

Title	Enterococcus faecalis demonstrates pathogenicity through increased attachment in an ex vivo polymicrobial pulpal infection
Authors	Nishio Ayre, Wayne;Melling, Genevieve;Cuveillier, Camille;Natarajan, Madhan;Roberts, Jessica L.;Marsh, Lucy L.;Lynch, Christopher D.;Maillard, Jean-Yves;Denyer, Stephen P.;Sloan, Alastair J.
Publication date	2018-02-26
Original Citation	Nishio Ayre, W., Melling, G., Cuveillier, C., Natarajan, M., Roberts, J. L., Marsh, L. L., Lynch, C. D., Maillard, J.-Y., Denyer, S. P. and Sloan, A. J. (2018) 'Enterococcus faecalis demonstrates pathogenicity through increased attachment in an ex vivo polymicrobial pulpal infection', Infection and Immunity, 86(5), e00871-17 (14pp). doi:10.1128/iai.00871-17
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1128/iai.00871-17
Rights	© 2018, American Society for Microbiology. All Rights Reserved.
Download date	2024-04-26 18:48:56
Item downloaded from	https://hdl.handle.net/10468/5676

Supplemental materials

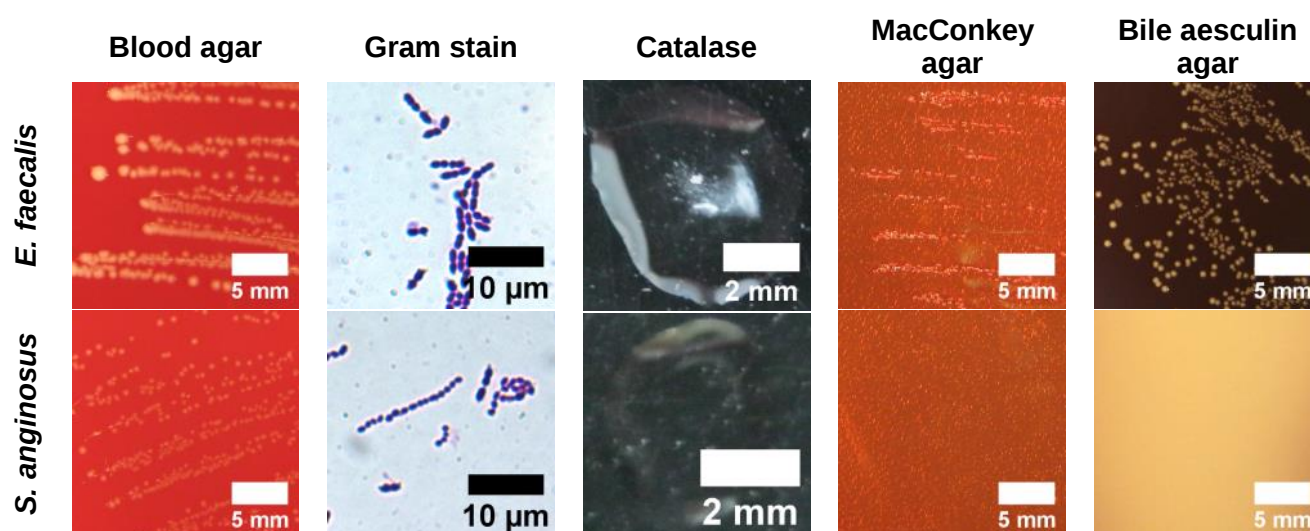


Fig. S1: Microbiology test results for the identification of *E. faecalis* and *S. anginosus*. The *E. faecalis* strain demonstrated large alpha haemolytic white colonies (1-2mm in size), chains of Gram-positive cocci approximately 1-2µm in diameter, was catalase negative, able to grow on MacConkey agar with a pink colony colour, able to grow on bile aesculin agar with a characteristic black agar colour change and belonged to the group D Lancefield grouping. The *S. anginosus* strain formed small alpha haemolytic colonies (<0.5mm) on blood agar, consisted of chains of Gram-positive cocci approximately 1-2µm in diameter, was catalase negative, unable to grow on MacConkey and bile aesculin agar and was positive for Group F Lancefield grouping. No growth of *S. anginosus* was detected after heat treatment and on bile aesculin agar supplemented with 6.5%w/w sodium chloride (data not shown).

A

E. faecalis clinical isolate D88 sequence showed a 97% sequence identity with *E. faecalis*

D88 (forward) sequence (query) alignment with *Enterococcus faecalis* strain CAU:180 16S ribosomal RNA gene, partial sequence (Accession number: MF369839.1; subject).

