Claims

1. A Cas12a protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 3 (CrmCas12a) and SEQ ID NO: 4 (SrmCas12a) or a Cas12a protein of the same clade as the Cas12a protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4; wherein the Cas12a protein comprises a maltose-binding protein (MBP) tag having an amino acid sequence of SEQ ID NO: 72.

2. The Cas12a protein of claim 1, wherein the Cas12a protein is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

3. The Cas12a protein of claim 1, wherein the MBP tag is encoded by a DNA sequence of SEQ ID NO: 71.

4. A CRISPR RNA (crRNA) for detecting Brucella, characterized in that the crRNA is selected from the group consisting of the following sequences:
S-BC1 – sequence shown in SEQ ID NO: 8;
S-BP1 – sequence shown in SEQ ID NO: 9;
S-OM1 – sequence shown in SEQ ID NO: 10;
C-BC1 – sequence shown in SEQ ID NO: 11;
C-BP1 – sequence shown in SEQ ID NO: 12;
C-OM1 – sequence shown in SEQ ID NO: 13;
S-BC2 – sequence shown in SEQ ID NO: 14;
S-BC3 – sequence shown in SEQ ID NO: 15;
S-BC4 – sequence shown in SEQ ID NO: 16;
S-BC5 – sequence shown in SEQ ID NO: 17;
S-BC6 – sequence shown in SEQ ID NO: 18;
S-BC7 – sequence shown in SEQ ID NO: 19;
S-BC8 – sequence shown in SEQ ID NO: 20;
C-BC2 – sequence shown in SEQ ID NO: 21;

C-BC3 – sequence shown in SEQ ID NO: 22;

C-BC4 – sequence shown in SEQ ID NO: 23;

C-BC5 – sequence shown in SEQ ID NO: 24;

C-BC6 – sequence shown in SEQ ID NO: 25;

C-BC7 – sequence shown in SEQ ID NO: 26;

C-BC8 – sequence shown in SEQ ID NO: 27;

S-BP2 – sequence shown in SEQ ID NO: 28;

S-BP3 – sequence shown in SEQ ID NO: 29;

S-BP5 – sequence shown in SEQ ID NO: 30;

S-BP6 – sequence shown in SEQ ID NO: 31;

S-BP7 – sequence shown in SEQ ID NO: 32;

C-BP2 – sequence shown in SEQ ID NO: 33;

C-BP3 – sequence shown in SEQ ID NO: 34;

C-BP5 – sequence shown in SEQ ID NO: 35;

C-BP6 – sequence shown in SEQ ID NO: 36;

C-BP7 – sequence shown in SEQ ID NO: 37;

S-OM2 – sequence shown in SEQ ID NO: 38;

S-OM3 – sequence shown in SEQ ID NO: 39;

S-OM4 – sequence shown in SEQ ID NO: 40;

C-OM2 – sequence shown in SEQ ID NO: 41;

C-OM3 – sequence shown in SEQ ID NO: 42;

C-OM4 – sequence shown in SEQ ID NO: 43;

S-BP8 – sequence shown in SEQ ID NO: 44;

S-BP9 – sequence shown in SEQ ID NO: 45;

C-BP8 – sequence shown in SEQ ID NO: 46; and

C-BP9 – sequence shown in SEQ ID NO: 47;
wherein the crRNA comprises a direct repeat (DR) and a guide domain (protospacer), wherein the DR and the protospacer are connected in the 5' to 3' direction of the crRNA, and the crRNA is configured to target a nucleic acid of Brucella with a predetermined target sequence and form a complex with the Cas12a protein of any one of claims 1-3.

5. The crRNA of claim 4, wherein the protospacer has 20-26 nucleotides; and

optionally wherein the nucleic acid of Brucella comprises a sequence selected from the group consisting of SEQ ID NO: 5 (BSCP31), SEQ ID NO: 6 (bp26), and SEQ ID NO: 7 (Omp2a).

6. A detection kit for Brucella, wherein the detection kit comprises the crRNA of claim 4 or 5.

7. The detection kit of claim 6, further comprising the Cas12a protein of any one of claims 1-3.

8. The detection kit of claim 7, further comprising a detecting agent comprising a probe nucleic acid, wherein the detecting agent generates a detectable signal when the probe nucleic acid is cleaved,

optionally wherein the probe nucleic acid is a fluorescent probe, wherein the 5' end of the fluorescent probe sequence is labeled with a fluorescent group, and the 3' end is labeled with a quenching group; or a colorimetric probe,

optionally wherein the fluorescent group is selected from the group consisting of FAM, VIC, HEX, TRT, Cy3, Cy5, ROX, JOE and Texas Red, and the quenching group is selected from the group consisting of IABKFQ, DABCYL, MGB, BHQ-1, BHQ-2 and BHQ-3;

optionally wherein the colorimetric probe comprises FAM and Biotin, and is for detection by a lateral flow strip; and

optionally wherein the Cas12a protein and the crRNA are in the form of a ribonucleoprotein complex.

