New insights into the role of ribonuclease P protein subunit p30 from tumor to internal reference

Junchao Wu¹,²†, Sijie Yu¹,²†, Yalan Wang¹,²†, Jie Zhu¹ and Zhenhua Zhang¹*

¹Institute of Clinical Virology, Department of Infectious Diseases, The Second Hospital of Anhui Medical University, Hefei, China, ²Department of Clinical Medicine, Anhui Medical University, Hefei, China

Ribonuclease P protein subunit p30 (RPP30) is a highly conserved housekeeping gene that exists in many species and tissues throughout the three life kingdoms (archaea, bacteria, and eukaryotes). RPP30 is closely related to a few types of tumors in human diseases but has a very stable transcription level in most cases. Based on this feature, increasing number of studies have used RPP30 as an internal reference gene. Here, the structure and basic functions of RPP30 are summarized and the likely relationship between RPP30 and various diseases in plants and human is outlined. Finally, the current application of RPP30 as an internal reference gene and its advantages over traditional internal reference genes are reviewed. RPP30 characteristics suggest that it has a good prospect of being selected as an internal reference; more work is needed to develop this research avenue.

Introduction

The ribonuclease P protein subunit P30 (RPP30) gene is included in the National Center for Biotechnology Information (NCBI ID#10556) database. RPP30 has been shown to be highly conserved in gene pool data, and many studies have shown that there are 16 homologous genes of RPP30 contained in many species from the three life kingdoms (archaea, bacteria, and eukaryotes) (1, 2). As a housekeeping gene, the protein encoded by RPP30 is one of shared protein subunits of ribonuclease P (RNase P) and ribonuclease MRP (RMRP), which are widely expressed in various tissues and participate in many life processes of microscopic and macroscopic organisms. It should be noted that as a protein subunit, detection of RPP30 in different tissues is not uniform and stable, possibly because of the complex modification process after translation (3–6).
In this review, the diseases associated with RPP30 and the factors that may influence its expression are introduced for reference in further studies and in quality control. Abnormal gene expression or mutation studies have made some progress with regard to botanical diseases (5–7). At present, studies on human diseases mainly involve tumors, but only a few of them have demonstrated RPP30 overexpression (8). In addition, RPP30 is associated with glioblastoma (GBM) pathogenesis and low bone mineral density (LBMD) (9).

Reports that RPP30 expression level is affected by other factors are very limited, such as aging (10). Given the relatively high and stable ribose nucleic acid (RNA) expression of RPP30 in human tissues, increasing studies have recently used RPP30 as an internal reference gene for many applications, including detection of pathogens, calculation of the number of tumor cells, diagnosis of tumors, and some childhood diseases are discussed. In particular, the application of this gene in nucleic acid detection of SARS-CoV-2 demonstrates its great value as an internal reference (11).

Finally, the advantages of RPP30 over conventional reference genes, such as β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are discussed. Ideal reference genes should be stably expressed in different tissues and different life cycles. With increased research and more applications, the expression of β-actin and GAPDH has been observed to be related to physiological/pathological states, experimental conditions, and tissue type (12–14). In contrast, changes in RPP30 expression seem less likely to be reported in the many conditions described above. These data suggest RPP30 may be used as an internal reference gene in further studies. Of course, the reliability of RPP30 as an internal reference is required to be verified by more comprehensive experiments.

**Gene and protein structure**

The highly conserved RPP30 genome sequence is located on human chromosome 10 (10Q23.31) at 90,871,974–90,908,556 and is 36,582 nucleotides in length, with 14 exons (https://www.ncbi.nlm.nih.gov/gene/). There are 16 homologous genes in primates, canine, bovine, Rodentia, Amphibia, Drosophila, Arthropoda, and Saccharomycetes. These data are obtained from NCBI (ID#10556). The highly conserved sequence and other characteristics of RPP30 are illustrated in a gene evolutionary tree in Figure 1A.

Human nuclear RNase P consists of 1 RNA subunit H1 and 10 conserved proteins, and the complex has a slender conformation similar to the overall shape of yeast RNase P shown by cryo-negative staining electron microscopy (2). The human RNase P protein consists of a single protein, Pop1, and three subcomplexes, which include the RPP20-RPP25 heterodimer, Pop5-RPP14-(RPP30)2-RPP40 heteropentamer, and RPP21-RPP29-RPP38 heterotrimer (2). The proteins are tightly attached to each other, forming a structure similar to a

![Figure 1A](https://www.frontiersin.org)
right-handed clip with three modules: finger, palm, and wrist. The POP5-RPP14-(RPP30)2-RPP40 heteropentamer becomes the palm module of the protein clamp. Two copies of the RPP30 molecule bind to the central POP5-RPP14 from opposite sides, forming a typical trisose phosphate isomerase (TIM) barrel fold. The molecule of RPP30 that interacts with RPP40 is called RPP30B, and the other molecule is called RPP30A. The secondary and tertiary structures of RPP30 have not been analyzed at this stage (2).

The RPP30 protein subunit is homologous to the RNase P protein subunit of archaea and other eukaryotes, as shown in Figure 1B. By comparing the amino acid sequence of homologous genes of RPP30, the domain and conserved site of the RPP30 protein may be identified, which will illustrate how conserved this protein is.

Main functions of RPP30

Life processes

RPP30 mainly functions in catalysis, nuclear localization, assembly, and/or regulation of holoenzyme activity (3). The GeneCards (https://www.genecards.org/) and Gene Ontology (GO; http://geneontology.org/) databases were searched for the RPP30 gene to identify its basic functions. The gene’s molecular functions include binding proteins, catalyzing reactions, and so on (15). In archaea, two RPP30 copies bind with ribonuclease P/MRP protein subunit Pop5 dimers to form the Pop5•RPP30 heterodimer. The Pop5•RPP30 heterodimer is anchored on the catalytic domain of RNase P RNA (RPR), which is necessary for pre-tRNA cleavage (16). Cellular components encoded by the gene include RNase P complex, RNase MRP complex, and the multimeric ribonuclease P complex. Biological processes mediated by the gene include rRNA and tRNA processing (15). RPP30 gene encodes a type of ribonuclease that achieves RNaseP RNA binding activity, contributes to ribonuclease P activity, and participates in the removal of tRNAs’ precursor, as well as the formation of polynuclease P complex, and ribonuclease MRP complex, which is necessary for the gene transcription of RNA polymerase III (17). RPP30 also facilitates immunity in rice and reproduction in Drosophila (5–7). Therefore, as the most conserved gene in various types of organisms, RPP30 is also involved in the most basic life processes, which drive almost all life functions and activities.

A common subunit joining RNase P and RMRP

RNase P and RMRP are both small nucleolar ribonucleoprotein complexes (snorRNPs) that are classified into three major classes (box H/ACA snorRNPs, box C/D snorRNPs, RNase P and RnaseMRP) (18). RMRP has only been found in eukaryotes, located mostly in the nucleolus, and has many functions, including cleaving the pre-rRNA at site A3 in vivo and in vitro to mature the 5’ end of the 5.8S rRNA, cleaving an RNA transcript to generate RNA primers for mitochondrial DNA duplication, cleaving the B-type cyclin, Clb2, mRNA, recognizing and cutting pre-tRNA, and is required to turn over cell cycle mRNA (19–21). RNase P, located both in the nucleoplasm and nucleolus, is necessary for Mg2+ dependent 5’ maturation of tRNAs in archaeal, bacterial, and eukaryotic kingdoms (22). RNase P may also act in the stress response and be a transcription factor that regulates polymerase I and III (23). RNAs containing N6 methyladenosine (m6A), 4.5S pre-rRNA, operon mRNAs, box C/D small nucleolar RNAs that reassemble tRNAs are also substrates of RNase P (15, 24–30). RNase P and RMRP have similar functional and structural characteristics (18, 31). These two enzymes share at least ten protein subunits, including RPP14, RPP20, RPP21, RPP25, RPP29, RPP30, RPP38, RPP40, Pop1, and Pop5 (4, 15). RPP30, with a highly conserved amino acid sequence, has an important role in joining the RNase P and RMRP complexes (32). RPP30, as one of the common subunits between the RNase P and RMRP complexes, contributes to an increased number of RNA substrates and atypical functions of eukaryotes (4, 33). RNase P H1 and RMRP RNAs may crosstalk with miRNAs that are related to stability and translation of mRNAs (34). Stolc and Altman reveal that reduction RPP1 (homologous to human RPP30) in S. cerevisiae causes disruptions in both RNase P and RMRP by inhibiting correct cleavage of the internal transcribed spacer I of rRNA surrounding the A3 site (35).