Query	12	GCGCT-CTATAATGCAGTCGACGCTTCTTTCTCCCGAGTGCTTGCACTCAATTGGAAG	70
Sbjct	8	GCGCTGCTATAATGCAGTCGACGCTTCTTTCTCCCGAGTGCTTGCACTCAATTGGAAG	67
Query	71	AGGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGATAAACT	130
Sbjct	68	AGGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGATAAACT	127
Query	131	TGGAAACAGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCG	190
Sbjct	128	TGGAAACAGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCG	187
Query	191	CTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGC	250
Sbjct	188	CTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGC	247
Query	251	TCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGA	310
Sbjct	248	TCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGA	307
Query	311	CACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCT	370
Sbjct	308	CACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCT	367
Query	371	GACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAACTCTGTTGTTAGAG	430
Sbjct	368	GACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAACTCTGTTGTTAGAG	427
Query	431	AAGAACAAGGACGTTAGTAAGTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGC	490
Sbjct	428	AAGAACAAGGACGTTAGTAAGTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGC	487
Query	491	TAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGG	550
Sbjct	488	TAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGG	547
Query	551	GCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGCTCAACCGGG	610
Sbjct	548	GCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGCTCAACCGGG	607
Query	611	GAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCATGTGTA	670
Sbjct	608	GAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCATGTGTA	667
Query	671	GCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAAGCGGCTCTCTGGTCTGT	730
Sbjct	668	GCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAAGCGGCTCTCTGGTCTGT	727
Query	731	AACTGACGCTGAGGCTCGAAAGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA	790
Sbjct	728	AACTGACGCTGAGGCTCGAAAGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA	787
Query	791	CGCCGT-AACGATGAGTGCTAAGTGTTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAA	849
Sbjct	788	CGCCGTAAACGATGAGTGCTAAGTG-TTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAA	846

Query	850	CGCATTAAGCACTCCGCCCTGGGGAGTACGACCGCAAG-T-GAACTTCAAAGGAA-TGAC	906
Sbjct	847	CGCATTAAGCACTCCG-CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGAC	905
Query	907	GGGGGCCCCGCCACA-GCGTTG-AGCATGGTGGATTTATTCGA-GCAACGCGA-GA-C-T-	959
Sbjct	906	GGGGGCCCCGC-ACAAGCGGTGGAGCATG-TGGTTTAATTCTGAAGCAACGCGAAGAACCTT	963
Query	960	AC-AG-TCTTGACACTCTCTTTGA	981
Sbjct	964	ACCAGGTCTTGACA-TC-CTTTGA	985

Query	732	GTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCGTCGCC	791
Sbjct	217	GTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCGCTTCGCC	158
Query	792	ACTGGTGTTCTCCATATATCTACGCATTTAC-GCTACACATGGAGT-C-ACTCT-CTC	847
Sbjct	157	ACTGGTGTTCTCCATATATCTACGCATTTACCGCTACACATGGAATTCCTCTCCTC	98
Query	848	TTCTGCACTCAAGTCTCC-AGTT-CCAATGAACCCCTCCC-GGTGAAGCCGGGGCCTTTCT	904
Sbjct	97	TTCTGCACTCAAGTCTCCAGTTTCCAATGA-CCCTCCCCGGTTGAGCCGGGGCCTTTCA	39
Query	905	CATCCCAACTTAA-AAACGCC-GCGCGTGCGCTT	936
Sbjct	38	CATCAGA-CTTAAGAAACGCCTGCGC-T-CGCTT	8

C

S. anginosus clinical isolate D88 sequence showed 98% sequence identity with *S. anginosus*

D88 (forward) sequence (query) alignment with *Streptococcus anginosus* strain ChDC B407-I 16S ribosomal RNA gene, partial sequence (Accession number KC569583.1; subject).

Query	8	GGCG-GGCT-ATA-ATGCA-GTAGGACGCACAGTTTATACCGTAGCTTGCTACACCATAG	63
Sbjct	22	GGCGTGCTTAATACATGCAGGTAGGACGCACAGTTTATACCGTAGCTTGCTACACCATAG	81
Query	64	ACTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTATTAGAGGGGGATAAC	123
Sbjct	82	ACTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTATTAGAGGGGGATAAC	141
Query	124	TATTGGAACGATAGCTAATACCGCATAACAGTATGTAACACATGTTAGATGCTTGAAAG	183
Sbjct	142	TATTGGAACGATAGCTAATACCGCATAACAGTATGTAACACATGTTAGATGCTTGAAAG	201
Query	184	ATGCAATTGCATCGCTAGTAGATGGACCTGCGTTGTATTAGCTAGTAGGTAGGGTAATGG	243
Sbjct	202	ATGCAATTGCATCGCTAGTAGATGGACCTGCGTTGTATTAGCTAGTAGGTAGGGTAATGG	261
Query	244	CCTACCTAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG	303
Sbjct	262	CCTACCTAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG	321
Query	304	ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGGAACCC	363
Sbjct	322	ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGGAACCC	381
Query	364	TGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTAAG	423
Sbjct	382	TGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTAAG	441
Query	424	GAAGAACGAGTGTGAGAATGGAAAGTTCATACTGTGACGGTACTTAACCAGAAAGGGACG	483
Sbjct	442	GAAGAACGAGTGTGAGAATGGAAAGTTCATACTGTGACGGTACTTAACCAGAAAGGGACG	501
Query	484	GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATT	543
Sbjct	502	GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATT	561
Query	544	GGGCGTAAAGCGAGCGCAGGCGGTTAGAAAAGTCTGAAGTGAAAGGCAGTGCGTCAACCA	603
Sbjct	562	GGGCGTAAAGCGAGCGCAGGCGGTTAGAAAAGTCTGAAGTGAAAGGCAGTGCGTCAACCA	621

