Clarida

geNorm and NormFinder analysis generated by Clarida's Reference Gene Finder

2026-11-03 09:24 CET · Analysis ID: example-demo-dataset · Source: demo dataset

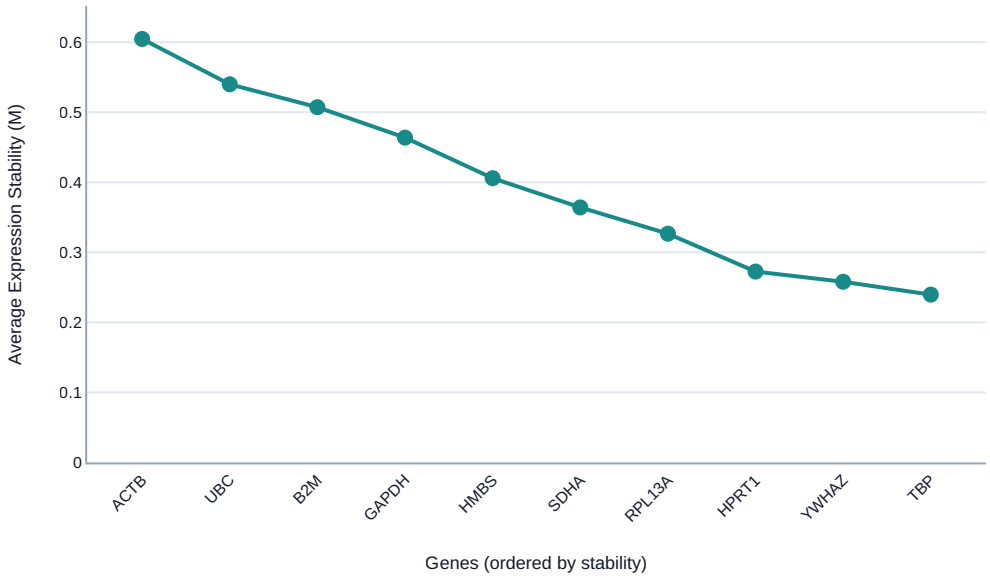
Recommended reference gene set: TBP, YWHAZ

Mean M of these genes 0.214 · 16 samples × 10 candidates (TBP, YWHAZ, HPRT1, RPL13A, SDHA, HMBS, GAPDH, B2M, UBC, ACTB)
NormFinder cross-check: Most stable gene: TBP. Best combination of two genes: TBP + YWHAZ. Both methods agree on the top 2 genes: TBP, YWHAZ.

Data health · Some checks need attention

- **Samples: 16 samples analysed** — geNorm
- **Candidate genes: 10 candidate genes analysed** — NormFinder
- **Sample groups: 2 groups with at least 8 samples each** · Normoxia 8, Hypoxia 8 — NormFinder
- **Missing data: No missing values** — Both methods
- **PCR efficiency: 100% efficiency (E = 2) assumed for 10 of 10 genes** · No PCR efficiency supplied for any gene — Both methods

Gene stability ranking (geNorm, average M)



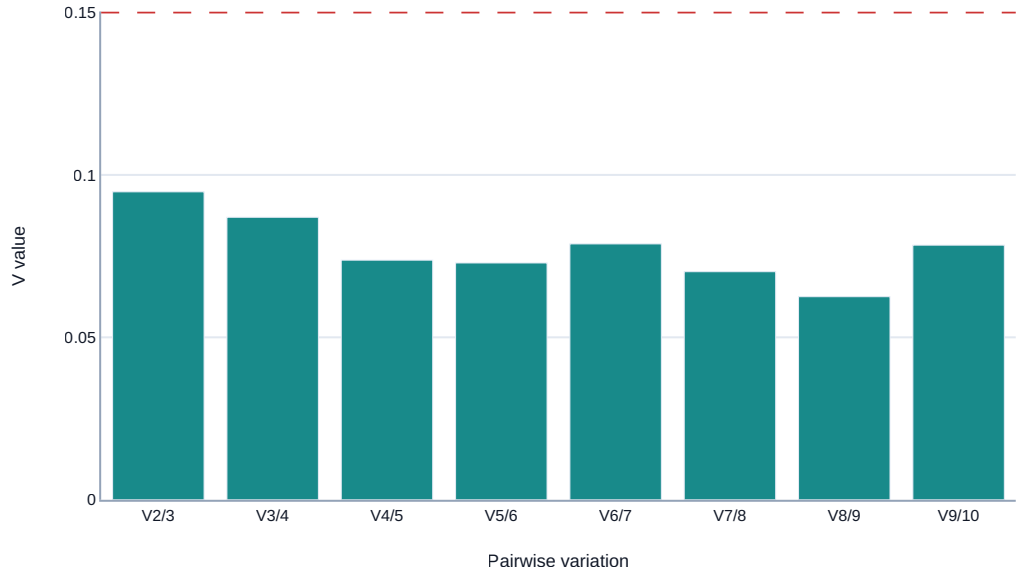
Rank	Gene	Average M	Gene M
1	TBP	0.240	0.240
2	YWHAZ	0.258	0.276
3	HPRT1	0.272	0.302
4	RPL13A	0.327	0.381
5	SDHA	0.364	0.420
6	HMBS	0.406	0.489