A cofactor acting with the RNA subunit and other protein subunits of RNase P or RMRP

Although RNase P RNA (RPR) is suggested to have activity in vitro, its activity in vivo requires protein cofactors (36). In 2006, Welting et al. used glycerol gradient sedimentation and coimmunoprecipitation to determine that RPP30 is related to the RNA subunit of RNase P and RMRP (18). UV–crosslinking studies also show that RPP30 interacts directly with H1 RNA, an RNA subunit of RNase P (37, 38). Isothermal titration calorimetry has been used to explore interactions among the protein subunits of RNase P and RMRP (22, 39, 40). In archaea, bacteria, and yeast, RPP30/RPP30 paired with Pop5/Pop5, may be functionally reconstituted with the phylogenetically-conserved core catalytic domain (C domain) of the RNA subunit to promote the assembly of RNase P providing substrate RNA binding sites and activating the RNA subunit (probably by RNA annealing and strand displacement (41) and stabilize ionic interactions with the RNA subunit or the substrate pre-tRNA at a relatively lower salt concentration (1, 22, 42–44). In the hyperthermophilic archaeon Pyrococcus horikoshii, PhoRPP30 is homologous to human RPP30.
and acts as a molecular chaperone of PhoPop5, which recognizes the stem-loop containing the P3 helix in PhopRNA (45). RPP30-Pop5 is a tight heterotetrameric complex that increases the affinity of the holoenzyme for Mg$^{2+}$ and protects the RNase P M1 RNA’s C domain from RNase T1 cleavage, especially near conserved nucleotides of RNase P in archaea whose RNase P protein is homologous to eukaryotic counterparts (36, 46–48). The RPP30-Pop5 complex also increases the RPR cleavage rate of pre-tRNA and may be activated by the RPP21-RPP29 complex reflecting indirect effects (36). In Dictyostelium discoideum, RPP30 adopts a TIM-barrel fold that stabilizes the structure and enhances the affinity of pre-tRNA of RNase P to promote the formation of a native fold (46, 49, 50). In humans, RPP30 interacts with RPP14, RPP40, RPP20, RPP21, Pop1, RPP29, Rpp38, and RPP30 itself (15, 37, 51). Moreover, Stolc and Altman have shown that the RPP30 and RPP38 cDNA code for proteins related to catalytic complexes of RNase P from HeLa cells (35). Additionally, RPP30 may interact with other RNAs; as an important subunit of RNase P, RPP30 may be involved in the cleavage of hepatitis C virus RNA (52).

**Regulation of biological procedures in other species**

In Arabidopsis, the RPP30 domain is present from 98–248 amino acids in gametophyte defective 1 (GAF1), which is important in female gametophyte development and male competence and has a universal contribution to plant development (5). In Drosophila, RPP30 is necessary for female oogenesis because of its relationship with tRNA processing, DNA replication, and piRNA transcription (7). RPP30 also positively regulates rice immunity by interacting with histone deacetylase 701 (HD701, RPP30 may be a substrate of HD701), which functions in suppressing innate immunity in rice and may upregulate expression of defense genes (6).

Although many functions of RPP30 have recently been identified, the specific role of RPP30 in basic life processes requires further research.

**Relationship between RPP30 and disease**

RNase P and RMRP play an important role in RNA or non-RNA processing that are universal programs closely related to many life activities. As an important subunit, the mutation and abnormal expression of RPP30 leads to many diseases.

**RPP30 mutations and reproductive diseases**

In Arabidopsis, GAF1 mutations result in decreased RPP30 levels that induce defects in mitosis during female gametophyte development, arrest embryo sacs at stages FG1–FG7 and also cause defects in male competence (5). In Drosophila, an isolated mutation that inserts the P-element (lacW)k01901 into RPP30 leads to complete sterility in females (7, 49). The pathogenic mechanisms that have been uncovered include a mutation in RPP30 that arrests oogenesis by decreasing tRNA processing, which leads to transcription-replication conflicts (7). This includes decreases in transposon expression, accumulation of the polymerase III subunit Brf, and the collapse of Proliferating Cell Nuclear Antigen (PCNA), which increases DNA replication stress and gene defense by small RNAs and activates several DNA duplication checkpoint proteins, including p53, clasin, and checkpoint kinase 2 that decrease piRNA transcription and piRNAclusterpopulations (7). piRNAs are native defenders of germline cell genomes whose mature structure called a “nuage” surrounds the nurse cells that provide nutrients to oocytes (53). Additionally, downregulation of piRNA levels leads to derepression of transposable elements and activates DNA checkpoints to promote positive feedback of defective oogenesis (7, 53–56).

**A factor that protects plants against pathogens**

Li et al. have identified OsRPP30, a cellular protein that may regulate the biological function of rice HDI701 (6). HDI701 negatively regulates defense mechanisms in rice by increasing histone H4 deacetylation and increasing the sensitivity to Magnaporthe grisea and Xanthomonas oryzae pv. oryzae (57). When rice is infected with Pyricularia oryzae (syn. Magnaporthe oryzae), RPP30 expression increases, which activates the transcription of defense genes (6). The overexpression of OsRPP30 in genetically modified rice increases expression of defense genome and the production of reactive oxygen species, resulting in resistance to Magnaporthe grisea and Xanthomonas oryzae. OsRPP30 is located at the top of the immune pathway triggered by HDI701-mediated pathogen-associated molecular patterns, which may overcome the negative effects of HDI701 and provide a new direction for the cultivation of pathogen-resistant food in the future (6).
Anti-RPP30 antibody and autoimmune diseases in humans

Anti-Th/To is one of the rarer antinuclear antibodies identified in patients with systemic sclerosis (SSc) and is composed of hPOP1, RPP25, RPP30, and RPP40 (58, 59). Researchers refer to “anti-Th” and “anti-To” in the cases of RNase MRP and RNase P, respectively (60, 61). Recombinant RPP30 and RPP38 cross-react with anti-Th/To antibodies of patients afflicted with SSc (3, 32). In addition, people with positive anti-RPP30 antibodies have a lower risk of tendon friction rubs and cancer, but more likely to have severe lung diseases and pulmonary hypertension (59, 62). However, the positivity of anti-RPP30 antibodies only represents the antigenicity of RPP30 protein, and does not suggest the existence of abnormal expression or a RPP30 gene defect, which requires further research.