Query	604	TTGTAGGCTTTGGAAACTGTTTAACTTGAGTGCAGAAGGGGAGAGTGGAAATTCATGTGT	663
Sbjct	622	TTGTAGGCTTTGGAAACTGTTTAACTTGAGTGCAGAAGGGGAGAGTGGAAATTCATGTGT	681
Query	664	AGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTG	723
Sbjct	682	AGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTG	741
Query	724	TAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC	783
Sbjct	742	TAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC	801
Query	784	ACGCCGT-AACGATGAGTGCTAGGTGTTGGGTCTTTCC-GGACTCAGTGCCGCAGCTA-	840
Sbjct	802	ACGCCGTAAACGATGAGTGCTAGGTGTTGGGTCTTTCCGGGACTCAGTGCCGCAGCTAA	861
Query	841	CGCA-TAAGCACTCCGCCCTGGGGGAGTACGACCGCAAGGTTTGTAACCTCAAAGGAATT	899
Sbjct	862	CGCATTAAGCACTCCGCC-T-GGGGAGTACGACCGCAAGGTT-GAAACT-CAAAGGAATT	917
Query	900	GTACCGGGGGGCCCCGC	915
Sbjct	918	G-AC-GGGGG-CCCCGC	930

Query	498	CTCTAGAAATAGCATCGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTA	557
Sbjct	1031	CTCTAGAAATAGCATCGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTA	972
Query	558	AACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCG	617
Sbjct	971	AACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCG	912
Query	618	GTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCGGCACTGAGTCCCGGAAAGGA	677
Sbjct	911	GTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCGGCACTGAGTCCCGGAAAGGA	852
Query	678	CCCAACACCTAGCACTCATCGTTTACGGCGTGACTACCAGGGTATCTAATCCTGTTTCGC	737
Sbjct	851	CCCAACACCTAGCACTCATCGTTTACGGCGTGACTACCAGGGTATCTAATCCTGTTTCGC	792
Query	738	TCCCCACGCTTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCTTTCGCCACCGGT	797
Sbjct	791	TCCCCACGCTTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCTTTCGCCACCGGT	732
Query	798	GTTCTCCATATATCTACGCATTTTAC-GCTACACATGGAAT-CCACTCTCCCC-TCTGC	854
Sbjct	731	GTTCTCCATATATCTACGCATTTTACCGCTACACATGGAATTCCTACTCTCCCCTTCTGC	672
Query	855	ACTCAAGT-AA-CAGTT-CCAA-GCCTACATGGGTTGAGGCACTGCGTT-CAATTGAAA	909
Sbjct	671	ACTCAAGTTAAACAGTTTCCAAAGCCTACAATGGTTGAGCCACTGCCTTTCCTTCAGAC	612
Query	910	TTTTCTAAC-GCCTGGGGCT-GCTTTTATAGTCC-ATAATTCCGGAAAA-GCACTGGGAC	965
Sbjct	611	TTTTCTAACCGCCTGCG-CTCGCTTT-AC-GCCCAATAAATCCGGACAACGCTC-GGGAC	556
Query	966	C-ACG 969	
Sbjct	555	CTACG 551	

Fig. S2: Sequence alignments for *E. faecalis* and *S. anginosus*. Bacterial 16S rRNA DNA sequence reactions were performed using DNA from clinical isolates of *E. faecalis* (A and B) and *S. anginosus* (C and D) with universal primers D88 (A and C) and E94 (B and D). The 16S rRNA sequencing revealed that the clinical isolates used in this study had an average of 97% sequence identity with *E. faecalis* and *S. anginosus* species respectively. The microbiological tests and the sequencing results confirmed that the clinically isolated species of bacteria used in this study were *E. faecalis* and *S. anginosus*

