Supplementary Information for

*Coxiella burnetii* inhibits host immunity by a protein phosphatase adapted from glycolysis

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Supplementary Information Materials and Methods

Immunoprecipitation

Transfected cells were collected and lysed with the RIPA buffer (Thermo Fisher Scientific) at 16-18 h post transfection. Immunoprecipitation was performed with cleared lysates of transfected cells using agarose beads coated with Flag- or HA-specific antibody (Sigma) by incubating on a rotatory shaker at 4°C for 4 h. Beads were washed three times with pre-chilled RIPA buffer. Samples solubilized with the Laemmli buffer were resolved by SDS–PAGE, transferred to nitrocellulose membranes and proteins of interest were detected by immunoblotting with appropriate antibodies.

Bacterial strains, infection, and protein translocation assay

*C. burnetii* (strain Nine Mile RSA493 phase II) was axenically grown in liquid ACCM-D or ACCM-D agarose at 37°C in 5% CO₂ and 2.5% O₂ as previously described (1, 2). When appropriate, kanamycin and chloramphenicol were added to ACCM-D at 300 µg/ml and 3 µg/ml, respectively. *L. pneumophila* strains derived from the intracellular replication competent strain Lp02 or strain Lp03 defective in the Dot/Icm transporter were cultured in ACES-buffered yeast extract broth or charcoal yeast extract extract agar (3). Thymidine autotrophic was used to maintain plasmids derived from pZL507 (4). Infection with *L. pneumophila* was performed with bacteria grown to post-exponential phase (OD₆₀₀=3.2-3.6). HEK293 cells transfected with the NFκB reporter plasmid pGL4.32 and pRL-TK Renilla were infected with the relevant *L. pneumophila* strains for 2 h and the induction of the reporter was measured by determining luciferase activities. A portion of the samples were used to probe for the level of IκBα and other relevant proteins by immunoblotting.

To examine protein translocation into host cells by *C. burnetii*, we constructed a plasmid that expresses the TEM1-Flag-CinF fusion by inserting sequentially the TEM-1 gene, a Flag tag and *cinF* into pJB-Kan-P1169-3xFLAG (5). This plasmid, designated as pTEM1-CinF was introduced into wild-type or the icmD::Tn mutant of *C. burnetii* (6) by electroporation, respectively. The resulting strains were cultured in ACCM-D medium for 5 d. Twelve hours before infection, 2×10⁵ HeLa cells were seeded in 24-well plates without antibiotics, bacteria were added to cells at an MOI of 100. The plates were then centrifugated immediately at 250g for 10 min at 25°C to facilitate bacterial internalization. Infections were allowed to proceed for
24 h at 37°C in a CO₂ incubator (5%). Cells were then washed 3 times with PBS, CCF2-AM was added to the cells at a final concentration of 1 µM and the cells were further incubated for 1 h. Protein translocation was examined by the emission of blue fluorescence signals by infected cells using an Olympus IX-83 fluorescence microscope. Images were acquired to determine the rates of FRET in the samples by counting the number of blue fluorescence cells.

To construct the knock down C. burnetii strain, the antisense sequence of cinF was synthesized and inserted into pJB-Kan-P1169-3xFLAG (5) as a Sacl/Sall fragment to give pJBCinFKD, in which its expression was driven by the CBU1169 promoter (5). To evaluate the growth of C. burnetii strains in bacteriological medium, saturated cultures of wild-type C. burnetii and the cinF KD strain were diluted 1:100 in ACCM-D medium, this point was considered Day 0. Cultures were incubated in a 5% CO₂ and 2.5% O₂ incubator and samples were withdrawn at 1-day intervals for 9 days. Genomic DNA extracted from the samples using the Illustra Bacteria GenomicPrep Mini Prep Kit (GE Healthcare, Piscataway, NJ) was used to quantify genomic equivalents by the outer membrane protein com1-specific qPCR (7, 8).

Gene synthesis and codon optimization was performed by the Nanjing Jinsirui biological technology company. To complement the cinF knockdown strain, the codon optimized cinF gene and its Y362A mutant under the control of the CBU1169 promoter was inserted pJBCinFKD as Sacl/Sall fragments to give pCinFcW and pCinFcM, respectively. Electrocompetent cells of C. burnetii were prepared using a previously described protocol (9). Plasmids were electroporated in Coxiella RSA493 with a Gene Pulse (Bio-Rad, Gene Pulser Xcell) using the following parameters: 1.8 kV, 400 Ω, 25 μF for 0.1 cm cuvettes.

Antibodies, immunoblotting and immunostaining

Antibodies specific for CinF were produced using a standard protocol (10, 11). Briefly, 1 mg of His₆-CinF was emulsified with equal volumes of complete Freund’s adjuvant and was injected intracutaneously into mice 4 times a month at 7-day intervals. Sera of the immunized mice that contain CinF-specific antibodies were used for affinity purification of IgG with an established protocol (12).

For immunoblotting, cells were lysed by the RIPA buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% sodium-deoxycholate, and 1% NP-40) supplemented with a protease inhibitor cocktail (Roche Molecular Biochemicals). The protein concentration was determined using the
BCA Protein Assay Kit (Thermo Scientific Pierce) prior to being denatured in the Laemmli buffer by boiling for 5 min. Samples were then resolved by SDS-PAGE, after which the proteins were transferred onto nitrocellulose membranes (Millipore). After blocking in 5% nonfat milk in TBST (150 mM NaCl, 20 mM Tris-HCl (pH 7.4, 0.1% Tween-20), the membranes were incubated with primary antibodies at the following dilutions: anti-Flag (Sigma, Cat# F1804, 1:3000); anti-IκBα (Cell signaling, Cat# 4814, 1:3000); anti-GAPDH (Cell signaling, Cat# 5174, 1:5000); anti-Phospho-IκBβ (Cell signaling, Cat# 4921S, 1:1000), anti-Phospho-IKKα/β (Cell signaling, Cat# 2697S, 1:3000); anti-IKKβ (Cell signaling, Cat# 8493S, 1:3000); anti-His (Sigma, Cat# H1029,1:3000); anti-GFP (Sigma, Cat# G7781 , 1:5000). Washed membranes were incubated in the same buffer containing IRDye 680- or 800-conjugated secondary antibodies (Abcam, 1:10,000), and after another 3x washes, signals were detected using an Odyssey infrared imaging system (Li-Cor’s Biosciences Lincoln, Nebraska, USA). Signal quantification was carried out using the same system following the instructions provided by the manufacturer.