RPP30 and human tumors

The nucleophosmin (NPM1) gene, located at human chromosome 5Q35, contains 12 exons and encodes a multifunctional shuttling protein that shuttles between the nucleolus and cytoplasm. NPM1 mutations happen in approximately one-third of acute myeloid leukemias (AMLs) (63). Martelli et al. have shown that the NPM1/RPP30 complex serves as one of three NPM1 rearrangements that have been found and analyzed in 13,979 AML samples (64). In patients with AML that have a NPM1 rearrangement, RPP30 is rearranged with NPM1 at exon 11, whereas the rearrangement of NPM1 with RPP30 is at the end of exon 9 (64). These data indicate RPP30 may help detect AML and monitor NPM1-mutated AML. A new study found that RPP30 may be a transcriptional regulator in glioblastoma (GBM) and the decreased RPP30 expression in elderly people could be a risk factor for GBM (10). This study showed that RPP30 was related to RNA and post-transcriptional modification in non-tumor tissues, and RNA modification in GBM. RPP30 regulates protein expression in GBM by affecting post-transcriptional modification of proteins and functional accumulation of these proteins indicates that these proteins are mainly involved in the activation of cancer signaling pathways (10). In addition, downregulation of RPP30 expression in human astrocyte (HA) cells promotes the proliferation of HA cells, while overexpression inhibits the activation of tumor-related pathways and the proliferation of HA cells, further confirming the close relationship between RPP30 and the occurrence and development of GBM (10). Correlation analysis of RPP30 expression levels with gene expression in cancer-related pathways, such as cancer, Wnt, and mitogen-activated protein kinase pathways in the Chinese Glioma Genome Atlas and The Cancer Genome Atlas databases show significant correlation (10). The Gene Expression Profiling Interactive Analysis (GEPIA2) database has been used to obtain broad knowledge of the relationship between RPP30 (Ensembl ID: ENSG00000148688.13) and tumors (Figure 2) (8). RPP30 expression was significantly different in tumor tissues (higher) and non-tumor tissues in diffuse large B-cell lymphoma, pancreatic adenocarcinoma (PAAD) and thymoma (THYM) (Figure 3). These data also show that there is no significant difference in RPP30 expression levels in different stages of those tumors while high expression of RPP30 is correlated with lower overall survival in PAAD using data from the GEPIA2 public database (http://geopia2.cancer-pku.cn/index). RPP30 gene expression is high under epidermal development, cell differentiation, and keratinocyte differentiation processes, which play important roles in the differentiation of gastric epithelial cells. Recently, Kan et al. used TGCA RNA-seq to explore the role of RPP30 expression in gastric cancer. They found that RPP30 protein expression was positively correlated with the number of T helper 2 cells, active dendritic cells, and T helper 1 cells, and negatively correlated with the number of T helper 17 cells. They also found that RPP30 RNA expression in gastric cancer (GC) tissue is higher than that in normal tissue and higher RPP30 RNA expression is related to worse overall survival (OS) at the T1, T2, and N0 stages of the tumor. The mechanism may be that RPP30 RNA expression is upregulation via the G alpha S signaling pathway, neuronal system, and olfactory transduction, in addition to increasing cAMP levels, which are tightly correlated with GC histopathology. RPP30 could regulate tRNA modification, transcriptional replication, DNA repair, replication fork stagnation, and protein expression, which are correlated with cancer cell proliferation (65, 66).

Other diseases

Lee et al. Have found that rpp30 may be related to genetic factors of LBMD through genome-wide association studies involving two signaling pathways of eight related diseases (9). No further association between rpp30 and LBMD has been reported. RPP30 is indirectly related to some diseases, including lung diseases and pulmonary hypertension, secondary to autoimmune diseases (62).

Currently, the research on genes and diseases is extremely in-depth and making rapid progress. Although rpp30 is involved in basic life activities, only a few human diseases have been confirmed to be related to rpp30, and even fewer have been confirmed to have abnormal expression. These results further reflect the stable expression of rpp30 and how well conserved it is.
RPP30 distribution and factors influencing RPP30 expression

Although \textit{RPP30} is associated with appellate disease, the expression of \textit{RPP30} in normal tissues and most tumor cells is stable. Approximately 3647 species have \textit{RPP30} subunits and 424 organisms have orthologs of human \textit{RPP30}. \textit{RPP30} RNA is widely expressed in 27 human tissues, including testis, heart, kidney, lung, thymus, and lymph nodes, and more, among which testes and lymph nodes show the most expression and pancreas shows the least expression using data from the NCBI, InterPro (https://www.ebi.ac.uk/interpro/), and GeneCards public databases.

FIGURE 2
Gene expression profile of ribonuclease P protein subunit p30 (\textit{RPP30}) in all tumor samples and paired normal tissues. Red words: significant difference; Black words: No significant difference. Abbreviations: ACC: Adrenocortical carcinoma, Bladder Urothelial Carcinoma, Breast invasive carcinoma, Cervical squamous cell carcinoma and endocervical adenocarcinoma, Cholangiocarcinoma, Colon adenocarcinoma, Diffuse Large B-cell Lymphoma, Esophageal carcinoma, Glioblastoma multiforme, Head and Neck squamous cell carcinoma, Kidney Chromophobe, Kidney renal clear cell carcinoma, Kidney renal papillary cell carcinoma, Acute Myeloid Leukemia, Brain Lower Grade Glioma, Liver hepatocellular carcinoma, Lung adenocarcinoma, Lung squamous cell carcinoma, Mesothelioma, Ovarian serous cystadenocarcinoma, Pancreatic adenocarcinoma, Pheochromocytoma and Paraganglioma, Prostate adenocarcinoma, Rectum adenocarcinoma, Sarcoma, Skin Cutaneous Melanoma, Stomach adenocarcinoma, Stomach and Esophageal carcinoma, Testicular Germ Cell Tumors, Thyroid carcinoma, Thymoma, Uterine Corpus Endometrial Carcinoma, Uterine Carcinosarcoma, Uveal Melanoma, in turn.
databases. In Figure 4A, although there are differences in RPP30 RNA expression levels calculated by different databases, RPP30 RNA expression levels of different organizations calculated by the same database are basically the same, which is consistent with the results obtained by Bgee involving gene expression data in animals. However, the expression of human RPP30 protein is not as stable as RPP30 RNA. There are differences in the expression of RPP30 protein among different tissues or cells and the RPP30 protein has weak expression in some tissues or cells, such as lymph node, brain, spinal cord, ovary, bone, colon, and liver secretion (Figure 4B). There have also been no reports using RPP30 protein as an internal reference for western blotting. To summarize, the expression level of RPP30 RNA in human tissue is relatively high and stable and is suitable to be used as an internal reference gene (11, 67, 68). Mouse RPP30 RNA is widely expressed in the central nervous system, bladder, brain, liver, and testis, etc., with higher expression in the central nervous system and lower expression in the adrenal gland and stomach.

At present, there are relatively few reports on factors affecting RPP30 expression levels. Li, Zhai (10) have found that RPP30 expression is affected by age-related factors. Using analysis of age-related genes, RPP30 expression was negatively correlated with increased age, indicating that the change in RPP30 expression may be related to cell senescence. Li, Xiong (6) have found that RPP30 expression is upregulated after rice has been infected with fungal and bacterial pathogens. Mattijssen, Welting (69) have speculated that the expression of housekeeping genes may be altered in the growth plates of patients with cartilage-hair hypoplasia.

**Application of RPP30 as an internal reference gene**

RPP30 has been used as an internal reference gene in the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronavirus disease 2019 (COVID-19) broke out in Wuhan, China in December 2019 and then spread widely around the world, with strong infectivity (70). Despite its reputation as the gold standard for the detection of SARS-CoV-2, RT-PCR often produces false negative results in detection and diagnosis (71). This may be related to sample quality or changes of primer/probe binding site sequences, but the latter is less likely (72, 73). RPP30 is a single copy sequence stably expressed in the human genome, which has a good amplification efficiency, shows 100% sensitivity and specificity, and is not affected by swabs and methodology (74). Compared to other internal parameters, only RPP30 exists in all types of SARS-COV-2 infection samples (67). Figure 5A is the flow of RT-PCR. Both RPP30 RNA and viral RNA were present in epithelial cells (Figure 5B), and RPP30 RNA levels were closely related to SARS-CoV-2 RNA levels in respiratory tract samples (Figure 5C). Thus, RPP30 RNA may be used to control sample quality and the RPP30 Ct cutoff value may effectively identify false negative results (11, 72), which may increase sensitivity and...
reduce the spread of SARS-CoV-2. In addition to evaluating the quality of the sample, RPP30 may also determine whether mRNA has been extracted successfully and whether there is inhibition in the PCR (75).