Rank	Gene	Average M	Gene M
7	GAPDH	0.464	0.608
8	B2M	0.507	0.637
9	UBC	0.540	0.654
10	ACTB	0.605	0.864

Average M: the mean M of all genes still in the pool when this gene was eliminated; this is the value in the chart and the one other geNorm tools report. Gene M: this gene's own stability value, its average pairwise variation with the other genes still in the pool; for the final three genes, its M when three genes remain. Rank follows the elimination order, so Gene M is not always in rank order.

Interpretation threshold (Hellemans et al. 2007): M < 0.5 is typically found in homogeneous samples; M < 1.0 is acceptable for heterogeneous samples. These guidelines apply to the mean M of the recommended genes, calculated within that set, not to individual genes or to Gene M.

Pairwise variation (V-values)



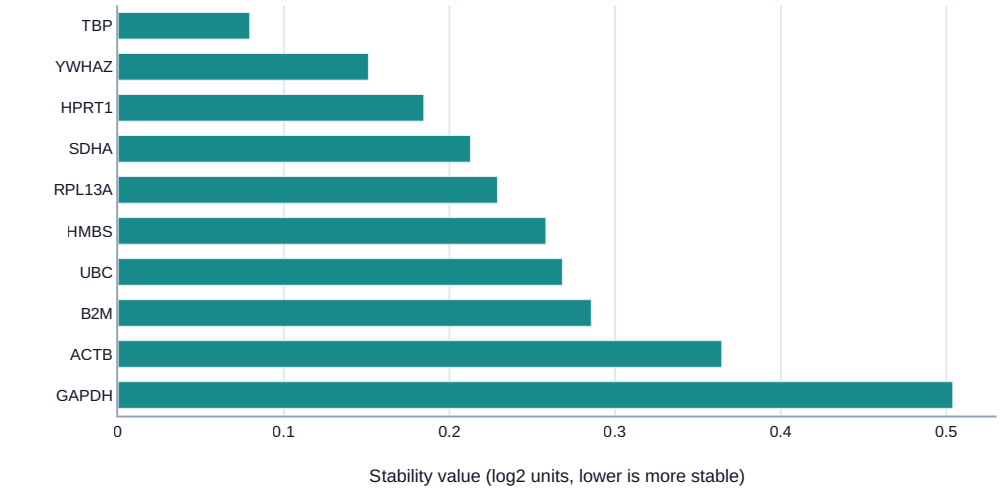
Optimal number of reference genes: **2**

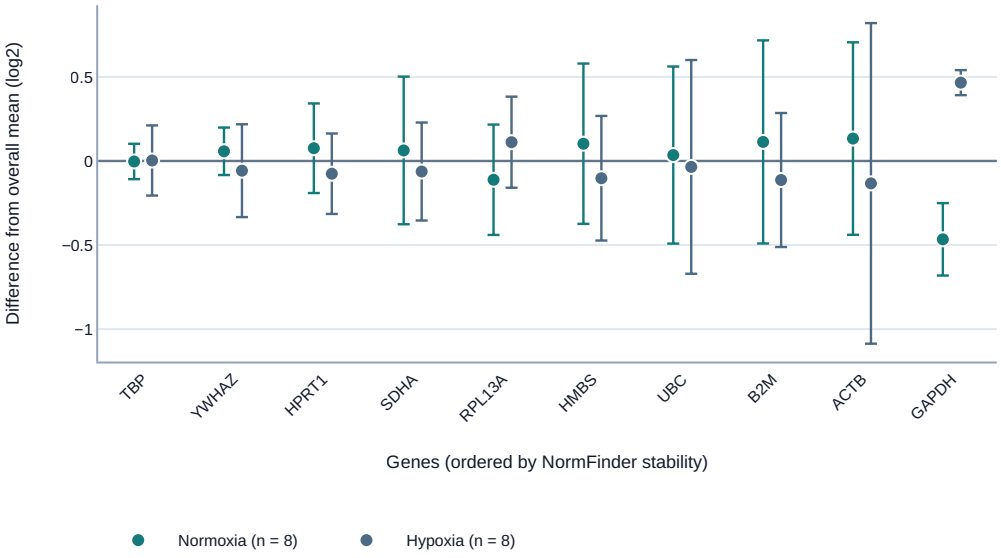
NormFinder stability

Most stable gene: TBP.