Immunostaining was performed as follows: 5x10⁴ MLE-12 cells seeded on glass coverslips placed in 24-well plates were transfected with 2 µg DNA of the plasmid carrying the gene of interest for 23 h. Samples transfected with the empty plasmid were established as positive controls. Transfected samples were treated with 100 ng PMA for 4 h. For staining experiments using infected cells, Hela cells seeded at 2×10⁴ per well in 24-well plates were infected with the relevant C. burnetii strains at an MOI of 100 for 72 h. Cells washed with PBS were fixed by 4% formaldehyde solution for 15 min at room temperature and were permeabilized with 0.1% Triton X-100 for 5 min. After blocking with 4% goat serum for 30 min at 37 °C, bacteria were stained with anti-Mouse C. burnetii sera at a dilution of 1:1,000 for 2 h. p65 was stained with the p65 specific antibody (Cell Signaling, Cat# 4764, 1:500), followed by staining with secondary antibodies conjugated to Alexa Fluor 594 and Alexa Fluor 488 (Thermo Fisher Scientific), respectively.

For transfected cells, samples were then fixed with 4% paraformaldehyde for 10 min at room temperature and were permeabilized with 0.5% Triton X-100 in PBS for 10 min. The samples were first stained for p65 as described above then for Flag-tagged proteins with the Flag-specific antibody (Sigma, Cat# F1804, 1:1000), followed by Alexa Flour 488-labeled secondary antibody (Invitrogen, Cat# A11001, 1:5000). In each case, nuclei were stained with Hoechst. Samples were inspected using an Olympus IX-83 microscope for image acquisition.
To quantitate the rates of p65 nuclear localization, at least 300 cells were scored for each sample.

**Protein expression and purification**

The coding sequence of *cinF* (cbu0513) amplified from *C. burnetii* genomic DNA with the appropriate primers (Table S2) was digested with *Bam*HI and *Sal*I and inserted into similarly digested pET-28a(+) (Novagen) to give pETcinF. The gene was inserted into pCMV4Flag (13) to give pFlagCinF for transient expression of Flag-CinF in mammalian cells. The gene coding for WipA (Lpg2718), a protein phosphatase from *L. pneumophila* (14) was similarly cloned into pET-28a(+) to give pET28aWipA. When needed, substitution mutations were introduced using the Quikchange kit (Agilent) and fusion PCR. Plasmids directing protein expression were individually introduced into the *E. coli* strain BL21(DE3) and the resulting strains were grown in 50-100 mL broth to saturation prior to being diluted at 1:50 to larger volumes of medium. The expression of the protein was induced with 0.2 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) when the diluted cultures reached an OD$_{600}$ of 0.6-0.8. The induction was allowed to proceed for 16 h at 18°C.

To purify the protein, cell harvested by centrifugation were resuspended in a lysis buffer (250 mM NaCl, 40 mM Tris-HCl, pH 8.0, 10 mM imidazole, 1 mM β-mercaptoethanol, 1 mM PMSF) and were lysed by passing through a cell homogenizer system twice (JN-mini, JNBIO, Guangzhou, China). The soluble fraction of the lysates obtained by centrifugation at 12,000g for 60 min was loaded onto HisTrap Chelating columns (GE Healthcare, Wisconsin, USA). After washing with the lysis buffer containing 10 mM imidazole, His-tagged proteins were eluted using an imidazole gradient (40 mM Tris-HCl, pH 8.0, 250 mM NaCl, 0-200 mM imidazole). When necessary, the protein was further purified on an AKTA system equipped with a Superdex 200 increase column, and the protein was eluted with gradient NaCl. All purified proteins were more than 95% purity as assessed by SDS-PAGE and Coomassie brilliant blue staining. Proteins were dialyzed and saved at −80°C in a storage buffer (20 mM Tris-HCl pH 8.0, 200 mM NaCl and 1 mM DTT, 10% glycerol). Protein concentration was determined by the Bradford method.
Enzymatic assays

Phosphatase activity was measured by using p-nitrophenyl phosphate (pNPP) (Aladdin, Shanghai, China) as the substrate. The assays were carried out in 96-well plates with purified His6-CinF, His6-ST3108, His6-WipA and their mutants. 5 μg (83.4 pmol) protein and 2.5 mM pNPP were mixed in wells containing 200 μl reaction buffer (100 mM NaCl, 25 mM Tris-HCl, pH 6.0). The reaction was allowed to proceed for 30 min at 37°C prior to determining the amounts of the product by measuring the absorbance at OD405 with a microplate reader (BioTek, Synergy H1).

FBP-dependent fructose-6-phosphate formation (FBP phosphatase activity) was measured by coupling the reaction with exogenous phosphoglucose isomerase and glucose-6-phosphate dehydrogenase, in which NADP⁺ is reduced to NADPH. NADPH formation was monitored at 340 nm (ε340 nm NADPH = 6,300 M⁻¹ cm⁻¹). The assay mixture (0.2 mL) consisted of 0.1 M Tris-HCl (pH 7.8), 20 mM MgCl₂, 20 mM DTT, 0.5 mM NADP⁺(Sigma-Aldrich, Cat#10128031001), 0.01-0.2 mM FBP and 1 U each of phosphoglucose isomerase from baker’s yeast (Sigma-Aldrich, Cat#P5381) and glucose-6-phosphate dehydrogenase from baker’s yeast (Sigma-Aldrich, Cat#G7877). The reactions were started by the addition of 20 μg purified protein enzymes in 96-well plates at 48°C, and the increase in absorption at 340 nm was monitored at a one minute interval by a Synergy H1 microplate reader (BioTek). Plot with the Lineweaver-Burk Equation to obtain Km and Kcat values.

Intracellular growth of C. burnetii

HeLa cells seeded at a density of 2x10⁴/well in 24-well plates for 24 h were infected with C. burnetii strains axenically grown to the stationary phase. The concentration of the bacterial cultures was determined by qPCR using com1 specific primers (15), and the bacterial cell density was adjusted with DMEM containing 2% FBS to achieve an MOI of 100. After incubation for 4 h, infected samples were washed once with PBS and incubated with fresh DMEM containing 2% FBS. This point was considered Day 0 and samples were collected to determine the inoculum. Infection lysate were collected at the following time points: 24 h (Day 1), 48 h (Day 2), 72 h (Day 3), 96 h (Day 4), 120 h (Day 5), 144 h (Day 6) and 168 h (Day 7). Genomic DNA extracted from the samples using the Illustra Bacteria GenomicPrep Mini Prep Kit (GE
Healthcare, Piscataway, NJ) was used to quantify genomic equivalents by com1-specific qPCR (7, 8).