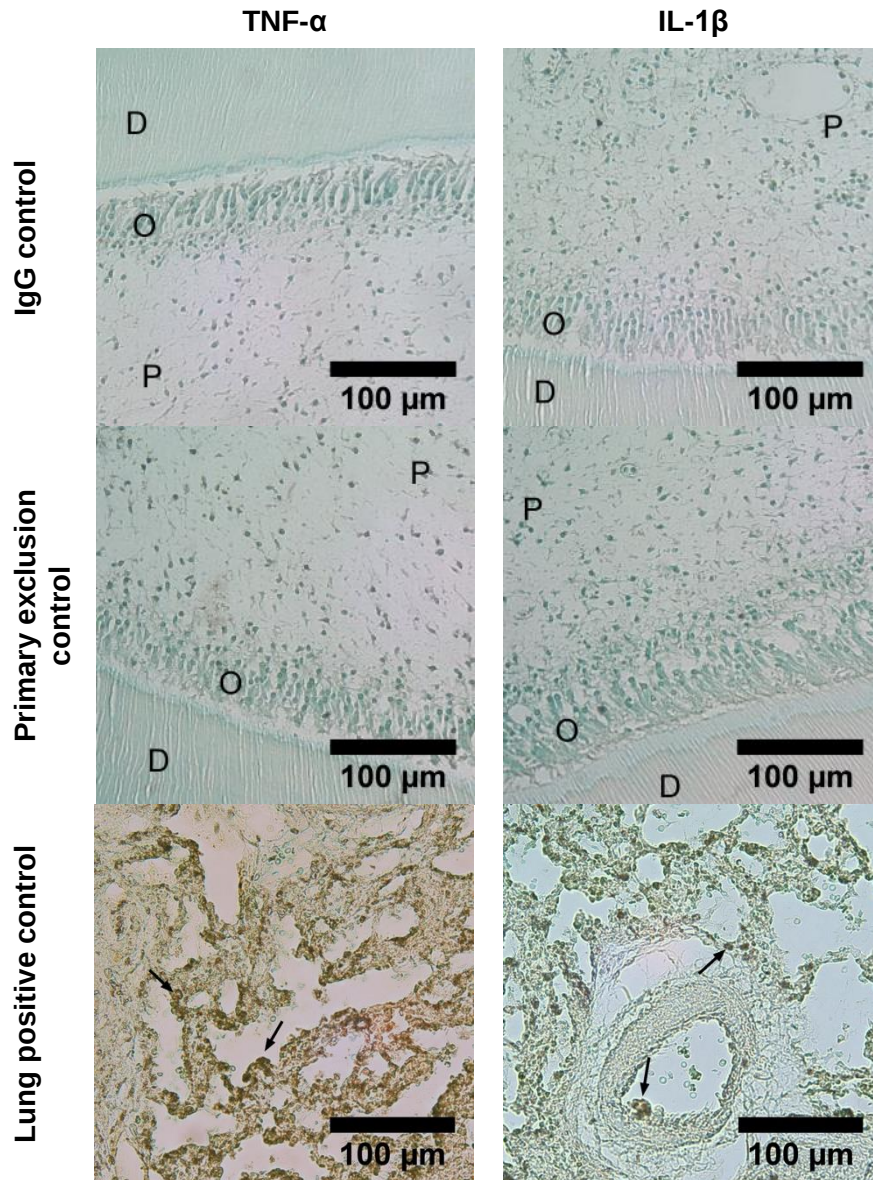


Fig. S3: Immunohistochemistry controls for TNF- α and IL-1 β for the mixed species infection experiment. P represents the dental pulp, O the odontoblast region and D the dentine. Representative images from three experimental repeats shown.

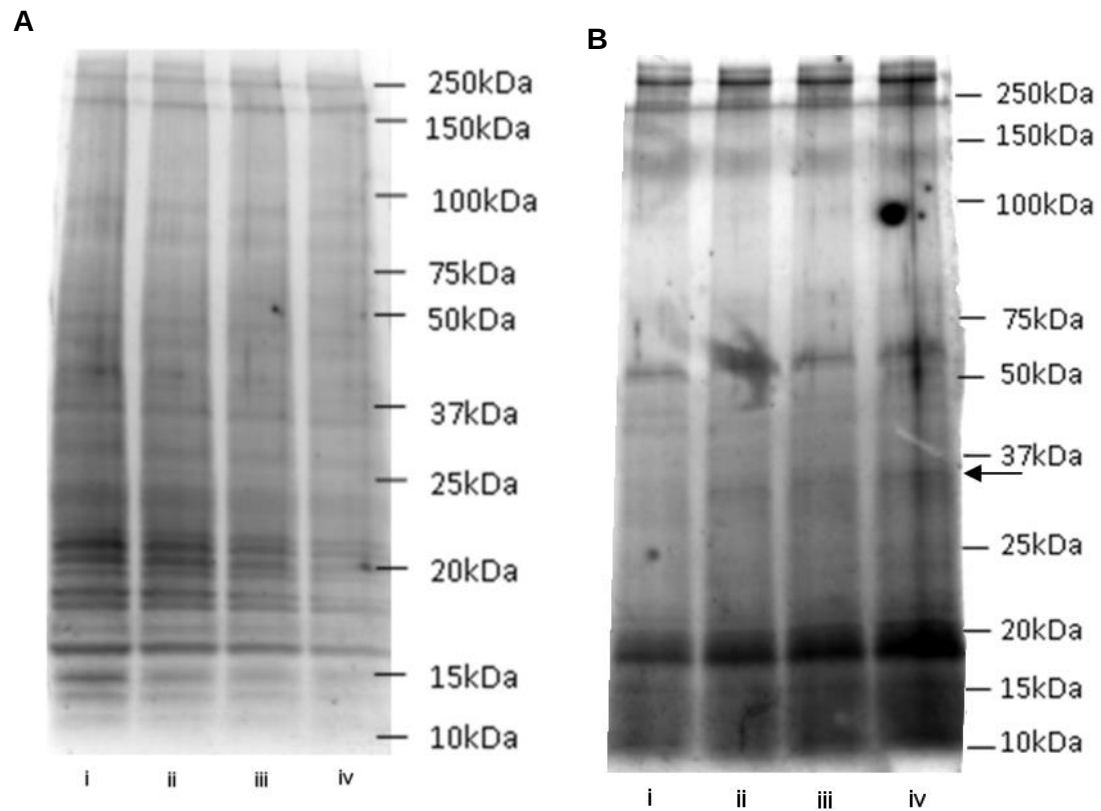


Fig. S4: SDS-PAGE and silver stain of bacterial (A) water soluble cell wall proteins and (B) culture supernatants: (i) *S. anginosus*, (ii) *E. faecalis*, (iii) 50:50 *S. anginosus* : *E. faecalis* and (iv) 90:10 *S. anginosus* : *E. faecalis*. Arrow highlights a difference in supernatant proteins at approximately 35kDa.

