded by the ACTB gene and is essential for cell integrity, structure, and motility. The ACTB gene has six exons and several introns, which causes alternative splicing to produce several transcript variations. This gene is a trustworthy housekeeping gene for gene expression research since it is well conserved and extensively expressed in several tissues. Beta-actin is frequently employed as a reference gene in quantitative PCR (qPCR) tests and as a loading control in Western blotting because of its steady expression level.[40-41]

The highly conserved protein beta-actin, which is expressed by the ACTB gene, is essential for a number of cellular processes, such as preserving cell shape, permitting motility, and promoting intracellular transport. On chromosome 7, the ACTB gene is situated between base pairs 5,566,782 and 5,603,415 on the reverse strand. Beta-actin is a protein consisting of 375 amino acids with a molecular weight of about 42 kDa. There are two types of it: polymeric filamentous actin (F-actin) and monomeric globular actin (G-actin). F-actin is created when G-actin polymerizes, and it then comes together to form a two-stranded helical filament. Beta-actin is found in the nucleus, where it controls gene transcription and takes part in DNA repair procedures, in addition to its functions in the cytoplasm.[42]

All cells constitutively express housekeeping genes, which preserve fundamental biological processes necessary for cell survival. Simple architectures with shorter exons and introns, which enable effective transcription and splicing, are their defining feature. Interestingly, their promoters frequently include GC content and lack TATA boxes, with CpG islands that help explain why they express themselves consistently in various tissues.

Additionally, the gene has transcription factor binding sites (TFBS) for NF-κB, AP-1, and SP1, among other proteins that aid in controlling activity in response to signals from cells. Furthermore, the promoter region's CpG islands affect transcription factor accessibility via

influencing DNA methylation, which in turn affects gene expression. The ACTB gene is a trustworthy reference gene in molecular biology research because these regulatory factors work together to guarantee its constitutive and steady expression.[43]

LIMITATION

It's possible that not all tissues express some housekeeping genes consistently. For example, genes that are frequently employed as internal controls, such as β-actin, have demonstrated expression variability across various tissues and clinical conditions, rendering them unreliable for normalization without previous validation.[44] Inaccurate findings may result from the usage of housekeeping genes without adequate validation. Normalization against unstable reference genes might give erroneous impressions of changes in gene expression or mask real biological differences. Therefore, before using housekeeping genes as internal controls, it is essential to confirm that they are stable under certain experimental settings.[45]

Variable Expression Across Conditions: Many housekeeping genes exhibit variable expression levels depending on the experimental or clinical setting, which is in contrast to the notion that expression is constant. Their expression may be influenced by variables such tissue type, developmental stage, cell cycle state, or outside stimuli, which could result in incorrect normalization in gene expression analysis.[46] The housekeeping gene group combines distinct evolutionary and genetic traits. For instance, they feature simpler sequence repetitions, shorter exons and introns, and a decreased propensity for nucleosome assembly in the 5′ region.[47]

APPLICATIONS

Biomarker Identification These genes act as internal controls to normalize gene expression data in biomarker identification, guaranteeing precise sample comparisons. For accurate biomarker analysis, the right housekeeping genes must be chosen. A study called "Validation of housekeeping genes as **internal controls** for studying endocrine disrupting chemicals in disk abalone" looked at the stability of expression of twelve potential housekeeping genes in tissues of abalone that had been exposed to these substances. In order to precisely measure biomarker expression in response to these drugs, the researchers found appropriate housekeeping gene.[48] **Drug Testing** These genes act as internal controls in drug testing, normalizing gene expression data and guaranteeing precise sample comparisons. For dependable drug testing experiments, choosing the right housekeeping genes is essential.

The study "Identification of stable housekeeping genes for induced pluripotent stem cells and -derived endothelial cells for drug testing" looked at the stability of putative housekeeping gene expression in both induced pluripotent stem cells (iPSCs) and endothelial cells produced from iPSCs. A panel of 15 potential housekeeping genes was discovered by the researchers, including well-known ones like B2M, GAPDH, GUSB, HMBS, and HPRT1. They assessed these genes' stability using techniques including RefFinder, normfinder, Genorm, Bestkeeper, and delta-Ct. The two most stable genes, RPL36AL and TMBIM6, were examined in iPSC-ECs both with and without genetic modification, as well as following telatinib treatment.

The gene expression of RPL36AL and TMBIM6 remained constant throughout medication treatment, according to quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). The significance of using stable housekeeping genes for precise gene expression monitoring in drug testing is highlighted by this study.[49]

Evolutionary Study These genes act as internal controls to normalize gene expression data in evolutionary investigations, guaranteeing precise sample comparisons. Reliable evolutionary analyses depend on the selection of suitable housekeeping genes. A study called "Mammalian Housekeeping Genes Evolve More Slowly than Tissue-Specific Genes" looked at how quickly housekeeping genes had evolved in comparison to tissue-specific genes. Housekeeping genes are useful for placental mammals with comparatively rapid rates of evolution were found and sequenced. These genes' usefulness in evolutionary research was demonstrated when they were utilized to recreate the phylogenetic links among eutherian mammals.[50] researching evolutionary relationships because they change more slowly and are subject to stronger selection, the researchers discovered. In a different study titled "Housekeeping Genes for **Phylogenetic Analysis** of Eutherian Relationships," eight housekeeping genes from 22 placental mammals with comparatively rapid rates of evolution were found and sequenced. These genes' usefulness in evolutionary research was demonstrated when they were utilized to recreate the phylogenetic links among eutherian mammals.[50]

CONCLUSION

In the conclusion, we successfully detected the beta actin gene in various biological samples, including whole blood, saliva, sputum menstrual blood and MTB biopsies. It also conclude, detection of the widely known housekeeping gene beta actin was constant in all sample types.

This demonstrates that it is a dependable control marker for guaranteeing the integrity and quality of DNA samples in a variety of biological materials.

Effective PCR amplification of the beta actin gene was made possible in all samples by the use of certain primers. Consistent beta actin amplification verifies the preparation of the material by confirming the presence of amplifiable DNA. It can be used as a reference gene to confirm the existence of genes particular to a pathogen. It guarantees the integrity of biological evidence in forensic science. Consequences and prospective courses. The results of this investigation show that the beta actin gene is a reliable control marker for DNA quality and PCR efficiency in a variety of biological samples.

It provides a benchmark for PCR processes and assessing the efficiency of DNA extraction, also ensure the reliability of diagnostic results. It has the ability to reliably detect the beta actin gene in diverse biological samples has significant implications for clinical diagnostics and forensic investigations. Furthermore, the precision and dependability of molecular studies in the domains of forensic science and clinical diagnostics may be improved by applying These discoveries in these sectors.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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