**qRT-PCR analysis of cytokine gene expression and determination of cytokines in culture supernatants**

To determine the expression of relevant cytokine genes, we extracted total RNA from THP-1 cells infected with the appropriate *C. burnetii* strains using the TRIzol reagent. Briefly, cell pellets suspended in 1 ml TRIzol were vortexed and incubated at room temperature (RT) for 10 min. After adding 200 μl chloroform, the mixtures were phase separated by centrifugation at 12,000g for 10 min at 4°C and the upper phase was transferred to a new set of tubes into which 500 μl isopropanol was added before precipitation by centrifugation at 12,000g for 10 min at 4°C. After carefully removing the supernatant, the pellets containing RNA were washed with 1 ml of 70% ice-cold ethanol. The samples were then centrifuged at 8,000g for 5 min at 4°C, followed by drying in a speed-vac for 5 min. The pellets were dissolved in 50 μl ddH₂O and the concentration of RNA and its purity was measured by spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific)(16). The total RNA was reverse transcribed into cDNA using the TIANGEN Quant One Step RT-PCR kit (KR113). PCR reactions were performed in 25 µl volumes using SYBR Green One-Step Kit (Bio-Rad, USA) and the amplification was assessed using the 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France). The following program was used for PCR: 3 min at 95°C followed by 40 cycles of 15 s at 95°C, 20 s at 60°C, and 20 s at 72°C. Cycle threshold (Ct) values for each transcript were normalized to the geometric mean of the expression of IL-1β, IL-6 and TNF-α, and GAPDH was used as control. The fold changes were determined by using the 2-ΔΔCt method (17). All samples were analyzed in triplicate, the primer pairs were listed in Table S2.

To quantitate cytokines released by THP-1 cells infected with the appropriate *C. burnetii* strains, supernatants were collected from infected THP-1 cells plated for 3 days. Similarly cultured uninfected cells were used as controls. The quantity of secreted cytokines was measured using an enzyme-linked immunosorbent assay (ELISA) by specific human ELISA kits (Elabscience Cat#E-EL-H0149c, E-EL-H0102c, E-EL-H0150c and E-EL-H0109c). The plates were read at 450 nm on a Biotek Synergy H1 plate reader. Values were calculated from a standard curve generated by two-fold dilutions of recombinant human IL-1β (1000 to 5 pg/ml),
IL-12 (2000 to 5 pg/ml), TNF-α (3000 to 50 pg/ml) according to the manufacturer’s recommendations.

**In vitro protein dephosphorylation assay**

To prepare of p-IκBα, cells were transfected with a plasmid that expresses Flag-IκBα for 16 h, and the samples were treated with 50 nM PMA for 4 h. Lysates of the cells were subjected to immunoprecipitation with agarose beads coated with the Flag-specific antibody (Sigma) by incubating at 4°C for 4 h. Beads were washed with pre-chilled Tris buffer (50 mM Tris, 150 mM NaCl, 10 mM MgCl₂, pH 7.5) for 3 times. To analyze p-IκBα dephosphorylation of, recombinant proteins of the indicated amounts were incubated with aliquots of the beads carrying the target protein in the Tris buffer. Unless otherwise noted, the reactions were allowed to proceed for 120 min at 37°C. Proteins of interest or its modification status were detected by immunoblotting with specific antibodies.
**Fig. S1 Comparison of CinF and ST0318.** The alignment of the two proteins was performed with the software Snapgene. Residues in blue background are identical and residues important for catalysis were highlighted in red background (left panel). The structure of CinF was modeled using the *pymol* software, the positioning the the predicted catalytic residues was highlighted as sticks (right panel).
Fig. S2 CinF does not detectably interact with IkBα

Combinations of plasmids that direct the expression of 4xFlag-CinF or HA-κBα were transfected into HEK293T cells for 16 cells and the cell lysates were subjected to immunoprecipitation with agarose beads coated with antibodies specific for either the Flag or HA tag. The presence of potential binding proteins was probed in the precipitates by immunoblotings. Note the successful enrichment of Flag-CinF by the antibody in the precipitates.
Fig. S3 Growth of *C. burnetii* strains in bacteriological medium

Saturated cultures of the RSA493 (WT) and the cinF KD strains were diluted at 1:100 in the ACCM-D medium in triplicate and the growth of the bacteria was monitored at the indicated time points by measuring the genome equivalents of the samples. The results shown were calculated as fold of growth using day 0 as the reference.
**Fig. S4** Comparison of the nucleotide sequences of the endogenous *cinF* gene and the one optimized to the codon of *E. coli*. Changed nucleotides are in red.
**Fig. S5 The impact of CinF on NFκB activation in cells infected with C. burnetii**

**A.** Nuclear translocation of p65 in cells infected with relevant strains at day 2 after bacterial uptake. Infected cells on cover slips were fixed and sequentially with antibodies specific for the bacteria, p65. Nuclei were stained with Hoechst. Samples were inspected under an Olympus IX-81 fluorescence scope for acquiring representative images. Nuclear localization of p65 was scored from samples each done in triplicate (Fig. 6E).

**B-E.** NFκB activation and CinF translocation at day 1, 2, 5 and 6 postinfection. Lysates of cells infected with the indicated C. burnetii strains were probed for p-κBa, κBa and CinF by immunoblotting with specific antibodies. GAPDH was detected as a loading control (lower panel for each data set). Data shown are one representative experiment from three independent experiments with similar results.
Fig. S6 The expression of four cytokine genes and their secretion by cells infected with relevant *C. burnetii* strains at the 3rd postinfection.

