SUPPLEMENTARY MATERIAL

Identification of Early Response Genes in Human Peripheral Leukocytes Infected with *Orientia tsutsugamushi*: The Emergent of a Unique Gene Expression Profile for Diagnosis of *O. tsutsugamush* Infection

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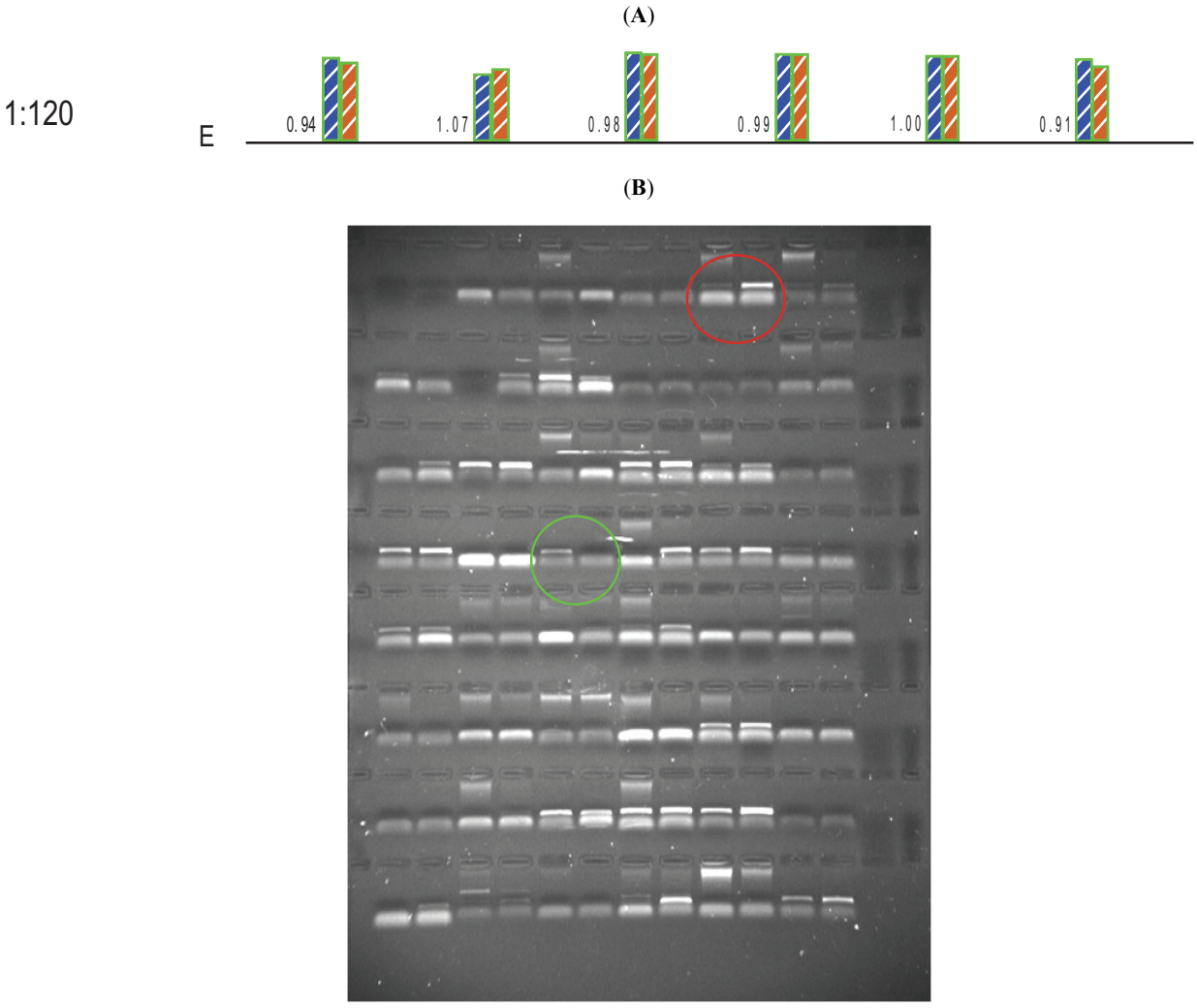
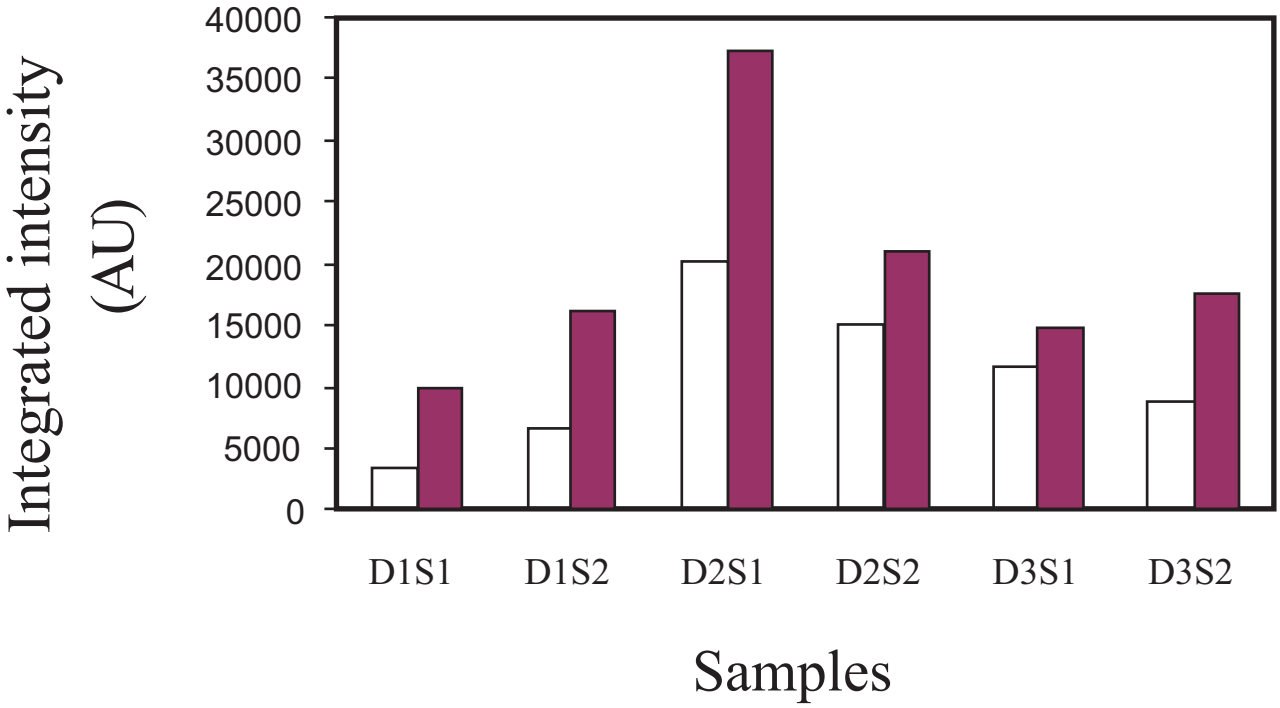
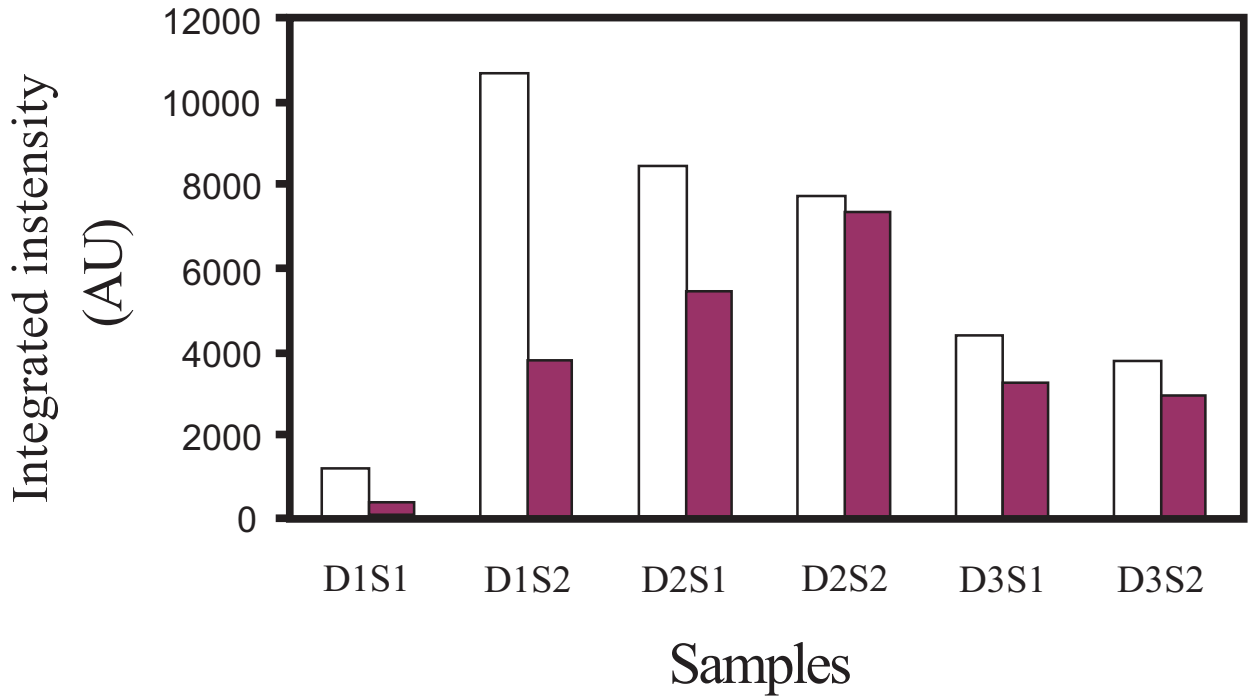