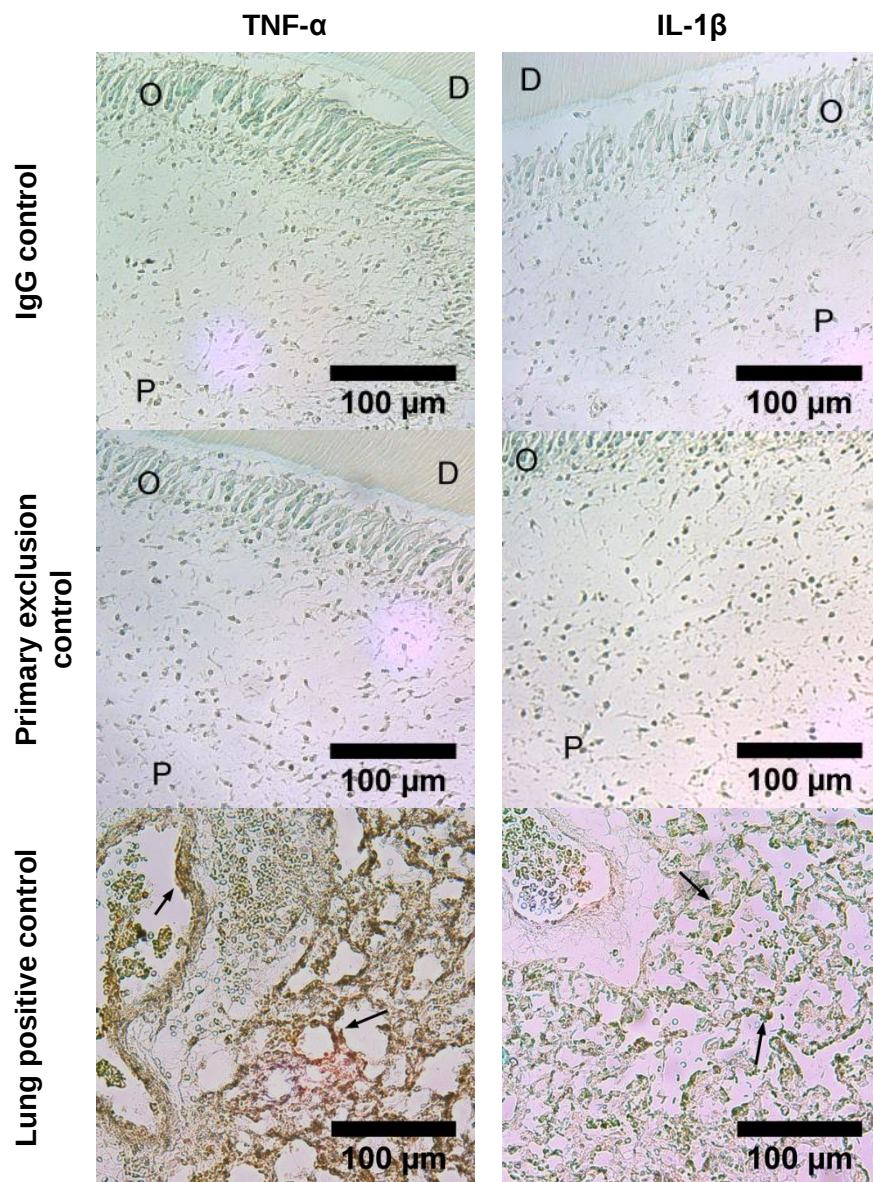


Fig. S5: Immunohistochemistry controls for TNF- α and IL-1 β for the *E. faecalis* heat killed and supernatant experiment. P represents the dental pulp, O the odontoblast region and D the dentine. Representative images from three experimental repeats shown.