9. A method of detecting a nucleic acid of Brucella in a sample using the detection kit of any one of claims 6-8, wherein the method comprises the following steps:

incubating the sample containing the nucleic acid of Brucella with the Cas12a protein and the crRNA, wherein when the nucleic acid of Brucella is present in the sample, collateral cleavage activity of the Cas12a protein is induced to generate a detectable signal when the probe nucleic acid is cleaved.

10. The method of claim 9, further comprising amplifying the nucleic acid of Brucella in the sample, using a technology selected from the group consisting of Polymerase Chain Reaction (PCR), Recombinase Polymerase Amplification (RPA), Loop-Mediated Isothermal Amplification (LAMP), and Rolling Circle Amplification (RCA);

or optionally wherein the method does not comprise amplifying the nucleic acid of Brucella in the sample.

11. The method of claim 9, further comprising using a detection method selected from the group consisting of a colorimetric detection method, a fluorescent detection method and an electrochemical detection method to detect the detectable signal which is a colorimetric signal, a fluorescent signal, or an electrochemical signal,

optionally wherein the colorimetric detection method uses a lateral flow strip and a colorimetric probe comprising FAM and Biotin;

optionally wherein the fluorescent detection method uses a fluorescent probe, wherein the fluorescence signal is measured using a fluorometer, a microplate reader, or Real-Time PCR;

optionally wherein the electrochemical detection method transduces CRISPR-Cas cleavage event to an electrochemical signal using a modified electrode or a nanoparticle-based sensor;

optionally wherein one or more of crRNAs of SEQ ID NO: 8-47 are used to detect Brucella;

optionally wherein both the Cas12a protein having an amino acid sequence of SEQ ID NOs: 3 (CrmCas12a) and the Cas12a protein having an amino acid sequence of SEQ ID NO: 4 (SrmCas12a) are used to detect Brucella;

optionally wherein the nucleic acid of Brucella comprises a sequence selected from the group consisting of SEQ ID NO: 5 (BSCP31), SEQ ID NO: 6 (bp26), and SEQ ID NO: 7 (Omp2a), and

optionally wherein the nucleic acid of Brucella is DNA, and the DNA is 10 copies/μL or less.

12. The method of any one of claims 9-11, wherein the Brucella is selected from the group consisting of *Brucella abortus, Brucella melitensis, Brucella suis, Brucella canis, Brucella ovis, Brucella neotomae, Brucella microti, Brucella ceti, Brucella pinnipedialis* or a combination thereof;

optionally wherein the sample is a biological sample obtained from a tissue sample, saliva, blood, plasma, milk, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, nasal swab specimen, or swab of skin or a mucosal membrane surface or a combination thereof, and

optionally wherein the biological sample is a crude sample and/or wherein the target molecule is not purified or amplified from the sample.

13. A CRISPR-Cas12a composition comprising the Cas12a protein of any one of claims 1-3, and a CRISPR RNA (crRNA), wherein the crRNA comprises a direct repeat (DR) and a guide domain (protospacer), wherein the DR and the guide domain are connected in the 5' to 3' direction of the crRNA, and the crRNA is configured to target a nucleic acid of a predetermined target sequence and form a complex with the Cas12a protein of any one of claims 1-3.

14. The CRISPR-Cas12a composition of claim 13, wherein the crRNA is the crRNA for detecting Brucella of claim 4 or 5;

optionally wherein the CRISPR-Cas12a composition further comprises a detecting agent comprising a probe nucleic acid, wherein the detecting agent generates a detectable signal when the probe nucleic acid is cleaved,

optionally wherein the probe nucleic acid is a fluorescent probe, wherein the 5' end of the fluorescent probe sequence is labelled with a fluorescent group, and the 3' end is labelled with a quenching group; or a colorimetric probe,

optionally wherein the fluorescent group is selected from the group consisting of FAM, VIC, HEX, TRT, Cy3, Cy5, ROX, JOE and Texas Red, and the quenching group is selected from the group consisting of IABKFQ, DABCYL, MGB, BHQ-1, BHQ-2 and BHQ-3;

optionally wherein the colorimetric probe comprises FAM and Biotin, and is for detection by a lateral flow strip; and

optionally wherein the Cas12a protein and the crRNA are in the form of a ribonucleoprotein complex.