Supplementary Fig. (1). Examples of semi-quantitative analysis of GAPDH and other genes in different samples. Panel **A** shows the integrated intensity of each band in a bar graph with blue bars representing uninfected samples and red bars representing infected samples. Panel **B** shows an example of a 96-well plate high throughput PCR on which 46 different genes from corresponding uninfected and infected samples along with duplicated positive and negative controls were compared.

(**A**)



(**B**)



Supplementary Fig. (2). Examples of semi-quantitative PCR analysis of gene expression. Duplicated cDNA samples 8 hours post infection from each of the 3 donors were used. The intensity of each band on agarose gel representing the expected amplicons was integrated as described earlier. Open bars represent the integrated intensity of samples without infection (control) and purple bars represent the infected samples. The X-axis contains the sample names (donors are labeled as D1, D2 and D3, the duplicate samples are labeled as S1 and S2) and the Y-axis represents the integrated intensity in arbitrary unit (AU). Panel **A** represents the results of NM_001838 for which the infected samples showed higher integrated intensity than the uninfected samples. Panel **B** represents the results of NM_000530 for which the infected samples had lower integrated intensity than uninfected ones.

Supplementary Table 1. Primers Used in SYBR Green Quantitative PCR[a]

Primer ID	Oligonucleotide Sequence
18S-F	5'-CTCGATGCTCTTAGCTGAGTGTC-3'
18S-R	5'-GAACGCCACTTGTCCCTCTAAG-3'
NM_000530-F	5'-CAATGGCACGTTCACTTGTGACG-3'
NM_000530-R	5'-CTTCTCACTGACAGCTTTGGTGC-3'
NM_000595-F	5'-ATCTTGCCCACAGCACCCTCAAAC-3'
NM_000595-R	5'-CAGCCCTGGATACACCATCTTCTG-3'
NM_000801-F	5'-ATGCTAGGCAAGCAGGAGGTGAT-3'
NM_000801-R	5'-GAAACAGAGGTGTCGGAAGCAAAG-3'
NM_001547-F	5'-AATAGGACACGCTGTGGCTCATC-3'
NM_001547-R	5'- CTCCTGAAGGAATGCCAAGACATG-3'
NM_001838-F	5'-CCAATGAAAAGCGTGCTGGTGGT-3'
NM_001838-R	5'-AAAGTGGACACCGAAGACCCAGG-3'
NM_002498-F	5'-GTCAGTCCATCTGAGGAAAGCCA-3'
NM_002498-R	5'-TGACCTCCATCAACACTGTCCGA-3'