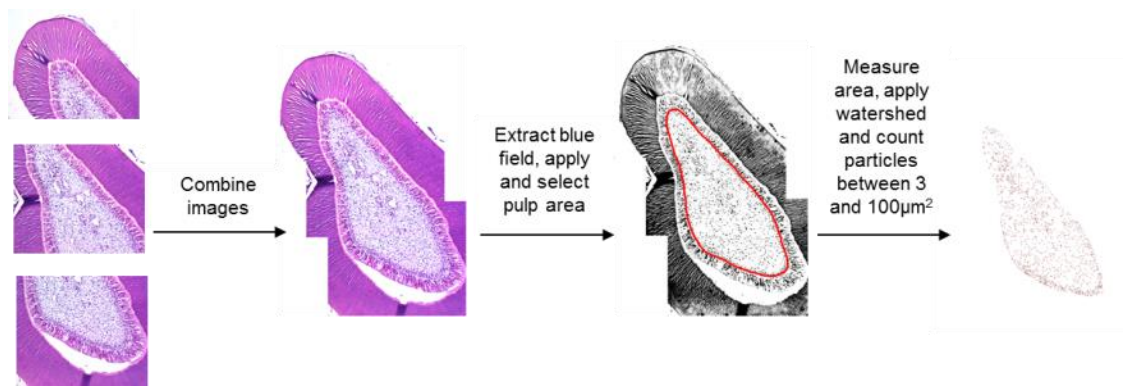
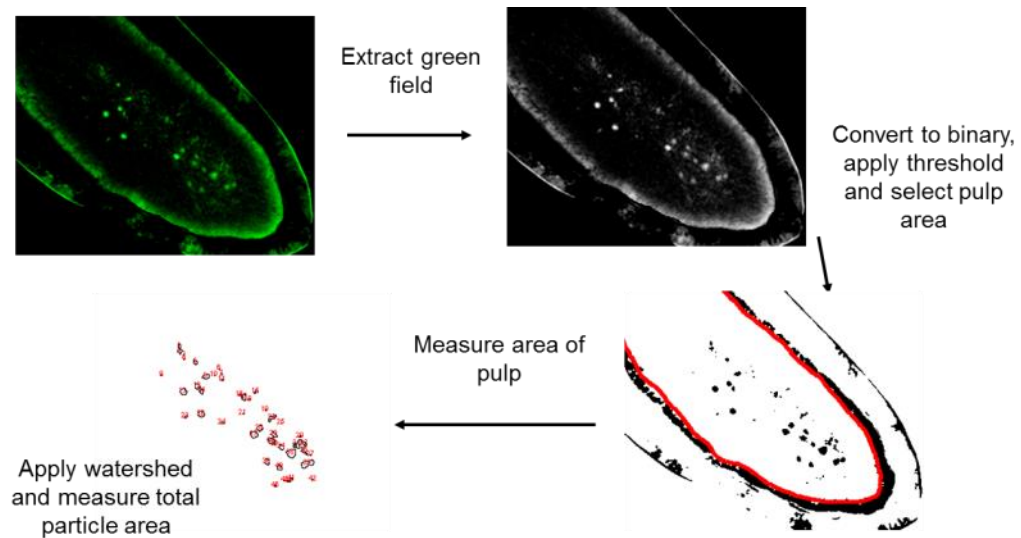


Fig. S6: Method of counting pulpal cells using ImageJ software.



$$\text{Percentage of pulp covered by bacteria} = (\text{measured area} / \text{area of pulp}) \times 100$$

Fig. S7: Method of fluorescence quantification using ImageJ software.

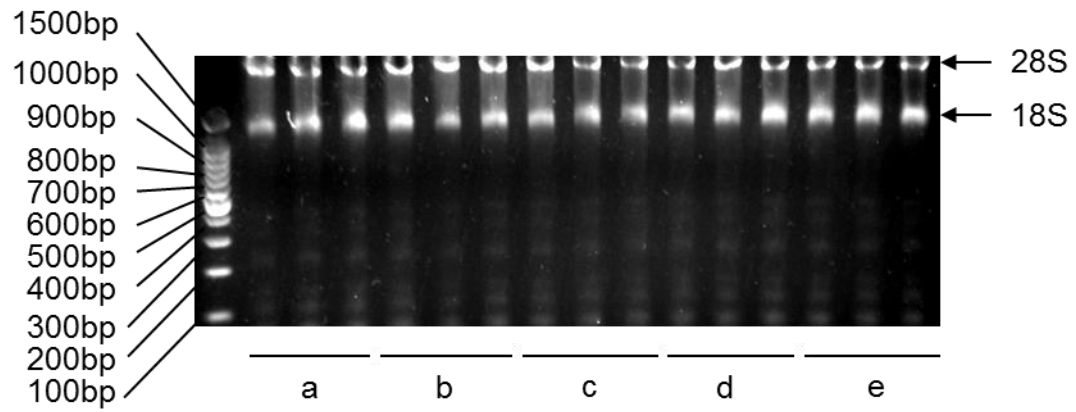


Fig. S8: RNA integrity for (a) Control, (b) *E. faecalis*, (c) *S. anginosus*, (d) 50:50 *S. anginosus*:*E. faecalis* and (e) 90:10 *S. anginosus*:*E. faecalis* samples showing 28S and 18S RNA bands.