RPP30 is used as an internal reference gene to determine the best effective drug concentration for tumor treatment (76). The efficacy of traditional antineoplastic drugs is evaluated by calculating the lethality of drugs to all cells in vitro, so it is impossible to measure the lethality of antineoplastic drugs to normal cells, which is often accompanied by unpredictable side effects. Because RPP30 is stably expressed in the vast majority of tumor cells and non-tumor cells, while the neurofibromatosis type 1 (NF1) gene loses heterozygosity in tumor cells, the number of tumor cells may be evaluated by the quantitative RT-PCR ratio of NF1 to RPP30, which may be used to evaluate the efficacy and side effects of tumor drugs, and may also be used in personalized adjuvant chemotherapy. Due to the different behavior of cells in vivo and in vitro, this method has some limitations (76–78). As an internal reference gene, RPP30 may also accurately and effectively evaluate the concentration of antiretroviral drugs in cells (79).

RPP30 is used as an internal reference gene to analyze the feasibility of HIV DNA detection in cerebrospinal fluid (CSF) (80). RPP30, as a housekeeper gene, is highly conserved and widely expressed in human tissues and may be used as an internal reference gene to detect the number of leukocytes in CSF. Using droplet digital PCR (dd-PCR) detection, the level of HIV DNA in CSF cells is not correlated with RPP30 levels, indicating that the detectability of HIV DNA does not depend entirely on the number of cells available in each sample. Then, the correlation between the level of HIV DNA in the CSF and the level of HIV RNA in peripheral blood cells, as well as the relationship between the virus inhibition and non-inhibition subgroups, may be analyzed to explore the detectability of HIV DNA level in CSF.

RPP30 is used as an internal reference gene in diagnostic experiments (68). At least 5.5% of all pathogenic genetic changes in humans are large genome deletions or duplicates (81). With the discovery of disease-related genes, dd-PCR has been used to quantify the copy number of genes to diagnose diseases (82). Because of its conserved sequence and stable expression in almost all cells, RPP30 is widely used as an internal reference gene (83). For example, RPP30 has been used as an internal reference in real-time fluorescent PCR or dd-PCR to quantify the survival of motor neuron 1 gene, T-cell receptor excision circles, and Kappa-deleting recombination excision circles; to screen neonatal spinal muscular atrophy, severe combined immunodeficiency disease, and detect immune remodeling of the thymus and bone marrow (84–86); and to quantify the sex-determining region Y gene to detect male/female chimerism, which may track chimerism after hematopoietic stem cell transplantation (87). RPP30 has also been used as an internal reference in single-cell dd-PCR to evaluate the genomic DNA of rare circulating fetal cells in peripheral blood samples of pregnant women with male fetuses and validate the concept of non-invasive prenatal diagnosis (88). There are also many reports on the use of RPP30 as an internal reference in different molecular biology techniques for the diagnosis of human diseases, as shown in Table 1.
Dyavar et al. (79) used human and rhesus macaque (RM) gDNA templates to quantitate RPP30 copies, and found a low coefficient of variation and strong correlation between human and RM gDNA templates and the number of RPP30 copies in intra-laboratory (R² = 0.996, p < 0.001; R² = 0.975, p < 0.001), inter-laboratory (R² = 0.997, p < 0.001; R² = 0.989, p < 0.001), and inter-operational (R² = 0.994, p < 0.001; R² = 0.986, p < 0.001) studies, which confirms the high accuracy and precision of the RPP30 dd-PCR assay. In addition, Profaizer and Slev (86) observed that RPP30 dd-PCR could detect 2 copies/µL of genes, which is more accurate than the previous 24 copies/µL for qPCR.

### Differences from other internal reference genes

Housekeeping genes are mainly involved in the maintenance of basic cell functions and are thought to be expressed in all cells (98). They are widely used as internal controls to standardize the expression of genes in western blotting, northern blotting, and RT-PCR. The ideal housekeeping gene should be expressed at the same level in all tissues (99). At present, frequently used housekeeping genes are β-actin and GAPDH, in which β-actin has a molecular weight of approximately 42–43 kDa and is composed of 375 amino acids. It is widely distributed in the cytoplasm and is involved in cell movement, structure, and integrity (100), whereas GAPDH is an enzyme with a molecular weight of approximately 37 kDa and is involved in glycolysis, DNA repair, tRNA output, membrane fusion, and transport (101). However, there are increasing reports that the RNA expression level of these genes is affected by the physiological/pathological state, experimental conditions, and tissue types (12–14). Thus, the factors affecting mRNA expression levels of RPP30, GAPDH, and β-actin were compared.

Table 2 shows that the length of the RPP30 amplification product is smaller than that of β-actin and GAPDH, which reduces errors and improves efficiency during the process of RPP30 amplification. Table 3 lists the pseudogenes found in β-actin and GAPDH, but, to date, no pseudogenes have been found in RPP30. The existence of pseudogenes reduces the amplification efficiency of genes and reduces the accuracy of their use as internal reference genes for standardization (122, 123). Therefore, using RPP30 as the internal reference gene may be more accurate.

Numerous reports suggest that gene expression levels of β-actin and GAPDH are affected by many factors under different pathological conditions, such as tumor cells and non-tumor cells (103–105, 124), steatosis and alcoholic hepatitis (106), and Alzheimer’s disease (107). Under different experimental conditions, expression levels of the traditional internal reference genes, β-actin and GAPDH, vary greatly, such as in serum-stimulated fibroblasts (108), miR-644a (109), dietary conditions (125), and other conditions (110–114, 119–121). In addition, β-actin has extensive variation in mouse lymphocytes and is not appropriate for use as an internal reference gene for the quantitative PCR analysis of mouse lymphocytes (126), since

### Table 1 Application of RPP30 as an internal reference.

| Molecular biology technology | Disease                      | Disease-related genes |
|------------------------------|------------------------------|-----------------------|
| Multiple dd-PCR              | Tumor disease                | BRCA1 (89)            |
| Nanofluid digital PCR array  | Lung cancer                  | EGFR (90)             |
| Real-time quantitative PCR   | Breast cancer                | ERBB2 (91)            |
| Dd-PCR                       | Childhood disease            | TREC (92)             |
| Real-time fluorescence       | Severe combined immunodeficiency | SMN1 (93)           |
| quantitative PCR             | Spinal muscular atrophy      |                       |
| Dd-PCR                       | Blood disease                | α-thalassemia         |
| Dd-PCR                       | Inflammatory disease         | Psoriasis, Chronic obstructive Pulmonary disease, Crohn’s disease and reproductive tract infections |
| Dd-PCR                       | Others                       | Pelizaeus-Merzbacher disease |
| Dd-PCR                       | Hearing loss                 | PLP1 (96)             |