Total RNA from THP-1 cells infected with the indicated *C. burnetii* strains for 3 days was isolated and used as templates for probing the expression of the 4 cytokine genes (A, IL1β; B, IL-6; C, IL-12; D, TNF-α). To detect the levels of these cytokines, culture supernatants of similarly infected THP-1 cells were collected and the levels of the indicated cytokines were measured using an enzyme-linked immunosorbent assay (ELISA) by specific human ELISA kits. Plates were read on a Biotek Synergy H1 at 450 nm to measure cytokine concentrations. In each case, similarly cultured cells without infection were included as controls. Data shown are one representative from an experiment done in triplicate. Similar results were obtained from two independent experiments.
Fig. S7 The expression of four cytokine genes in cells infected with relevant *C. burnetii* strains at several other infection time points. Infections were performed in THP-1 cells as described in Fig. S6 and samples collected at the indicated time points (A, 1 day; B, 2 day; C, 3 day; D, 5 day and E, 6 day) were probed for the expression of the four cytokine genes by qPCR. Data shown were one representative experiment done in triplicate from two independent experiments. Uninfected cells were used as controls.
Fig. S8 The levels of four cytokines secreted by cells infected with relevant C. burnetii strains at the $1^{st}$, $2^{nd}$, $5^{th}$ and $6^{th}$ days. Infections were performed in THP-1 cells as described in Fig. S7 and samples collected at the indicated time points (A, 1 day; B, 2 day; C, 3 day; D, 5 day and E, 6 day) were detected for the four cytokines by ELISA. Data shown were one representative experiment done in triplicate from two independent experiments. Uninfected cells were used as controls.
### Table S1 Plasmids used in this study