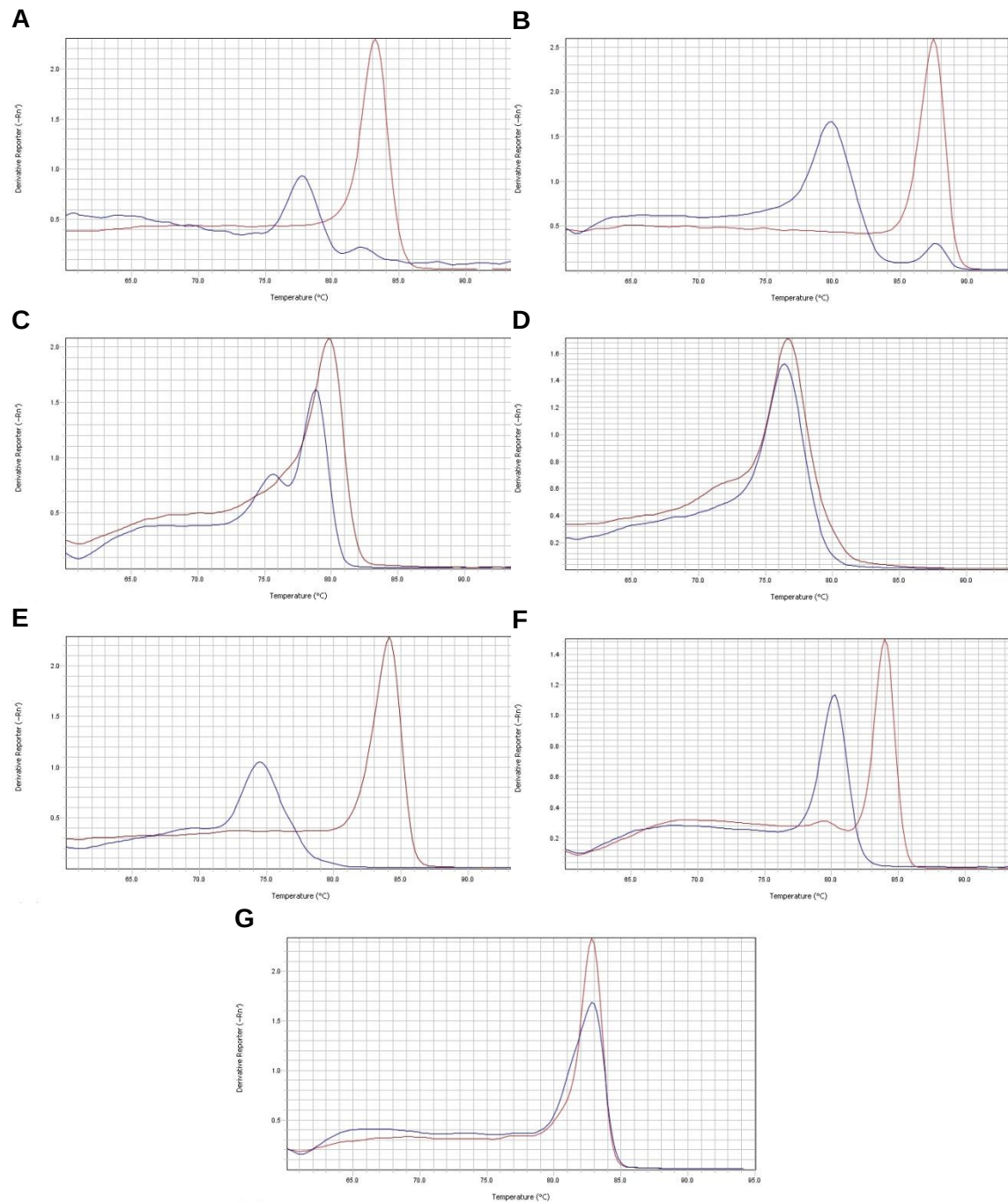


Fig. S9: Representative melt curve peaks for (A) GAPDH, (B) β -actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF- α and (G) IL-1 β (red line represents a samples whilst the blue line represents a no template control).

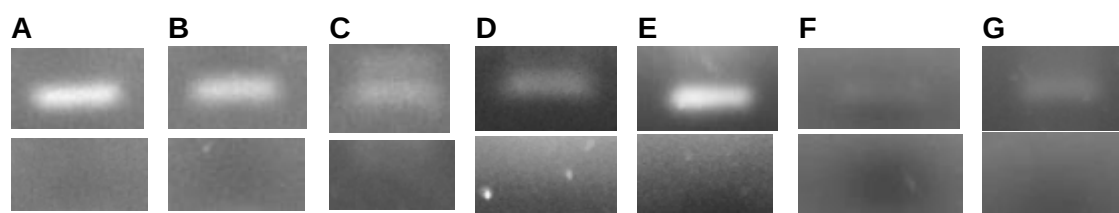


Fig. S10: Representative qPCR products run on gel for one *E. faecalis* infected tooth sample (top row) and the no template control (bottom row): (A) GAPDH, (B) β -actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF- α and (G) IL-1 β .