### Table 2 Internal reference gene primer sequences.

| Target gene | Forward primers | Reverse primers | Product length (bp) | Reference |
|-------------|-----------------|-----------------|---------------------|-----------|
| RPP30       | 5′-GGGCAAGATCGGTGTGCTGA-3′ | 5′-GGGCTTCTCCACAAAGT-3′ | 62       | (83, 96) |
| β-actin     | 5′-GGGCAAGATCGGTGTGCTGA-3′ | 5′-GGGCTTCTCCACAAAGT-3′ | 183      | (13)     |
| GAPDH       | 5′-GGGCAAGATCGGTGTGCTGA-3′ | 5′-GGGCTTCTCCACAAAGT-3′ | 201      |          |
such changes may lead to data divergence and inaccuracy. At present, there are few reports on factors affecting RPP30 expression levels, which may be related to the existence of RPP30 in all three fields of life (archaea, bacteria, and eukaryotes), and because it is widely expressed in different tissues whose gene sequences are conserved and homologous, such as in humans, chimpanzees, rhesus monkeys, mice, fruit flies, Saccharomyces cerevisiae, and archaea. In addition, currently, research on RPP30 is scant. The mRNA of β-actin and GAPDH are not highly expressed in all cell types or tissues of chicken embryos, and the expression levels are different in different tissues (115), which is similar to the 15-fold difference between the highest and lowest expression levels of GAPDH in different human tissues observed by Barber et al. (128). GAPDH expression levels also vary in different varieties of the same plant (129). Furthermore, β-actin and GAPDH expression levels fluctuate significantly at different stages of lymphocyte activation (116), which may be related to their participation in other cellular biology functions. RPP30 mRNA expression in different tissues is more stable than those of β-actin and GAPDH. In addition, RPP30 is widely expressed in 27 human tissues, is relatively conserved in structure and function, is not correlated with DNA content in the sample, and is not affected by the content of genes to be tested, resulting in high application value in a series of samples with scarce and uneven DNA content (91). Currently, to reduce the inaccurate data caused by differences in the expression of internal reference genes among different tissue types, RPP30 has become the main internal reference gene for quantitative detection of genes (130).

Moreover, the expression level of the three genes is affected by age (10, 117); their expression level decrease with age, but it is not known whether the specific mechanism is the same. There are also differences in the expression level of β-actin at different developmental stages (131). The factors affecting the expression level of RPP30 RNA in different pathological states, experimental conditions, and tissue types is lower than that of the commonly used internal reference genes, β-actin and GAPDH. RPP30 has good amplification efficiency and may be better used in RT-PCR experiments. Currently, there is no housekeeping gene that has stable expression, is abundant, and consistent under any experimental conditions. Currently, there is no housekeeping gene that has stable expression, is abundant, and consistent under any experimental conditions. Therefore, specific reference genes should be verified and selected according to experimental conditions and sample type (102, 118).

**Conclusion**

RPP30 is a highly conserved gene that has homologous genes in 16 species. Although RPP30 is a housekeeping gene and its encoding protein is a key subunit that maintains basic life activities, it is rarely reported to be associated with human diseases, and is overexpressed only in a few patients with cancer. In addition, compared to traditional reference genes, RPP30 has advantages of short sequence length, is widely and uniformly expressed in various tissues, and its expression level is rarely disturbed by external factors. Overall, RPP30 has great prospects and value as an internal reference gene. To date, RPP30 has been used as an internal reference for nucleic acid tests of Sars-CoV-2, evaluation of therapeutic drugs and drug side effects, analysis of the feasibility of HIV detection, and many other diagnostic experiments. However, due to the unstable detection results of the RPP30 protein, there are no studies that have used RPP30 as a reference in western blotting.

In such conditions, RPP30 may not be the first choice as a reference gene for these tests. Regardless of which kind of reference that is chosen, it may be affected by a few inevitable

---

**TABLE 3 Comparing RPP30 with other internal parameters.**

| Gene   | The function of encoding proteins                                                                 | Pseudogenes | Expression level of mRNA | Experimental conditions | Tissue types | Other                  |
|--------|--------------------------------------------------------------------------------------------------|-------------|--------------------------|-------------------------|--------------|------------------------|
| RPP30  | Realize RNase P RNA binding activity and participate in the excision of tRNA 5-precursor         | No found    | Rice infection ↑ (6)     | The expression between different tissues of human body is relatively stable | Age ↑ (10)   |                        |
| β-actin| Participate in the movement, structure and integrity of cells                                    | Exist (102) | Tumor ↑ (103-105),       | Unstable in different tissues of human body and at the stage of lymphocyte activation (115, 116) | Age ↑ (117)  |                        |
|        |                                                                                                 |             | Steatosis ↑ (106),       |                         |              |                        |
|        |                                                                                                 |             | Alcoholic hepatitis ↑ (107) |                         |              |                        |
| GAPDH  | Involved in glycolysis, DNA repair, tRNA output, membrane fusion and transport                   | Exist (118) | Tumor ↑ (103, 104),      |                         | Age ↑ (117)  |                        |
|        |                                                                                                 |             | Steatosis ↓, Alcoholic hepatitis ↑ (106) |                         |              |                        |

↑ indicates that the expression level is higher than that of the normal control in this cell. ↓ indicates that the expression level is lower than that of the normal control in this cell. AD, Alzheimer’s disease; HSV-1, herpes simplex virus; NO, nitric oxide.
conditions. Thus, the correct reference to be used for these tests should be further explored.

Author contributions

JW, SY and YW collected literature and wrote the original draft and prepared the figures and tables. JZ revised the original draft. ZZ conceived the idea and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by Anhui Provincial Natural Science Foundation (grant number 2108085MH298) and the Scientific research project of Anhui Medical University (grant number 2019GMFY02, 2021lxxk027). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Pulakkunat DK, Gopalan V. Studies on methanocaldococcus jannaschi rnase p reveal insights into the roles of rna and protein cofactors in rnase p catalysis. *Nucleic Acids Res* (2008) 36(12):e472–80. doi: 10.1093/nar/gkn360

2. Wu J, Niu S, Tan M, Huang C, Li M, Song Y, et al. Cryo-em structure of the human ribonuclease p holoenzyme. *Cell* (2018) 175(5):1393–404.e11. doi: 10.1016/j.cell.2018.10.003

3. Jarrous N, Eder PS, Guerrier-Takada C, Hoog C, Altman S. Autoantigenic properties of some protein subunits of catalytically active complexes of human ribonuclease p. *Rna* (1998) 4(4):407–17.

4. Jarrous N, Gopalan V. Archaeal/eukaryal rnase p: Subunits, functions and rna diversification. *Nucleic Acids Res* (2010) 38(22):7855–94. doi: 10.1093/nar/gkq1001

5. Wang SQ, Shi DQ, Long YP, Liu J, Yang WC. Gametophyte defective 1, a putative subunit of rnases P/Mrp, is essential for female gametogenesis and Male competence in arabidopsis. *Plant Biotechnol J* (2021) 19(10):1988–99. doi: 10.1111/pbi.13612

6. Li W, Xiong Y, Liu LB, Zhang K, Li Z, Kang H, et al. The rice rnase p protein subunit RPP30 confers broad-spectrum resistance to fungal and bacterial pathogens. *Plant Biotechnol J* (2021) 19(10):1988–99. doi: 10.1111/pbi.13612

7. Molla-Herman A, Valles AM, Ganem-Elbaz C, Antoniewski C, Huyhn JR. Trna processing defects induce replication stress and Chk2-dependent disruption of pirs transcription. *EMBO J* (2015) 34(24):3009–27. doi: 10.15252/embj.201519006

8. Tang Z, Kang B, Li C, Chen T, Zhang Z. Gepia2: An enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* (2019) 47(W1):W536–w60. doi: 10.1093/nar/gkz430

9. Lee YH, Choi SJ, Ji JD, Song GG. Pathway analysis of genome-wide association study for bone mineral density. *Med Biol Rep* (2012) 39(8):8099–106. doi: 10.1134/S0131023912070012

10. Li G, Zhai Y, Liu H, Wang Z, Huang R, Jiang H, et al. RPP30, a transcriptional regulator, is a potential pathogenic factor in globastoma. *Aging (Albany NY)* (2020) 12(16):16155–71. doi: 10.18632/aging.103596

