RNA interference and validation of reference genes for gene expression analyses using RT-qPCR in southern pine beetle, *Dendroctonus frontalis*

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9 **Supplemental Materials**

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11 **Final ranking of candidate reference genes from light treated beetles according to values**
12 **given by GeNorm, NormFinder, BestKeeper, and delta Ct and a comprehensive ranking by**
13 **RefFinder. <u>M: gene expression stability; R: ranking; SV stability value; SD: standard</u>**
14 **<u>deviation; GM: Geomean value.</u>**

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Gene	GeNorm		NormFinder		BestKeeper		delta-CT		Comprehensive	
	M	R	SV	R	SD	R	SD	R	GM	R
rps18	0.349	1	0.424	3	0.22	1	0.63	3	1.73	2
ef1a	0.522	4	0.271	2	0.54	5	0.56	2	3.16	4
rpl13	0.435	2	0.441	4	0.23	2	0.63	4	3.13	3
ak	0.619	6	0.662	8	0.76	8	0.77	7	7.48	8
sdf	0.578	5	0.489	5	0.65	7	0.67	5	5.69	6
rpl32	0.349	1	0.259	1	0.25	3	0.55	1	1.32	1
tub	0.476	3	0.514	6	0.37	4	0.68	6	4.9	5
ubiq	0.659	7	0.651	7	0.55	6	0.78	8	7.2	7

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Final ranking of candidate reference genes from temperature treated beetles according to values given by GeNorm, NormFinder, BestKeeper, and delta Ct and a comprehensive ranking by RefFinder. <u>M: gene expression stability; R: ranking; SV stability value; SD: standard deviation; GM: Geomean value.</u>

Gene	GeNorm		NormFinder		BestKeeper		delta-CT		Comprehensive	
	M	R	SV	R	SD	R	SD	R	GM	R
rps18	0.177	1	0.256	2	0.72	1	0.43	2	1.57	1
ef1a	0.414	6	0.423	7	1.15	8	0.51	5	7.24	8
rpl13	0.27	2	0.205	1	0.78	4	0.42	1	1.86	2
ak	0.386	4	0.285	4	1.01	6	0.42	1	3.94	4
sdf	0.366	3	0.289	5	0.99	5	0.44	3	4.73	5
rpl32	0.472	7	0.6	8	0.75	3	0.65	6	6.26	7
tub	0.177	1	0.282	3	0.73	2	0.44	3	2.21	3
ubiq	0.402	5	0.355	6	1.02	7	0.47	4	6.24	6

23	**Final ranking of candidate reference genes from male and female beetles according to**
24	**values given by GeNorm, NormFinder, BestKeeper, and delta Ct and a comprehensive**
25	**ranking by RefFinder.** <u>**M: gene expression stability; R: ranking; SV stability value; SD:**</u>
26	<u>**standard deviation; GM: Geomean value.**</u>

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Gene	GeNorm		NormFinder		BestKeeper		delta-CT		Comprehensive	
	M	R	SV	R	SD	R	SD	R	GM	R
rps18	0.329	2	0.167	1	0.55	2	0.59	1	1.57	1
ef1a	0.356	3	0.305	2	0.79	6	0.62	4	3.72	5
rpl13	0.449	5	0.364	5	0.41	1	0.72	5	3.5	4
ak	0.317	1	0.317	3	0.67	3	0.61	2	2.06	2
sdf	0.376	4	0.607	7	0.91	8	0.73	6	6.4	6
rpl32	0.317	1	0.341	4	0.7	4	0.62	3	2.63	3
tub	0.525	6	0.524	6	0.83	7	0.81	7	6.74	7
ubiq	0.784	7	1.521	8	0.72	5	1.56	8	7.11	8

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29 **Final ranking of candidate reference genes from all treatments according to values given by**
30 **GeNorm, NormFinder, BestKeeper, and delta Ct and a comprehensive ranking by**
31 **RefFinder. <u>M: gene expression stability; R: ranking; SV stability value; SD: standard</u>**
32 **<u>deviation; GM: Geomean value.</u>**

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Gene	GeNorm		NormFinder		BestKeeper		delta-Ct		Comprehensive	
	M	R	SV	R	SD	R	SD	R	GM	R
rps18	0.475	4	0.297	1	0.59	1	0.61	2	1.68	1
ef1a	0.362	2	0.314	2	0.8	5	0.6	1	2.34	2
rpl13	0.505	3	0.348	3	0.53	2	0.63	3	3.08	3
ak	0.312	1	0.43	4	0.83	6	0.64	4	3.13	4
sdf	0.312	1	0.464	6	0.92	8	0.66	5	4.12	5
rpl32	0.517	5	0.434	5	0.64	4	0.66	5	4.95	6
tub	0.556	6	0.485	7	0.63	3	0.71	6	5.66	7
ubiq	0.71	7	1.103	8	0.89	7	1.17	7	7.74	8

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