

Bone to pick: the importance of evaluating reference genes for RT-qPCR quantification of gene expression in craniosynostosis and bone-related tissues and cells

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Additional file 1.pdf

Simple and brief survey of the literature for articles that presented bone-related RT-qPCR data in 2011-2012.

Aim

We wished to determine how prevalent the use of validated reference genes was in current bone-related RT-qPCR studies, and also whether single or multiple reference genes were used.

Method

Using the PubMed search engine, we limited the searches to the year 2011-2012 (year to date) and to articles that were open access and in English. The search was refined by using search terms:

Search A: osteogenesis AND gene AND expression AND PCR AND real-time

Search B: gene expression OR RT-PCR OR quantitative PCR - limited to the journal "Bone"

The two different searches were carried out to survey trends in bone-related literature in journals in general and in literature from a bone specific journal. We manually identified the reference gene/s used for normalization and noted if evidence of validation had been presented. This was done for the 15 most recent articles as defined by each search pattern and the findings are presented in table form.

Results

Presented in Tables 1 and 2 on the following pages.

Conclusions

1. 93% (23/30) of RT-qPCR studies surveyed used single, non-validated genes to normalize data.
2. The classical housekeeping gene *GAPDH* was the most commonly used (57%), followed by *18S rRNA* (13%) and *ACTB* (13%).

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Table 1

	Search A	PMID:	Reference gene	Validation	Multiple reference genes
1	J Oral Sci. 2011 Dec;53(4):509-16	22167038	GAPDH	no	no
2	Gene. 2012 Jan 10;491(2):187-93. Epub 2011 Oct 12	22019432	GAPDH	no	no
3	Cell Prolif. 2011 Oct;44(5):420-7.	21951285	GAPDH	no	no
4	Clin Oral Investig. 2011 Sep 22 [Epub ahead of print]	21938481	GAPDH	no	no
5	Int J Biol Sci. 2011;7(7):968-77. Epub 2011 Aug 7	21850206	18S rRNA	no	no
6	Lasers Med Sci. 2011 Aug 13. [Epub ahead of print]	21842225	GAPDH	no	no
7	J Biol Chem. 2011 Sep 23;286(38):32995-3002. Epub 2011 Aug 2	21828043	GAPDH	no	no
8	Biochem Biophys Res Commun. 2011 Aug 19;412(1):143-9. Epub 2011 Jul 22	21806974	RPL13A	no	no
9	Biochem Biophys Res Commun. 2011 Aug 12;411(4):690-4. Epub 2011 Jul 13	21772583	GAPDH	no	no
10	Eur Cell Mater. 2011 Jul 15;22:68-82; discussion 83	21761393	GAPDH	no	no
11	Platelets. 2011;22(6):452-60. Epub 2011 Jul 11	21740339	GAPDH	no	no
12	Biomaterials. 2011 Oct;32(29):6900-11/ Epub 2011 Jul 5	21733571	GAPDH	no	no
13	Mol Cell Biochem. 2011 Dec;358(1-2):37-44. Epub 2011 Jun 23	21698346	GAPDH	no	no
14	Biotechnol Lett. 2011 Oct;33(10):1933-8. Epub 2011 Jun 10	21660578	ACTB	no	no
15	Bone. 2011 Sep;49(3):463-72. Epub 2011 Apr 27.	21549232	GAPDH	no	no

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Table 2

	Search B	PMID:	Reference gene	Validati on	Multiple reference genes
1	Bone. 2012 Feb;50(2):467-76. Epub 2011 Oct 14.	22019459	GAPDH	no	no
2	Bone. 2012 Feb;50(2):499-509. Epub 2011 Aug 2.	21839191	L24/ACTB/cyclophilin	no	yes
3	Bone. 2012 Feb;50(2):546-52. Epub 2011 Jun 24	21723971	18S rRNA or FABP4/aP2	no	no
4	Bone. 2012 Jan 21. [Epub ahead of print]	22285620	ACTB	no	no
5	Bone. 2012 Jan 16. [Epub ahead of print]	22270057	GAPDH	no	no
6	Bone. 2012 Jan;50(1):364-72. Epub 2011 Nov 12.	22108137	ACTB	no	no
7	Bone. 2012 Jan;50(1):337-42. Epub 2011 Nov 10.	22100507	YWHAZ, UBC and TOP1	yes	yes
8	Bone. 2012 Jan;50(1):265-75. Epub 2011 Nov 4.	22079283	ACTB	yes	no
9	Bone. 2012 Jan;50(1):209-17. Epub 2011 Oct 30.	22075208	ChoB	no	no
10	Bone. 2012 Jan;50(1):171-81. Epub 2011 Oct 18.	22037351	U6 snRNA or GAPDH	no	no
11	Bone. 2012 Feb;50(2):467-76. Epub 2011 Oct 14.	22019459	GAPDH	no	no
12	Bone. 2011 Dec;49(6):1311-6. Epub 2011 Sep 22.	21964323	GAPDH	no	no
13	Bone. 2012 Jan;50(1):54-62. Epub 2011 Sep 16.	21964322	18s rRNA	no	no
14	Bone. 2012 Jan;50(1):79-84. Epub 2011 Sep 10.	21930256	18s rRNA	no	no
15	Bone. 2012 Jan;50(1):28-41. Epub 2011 Aug 31.	21911092	HPRT1	no	no