

PDF S3

Reference gene validation (pilot scale):

	1	2	3	4	5	6	7	8	9	10	11	12
A	C-1, A.1	C-1, A.2	C-1, A.3	C-2, B.1	C-2, B.2	C-2, B.3	C+1, C.1	C+1, C.2	C+1, C.3	C+2, D.1	C+2, D.2	C+2, D.3
B	FU1, E.1	FU1, E.2	FU1, E.3	FU2, F.1	FU2, F.2	FU2, F.3	WF1, G.1	WF1, G.2	WF1, G.3	WF2, H.1	WF2, H.2	WF2, H.3
C	RF1, I.1	RF1, I.2	RF1, I.3	RF4, J.1	RF4, J.2	RF4, J.3	AE1, K.1	AE1, K.2	AE1, K.3	AE2, L.1	AE2, L.2	NTC
D												
E												
F	C-1, A.1	C-1, A.2	C-1, A.3	C-2, B.1	C-2, B.2	C-2, B.3	C+1, C.1	C+1, C.2	C+1, C.3	C+2, D.1	C+2, D.2	C+2, D.3
G	FU1, E.1	FU1, E.2	FU1, E.3	FU2, F.1	FU2, F.2	FU2, F.3	WF1, G.1	WF1, G.2	WF1, G.3	WF2, H.1	WF2, H.2	WF2, H.3
H	RF1, I.1	RF1, I.2	RF1, I.3	RF4, J.1	RF4, J.2	RF4, J.3	AE1, K.1	AE1, K.2	AE1, K.3	AE2, L.1	AE2, L.2	NTC

Actb gene

B2m gene

Figure 1. Distal colon samples from different rats from different treatments (see Fig. S1) analysed.

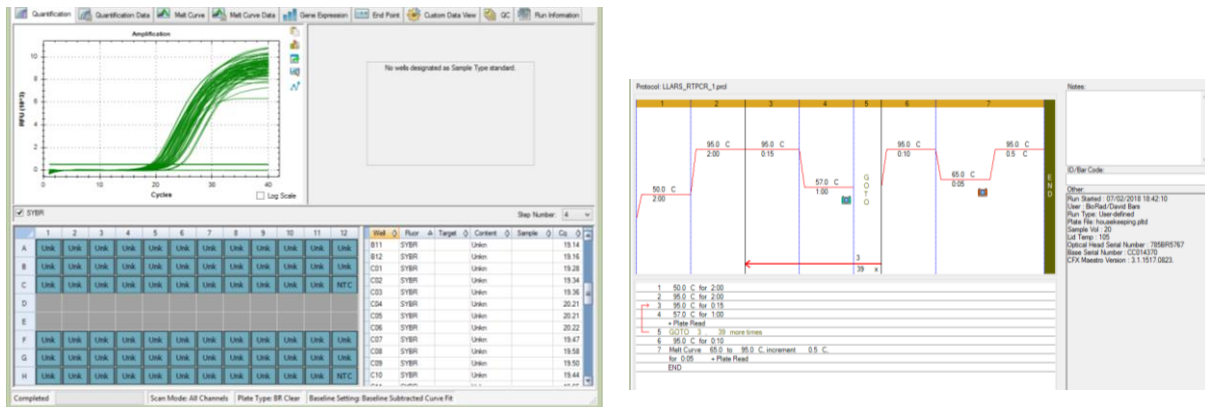


Figure 2. Output of the results obtained once analysed through Bio-Rad CFX96 real-time PCR system.

Two software resources have been used to decide if one of the two reference assayed within different experimental conditions are better or not.

Tool 1)

GeNorm algorithm

Bio-Rad CFX Maestro 1.1. software has a tool (based on geNorm algorithm) to check the stability (aptitude) of the candidate reference genes.