NM_002922-F	5'-GAGTTCTGGCTGGCTTGTGAAGAC-3'
NM_002922-R	5'-GGAGCCATACTGGCACATTCCTTC-3'
NM_002946-F	5'-ATGACAGCTGCACCCATGGACG-3'
NM_002946-R	5'-CCTTCAGGTCTTGGACAAGCCTT-3'
NM_002983-F	5'-GGTGTCATCTTCCTAACCAAGCG-3'
NM_002983-R	5'-GCTGATGACAGCCACTCGGTTG-3'
NM_003205-F	5'-TCTCCTGACCATACCAGCAGTAG-3'
NM_003205-R	5'-AGACTGACAGAGTCTTCCCGATG-3'
NM_003404-F	5'-TCGGCTGTGGATAGAGAAGCAGG-3'
NM_003404-R	5'-CACCTTACTTTCTGGTTGTGTAGC-3'
NM_003906-F	5'-CAGTTCCTGGCTTCTGTGGTGTC-3'
NM_003906-R	5'-CTTGCTCTTCCACCTACAGTAGG-3'
NM_004551-F	5'-CAACCTGTTGTCTCTGCGCTTCA-3'
NM_004551-R	5'-TTGGCGATAGACTGGGAAAGCCT-3'
NM_005082-F	5'-CCAAGTCCAGACCTGAGCTCCT-3'
NM_005082-R	5'-GTGGTCACAGTTGAGAAGCACGC-3'
NM_005623-F	5'-CAAGGAGAGATGGGTCAGGGATT-3'
NM_005623-R	5'-CCCACAACACTACAGACAGGTAG-3'
NM_006187-F	5'-TGGCTCTTCAGCCAAAGGCACAG-3'
NM_006187-R	5'-GCTCTGTGAAGCAGGTGGAGTAC-3'
NM_007215-F	5'-TTCTTCACGGTGCCTTGGAACAC-3'
NM_007215-R	5'-CGTGATCTCCTAAGTTCCACAGG-3'
NM_015369-F	5'-CGATTTCCTGTCAGCCAACAAAGG-3'
NM_015369-R	5'-TCTGTCTCTTCCCGCTTTTCCTC -3'
NM_021991-F	5'-CTGGTGCAGAACTGCCTGTGGA -3'
NM_021991-R	5'-GGATGCCATAGTTGAGACGCACA -3'
NM_033340-F	5'-GTGATCTCGGAAGACTGCAACCT -3'
NM_033340-R	5'- AGAGTTCCTTGGTGAGCATGGAG-3'
NM_080657-F	5'-CGCCACAAAGAAGTGTCCTGCTTG -3'
NM_080657-R	5'-GACCACAGGTAATCAGATGCCACG -3'
NM_152998-F	5'-TGTGGAGTTGGTGAATGCCCTTG-3'
NM_152998-R	5'-ACATCGCCTACAGAAAAGCGTATG-3'

[a] NM_xxxxxx-F represents forward primer for the gene with given reference number and NM_xxxxxx-R represents reverse primer for the same gene.

Supplementary Table 2. Primers and Probes Used in Quantitative Real-Time PCR[a]

Primer ID	Oligonucleotide Sequence
18S-F	5’-CTTTCGATGGTAGTCGCCGT-3’
18S-R	5’-TTGGAGCTGGAATTACCGCG-3
18S-P	5'FAM-CCACATCCAAGGAAGGCAGCAGGC-3'TAMRA
NM_001547-F	5’-TTCACCTCTGGACTGGCAA-3’
NM_001547-R	5’- TTCAGAGCCAGGAGGACTT –3’
NM_001547-P	5'FAM-CCATTGACCCTCTGAGGCAAGCCA-3'TAMRA
NM_006187-F	5’-GCTTTCTGAGTAGAGACGG-3’
NM_006187-R	5’-CACTGATGAACTTGTCAAGG-3’
NM_006187-P	5'FAM-ATGTGATGCCAGCCCTCCTTTACCAAA-3'TAMRA

[a] Genes follow by F, R and P represent forward primer, reverse primer and probe used for the experiment.