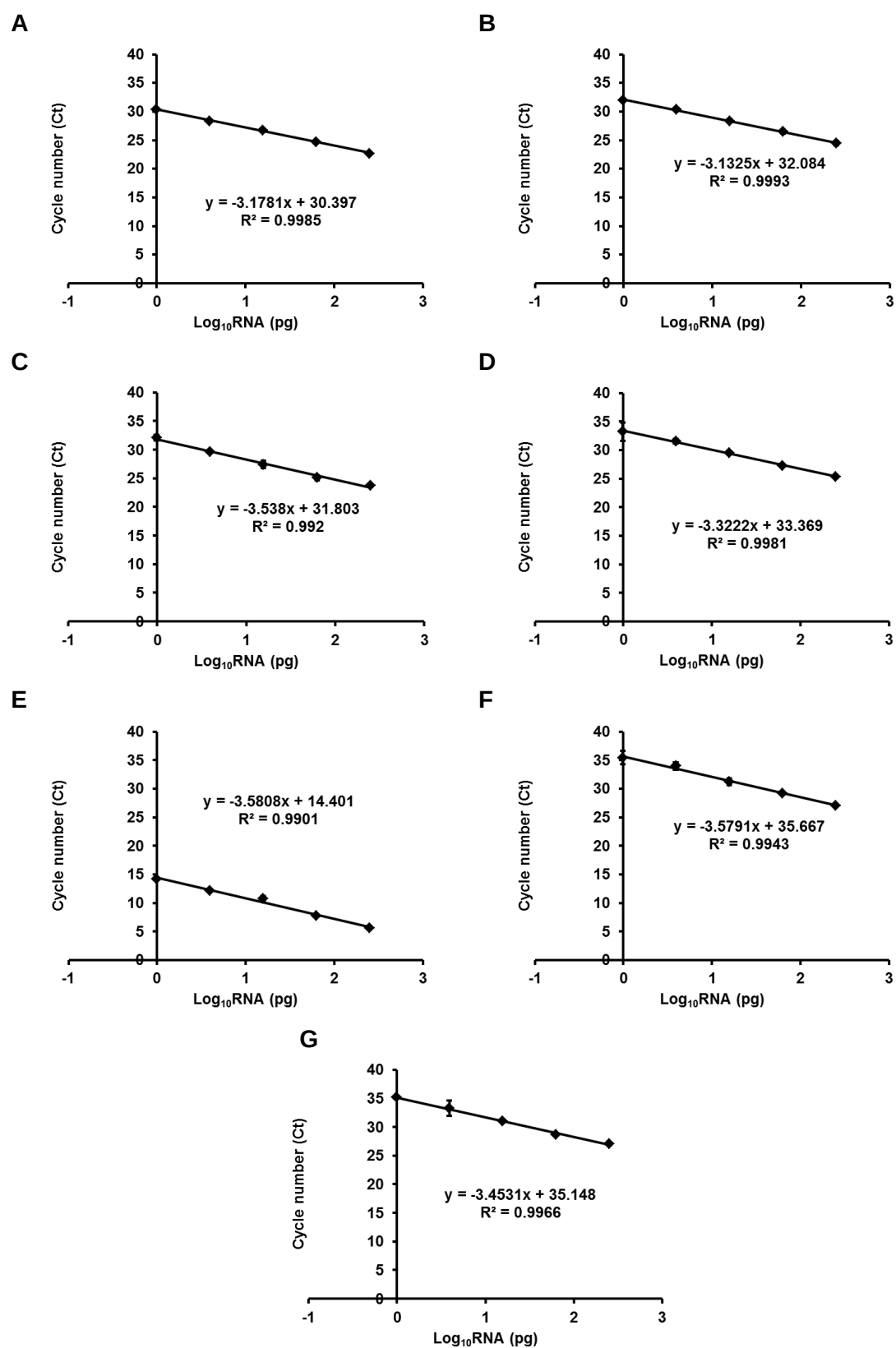


Fig. S11: Primer efficiency curves for (A) GAPDH, (B) β -actin, (C) HPRT-1, (D) RPL13a, (E) 18s rRNA, (F) TNF- α and (G) IL-1 β .

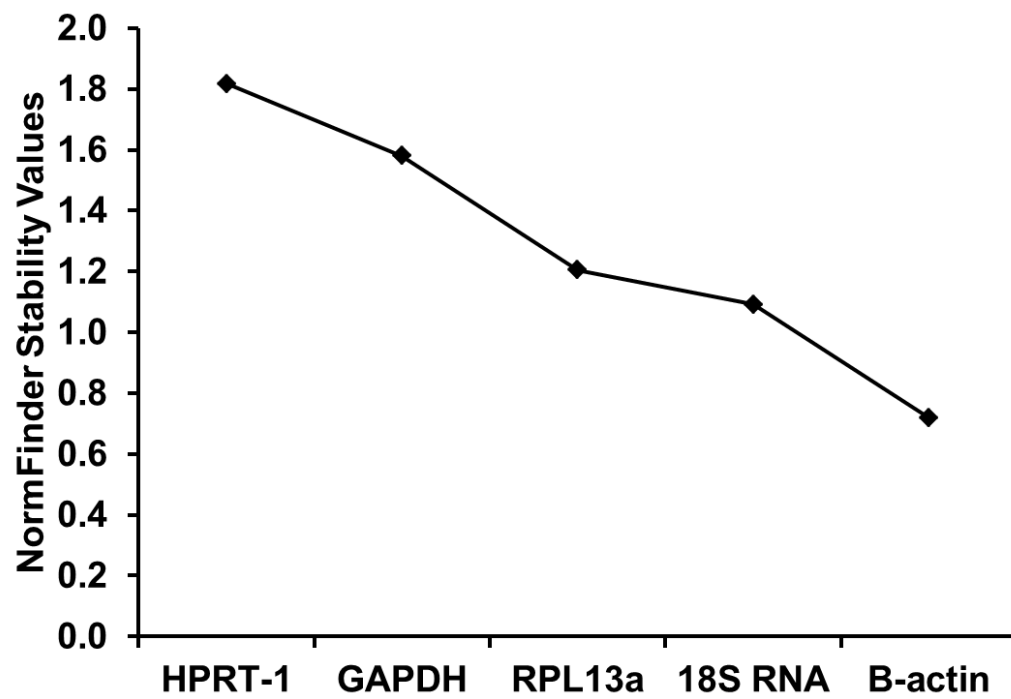


Fig. S12: NormFinder results for qPCR reference gene selection.