15. A method of detecting a nucleic acid having a predetermined target sequence in a sample using the CRISPR-Cas12a composition of claim 13 or 14, comprising: incubating the sample with the CRISPR-Cas12a composition, wherein when the nucleic acid having the predetermined target sequence is present in the sample, the collateral cleavage activity of the CRISPR-Cas12a composition is induced to generate a detectable signal when the probe nucleic acid is cleaved.

16. The method of claim 15, further comprising amplifying the nucleic acid having a predetermined target sequence in the sample, using a technology selected from the group consisting of Polymerase Chain Reaction (PCR), Recombinase Polymerase Amplification (RPA), Loop-Mediated Isothermal Amplification (LAMP), and Rolling Circle Amplification (RCA); or

optionally wherein the method does not comprise amplifying the nucleic acid having a predetermined target sequence in the sample.

17. The method of claim 15, further comprising using a detection method selected from the group consisting of a colorimetric detection method, a fluorescent detection method and an electrochemical detection method to detect the detectable signal which is a colorimetric signal, a fluorescent signal, or an electrochemical signal,

optionally wherein the colorimetric detection method uses a lateral flow strip and a colorimetric probe comprising FAM and Biotin;

optionally wherein the fluorescent detection method uses a fluorescent probe, wherein the fluorescence signal is measured using a fluorometer, a microplate reader, or Real-Time PCR; and

optionally wherein the electrochemical detection method transduces CRISPR-Cas cleavage event to an electrochemical signal using a modified electrode or a nanoparticle-based sensor.

18. The method of any one of claims 15-17, wherein one or more crRNAs are used to detect the nucleic acid having a predetermined target sequence;

optionally wherein both the Cas12a protein having an amino acid sequence of SEQ ID NOs: 3 (CrmCas12a) and the Cas12a protein having an amino acid sequence of SEQ ID NO: 4 (SrmCas12a) are used to detect the nucleic acid having a predetermined target sequence,

optionally wherein the nucleic acid is DNA, and

optionally wherein the DNA is 10 copies/µL or less.

19. The method of claim 15, wherein the sample is a biological sample obtained from a tissue sample, saliva, blood, plasma, milk, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, nasal swab specimen, or swab of skin or a mucosal membrane surface or a combination thereof,

optionally wherein the biological sample is a crude sample and/or wherein the target molecule is not purified or amplified from the sample;

optionally wherein nucleic acid in the sample belongs to a microbe selected from a bacterium, a virus, an archaea, a protozoa, a fungi, an algae, a slime mode, a lichen, and a prion,

optionally wherein the virus is selected from a group consisting of a double-stranded DNA virus, a single-stranded DNA virus, a double-stranded RNA virus, a positive sense RNA virus, a negative sense RNA virus, and a retrovirus,

optionally wherein the virus is selected from a group consisting of a coronavirus, an Ebola virus, measles, SARS, Chikungunya virus, Marburg, MERS, Dengue, Lassa, influenza, rhabdovirus, HIV, a hepatitis virus (including hepatitis A, B, C, D, or E), an influenza virus (including an influenza A or influenza B), a human respiratory syncytial virus, Sudan ebola virus, Bundibugyo virus, Tai Forest ebola virus, Reston ebola virus, Achimota virus, Aedes flavivirus, Aguacate virus, Akabane virus, Alethinophid reptarenavirus, Allpahuayo mammarenavirus, Amapari mmarenavirus, Andes virus, Apoi virus, Aravan virus, Aroa virus, Arumwot virus, Atlantic salmon paramyxovirus, Australian bat lyssavirus, Avian bomavirus, Avian metapneumovirus, Avian paramyxoviruses, penguin or Falkland Islandsvirus, BK polyomavirus, Bagaza virus, Banna virus, Bat herpesvirus, Bat sapovirus, Bear Canon mammarenavirus, Beilong virus, Betacoronavirus, Betapapillomavirus 1-6, Bhanja virus, Bokeloh bat lyssavirus, Boma disease virus, Bourbon virus, Bovine hepacivirus, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Brazoran virus, Bunyamwera virus, Caliciviridae virus. California encephalitis virus, Candiru virus, Canine distemper virus, Canine pneumovirus, Cedar virus, Cell fusing agent virus, Cetacean morbillivirus, Chandipura virus, Chaoyang virus, Chapare mammarenavirus, Colobus monkey papillomavirus, Colorado tick fever virus, Cowpox virus, Crimean-Congo hemorrhagic fever virus, Culex flavivirus, Cupixi mammarenavirus, Dengue virus, Dobrava-Belgrade virus, Donggang virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Entebbe bat virus, Enterovirus A-D, European bat lyssavirus 1-2, Eyach virus, Feline morbillivirus, Fer-de- Lance paramyxovirus, Fitzroy River virus, Flaviviridae virus, Flexal mammarenavirus, GB virus C, Gairo virus, Gemycircularvirus, Goose paramyxovirus SF02, Great Island virus, Guanarito mammarenavirus, Hantaan virus, Hantavirus