| name                                    | source                                      |
|-----------------------------------------|---------------------------------------------|
| pET28a-CinF                             | This paper                                  |
| pET28a-CinF<sub>Y362A</sub>            | This paper                                  |
| pET28a-ST0318                           | This paper                                  |
| pET28a-ST0318<sub>Y347T</sub>           | This paper                                  |
| pET28a-WipA                             | This paper                                  |
| pET28a-WipA<sub>D180A</sub>             | This paper                                  |
| pJB-Kan-P1169-3xFLAG                    | (5)                                          |
| pTEM1-CinF                              | This paper                                  |
| pJB-CinFKD                              | This paper                                  |
| pCinFcW                                 | This paper                                  |
| pCinFcM                                 | This paper                                  |
| pcDNA3Flag-TRAF2 (1087)                 | Addgene, Cat# 66931 (a gift from Michael Karin) |
| pcDNA3-TAK1                             | (18)                                        |
| pCMV2 Flag-IKKβ                         | (19)                                        |
| pCMV4-p65                               | (20)                                        |
| pGL4.32 (NFκB reporter)                 | (21, 22)                                    |
| pRL-SV40-Renilla                        | Promega                                     |
| pEGFPC1-LegK1                           | This paper                                  |
| pCMV4xFlag                              | (13)                                        |
| pCMV-Flag-CinF                          | This paper                                  |
| pCMV-Flag-ST0318                         | This paper                                  |
| 2X_pX458_pSpCas9(BB)-2A-GFP             | a gift from Alexander Meissner (Addgene Cat # 172221) (23) |
| 2X_pX458_pSpCas9(BB)-2A-GFP_p65KO-1     | This paper                                  |
| 2X_pX458_pSpCas9(BB)-2A-GFP_p65KO-2     | This paper                                  |
| pCMV-Flag-Cbu0388                        | This paper                                  |
| pCMV-Flag-Cbu1757                        | This paper                                  |
| pCMV-Flag-Cbu1576                        | This paper                                  |
| pCMV-Flag-Cbu0021                        | This paper                                  |
| pCMV-Flag-Cbu0487                        | This paper                                  |
| pCMV-Flag-Cbu1686                        | This paper                                  |
| pCMV-Flag-Cbu1724                        | This paper                                  |
| pCMV-Flag-Cbu0069                        | This paper                                  |
| pCMV-Flag-Cbu0041                        | This paper                                  |
| pCMV-Flag-Cbu0626                        | This paper                                  |
| pCMV-Flag-Cbu2035                        | This paper                                  |
| pCMV-Flag-Cbu1379                        | This paper                                  |
| pCMV-Flag-Cbu1457                        | This paper                                  |
| pCMV-Flag-Cbu1213                        | This paper                                  |
| pCMV-Flag-Cbu1790 | This paper |
|-------------------|------------|
| pCMV-Flag-Cbu0814 | This paper |
| pCMV-Flag-Cbu1863 | This paper |
| pCMV-Flag-Cbu1665 | This paper |
| pCMV-Flag-Cbu1556 | This paper |
| pCMV-Flag-Cbu1493 | This paper |
| pCMV-Flag-Cbu1569 | This paper |
| pCMV-Flag-Cbu2059 | This paper |
| pCMV-Flag-Cbu0062 | This paper |
| pCMV-Flag-Cbu1217 | This paper |
| pCMV-Flag-Cbu0635 | This paper |
| pCMV-Flag-Cbu0295 | This paper |
| pCMV-Flag-Cbu0937 | This paper |
| pCMV-Flag-Cbu1685 | This paper |
| pCMV-Flag-Cbu1063 | This paper |
| pCMV-Flag-Cbu0794 | This paper |
| pCMV-Flag-Cbu0425 | This paper |
| pCMV-Flag-Cbu0372 | This paper |
| pCMV-Flag-Cbu0886 | This paper |
| pCMV-Flag-Cbu1751 | This paper |
| pCMV-Flag-Cbu0270 | This paper |
| pCMV-Flag-Cbu0072 | This paper |
| pCMV-Flag-Cbu0534 | This paper |
| pCMV-Flag-Cbu1639 | This paper |
| pCMV-Flag-Cbu2007 | This paper |
| pCMV-Flag-Cbu1636 | This paper |
| pCMV-Flag-Cbu0885 | This paper |
| pCMV-Flag-Cbu1819 | This paper |
| pCMV-Flag-Cbu2013 | This paper |
| pCMV-Flag-Cbu1676 | This paper |
| pCMV-Flag-Cbu1130 | This paper |
| pCMV-Flag-Cbu1823 | This paper |
| pCMV-Flag-Cbu0006 | This paper |
| pCMV-Flag-Cbu0016 | This paper |
| pCMV-Flag-Cbu0781 | This paper |
| pCMV-Flag-Cbu0665 | This paper |
| pCMV-Flag-Cbu1370 | This paper |
| pCMV-Flag-Cbu2052 | This paper |
| pCMV-Flag-Cbu0009 | This paper |
| pCMV-Flag-Cbu1789 | This paper |
| pCMV-Flag-Cbu0020 | This paper |
| pCMV-Flag-Cbu1409 | This paper |
| pCMV-Flag-Cbu1794 | This paper |
| pCMV-Flag-Cbu0012 | This paper |
| pCMV-Flag-Cbu0077 | This paper |
| pCMV-Flag-Cbu1460          | This paper |
|----------------------------|------------|
| pCMV-Flag-Cbu0637          | This paper |
| pCMV-Flag-Cbu2028          | This paper |
| pCMV-Flag-Cbu0175          | This paper |
| pCMV-Flag-Cbu1566          | This paper |
| pCMV-Flag-Cbu0013          | This paper |
| pCMV-Flag-Cbu0375          | This paper |
| pCMV-Flag-Cbu0023          | This paper |
| pCMV-Flag-Cbu1150          | This paper |
| pCMV-Flag-Cbu0015          | This paper |
| pCMV-Flag-Cbu0881          | This paper |
| pCMV-Flag-Cbu2056          | This paper |
| pCMV-Flag-Cbu1387          | This paper |
| pCMV-Flag-Cbu1314          | This paper |
| pCMV-Flag-Cbu1769          | This paper |
| pCMV-Flag-Cbu1079          | This paper |
| pCMV-Flag-Cbu1754          | This paper |
| pCMV-Flag-Cbu2016          | This paper |
| pCMV-Flag-Cbu1543          | This paper |
| pCMV-Flag-Cbu0447          | This paper |
| pCMV-Flag-Cbu1198          | This paper |
| pCMV-Flag-Cbu0773          | This paper |
| pCMV-Flag-Cbu0978          | This paper |
| pCMV-Flag-Cbu1434          | This paper |
| pCMV-Flag-Cbu1425          | This paper |
| pCMV-Flag-Cbu0801          | This paper |
| pCMV-Flag-Cbu1607          | This paper |
| pCMV-Flag-Cbu1825          | This paper |
| pCMV-Flag-Cbu2076          | This paper |
| pCMV-Flag-Cbu0183          | This paper |
| pCMV-Flag-CbuA0025         | This paper |
| pCMV-Flag-CbuA0014         | This paper |
| pCMV-Flag-Cbu0113          | This paper |