11. Zhang Y, Wang C, Han M, Ye J, Gao Y, Liu Z, et al. Discrimination of false negative results in rt–pcr detection of sars-cov-2 rnas in clinical specimens by using an internal reference. *Virol Sin* (2020) 35(6):758–70. doi: 10.1007/s12250-021-00273-8

12. Thellin O, Zerzi W, Lakaye B, De Borman B, Coumans B, Hennen G, et al. Housekeeping genes as internal standards. Use and limits. *J Biotechnol* (1999) 75(2–3):291–5. doi: 10.1016/s0168-1656(99)00163-7

13. Yin R, Liu X, Liu C, Ding Z, Zhang X, Tian F, et al. Systematic selection of housekeeping genes for gene expression normalization in chicken embryos fibroblasts infected with Newcastle disease virus. *Biochem Biophys Res Commun* (2011) 413(4):537–40. doi: 10.1016/j.bbrc.2011.08.131

14. Li R, Shen Y. An old method facing a new challenge: Re-visiting housekeeping proteins as internal reference control for neuroscience research. *Life Sci* (2013) 92(13):747–51. doi: 10.1016/j.lfs.2013.02.034

15. Jarrous N. Roles of rnase p and its subunits. *Trends Genet* (2017) 33(9):594–603. doi: 10.1016/j.tig.2017.06.006

16. Phan HD, Norris AS, Du C, Stachowski K, Kharinusa BH, Sidharthan V, et al. Elucidation of structure-function relationships in methanocaldococcus jannaschi rnase p, a multi-subunit catalytic ribonucleoprotein. *Nucleic Acids Res* (2022) 50(14):e184–7. doi: 10.1093/nar/gkac595

17. Reiner R, Ben-Asooli Y, Krivolevsky J, Jarrous N. A role for the catalytic ribonucleoprotein rs p in rna polymerase iii transcription. *Genes Dev* (2006) 20(12):1621–35. doi: 10.1101/gad.136706

18. Welting TJ, Kikker BJ, van Ventrooij WJ, Pruijn GI. Differential association of protein subunits with the human mae rnp and mae p complexes. *RNA* (2006) 12(7):1373–82. doi: 10.1261/rna.2293906

19. Chang DD, Clayton DA. A mammalian mitochondrial rna processing activity contains nucleus-encoded rna. *Science* (1987) 235(4793):1178–84. doi: 10.1126/science.2434997

20. Lygerou Z, Allmann C, Torelledy D, Seraphin B. Accurate processing of a eukaryotic precursor ribosomal rna by ribonuclease mpr in vitro. *Science* (1996) 272(5259):268–70. doi: 10.1126/science.272.5259.268

21. Cai T, Auld J, Gill T, Cerio M, Schmitt ME. The saccharomyces cerevisiae rna mitochondrial rna processing is critical for cell cycle progression at the end of mitosis. *Genetics* (2002) 161(3):1029–42. doi: 10.1093/genetics/161.3.1029

22. Guerriettakada C, Gartner K, Marsh T, Pace N, Altman S. The rna moiety of ribonuclease-p is the catalytic subunit of the enzyme. *Cell* (1983) 35(3):849–57. doi: 10.1016/0092-8674(83)90174-7

23. Li N, Yuan L, Liu N, Shi D, Li X, Tang Z, et al. Slow Walker2, a Noc1/Mak21 homologue, is essential for coordinated cell cycle progression during female gametophyte development in arabidopsis. *Plant Physiol* (2009) 151(3):1486–97. doi: 10.1104/pp.109.142414

24. Guerrier-Takada C, Altman S. Catalytic activity of an rna molecule prepared by transcription in vitro. *Science* (1984) 228(4683):285–6. doi: 10.1126/science.6199841

Acknowledgments

We would like to thank Editage (www.editage.cn) for English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

We would like to thank Editage (www.editage.cn) for English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.
functional cooperation among protein subunits in archaeal rna-seq, a catalytic
j.mol.biol.2009.08.068

of polycistronic operon mrnas.
Proc Natl Acad Sci U.S.A.
cerevisiae.
Mol Cell

ckse and stolc v, altman s. characterization of two sderceral autoimmune antigens that copurify with human ribonuclease p. Proc Natl Acad Sci U.S.A. (1997) 94(1):1101–6. doi:10.1073/pnas.94.4.1101

engelke dr, fischer ca. the evolution of rna-splice rna. Proc Natl Acad Sci U.S.A. (2015) 12(14):517–8. doi:10.1073/pnas.1509732115

shao y, ye m, li q, sun w, ye g, zhang x, et al. lnclna-rnmp promotes carcinogenicity by acting as a mir-206 sponge and is used as a novel biomarker for gastric cancer. Oncotarget (2016) 7(25):37812–9. doi:10.18632/oncotarget.9336

stolc v, altman s, rpp1, an essential protein subunit of nuclear rna-splice rna required for processing of precursor rna and 35s precursor rna in saccharomyces cerevisiae. Genes Dev (1997) 11(21):2926–37. doi:10.1101/gad.11.21.2926

chen wy, pulukkunat dk, cho im, tsai hy, gopalan v. dissecting functional cooperation among protein subunits in archaeal rna-splice rna, a catalytic ribonucleoprotein complex. Nucleic Acids Res (2010) 38(22):8316–27. doi:10.1093/nar/gqh368

jiang t, altman s. protein-protein interactions with subunits of human nuclear rna-splice rna. Proc Natl Acad Sci U.S.A. (2001) 98(3):920–5. doi:10.1073/pnas.98.3.920

jiang t, guerrer-takada c, altman s. protein-rna interactions in the subunits of human nuclear rna-splice rna. Proc Natl Acad Sci U.S.A. (2001) 98(7):3937–41. doi:10.1073/pnas.1355838100

altman s. a view of rna. Mol Biol (2007) 3(9):604–7. doi:10.1039/b707850c

xu y, oraganti sv, gopalan v, foster mp. thermodynamics of coiled folding in the interaction of archaeal rna-splice rna proteins rpp21 and rpp29. Biochemistry (2012) 51(16):926–35. doi:10.1021/bi201674d

ishihara m, nishimoto e, yamashita s, kakuta y, kimura m. a distinct binding mode of archaeal ribonuclease p proteins to rna. Biosci Biootechnol Biochem (2012) 76(12):2335–7. doi:10.1271/bbb.120546

ziehler wa, day jj, fischer ca, engelke dr. effects of 5’-leader and 3’-trailer structures on pre-rna processing by nuclear rna. Biochemistry (2000) 39(32):9909–16. doi:10.1021/b000603n

wilson rc, bohlen cj, foster mp, bell ce. structure of the pfu pop5, an archaeal rna-splice rna protein. Proc Natl Acad Sci U.S.A. (2006) 103(4):873–8. doi:10.1073/pnas.0508004103

amero cd, boomershine wp, xu y, foster m. solution structure of pyrococcus furiosus rpp21, a component of the archaeal rna-splice rna holoenzyme, and interactions with its rpp29 protein partner. Biochemistry (2008) 47(45):11704–10. doi:10.1021/bi0804103

hamasaki m, hanzaya k, ibusaki f, ueda t, nakashima t, kakuta y, et al. functional implication of archaeal homologues of human rna-splice rna protein pair rpp5 and rpp30. J Biochem (2016) 159(1):31–40. doi:10.1093/jb/mmv067

smith jk, hsieh j, fischer ca. importance of rna-protein interactions in bacterial ribonuclease p structure and catalysis. Biopolymers (2007) 87(5-6):329–38. doi:10.1002/bip.20846

