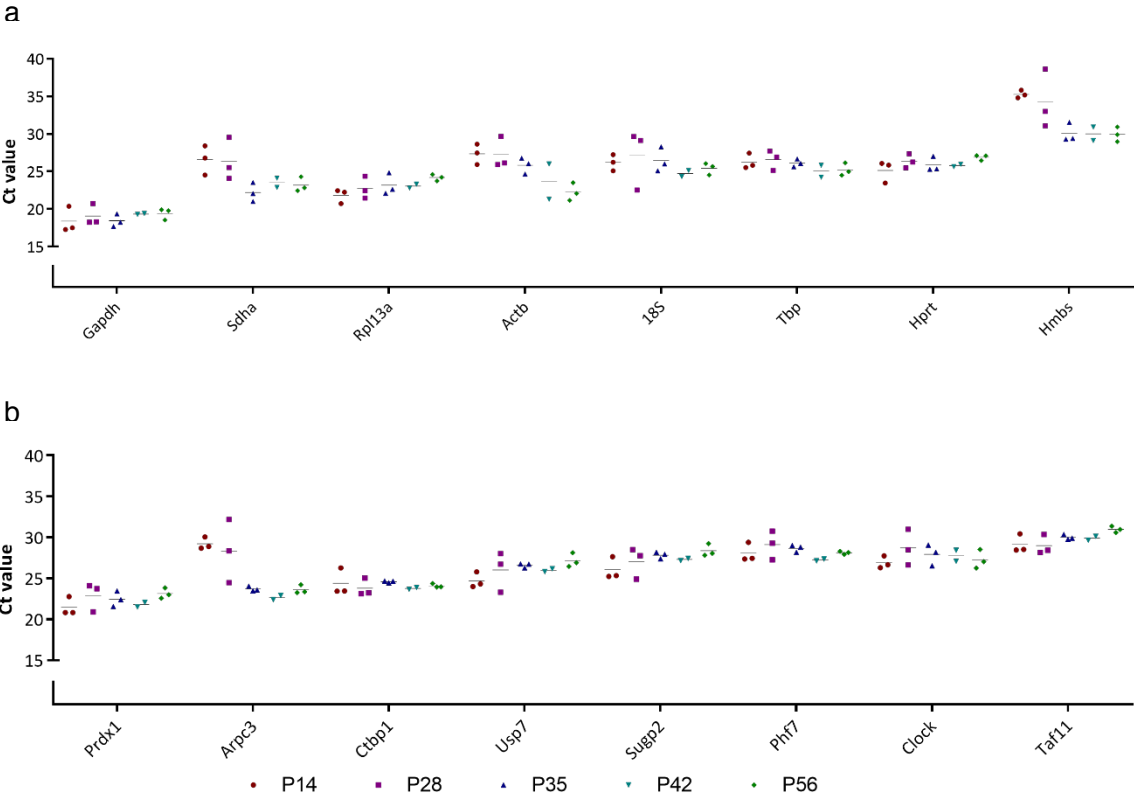


# **Identification of reliable reference genes for qRT-PCR studies of the developing mouse mammary gland**

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## **Supplementary Files**

- [Supplementary Figure S1 \(this PDF\)](#)
- [Supplementary Figure S2 \(this PDF\)](#)
- [Supplementary Table S1 \(uploaded separately as an .xlsx file\)](#)
- [Supplementary Table S2 \(this PDF\)](#)



**Supplementary figure S1. Ct values and reference gene ranking for #3 mammary gland.** Scatter plot depicting spread in expression of candidate reference genes in different stages of the #3 mouse mammary gland. a) Traditional reference genes b) Novel candidate reference genes.

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(A)

Ranking Order (Better--Good--Average)								
Method	1	2	3	4	5	6	7	8
<a href="#">Delta CT</a>	Tbp	Rpl13a	Hprt	18S	Gapdh	Sdha	Actb	Hmbs
<a href="#">BestKeeper</a>	Rpl13a	Tbp	Hprt	Gapdh	18S	Sdha	Actb	Hmbs
<a href="#">Normfinder</a>	Tbp	Rpl13a	Hprt	18S	Sdha	Gapdh	Actb	Hmbs
GeNorm qBase	Rpl13a	Hprt	Gapdh	Tbp	18S	Sdha	Actb	Hmbs
<a href="#">Recommended comprehensive ranking</a>	<b>Rpl13a</b>	<b>Tbp</b>	<b>Hprt</b>	<b>Gapdh</b>	<b>18S</b>	<b>Sdha</b>	<b>Actb</b>	<b>Hmbs</b>