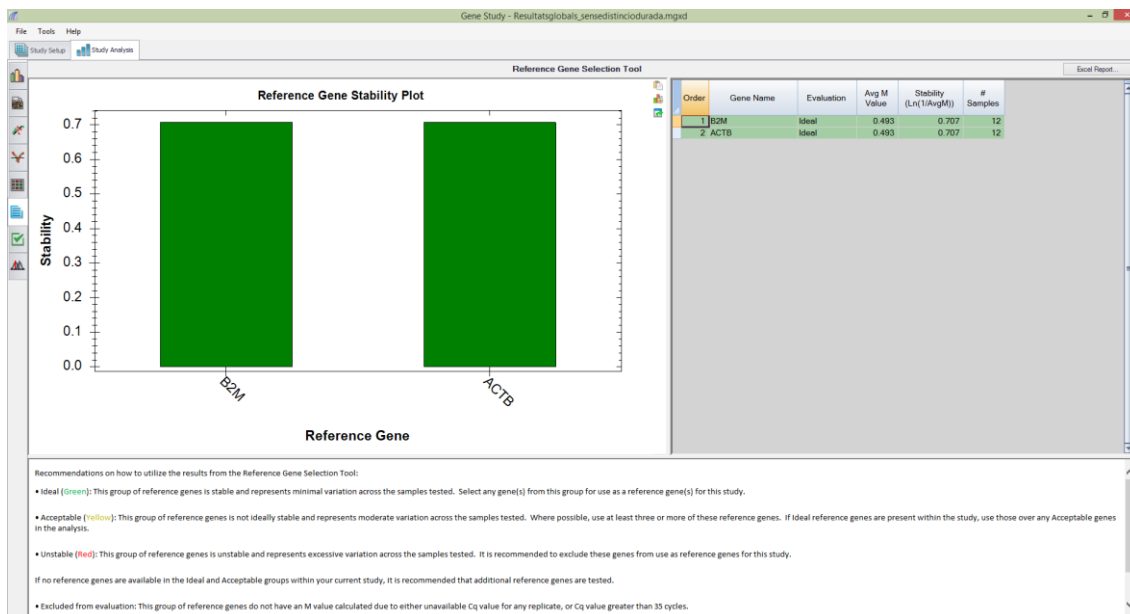


Figure 3. Output results of Bio-Rad CFX Maestro 1.1. software.

Both of them are stable along the treatments studied. Therefore, both are good candidates.

Tool 2)

Web-based RefFinder platform

<http://150.216.56.64/referencegene.php?type=reference>

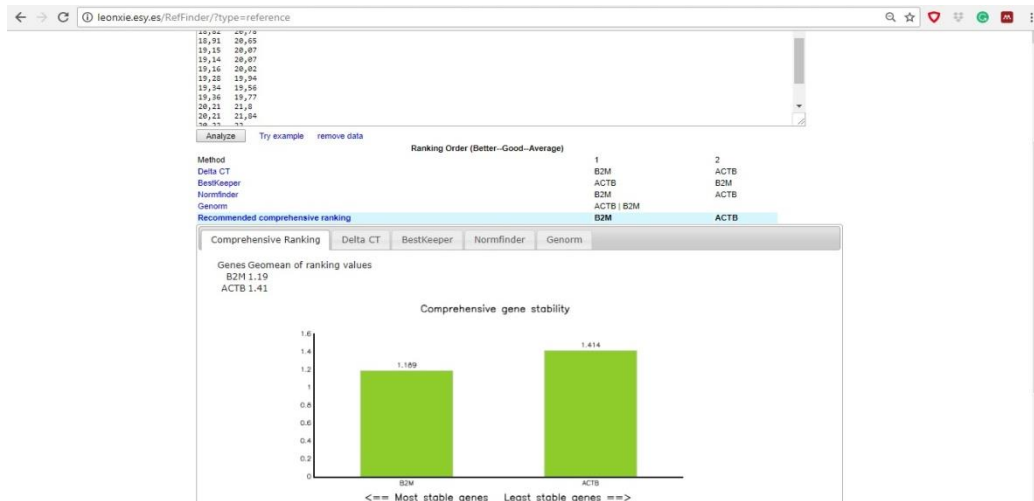


Figure 4. Output results of RefFinder platform.

RefFinder recommended us *B2M* gene instead of *Actb* gene by minor differences in geomean of ranking values, as it can be seen in the screenshot.