Z10, Heartland virus, Hendra virus, Hepatitis A/B/C/E, Hepatitis delta virus, Human bocavirus, Human coronavirus, Human endogenous retrovirus K, Human enteric coronavirus, Human genital-associated circular DNA virus- 1, Human herpesvirus 1-8, Human mastadenovirus A-G, Human papillomavirus, Human parainfluenza virus 1-4, Human paraechovirus, Human picomavirus, Human smacovirus, Ikoma lyssavirus, Ilheus virus, Influenza A-C, Ippy mammarenavirus, Irkut virus, J-virus, JC polyomavirus, Japanese encephalitis virus, Junin mammarenavirus, KI polyomavirus, Kadipiro virus, Kamiti River virus, Kedougou virus, Khujand virus, Kokobera virus, Kyasanur forest disease virus, Lagos bat virus, Langat virus, Lassa mammarenavirus, Latino mammarenavirus, Leopards Hill virus, Liao ning virus, Ljungan virus, Lloviu virus, Louping ill virus, Lujo mammarenavirus, Luna mammarenavirus, Lunk virus, Lymphocytic choriomeningitis mammarenavirus, Lyssavirus Ozemoe, MSSI2Y225 virus, Machupo mammarenavirus, Mamastrovirus 1, Manzanilla virus, Mapuera virus, Marburg virus, Mayaro virus, Measles virus, Menangle virus, Mercadeo virus, Merkel cell polyomavirus, Middle East respiratory syndrome coronavirus, Mobala mammarenavirus, Modoc virus, Moijang virus, Mokolo virus, Monkeypox virus, Montana myotis leukoenchalitis virus, Mopeia lassa virus reassortant 29, Mopeia mammarenavirus, Morogoro virus, Mossman virus, Mumps virus, Murine pneumonia virus, Murray Valley encephalitis virus, Nariva virus, Newcastle disease virus, Nipah virus, Norwalk virus, Norway rat hepacivirus, Ntaya virus, O'nyong-nyong virus, Oliveros mammarenavirus, Omsk hemorrhagic fever virus, Oropouche virus, Parainfluenza virus 5, Parana mammarenavirus, Parramatta River virus, Peste-des-petits- ruminants virus, Pichande mammarenavirus, Picomaviridae virus, Pirital mammarenavirus, Piscihepevirus A, Porcine parainfluenza virus 1, porcine rubulavirus, Powassan virus, Primate T-lymphotropic virus 1-2, Primate erythroparvovirus 1, Punta Toro virus, Puumala virus, Quang Binh virus, Rabies virus, Razdan virus, Reptile bomavirus 1, Rhinovirus A-B, Rift Valley fever virus, Rinderpest virus, Rio Bravo virus, Rodent Torque Teno virus, Rodent hepacivirus, Ross River virus, Rotavirus A-I, Royal Farm virus, Rubella virus, Sabia mammarenavirus, Salem virus, Sandfly fever Naples virus, Sandfly fever Sicilian virus, Sapporo virus, Sathuperi virus, Seal anellovirus, Semliki Forest virus, Sendai virus, Seoul virus, Sepik virus, Severe acute respiratory syndrome-related