xu y, amero cd, pulukkunat dk, gopalan v, foster mp. solution structure of an archaeal rna-splice rna protein complex. formation of the 30-kda complex between pyrococcus furiosus rpp21 and rpp29 is accompanied by coupled protein folding and highlights critical features for protein-protein and protein-rna interactions. J Mol Biol (2009) 393(5):1043–55. doi:10.1016/j.jmb.2009.08.068

crowe bl, bohlen cj, wilson rc, gopalan v, foster mp. assembly of the complex between archaeal rna-splice rna proteins rpp30 and rpp5. Archaea (2011) 2011:895131. doi:10.1155/2011/895131

vourkakis a, kavaliouziot j, zarkasid ik, spyroulias ga, stathopoulos c, drainas d. a 40.7 kda rpp30/rpp1 homologue is a protein subunit of dicer/dsoduum rna-splice rna holoenzyme. Biochimie (2007) 89(3):301–10. doi:10.1016/j.biochi.2006.11.006

honda t, hara t, nan j, zhang x, kimura m. archaeal homologues of human rna-splice rna protein pairs pop5 with rpp30 and rpp29 work on distinct functional domains of the rna-subunit. Biosci Biotechnol Biochem (2010) 74(2):266–72. doi:10.1271/bbb.090550

welting tj, van venrooij wj, pruin gj. mutual interactions between subunits of the human rna-splice ribonuclease p complex. Nucleic Acids Res (2004) 32(7):2138–46. doi:10.1093/nar/gdh539

ariza-mateos a, briones c, perales d, domingo e, gómez j. the archaeology of coding rna. Biochimie (2019) 144(71):119–34. doi:10.1011/nyasa.14173

chambyeron s, popkova a, payen-groschen g, brun c, laouini d, pelisson a, et al. pirna-mediated nuclear accumulation of retrotransposon transcripts in the drosophila female germline. Proc Natl Acad Sci U.S.A. (2008) 105(39):14964–9. doi:10.1073/pnas.0805943105

bremerke j, aravin aa, stark a, dus m, kelli s, sachidanandam r, et al. discrete small rna-generating loci as master regulators of transposon activity in drosophila. Cell (2007) 128(6):1089–103. doi:10.1016/j.cell.2007.01.043

paine a, wehr k, schupbach t. zucchini and squash encode two putative nucleases required for rsarna production in the drosophila germline. Dev Cell (2007) 12(6):851–62. doi:10.1016/j.devcel.2007.03.022

zhang f, wang j, xu j, zhang z, koppetsch bs, schultz n, et al. upap coops pirna clusters to the peripheral transposon silencing machinery. Cell (2012) 151(4):871–84. doi:10.1016/j.cell.2012.09.040

ding b, bellazzi ml, rind n, ying y, meyers bc, wang gl. h7d’r01, a histone h4 deacetylase, negatively regulates plant innate immunity by modulating histone h4 acetylation of defense-related genes in rice. Plant Cell (2012) 24(9):3783–94. doi:10.1105/tpc.112.103172

okano y, medger t.d.t. autoantibody to th ribonuclease (nuclear 7.2 rna-protein particle) in patients with systemic sclerosis. Arthritis Rheum (1990) 33(12):1822–8. doi:10.1002/art.1780331210

meccoli ca, adler bl, yang q, hummers l, rosen a, casciola-rosen l, et al. cancer in systemic sclerosis. analysis of antibodies against components of the 7.2 rna-protein particle. J Autoimmun (1992) 7:21–147. doi:10.1016/j.jautrev.1988.01.058

reddy r, tan em, hennig d, nöhga k, busch h. detection of a nuclear 7-2 ribonucleoprotein and a cytoplasmic 8-2 ribonucleoprotein with autoantibodies from patients with scleroderma. J Biol Chem (2003) 278(3):1833–8. doi:10.1074/jbc.m207136200

nymaes j, pulukkunat dk, gopalan v, foster mp. assembly of the complex between archaeal rna-splice rna proteins rpp30 and rpp5. rpp30 and rpp29 work on distinct functional domains of the rna-subunit. biosci biotechnol biochem (2010) 74(2):266–72. doi:10.1271/bbb.090550
control gene. *Appl Immunohistochem Mol Morphol* (2016) 24(3):179–87. doi:10.1097/PAI.0000000000000100
92. Vidal-Folch N, Malasevic D, Majumdar R, Gavrilov L, Matern D, Raymond K, et al. A droplet digital pcr method for severe combined immunodeficiency newborn screening. *J Mol Diagn* (2017) 19(5):755–65. doi:10.1016/j.jmoldx.2017.05.011
93. Adams SP, Gravett E, Kent N, Kricks S, Iederso A, Mesto C, et al. Screening of neonatal UK dried blood spots using a duplex 5nm1 screening assay. *Int J Neonatal Screen* (2021) 7(4):69. doi:10.3390/ijns7040069
94. Bao X, Qin D, Ma J, Zhou X, Wang J, Yao C, et al. Accurate detection of et- globin gene copy number variants with two reactions using droplet digital pcr. *Hematology* (2022) 27(1):198–203. doi:10.1007/s10587-022-000885
95. Wen T, Zhang X, Lippuner C, Schuff M, Stuber F. Development and evaluation of a droplet digital pcr assay for 8p23 beta-defensin cluster copy number determination. *Mol Diagn Ther* (2021) 25(5):607–15. doi:10.1007/s12029-021-00546-2
96. Imaiuzumi T, Yamamoto-Shimoijima K, Yamamoto T. Advantages of ddPCR in detection of P1dup. *Intractable Rare Dis Res* (2019) 8(3):198–202. doi:10.5582/irdr.2019.01067
97. Ito T, Kawashima Y, Fujikawa T, Honda K, Makabe A, Kitamura K, et al. Rapid screening of copy number variations in strep by droplet digital pcr in patients with mild-to-moderate hearing loss. *Hum Genome Var* (2019) 6:41. doi:10.1038/s41388-019-0007-5
98. Chang CW, Cheng WC, Chen CR, Shu WT, Tsai ML, Huang CL, et al. Identification of human housekeeping genes and tissue-specific genes by microarray meta-analysis. *PloS One* (2011) 6(7):e22859. doi:10.1371/journal.pone.0022859
99. Mehta R, Birerdic A, Hossain N, Afendy A, Chandhoke V, Younossi Z, et al. Validation of endogenous reference genes for qrt-pcr analysis of human visceral adipose samples. *BMC Mol Biol* (2010) 11:39. doi:10.1186/1471-2199-11-39
100. Ampe C, Van Trosy M. Mammalian actins: Isoform-specific functions and diseases. *Handb Exp Pharmacol* (2017) 251:1–37. doi:10.1007/164_2016_43
101. Tristan C, Shaltani N, Sedlak TW, Sawa A. The diverse functions of g3pdh. Views from different subcellular compartments. *Cell Signal* (2011) 23(2):317–23. doi:10.1016/j.cellsig.2010.08.003
102. Sun Y, Li Y, Luo D, Liao DJ. Pseudogenes as weaknesses of actb (Actb) and g3pdh (G3PDH) used as reference genes in real-time reverse transcription and polymerase chain reactions. *PloS One* (2012) 7(8):e41659. doi:10.1371/journal.pone.0041659
103. Chang TJ, Juan CC, Yin PH, Chi CW, Tsay HY. Up-regulation of beta- actin, cyclophilin and g3pdh in N1s1 rat hepatoma. *Oncol Rep* (1998) 5(2):469–71. doi:10.3892/or.5.2.469
104. Ferguson RE, Carroll HP, Harris A, Maher ER, Selby PJ, Banks RE. Housekeeping proteins: A preliminary study illustrating some limitations as useful references in protein expression studies. *Proteomics* (2005) 5(2):566–71. doi:10.1002/pro.200409941
105. Ruan W, Lai M. Actin, a reliable marker of internal control? *Clin Chim Acta* (2007) 385(1–2):1–5. doi:10.1016/j.cca.2007.07.003
106. Boujedjali H, Bouchet-Debois L, Cassard-Doulcier AM, Nijm-Keske M, Meure S, Prevot S, et al. Housekeeping gene variability in the liver of alcoholic patients. *Alcohol Clin Exp Res* (2012) 36(2):258–66. doi:10.1111/j.1460-244X.2011.04267.x
107. Gebhardt FM, Scott HA, Dodd PR. Housekeepers for accurate transcript expression analysis in alzheimer’s disease autopsy brain tissue. *Alzheimers Dement* (2010) 6(1):465–74. doi:10.1016/j.jalz.2009.11.002
108. Schmittgen TD, Zakraje A. Effect on real-time rt-pcr to housekeeping gene expression: Validation by real-time, quantitative rt-pcr. *J Biochem Biophys Methods* (2000) 46(1–2):69–81. doi:10.1016/s0165-022x(00)00229-9
109. Sankar K, Singh J, Ebron JY, Shulka GC. Housekeeping gene selection advisory: Glyceraldehyde-3-Phosphate dehydrogenase (Gapdh) and beta-actin are targets of mir-644a. *PloS One* (2012) 7(10):e47510. doi:10.1371/journal.pone.0047510
110. Wang XT, Cheng K, Zhu L. [Hyperxia accelerate β-actin expression through transcriptional activation of actb by nuclear respiratory factor-1]. *Mol Biol (Mosk)* (2021) 55(3):460–7. doi:10.1134/s1065773621030186
111. Nyström K, Biller M, Grahn A, Lindh M, Larson G, Olofsson S. Real time pcr for monitoring regulation of host gene expression in herpes simplex virus type 1 infected human diploid cells. *J Virol Methods* (2004) 118(2):83–94. doi:10.1016/j.jviromet.2004.01.019
112. Jenmiolo B, Trappe S. Single muscle fiber gene expression in human skeletal muscle: Validation of internal control with exercise. *Biochem Biophys Res Commun* (2004) 320(3):1043–50. doi:10.1016/j.bbrc.2004.05.223
113. Yamada H, Chen D, Monstein HJ, Håkanson R. Effects of fasting on the expression of gastrin, cholecystokinin, and somatostatin genes and of various housekeeping genes in the pancreas and upper digestive tract of rats. *Biochem Biophys Res Commun* (1997) 231(3):835–8. doi: 10.1006/bbrc.1997.6198