| pCMV-Flag-Cbu0469          | This paper |
| pCMV-Flag-Cbu0590          | This paper |
| pCMV-Flag-Cbu1634          | This paper |
| pCMV-Flag-Cbu0080          | This paper |
| pCMV-Flag-Cbu0129          | This paper |
| pCMV-Flag-Cbu0212          | This paper |
| pCMV-Flag-Cbu0329          | This paper |
| pCMV-Flag-Cbu0376          | This paper |
| pCMV-Flag-Cbu0393          | This paper |
| pCMV-Flag-Cbu0414          | This paper |
| pCMV-Flag-Cbu0606          | This paper |
| pCMV-Flag-Cbu1045          | This paper |
| pCMV-Flag-Cbu1102 | This paper |
|------------------|-----------|
| pCMV-Flag-Cbu1108| This paper |
| pCMV-Flag-Cbu1532| This paper |
| pCMV-Flag-Cbu1599| This paper |
| pCMV-Flag-Cbu1620| This paper |
| pCMV-Flag-Cbu1776| This paper |
| pCMV-Flag-Cbu1963| This paper |
| pCMV-Flag-Cbu2064| This paper |
| pCMV-Flag-CbuA0019| This paper |
| Primer name  | Sequence (Restriction enzyme sites are underlined) | Note |
|--------------|---------------------------------------------------|------|
| CinF-F       | CGCGGATCCATGAAAATCTACCTAAAGGCT                   | CinF 5’ BamH I |
| CinF-R       | CTAGTCGACTTATCATCCATTTTTCGCAAT                   | CinF 3’ Sal I |
| CinF<sub>Y362A</sub>-F | CGTATCCTAAATGCGGCGCATTTTCTGCTTCG | CinF mutant Y362A |
| CinF<sub>Y362A</sub>-R | GCAGGAAGCAGAAATTGCGCCACTTTAGTGGA | |
| ST0318-F     | CGCGGATCCATGAAAATCTACCTAAAGGCT                   | ST0318 5’ BamH I |
| ST0318-R     | CTAGTCGACTTATCATCCATTTTTCGCAAT                   | ST0318 3’ Sal I |
| ST0318<sub>Y347T</sub>-F | GTTTTTCTAAAAATGAAGTTAGTTATCTCTCTCTGCTTT    | ST0318 mutant Y347T |
| ST0318<sub>Y347T</sub>-R | GCAGGAAGCAGAAATTGCGCCACTTTAGTGGA | |
| WipA-F       | CGCGGATCCATGAAAATCTACCTAAAGGCT                   | WipA 5’ BamH I |
| WipA-R       | CTAGTCGACTTATCATCCATTTTTCGCAAT                   | WipA 3’ Sal I |
| Wip<sub>D180A</sub>-F | GATCGGCCACCTCAGCACCCAGCAATCGA | WipA mutant D180A |
| Wip<sub>D180A</sub>-R | TCAGTAATGATACTTTGGCAGCGTATTG | |
| P1169<sub>Promoter-F</sub> | CTGCTCGAGATGGGCTTTCCGCAAGCG | P1169 Promoter 5’ Xho I |
| P1169<sub>Promoter-R</sub> | CTGAGCTCCTCCCTCTTGTATAGGATTA | P1169 Promoter 3’ Sac I |
| TEM1-F       | GTCGTCGCCAACCAGAAAACGTGCTGTAAGGT | TEM1 5’ Sal I |
| TEM1-R       | CTGGGATCTTACCAATGCTTTACATCGTGAG | TEM1 3’ BamH I |
| Com1-F       | AAAACCTCGCCGTGCTCTTCA | Quantify the amount of C. burnetii genomic |
| Com1-R       | GCTAATGATACTTTGGCAGCGTATTG | |
| Com1-probe   | AGAACTGCTCCCATTTTTGCGCGGCA                     | 5’6-FAM, 3’BHQ1 |
| IL1β-F       | CCAGCTAGAATCTCCGACC                           | qRT-PCR analysis of Cytokine IL1β |
| IL1β-R       | TCTCCGTAGGAAGTTGCTGGG                         | |
| IFNγ-F       | TTCAAGCCCACCACAACTG                         | qRT-PCR analysis of Cytokine IFNγ |
| IFNγ-R       | GTGACTCTTTCAAGGCGTCC                     | |
| TNFα-F       | GGCCTGAGGCTGAGATAAC                        | qRT-PCR analysis of Cytokine TNFα |
| TNFα-R       | GGTGTGGGTGAGGACACAT                         | |
| LegK1-F      | CTGAGTGAGCTGCTGACTGTTA | LegK1 5’ BamH I |
| LegK1-R      | CTGAGTGACTGCTGACTGTTA | LegK1 3’ Sal I |
| 1’<sub>p65</sub>-sgRNA-F | CCACGGGCCGCTCCGGCTACAAGTGAG | p65-sgRNA 5’ Bbsl |
| 1’<sub>p65</sub>-sgRNA-R | AAACCGCAGGCTGCTGCTGACGCGG | p65-sgRNA 3’ Bbsl |
| 2’<sub>p65</sub>-sgRNA-F | CCACGGCTGCTGGCTGCTGACGCGG | p65-sgRNA 5’ Bbsl |
| 2’<sub>p65</sub>-sgRNA-R | AAACCGCAGGCTGCTGCTGACGCGG | p65-sgRNA 3’ Bbsl |
| CBU0388-F    | CTGGGATCTGAGATCATGGTTGCG | CBU0388 5’ BamH I |
| CBU0388-R    | CTGGGATCTGAGATCATGGTTGCG | CBU0388 3’ Sal I |
| CBU1525-F    | CTGGGATCTGAGATCATGGTTGCG | CBU1525 5’ BamH I |
| CBU1525-R    | CTGGGATCTGAGATCATGGTTGCG | CBU1525 3’ Sal I |
| CBU1686-F    | CTGGGATCTGAGATCATGGTTGCG | CBU1686 5’ BamH I |
| CBU1686-R    | CTGGGATCTGAGATCATGGTTGCG | CBU1686 3’ Sal I |
| Gene     | Forward Primer | Reverse Primer | Restriction Site |
|----------|----------------|----------------|------------------|
| CBUD1108 | CGGGATCCATGGAATAATTTTCTTA | CBUD1108 5’ BamH I |
| CBUD1108-R | CTGGTCGACTTAAATAACGATTTTTGGT | CBUD1108 3’ Sal I |
| CBU0041-F | CTGGGATCCATGAGGAGGATGGCACTACA | CBU0041 5’ BamH I |
| CBU0041-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0041 3’ Sal I |
| CBUD2035-F | CTGGGATCCCCTGGGACAAATACACG | CBUD2035 5’ BamH I |
| CBUD2035-R | CTGGTCGACCTTTAAGCTAAGCAAGGCTT | CBU1819 3’ Sal I |
| CBU1457-F | CTGGGATCCATGGATGACTGCAAAAAACATA | CBU1493 5’ BamH I |
| CBU1457-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1493 3’ Sal I |
| CBU2059-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU2059 5’ BamH I |
| CBU2059-R | CTGGTGCACTTATCCCTACGAAGATG | CBU2059 3’ Sal I |
| CBU1217-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1217 5’ BamH I |
| CBU1217-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1217 3’ Sal I |
| CBU0270-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0270 5’ BamH I |
| CBU0270-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0270 3’ Sal I |
| CBU0295-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0295 5’ BamH I |
| CBU0295-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0295 3’ Sal I |
| CBU1819-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1819 5’ BamH I |
| CBU1819-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1819 3’ Sal I |
| CBU1063-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1063 5’ BamH I |
| CBU1063-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1063 3’ Sal I |
| CBU0425-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0425 5’ BamH I |
| CBU0425-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0425 3’ Sal I |
| CBUD0886-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBUD0886 5’ BamH I |
| CBUD0886-R | CTGGTGCACTTATCCCTACGAAGATG | CBUD0886 3’ Sal I |
| CBU0270-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0270 5’ BamH I |
| CBU0270-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0270 3’ Sal I |
| CBU0354-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0354 5’ BamH I |
| CBU0354-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0354 3’ Sal I |
| CBU1819-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1819 5’ BamH I |
| CBU1819-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1819 3’ Sal I |
| CBU1676-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1676 5’ BamH I |
| CBU1676-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1676 3’ Sal I |
| CBU1823-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1823 5’ BamH I |
| CBU1823-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1823 3’ Sal I |
| CBU0006-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0006 5’ BamH I |
| CBU0006-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0006 3’ Sal I |
| CBU0781-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU0781 5’ BamH I |