Best combination of two genes: TBP + YWHAZ.

geNorm recommends using 2 reference genes.





Intergroup difference per gene with the intragroup SD as error bars. Points far from zero mean the gene's level shifts between groups.

Rank	Gene	Stability	Within-group SD	Group difference	Normoxia (n = 8): difference ± SD	Hypoxia (n = 8): difference ± SD
1	TBP*	0.079	0.165	0.006	-0.003 ± 0.105	+0.003 ± 0.209
2	YWHAZ	0.151	0.219	0.115	+0.058 ± 0.141	-0.058 ± 0.276
3	HPRT1	0.185	0.253	0.152	+0.076 ± 0.267	-0.076 ± 0.239
4	SDHA	0.213	0.373	0.125	+0.063 ± 0.439	-0.063 ± 0.291
5	RPL13A	0.229	0.301	0.223	-0.112 ± 0.328	+0.112 ± 0.271
6	HMBS	0.258	0.427	0.205	+0.103 ± 0.477	-0.103 ± 0.371
7	UBC	0.268	0.584	0.070	+0.035 ± 0.527	-0.035 ± 0.636
8	B2M	0.286	0.512	0.227	+0.113 ± 0.604	-0.113 ± 0.399
9	ACTB	0.364	0.787	0.267	+0.133 ± 0.573	-0.133 ± 0.954
10	GAPDH	0.504	0.161	0.932	-0.466 ± 0.215	+0.466 ± 0.074

* For this gene a variance estimate came out negative, so NormFinder used its fallback variance (as the original NormFinder does). The ranking stays valid; the value is less precise.

Values are in log2 units (Cq scale), comparable to geNorm M. The original NormFinder Excel add-in reports natural-log values, which are 0.693× these; the ranking is identical.

NormFinder has no cut-off for an acceptable stability value: its authors use the value to rank candidates, not to accept or reject them. The number of reference genes comes from geNorm's pairwise variation V.

Best combinations of two genes

Rank	Genes	Stability of the pair
1	TBP + YWHAZ	0.090
2	HPRT1 + TBP	0.107
3	HPRT1 + RPL13A	0.107
4	RPL13A + YWHAZ	0.109
5	SDHA + TBP	0.117
6	HMBS + RPL13A	0.131
7	RPL13A + TBP	0.132
8	RPL13A + SDHA	0.134
9	TBP + UBC	0.140
10	HMBS + TBP	0.140

Method comparison

Both methods agree on the top 2 genes: TBP, YWHAZ.

Gene	geNorm rank	NormFinder rank	Difference	
TBP	1	1	0	
YWHAZ	2	2	0	
HPRT1	3	3	0	
RPL13A	4	5	+1	
SDHA	5	4	-1	
HMBS	6	6	0	
GAPDH	7	10	+3	methods disagree
B2M	8	8	0	
UBC	9	7	-2	
ACTB	10	9	-1	

Highlighted: the ranks differ by 3 or more positions, or the gene is in one method’s top 2 but not the other’s. This is a display heuristic, not a statistical test. No combined rank is computed: where the methods disagree, that is information about the data.

Data preparation

- 16 rows submitted; 16 samples analysed by both methods.
- No technical replicates: each of the 16 rows is one sample.
- Amplification efficiency: none supplied, so E = 2 (100%) was assumed for all 10 genes.
- Automatic exclusions (missing data): none.
- Excluded by you: none.
- Sample groups: Normoxia (8), Hypoxia (8).

Interpretation

The recommended reference gene set has a mean M value of 0.214 (calculated within the set); interpretation should follow the MIQE guidelines (Bustin et al. 2025)[4] alongside the threshold convention from Hellemans et al. 2007[3]. Consider sample-panel homogeneity, tissue-specific validation, and possible co-regulation among the selected genes before using this set as an internal control. Validate the recommended genes in a new experiment when extending this analysis to a new tissue or condition.

