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(54) Title: METHODS FOR DIAGNOSIS OF *PSEUDOPERONOSPORA CUBENSIS* INFECTION AND SELECTION OF PLANT RESISTANCE GENES TO THE SAME

(57) Abstract: The invention is directed to methods of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising detecting at least one nucleotide sequence selected from the group of nucleotide sequences of SEQ ID NOs:1-52, or any combination thereof, in a sample from a cucurbit plant or in an environmental sample, thereby diagnosing a *P. cubensis* infection. Also provided is a method of selecting a cucurbit plant comprising at least one resistance gene, the method comprising introducing into the cucurbit plant a nucleotide sequence of any one of SEQ ID NOs:1-52, wherein the expression of the nucleotide sequence produces a hypersensitive response in a cucurbit plant comprising a resistance gene, thereby identifying the cucurbit plant as comprising a resistance gene; and selecting the plant having the hypersensitive response and identified as comprising said resistance gene.



METHODS FOR DIAGNOSIS OF *PSEUDOPERONOSPORA CUBENSIS* INFECTION AND SELECTION OF PLANT RESISTANCE GENES TO THE SAME

STATEMENT OF FEDERAL SUPPORT

5 This invention was made with government support under grant number 13-8130-0254-CA and grant number 12-25-B-1688 awarded by USDA/APHIS. The government has certain rights to this invention.

RELATED APPLICATION

10 This application claims the benefit of United States Provisional Patent Application Serial No. 62/384,817, filed September 8, 2016, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

15 The invention relates to methods for diagnosis and treatment of *Pseudoperonospora cubensis* (*P. cubensis*) infection, and methods for distinguishing between different types of *P. cubensis*, as well as methods for selecting genes conferring resistance to *P. cubensis*.

BACKGROUND OF THE INVENTION

20 In the past decade, a number of plant pathogens have emerged in the United States causing significant damage in agricultural and natural ecosystems and threatening food security (Fisher et al. 2012). Several recent epidemics have been caused by oomycete pathogens such as the hemibiotroph *Phytophthora infestans* (Fry et al. 2015), the causal agent of late blight of potato and tomato, and obligate plant pathogens within the family Peronosporaceae that cause downy
25 mildew (Cohen et al. 2015; Gascuel et al. 2015; Holmes et al. 2015). Downy mildew pathogens include diverse genera such as *Pseudoperonospora* spp. (Gent and Ocamb 2009; Holmes et al. 2015), *Plasmopara* spp. (Gascuel et al. 2015), *Peronospora* spp. (Roberts et al. 2009; Testen et al. 2013), and *Bremia* spp. (Michelmore and Wong 2008) that infect economically important crops and are usually host-specific due to their biotrophic nature. Diagnostic tools for oomycete pathogens
30 range from visual inspection to culturing, serology, and PCR-based tests (Martin et al. 2012). Due to their inability to grow in media and, consequentially, a lack of genomic resources for marker development, downy mildew pathogen diagnostics has mainly been performed by visual inspection of pathogen structures on infected plants (Holmes et al. 2015). However, signs of the pathogen and symptoms of the disease are usually detected when the disease is at advanced stages (>30%
35 severity, **Fig. 1**) and control strategies, such as fungicide applications, are less effective (Gent et al. 2015; Homa et al. 2014; Ojiambo et al. 2010). In addition, while the main dispersal mechanism of downy mildew pathogens is through airborne sporangia that can travel several miles on wind currents, several downy mildew pathogens are known to be seedborne (Cohen et al. 2014; Gascuel et al. 2015; Testen et al. 2013).

Increasing diagnostic and airborne inoculum monitoring capabilities for downy mildew pathogens could aid in restricting movement of infected plant material (Djalali Farahani-Kofoet et al. 2012) and improving forecasting and alert systems that predict inoculum dispersal (Ojiambo et al. 2015). Recently, PCR assays have been developed for detection of the downy mildew pathogens of cucurbits (*Pseudoperonospora cubensis*) (Summers et al. 2015), hop (*Pseudoperonospora humuli*) (Gent et al. 2009), basil (*Peronospora belbahrii*) (Djalali Farahani-Kofoet et al. 2012), spinach (*Peronospora effusa*), and beet (*Peronospora schachtii*) (Klosterman et al. 2014) on seed or from air samples to develop alert systems based on primary inoculum levels. PCR primers and markers in these studies were developed from conserved regions such as the internal transcribed spacer (ITS) or mitochondrial genes, resulting in nonspecific amplification of closely related species (Gent et al. 2009; Klosterman et al. 2014). Such assays have limitations for monitoring inoculum in environmental samples since different downy mildew species may be present at the time of sampling due to production of a variety of susceptible crops in the same region (Gent et al. 2009; Klosterman et al. 2014). Disease alert systems based on airborne inoculum require species-specific diagnostic assays for timely initiation of disease control measures for a particular crop that is susceptible to its corresponding downy mildew pathogen (Gent et al. 2009; Klosterman et al. 2014).

Thus, the present invention overcomes previous shortcomings in the art by providing species-specific diagnostic tools for *P. cubensis* as well as tools for identifying genes useful for producing plants having resistance to *P. cubensis*.

SUMMARY OF THE INVENTION

A first aspect of the invention provides a method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising detecting at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, in a sample from the cucurbit plant, thereby diagnosing a *P. cubensis* infection.