(B)

Ranking Order (Better--Good--Average)								
Method	1	2	3	4	5	6	7	8
<a href="#">Delta CT</a>	Prdx1	Sugp2	Ctbp1	Phf7	Usp7	Taf11	Clock	Arpc3
<a href="#">BestKeeper</a>	Prdx1	Ctbp1	Phf7	Sugp2	Taf11	Usp7	Clock	Arpc3
<a href="#">Normfinder</a>	Prdx1	Phf7	Ctbp1	Sugp2	Clock	Usp7	Taf11	Arpc3
GeNorm qBase	Phf7	Prdx1	Ctbp1	Sugp2	Usp7	Taf11	Clock	Arpc3
<a href="#">Recommended comprehensive ranking</a>	<b>Prdx1</b>	<b>Phf7</b>	<b>Ctbp1</b>	<b>Sugp2</b>	<b>Usp7</b>	<b>Taf11</b>	<b>Clock</b>	<b>Arpc3</b>

**Supplementary Figure S2.**

Comprehensive ranking of the old/traditional (A) and novel candidate reference genes (B) using RefFinder (available at <http://fulxie.0fees.us/?type=reference>). A combined comprehensive ranking of both the traditional and novel candidate genes is presented in Table 1.

Gene name	Accession Number	Primer sequence (5' – 3')	Amplicon length	PCR Efficiency %
<b>Reference genes</b>				
<i>18S</i>	NR_003278	F: CCGCCGCCATGTCTCTAGT R: CTTTCCTCAACACCACATGAGC	150 bp	90
<i>Arpc3</i>	NM_019824	F: TGAAGCGGACAGGACATTGAT R: AGTTGGTGATTCTAGCGTGT	122 bp	92
<i>Actb</i>	NM_007393	F: GAGCTGCCTGACGGCCAGGT R: TACTCCTGCTTGCTGATCCA	365 bp	72
<i>Clock</i>	NM_001289826	F: TTTACAGGCGTTGTTGATTGGA R: ACGCAAGGCCGTCTTCTG	92 bp	97
<i>Ctbp1</i>	NM_001198859	F: GTGCCCTGATGTACCATACCA R: GCCAATTCGGACGATGATTCTA	83 bp	92
<i>Gapdh</i>	NM_001289726	F: CTGGTGCTGCCAAGGCT R: CTGCTTCACCACCTTCTTGATGTC ATCATA	161 bp	99
<i>Hmbs</i>	NM_013551	F: GCCCATGTGCCTTCAGTC R: TCTTTGAGCCGTTTTCTCCGC	151 bp	101
<i>Hprt</i>	NM_013556	F: TGTTGTTGGATATGCCCTTG R: TTGCGCTCATCTTAGGCTTT	108 bp	97
<i>Phf7</i>	NM_027949	F: TGTTTGCCTTCTATGCCTTCAA R: GGCAGTCTGCTAGACAGGATAAG	114 bp	88
<i>Prdx1</i>	NM_011034	F: AATGCAAAAATTGGGTATCCTGC R: CGTGGGACACACAAAAGTAAAGT	150 bp	96
<i>Rpl13a</i>	NM_009438	F: CCCTCCACCCTATGACAAGA R: GCCCCAGGTAAGCAAACCTT	95 bp	101
<i>Sdha</i>	NM_023281	F: GTTGCTGTGTGGCTGATCG R: GCACAGTGCAATGACACCAC	149 bp	89
<i>Sugp2</i>	NM_001168290	F: TGACCAGAGGCCGATCTCTAA R: GGAAGTAATACGACTGGCAACAG	158 bp	105
<i>Tbp</i>	NM_013684	F: GTCATTTTCTCCGAGTGCC R: GCTGTTGTTCTGGTCCATGAT	151 bp	94
<i>Taf11</i>	NM_026836	F: GATGGAGACGCGGACTTGAAA R: GCAGGAGTAAGTAAGGACGAGTT	113 bp	99
<i>Usp7</i>	NM_001003918	F: CCACAAGGAAAACGACTGGG R: GTAACACGTTGCTCCCTGATT	196 bp	88
<b>Target genes</b>				
<i>Wnt4</i>	NM_009523	F: ACTGGACTCCCTCCCTGTCT R: TGCCCTTGCTACTGCAAA	109 bp	97

**Supplementary table S2. Overview of all genes and primer sequences used in this study.** The length of the amplicon and the efficiency of the primers is also listed.