Methods text (for your manuscript)

Reference gene stability was assessed across 16 samples and 10 candidate reference genes using the geNorm algorithm (Vandesompele et al., 2002)[2], as implemented in the Clarida Reference Gene Finder (v2026.10.0)[1]. Cq values were converted to relative quantities assuming 100% amplification efficiency (E = 2) for all 10 genes. Expression stability was also assessed on the same data with NormFinder (Andersen et al., 2004)[5], with 2 sample groups (Normoxia, Hypoxia); NormFinder values are reported in log2 units. The number of reference genes was chosen with geNorm's pairwise variation (V), which indicated an optimal number of 2. The recommended reference gene set was TBP, YWHAZ, with a mean stability value (M, calculated within the recommended set) of 0.214, below the threshold of 0.5 for homogeneous samples; M-value interpretation thresholds according to (Hellemans et al., 2007)[3].

References

1. Clarida. Reference gene finder [Internet]. Version 2026.10.0. 2026 [cited 2026 Nov 3]. Available from: https://app.clarida.bio/reference-gene-finder/genorm
2. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7):RESEARCH0034.
3. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007;8(2):R19.
4. Bustin SA, Ruijter JM, van den Hoff MJB, Kubista M, Pfaffl MW, Shipley GL, Tran N, Rödiger S, Untergasser A, Mueller R, Nolan T, Milavec M, Burns MJ, Huggett JF, Vandesompele J, Wittwer CT. MIQE 2.0: Revision of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines. Clin Chem. 2025;71(6):634-51.
5. Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004;64(15):5245-50.

Supplementary table S1

Stability of every candidate reference gene in this analysis. Suitable for MIQE 2.0 supplementary material.

Gene	geNorm average M	geNorm rank	NormFinder stability (log2)	NormFinder rank
TBP	0.240	1	0.079	1
YWHAZ	0.258	2	0.151	2
HPRT1	0.272	3	0.185	3
RPL13A	0.327	4	0.229	5
SDHA	0.364	5	0.213	4
HMBS	0.406	6	0.258	6
GAPDH	0.464	7	0.504	10
B2M	0.507	8	0.286	8
UBC	0.540	9	0.268	7
ACTB	0.605	10	0.364	9

Lower is more stable for both measures. geNorm average M: Vandesompele et al. 2002; NormFinder stability: Andersen et al. 2004, in log2 units (natural-log values are 0.693× these). 16 samples.

Cq matrix

Sample	Group	ACTB	B2M	GAPDH	HMBS	HPRT1	RPL13A	SDHA	TBP	UBC	YWHAZ
Nx-01	Normoxia	17.18	18.39	19.33	27.16	24.79	16.53	23.76	27.81	22.18	21.80
Nx-02	Normoxia	18.18	18.47	19.83	26.69	25.78	17.84	25.33	28.33	22.04	22.27
Nx-03	Normoxia	17.82	19.20	19.34	25.92	25.11	16.74	24.11	27.83	21.63	21.46
Nx-04	Normoxia	17.31	17.83	18.17	25.96	23.91	15.40	23.08	26.65	19.71	20.80
Nx-05	Normoxia	16.19	17.53	18.84	26.13	24.43	16.53	23.83	27.03	20.00	21.27
Nx-06	Normoxia	17.88	19.56	19.26	26.65	24.46	16.71	23.80	27.57	21.40	21.79
Nx-07	Normoxia	17.09	18.80	18.50	26.38	24.88	16.54	23.40	27.47	20.96	21.20
Nx-08	Normoxia	16.82	19.02	19.71	27.08	24.89	16.65	24.74	27.80	21.09	22.01
Hx-01	Hypoxia	14.95	17.94	16.73	25.47	23.80	15.14	22.77	26.33	20.28	20.28
Hx-02	Hypoxia	17.53	19.70	19.00	27.32	25.40	17.32	24.71	28.68	22.36	22.67
Hx-03	Hypoxia	17.40	19.14	18.68	26.79	25.30	17.32	24.42	28.07	22.34	22.29
Hx-04	Hypoxia	16.84	17.93	17.11	25.60	24.00	15.19	23.25	26.54	20.09	21.02
Hx-05	Hypoxia	17.43	17.70	17.72	26.90	24.91	15.47	23.89	27.16	19.45	21.31
Hx-06	Hypoxia	18.18	17.90	17.56	26.45	24.21	15.63	23.70	26.71	20.75	20.89
Hx-07	Hypoxia	17.05	18.88	18.07	25.95	24.48	16.02	23.16	27.11	20.73	21.32
Hx-08	Hypoxia	18.90	19.10	18.33	26.81	25.04	16.74	24.83	27.52	21.25	21.42