| CBU0781-R | CTGGTGCACTTATCCCTACGAAGATG | CBU0781 3’ Sal I |
| CBU1370-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1370 5’ BamH I |
| CBU1370-R | CTGGTGCACTTATCCCTACGAAGATG | CBU1370 3’ Sal I |
| CBUDA0009-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBUDA0009 5’ BamH I |
| CBUDA0009-R | CTGGTGCACTTATCCCTACGAAGATG | CBUDA0009 3’ Sal I |
| CBU1409-F | CTGGGATCCATGGAACAAGCAAAACAATCT | CBU1409 5’ BamH I |
| CBU1409-R | CTGGTCGACTCAATCGCAGCTCCATAC | CBU1409 3' Sal I |
| CBU1794-F | CTGGGATCCATGGAGCTGTATCATG | CBU1794 5' BamH I |
| CBU1794-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1794 3' Sal I |
| CBU0077-F | CTGGGATCATGAGACAACTCCTTCA | CBU0077 5' BamH I |
| CBU0077-R | CTGGTCGACTTACATAAGACACCC | CBU0077 3' Sal I |
| CBUA0013-F | CTGGGATCATGACCATATTITTTITACA | CBUA0013 5' BamH I |
| CBUA0013-R | CTGTTCGACCTATCGATGACTCGTTAA | CBUA0013 3' Sal I |
| CBUA0023-F | CTGGGATCATGAGACAACTCCTTCA | CBUA0023 5' BamH I |
| CBUA0023-R | CTGTTCGACCTATCGATGACTCGTTAA | CBUA0023 3' Sal I |
| CBUA0015-F | CTGGGATCATGACCATATTITTTITACA | CBUA0015 5' BamH I |
| CBUA0015-R | CTGTTCGACCTATCGATGACTCGTTAA | CBUA0015 3' Sal I |
| CBU2056-F | CTGGGATCCATGTTAGTATTTTATTTT | CBU2056 5' BamH I |
| CBU2056-R | CTGGTCGACCTATCGATGACTCGTTAA | CBU2056 3' Sal I |
| CBU1314-F | CTGGGAATCTCGTATCCCAATTCGACG | CBU1314 5' BamH I |
| CBU1314-R | CTGTTCGATCCCTGATTTGACGC | CBU1314 3' Sal I |
| CBU1769-F | CTGGGATCATGAGACAACTCCTTCA | CBU1769 5' BamH I |
| CBU1769-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1769 3' Sal I |
| CBU1754-F | CTGGGATCATGAGACAACTCCTTCA | CBU1754 5' BamH I |
| CBU1754-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1754 3' Sal I |
| CBU1543-F | CTGGGATCATGAGACAACTCCTTCA | CBU1543 5' BamH I |
| CBU1543-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1543 3' Sal I |
| CBU1198-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU1198 5' BamH I |
| CBU1198-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1198 3' Sal I |
| CBU0773-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU0773 5' BamH I |
| CBU0773-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU0773 3' Sal I |
| CBU1434-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU1434 5' BamH I |
| CBU1434-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1434 3' Sal I |
| CBU1425-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU1425 5' BamH I |
| CBU1425-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1425 3' Sal I |
| CBU0801-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU0801 5' BamH I |
| CBU0801-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU0801 3' Sal I |
| CBU1607-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU1607 5' BamH I |
| CBU1607-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1607 3' Sal I |
| CBUDA0023-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBUDA0023 5' BamH I |
| CBUDA0023-R | CTGTTCGACCTATCGATGACTCGTTAA | CBUDA0023 3' Sal I |
| CBU2076-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU2076 5' BamH I |
| CBU2076-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU2076 3' Sal I |
| CBUA0025-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBUA0025 5' BamH I |
| CBUA0025-R | CTGTTCGACCTATCGATGACTCGTTAA | CBUA0025 3' Sal I |
| CBU0113-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU0113 5' BamH I |
| CBU0113-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU0113 3' Sal I |
| CBU0469-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU0469 5' BamH I |
| CBU0469-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU0469 3' Sal I |
| CBU1634a-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU1634a 5' BamH I |
| CBU1634a-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1634a 3' Sal I |
| CBU1576-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU1576 5' BamH I |
| CBU1576-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU1576 3' Xho I |
| CBU0021-F | CTGGGATCCATGATTTAAGAGGCCTAACT | CBU0021 5' Bgl II |
| CBU0021-R | CTGTTCGACCTATCGATGACTCGTTAA | CBU0021 3' Sal I |
| Gene Name      | Primer 1 Sequence                  | Primer 1 Restriction Site | Gene Name      | Primer 2 Sequence                  | Primer 2 Restriction Site |
|---------------|------------------------------------|---------------------------|---------------|------------------------------------|---------------------------|
| CBU1863-F     | CTGAGATCTATGCGAAATGTAGTAGAT        | 5' Bgl II                 | CBU1863-R     | CTGTCGACTCTGTACGATGAGCAGA          | 3' Sal I                 |
| CBU1863-R     | CTGTCGACTCTGTACGATGAGCAGA          | 3' Sal I                  | CBU0410-F     | CTGAGATCTATGAAACAGAAGCATG          | 5' Bgl II                 |
| CBU0410-R     | CTGAGATCTATGAAACAGAAGCATG          | 5' Bgl II                 | CBU0410-R     | CTGTCGAGCTTGTACGACAGCTAA          | 3' Xho I                 |
| CBU2007-F     | CTGATGCGACAGCTGAGTTGTACGAGAAG     | 5' BamH I                 | CBU2007-R     | CTGTCGAGCTTGTACGACAGCTAA          | 3' Xho I                 |
| CBU2007-R     | CTGTCGAGCTTGTACGACAGCTAA          | 3' Xho I                  | CBU0080-F     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0080-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0080-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU0129-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0129-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0129-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0329-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU0329-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0372-F     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0372-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0376-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU0376-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0487-F     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0487-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0794-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU0794-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU1569-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1569-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU1685-F     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU1685-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU1724-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1724-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0175-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0175-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU1213-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1213-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0626-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0626-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU1556-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1556-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU1685-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1685-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0937-F     