114. Bémeur C, Ste-Marie L, Desjardins P, Hazell AS, Vachon L, Butterworth R, et al. Decreased beta-actin mrna expression in hyperglycemic focal cerebral ischemia in the rat. *Neurosci Lett* (2004) 357(3):211–4. doi: 10.1016/j.neulet.2003.12.081

115. Lin J, Redies C. Histological evidence: Housekeeping genes beta-actin and gapdh are of limited value for normalization of gene expression. *Exp Genes Dev* (2012) 222(6):369–76. doi: 10.1074/ed2.012-0420.x

116. Bas A, Forsberg G, Hammarström S, Hammarström ML. Utility of the housekeeping genes 18s rna, beta-actin and glyceraldehyde-3-phosphate-dehydrogenase for normalization in real-time quantitative reverse transcriptase-polymerase chain reaction analysis of gene expression in human T lymphocytes. *Scand J Immunol* (1999) 50(2):193–7. doi: 10.1046/j.1365-3083.1999.00227.x

117. Hernandez-Segura A, Rubingh R, Demaria M. Identification of stable senescence-associated reference genes. *Aging Cell* (2019) 18(2):e12911. doi: 10.1111/ace.12911

118. Liu YJ, Zheng D, Balasubramanian S, Carriero N, Robilotto R, et al. Comprehensive analysis of the pseudogenes of glycolytic enzymes in vertebrates: The anomalously high number of gapdh pseudogenes highlights a recent burst of retrotranspositional activity. *BMC Genomics* (2009) 10:480. doi: 10.1186/1471-2164-10-480

119. Alexander MC, Lomanto M, Nasrin N, Ramaika C. Insulin stimulates glyceraldehyde-3-phosphate dehydrogenase gene expression through cis-acting DNA sequences. *Proc Natl Acad Sci U.S.A.* (1988) 85(14):5092–6. doi: 10.1073/pnas.85.14.5092

120. Graven KK, Troxler RF, Kornfeld H, Panchenko MV, Farber HW. Regulation of endothelial cell glyceraldehyde-3-phosphate dehydrogenase expression by hypoxia. *J Biol Chem* (1994) 269(39):24446–53

121. Bereta J, Bereta M. Stimulation of glyceraldehyde-3-phosphate dehydrogenase mrna levels by endogenous nitric oxide in cytokine-activated endothelium. *Biochem Biophys Res Commun* (1995) 217(1):363–9. doi: 10.1006/bbrc.1995.2785

122. Wimmer K, Wernstedt A. Pms2 gene mutational analysis: Direct cdna sequencing to circumvent pseudogene interference. *Methods Mol Biol* (2014) 1167:289–302. doi: 10.1007/978-1-4939-0835-6_20

123. Balaji S, Vanniarajan A. Implication of pseudo reference genes in normalization of data from reverse transcription-quantitative pcr. *Gene* (2020) 757:144948. doi: 10.1016/j.gene.2020.144948

124. Zhang JY, Zhang F, Hong CQ, Giuliano AE, Cui XJ, Zhou GJ, et al. Critical protein gapdh and its regulatory mechanisms in cancer cells. *Cancer Biol Med* (2015) 12(1):10–22. doi: 10.7497/jissn.2095-3941.2014.0019

125. Janovick-Guretzky NA, Dann HM, Carlson DB, Murphy MR, Loor JJ, Drackley JK. Housekeeping gene expression in bovine liver is affected by physiological state, feed intake, and dietary treatment. *J Dairy Sci* (2007) 90(5):2246–52. doi: 10.3168/jds.2006-6640

126. Abershaid TC, Iritani BM, Ruddell A. Evaluation of reference genes for quantitative pcr analysis of mouse lymphocytes. *J Immunol Methods* (2012) 384(1-2):156–9. doi: 10.1016/j.jim.2012.07.020

127. Jarrous N. Human ribonuclease p: Subunits, function, and intranuclear localization. *Rna* (2002) 8(1):1–7. doi: 10.1017/s1355838202011184

128. Barber RD, Harmer DW, Coleman RA, Clark BJ. Gapdh as a housekeeping gene: Analysis of gapdh mrna expression in a panel of 72 human tissues. *Physiol Genomics* (2005) 21(3):389–95. doi: 10.1152/physiolgenomics.00025.2005

129. Kim BR, Nam HY, Kim SU, Kim SI, Chang YJ. Normalization of reverse transcription quantitative-pcr with housekeeping genes in rice. *Biotechnol Lett* (2003) 25(21):1869–72. doi: 10.1023/a:1026298032009

130. Mancini C, Messana E, Turco F, Brusino A, Brusco A. Gene-targeted embryonic stem cells: Real-time pcr assay for estimation of the number of neomycin selection cassettes. *Biol Proc Online* (2011) 13:10. doi: 10.1186/1480-9222-13-10

131. Sellers MJ, Vuocolo T, Leeton LA, Conan GJ, Degenman BM, Preston NP. Real-time rt-pcr quantification of kuruma shrimp transcripts: A comparison of relative and absolute quantification procedures. *J Biotechnol* (2007) 129(3):391–9. doi: 10.1016/j.jbiotec.2007.01.029

132. Derveaux S, Vandesompele J, Hellemans J. How to do successful gene expression analysis using real-time pcr. *Methods* (2010) 50(4):227–30. doi: 10.1016/j.ymeth.2009.11.001