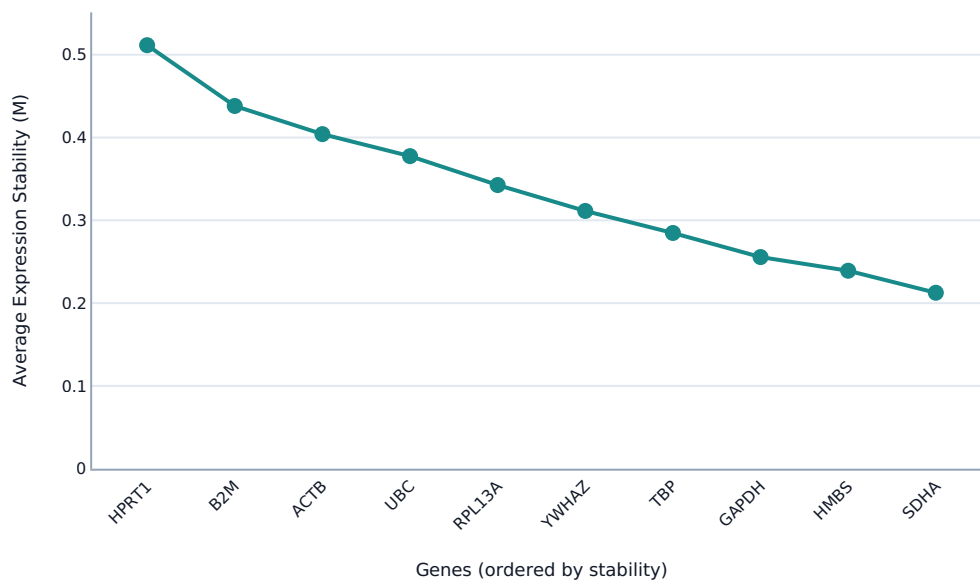


**Recommended reference gene set: SDHA, HMBS**

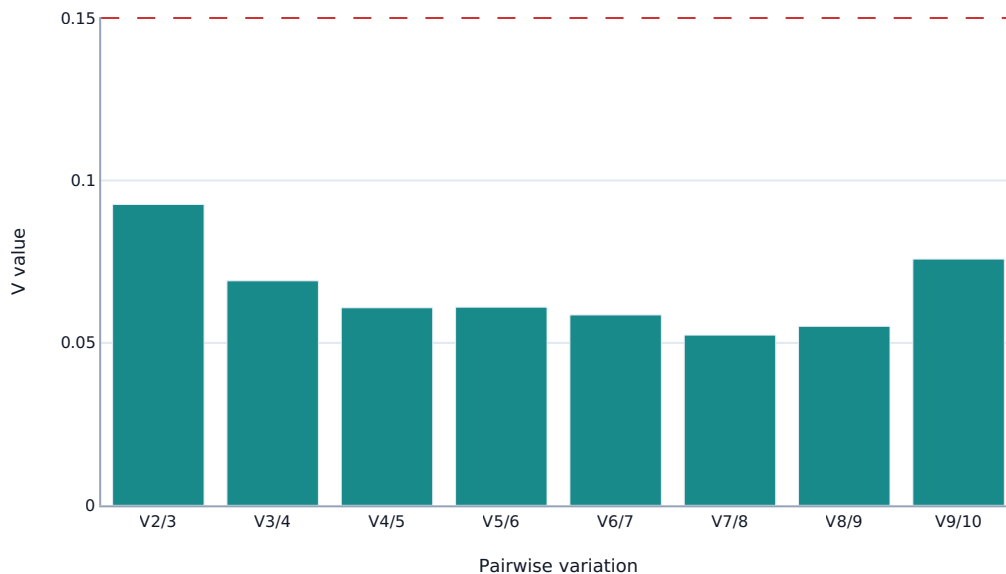
Mean M-value 0.226 · 10 samples × 10 candidates (SDHA, HMBS, GAPDH, TBP, YWHAZ, RPL13A, UBC, ACTB, B2M, HPRT1)

**Gene stability (M-values)**


Rank	Gene	M-value
1	SDHA	0.212
2	HMBS	0.239
3	GAPDH	0.256
4	TBP	0.285
5	YWHAZ	0.311
6	RPL13A	0.343
7	UBC	0.378
8	ACTB	0.404
9	B2M	0.438
10	HPRT1	0.511

Interpretation threshold (Hellemans et al. 2007):  $M < 0.5$  is typically found in homogeneous samples;  $M < 1.0$  is acceptable for heterogeneous samples.

## Pairwise variation (V-values)



Optimal number of reference genes: **2**

## Interpretation

The recommended reference gene set has a mean M value of 0.226; interpretation should follow the MIQE guidelines (Bustin et al. 2025)<sup>[4]</sup> alongside the threshold convention from Hellemans et al. 2007<sup>[3]</sup>. 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 10 samples and 10 candidate reference genes using the geNorm algorithm (Vandesompele et al., 2002)<sup>[2]</sup>, as implemented in the Clarida Reference Gene Finder (v2026.06.1)<sup>[1]</sup>. Pairwise variation analysis indicated an optimal number of 2 reference genes. The recommended reference gene set was SDHA, HMBS, with a mean stability value (M) of 0.226, below the threshold of 0.5 for homogeneous samples; M-value interpretation thresholds according to (Hellemans et al., 2007)<sup>[3]</sup>.

## References

1. Clarida. Reference gene finder [Internet]. Version 2026.06.1. 2026 [cited 2026 Jun 22]. 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.