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0937-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0635-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU0635-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0372-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0372-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0372-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU0372-F     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU1715-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU1715-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU1751-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1751-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0175-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0175-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 | CBU0175-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                 |
| CBU1751-R     | CTGGATCTCTGGCTACGAGAA             | 3' Sal I                  | CBU0175-R     | CTGGATCTCTGGCTACGAGAA             | 5' BamH I                 |
| CBU0072-F | CTGGGATCCTTCGAGGCGGACCGCCGT | CBU0072 5' BamH I |
| CBU0072-R | CTGGTGCAGTTAAACAGTGTCGGGGGCC | CBU0072 3' Sal I |
| CBU1639-F | CTGGGATCCATGATGAGTCAGTGCTCCTT | CBU1639 5' BamH I |
| CBU1639-R | CTGGTGCAGTTGAGTCTCAGCCTTCG | CBU1639 3' Sal I |
| CBU1636-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1636 5' BamH I |
| CBU1636-R | CTGGTGCAGTTAAAGACGACGGCCTT | CBU1636 3' Sal I |
| CBU2013-F | CTGGGATCCATGACCTGGAAATTAAT | CBU2013 5' BamH I |
| CBU2013-R | CTGGGATCCATGACCTGGAAATTAAT | CBU2013 3' Sal I |
| CBUK1130-F | CTGGGATCCATGACCTGGAAATTAAT | CBUK1130 5' BamH I |
| CBUK1130-R | CTGGGATCCATGACCTGGAAATTAAT | CBUK1130 3' Sal I |
| CBUA0016-F | CTGGGATCCATGACCTGGAAATTAAT | CBUA0016 5' BamH I |
| CBUA0016-R | CTGGGATCCATGACCTGGAAATTAAT | CBUA0016 3' Sal I |
| CBU0685-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0685 5' BamH I |
| CBU0685-R | CTGGGATCCATGACCTGGAAATTAAT | CBU0685 3' Sal I |
| CBU2052-F | CTGGGATCCATGACCTGGAAATTAAT | CBU2052 5' BamH I |
| CBU2052-R | CTGGGATCCATGACCTGGAAATTAAT | CBU2052 3' Sal I |
| CBU1789-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1789 5' BamH I |
| CBU1789-R | CTGGGATCCATGACCTGGAAATTAAT | CBU1789 3' Sal I |
| CBUA0020-F | CTGGGATCCATGACCTGGAAATTAAT | CBUA0020 5' BamH I |
| CBUA0020-R | CTGGGATCCATGACCTGGAAATTAAT | CBUA0020 3' Sal I |
| CBU1460-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1460 5' BamH I |
| CBU1460-R | CTGGGATCCATGACCTGGAAATTAAT | CBU1460 3' Sal I |
| CBU2028-F | CTGGGATCCATGACCTGGAAATTAAT | CBU2028 5' BamH I |
| CBU2028-R | CTGGGATCCATGACCTGGAAATTAAT | CBU2028 3' Sal I |
| CBU1566-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1566 5' BamH I |
| CBU1566-R | CTGGGATCCATGACCTGGAAATTAAT | CBU1566 3' Sal I |
| CBU0881-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0881 5' BamH I |
| CBU0881-R | CTGGGATCCATGACCTGGAAATTAAT | CBU0881 3' Sal I |
| CBU1387-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1387 5' BamH I |
| CBU1387-R | CTGGGATCCATGACCTGGAAATTAAT | CBU1387 3' Sal I |
| CBU1079-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1079 5' BamH I |
| CBU1079-R | CTGGGATCCATGACCTGGAAATTAAT | CBU1079 3' Sal I |
| CBU2016-F | CTGGGATCCATGACCTGGAAATTAAT | CBU2016 5' BamH I |
| CBU2016-R | CTGGGATCCATGACCTGGAAATTAAT | CBU2016 3' Sal I |
| CBU0447-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0447 5' BamH I |
| CBU0447-R | CTGGGATCCATGACCTGGAAATTAAT | CBU0447 3' Sal I |
| CBU0978-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0978 5' BamH I |
| CBU0978-R | CTGGGATCCATGACCTGGAAATTAAT | CBU0978 3' Sal I |
| CBU1825-F | CTGGGATCCATGACCTGGAAATTAAT | CBU1825 5' BamH I |
| CBU1825-R | CTGGGATCCATGACCTGGAAATTAAT | CBU1825 3' Sal I |
| CBU0183-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0183 5' BamH I |
| CBU0183-R | CTGGGATCCATGACCTGGAAATTAAT | CBU0183 3' Sal I |
| CBUA0014-F | CTGGGATCCATGACCTGGAAATTAAT | CBUA0014 5' BamH I |
| CBUA0014-R | CTGGGATCCATGACCTGGAAATTAAT | CBUA0014 3' Sal I |
| CBUA0034-F | CTGGGATCCATGACCTGGAAATTAAT | CBUA0034 5' BamH I |
| CBUA0034-R | CTGGGATCCATGACCTGGAAATTAAT | CBUA0034 3' Sal I |
| CBU0590-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0590 5' BamH I |
| CBU0590-R | CTGGGATCCATGACCTGGAAATTAAT | CBU0590 3' Sal I |
| CBU0393-F | CTGGGATCCATGACCTGGAAATTAAT | CBU0393 5' BamH I |
| Gene     | Primer  | Sequence                        | Digestion Site |
|----------|---------|---------------------------------|----------------|
| CBU0393-R| CTGGTCGA | CTGGTCGACTTACCAGAGGGCGGTTTT     | CBU0393 3’ Sal I |
| CBU0414-F| CTGGGATC | CTGGGATCCATGGGAGAAAAGTCAGGAGAG | CBU0414 5’ BamHI |
| CBU0414-R| CTGGTCGA | CTGGTCGACTTACGCGGAGAAAGCGG     | CBU0414 3’ Sal I |
| CBU0606-F| CTGGGATC | CTGGGATCCATGAACGCTTACTAC       | CBU0606 5’ BamHI |
| CBU0606-R| CTGGTCGA | CTGGTCGACTTACGCGGTTTTACTAC     | CBU0606 3’ Sal I |
| CBU1045-F| CTGGGATC | CTGGGATCCCTGGGCTGATTAGTGG      | CBU1045 5’ BamHI |
| CBU1045-R| CTGGTCGA | CTGGTCGACTTACGCGGTTTTACTAC     | CBU1045 3’ Sal I |
| CBU1102-F| CTGGGATC | CTGGGATCCCTGGCAGCCCTACTCTCAA   | CBU1102 5’ BamHI |
| CBU1102-R| CTGGTCGA | CTGGTCGACTTACGCGGTTTTACTAC     | CBU1102 3’ Sal I |
| CBU1108-F| CTGGGATC | CTGGGATCCCTTTAGTTATTCATTTTT    | CBU1108 5’ BamHI |
| CBU1108-R| CTGGTCGA | CTGGTCGACCTTTTGTTATATTTT       | CBU1108 3’ Sal I |
| CBU1532-F| CTGGGATC | CTGGGATCCCTTTGCGAGGCAATGCGCA   | CBU1532 5’ BamHI |
| CBU1532-R| CTGGTCGA | CTGGTCGACTTACGCGGTTTTACTAC     | CBU1532 3’ Sal I |
| CBU1599-F| CTGGGATC | CTGGGATCCCTTTGAGTGAAGAAGACCCT  | CBU1599 5’ BamHI |
| CBU1599-R| CTGGTCGA | CTGGTCGACCTTTGAGTGAAGAAGACCCT  | CBU1599 3’ Sal I |
| CBU1620-F| CTGGGATC | CTGGGATCCCTTTGAGTGAAGAAGACCCT  | CBU1620 5’ BamHI |
| CBU1620-R| CTGGTCGA | CTGGTCGACCTTTGAGTGAAGAAGACCCT  | CBU1620 3’ Sal I |
| CBU1776-F| CTGGGATC | CTGGGATCCCTTTGAGTGAAGAAGACCCT  | CBU1776 3’ Sal I |
| CBU1776-R| CTGGTCGA | CTGGTCGACCTTTGAGTGAAGAAGACCCT  | CBU1776 5’ BamHI |
| CBU1963-F| CTGGGATC | CTGGGATCCCTTTGAGTGAAGAAGACCCT  | CBU1963 5’ BamHI |
| CBU1963-R| CTGGTCGA | CTGGTCGACCTTTGAGTGAAGAAGACCCT  | CBU1963 3’ Sal I |
| CBU2064 -F| CTGGGATC | CTGGGATCCCTTTGAGTGAAGAAGACCCT  | CBU2064 5’ BamHI |
| CBU2064-R| CTGGTCGA | CTGGTCGACCTTTGAGTGAAGAAGACCCT  | CBU2064 3’ Sal I |
| CBUA0019-F| CTGGGATC | CTGGGATCCCTTTGAGTGAAGAAGACCCT  | CBUA0019 5’ BamHI |
| CBUA0019-R| CTGGTCGA | CTGGTCGACCTTTGAGTGAAGAAGACCCT  | CBUA0019 3’ Sal I |
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