coronavirus, Severe fever with thrombocytopenia syndrome virus, Shamonda virus, Shimoni bat virus, Shuni virus, Simbu virus, Simian torque teno virus, Simian virus 40-41, Sin Nombre virus, Sindbis virus, Small anellovirus, Sosuga virus, Spanish goat encephalitis virus, Spondweni virus, St. Louis encephalitis virus, Sunshine virus, TTV-like mini virus, Tacaribe mammarenavirus, Taila virus, Tamana bat virus, Tamiami mammarenavirus, Tembusu virus, Thogoto virus, Thottapalayam virus, Tick-borne encephalitis virus, Tioman virus, Togaviridae virus, Torque teno canis virus, Torque teno douroucouli virus, Torque teno felis virus, Torque teno midi virus, Torque teno sus virus, Torque teno tamarin virus, Torque teno virus, Torque teno zalophus virus, Tuhoko virus, Tula virus, Tupaia paramyxovirus, Usutu virus, Uukuniemi virus, Vaccinia virus, Variola virus, Venezuelan Vesicular stomatitis Indiana virus, WU Polyomavirus, Wesselsbron virus, West Caucasian bat virus, West Nile virus, Western equine encephalitis virus, Whitewater Arroyo mammarenavirus, Yellow fever virus, Yokose virus, Yug Bogdanovac virus, Zaire ebolavirus, Zika virus, or Zygosaccharomyces bailii virus Z viral sequence, Canine Parvovirus, Canine Distemper Virus, Canine Coronavirus, Canine Influenza Virus, Feline Herpesvirus, Feline Calicivirus, Feline Leukemia Virus, Feline Immunodeficiency Virus, Canine Adenovirus - Type 1 and Type 2, Canine Herpesvirus, Tobacco Mosaic Virus, Potato Virus Y, Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Potato Virus X, Barley Yellow Dwarf Virus, Bean Common Mosaic Virus, Maize Dwarf Mosaic Virus, Soybean Mosaic Virus, Citrus Tristeza Virus, Rice Yellow Mottle Virus, Plum Pox Virus, Turnip Mosaic Virus, Aphid-Transmitted Yellow Dwarf Viruses, Cotton Leaf Curl Virus, Papaya Ringspot Virus, Cassava Mosaic Virus, Rice Tungro Virus, Apple Mosaic Virus, Sugarcane Mosaic Virus or a combination thereof; and

optionally wherein the bacterium is a Gram-positive bacterium or a Gram-negative bacterium selected from the group consisting of *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis, Enterococcus faecium, Bacillus anthracis, Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Clostridium difficile and Clostridium tetani, Corynebacterium diphtheria, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Chlamydia trachomatis,*

Yersinia pestis, Neisseria gonorrhoeae, Neisseria meningitidis, Moraxella catarrhalis, Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens, Helicobacter pylori, Salmonella enteritidis, Salmonella typhi, and Vibrio cholera, Brucella abortus, Brucella melitensis, Brucella suis, Brucella canis, Brucella ovis, Brucella neotomae, Mycoplasma mycoides, Clostridium perfringens, Mycobacterium avium Salmonella spp., Pasteurella multocida, Haemophilus parasuis, Actinobacillus pleuropneumoniae, Yersinia pestis, Francisella tularensis, Bacillus anthracis, Coxiella burnetiid, Listeria monocytogenes, Clostridium chauvoei, Campylobacter spp., Chlamydia psittaci, Anaplasma marginale, Leptospira spp. Causes leptospirosis, Bordetella bronchiseptica, Clostridium perfringens, Staphylococcus intermedius, Bartonella henselae, Chlamydophila felis, Mycoplasma spp, Pasteurella multocida, Erwinia amylovora, Xanthomonas spp., Xanthomonas axonopodis, Ralstonia solanacearum, Pectobacterium and Dickeya spp., Agrobacterium tumefaciens, Pseudomonas syringae, Pseudomonas syringae pv. lachrymans, Xanthomonas campestris pv. campestris, Xanthomonas oryzae, Clavibacter michiganensis, Xylella fastidiosa, Pantoea spp., Burkholderia glumae, Xanthomonas citri, Xanthomonas translucens, Xanthomonas fragariae, Xanthomonas hortorum, Clavibacter michiganensis, Pseudomonas syringae or a combination thereof.

20. The method of claim 15, wherein the nucleic acid is a RNA or a DNA from a virus of claim 19, or a RNA or a DNA from a bacteria of claim 19; wherein the RNA is reverse-transcribed to complementary DNA (cDNA), and then isothermally amplified using methods such as Recombinase Polymerase Amplification (RPA) or Loop-Mediated Isothermal Amplification (LAMP), before subjected to the method of claim 15;

optionally wherein the method is used for identifying and characterising genetic variations selected from the group consisting of single nucleotide polymorphism (SNP) analysis and allelic discrimination.