A second aspect provides a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, thereby diagnosing an infection by the type of *P. cubensis* that infects cucumber and cantaloupe.

A third aspect provides a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of

SEQ ID NO:16, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash, thereby diagnosing an infection by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

A fourth aspect provides a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, thereby diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis* that infects cucumber and cantaloupe.

A fifth aspect provides a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of the amplification product identifies the *P. cubensis* as the type that infects pumpkin, watermelon, and squash,, thereby diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, loupe.

A sixth aspect provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: detecting at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, in a sample from a plant or in an environmental sample, wherein the presence of the at least one polynucleotide indicates the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak.

A seventh aspect of the invention provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment

thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and detecting in the sample whether the nucleotide sequence of **SEQ ID NO:16** and/or the fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, indicates the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe.

An eighth aspect provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and detecting in the sample whether the nucleotide sequence of **SEQ ID NO:52** and/or the fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

A ninth aspect provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe.

A tenth aspect provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting in the sample whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product indicates

the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

An additional aspect provides a method of selecting a treatment regimen for a *P. cubensis* infection, comprising: detecting in a sample from the plant at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof, thereby identifying a *P. cubensis* infection; and selecting a treatment regimen for the *P. cubensis* infection.

A further aspect provides a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe; and selecting a treatment regimen effective against the type of *P. cubensis* that infects cucumber and cantaloupe.

A still further aspect provides a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and selecting a treatment regimen effective against the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

An additional aspect provides a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe; and selecting a treatment regimen effective against the type of *P. cubensis* that infects cucumber and cantaloupe.

In a further aspect a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting in the sample whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects pumpkin, watermelon, and squash; and selecting a treatment regimen effective against the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In a still further aspect, the invention provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: detecting in a sample from a cucurbit plant or in an environmental sample at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52** and/or a fragment thereof, thereby identifying the presence of *P. cubensis* in the sample; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

An additional aspect provides a method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe; and implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe, thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe.

A further aspect provides a method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and implementing a treatment regimen against the type of *P. cubensis* that

infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects pumpkin, watermelon, and squash.

Another aspect of the invention provides a method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe; and implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe.

In another aspect, a method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects pumpkin, watermelon, and squash; and implementing a treatment regimen against the type of *P. cubensis* that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects pumpkin, watermelon, and squash.

In an additional aspect, a method of selecting a cucurbit plant comprising at least one gene that confers resistance to *P. cubensis* is provided, comprising introducing into the cucurbit plant at least one nucleotide sequence selected from the group of (a) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; (b) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of (a); (c) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any of (a) and/or (b), or a complement thereof; and (d) a nucleotide sequence that differs from the nucleotide sequence of any of (a) to (c) above due to the degeneracy of the genetic code, wherein the expression of the at least one nucleotide sequence produces a hypersensitive response in a cucurbit plant comprising a gene that confers resistance to *P. cubensis*, thereby identifying the cucurbit plant as comprising a gene that confers resistance to *P. cubensis*; and

selecting the plant having the hypersensitive response and identified as comprising the gene that confers resistance to *P. cubensis*.

Further provided are methods of producing a plant having increased resistance to *P. cubensis*, comprising: (a) selecting a cucurbit plant according to the methods of the invention; (b) crossing the selected plant with a second cucurbit plant; and (c) selecting progeny having increased resistance to *P. cubensis*, thereby producing a plant having increased resistance to *P. cubensis*.

In a further aspect, the invention provides an isolated nucleic acid molecule comprising: ((a) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; (b) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of (a); (c) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any of (a) and/or (b), or a complement thereof; (d) a nucleotide sequence that differs from the nucleotide sequence of any of (a) to (c) above due to the degeneracy of the genetic code; and (e) a fragment of a nucleotide sequence of any of (a) to (d) above.

In an additional aspect of the invention, a recombinant nucleic acid construct is provided, the recombinant nucleic acid construct comprising at least one an isolated nucleic acid molecule of the invention.

In a further aspect, an isolated polypeptide is provided comprising: (a) an amino acid sequence encoded by a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; and (b) an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of (a); and a fragment of any of (a) and/or (b). Additionally provided is a polyclonal or monoclonal antibody specifically reactive with the isolated polypeptides of the invention.

A further aspect of the invention provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or from an environmental sample with an oligonucleotide that hybridizes to an isolated nucleic acid molecule and/or an antibody of the invention; and detecting whether the isolated nucleic acid molecule, or fragment thereof, is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, or fragment thereof, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule, or fragment thereof, is present by detecting binding between the antibody and the polypeptide, wherein the presence of the isolated nucleic acid molecule and/or the polypeptide indicates the presence of *P. cubensis* in the sample and the risk of a *P. cubensis* outbreak.

In another aspect, the invention provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with a pair oligonucleotides that hybridize to an isolated nucleic acid molecule of the invention, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak.

A further aspect provides a method of selecting a treatment regimen for a *P. cubensis* infection of a cucurbit plant, comprising: contacting a sample from the cucurbit plant with an

oligonucleotide that hybridizes to the isolated nucleic acid molecule to an isolated nucleic acid molecule and/or an antibody of the invention; detecting whether the isolated nucleic acid molecule and/or a fragment thereof is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule and/or a fragment thereof is present by detecting binding between the antibody and the polypeptide, thereby identifying a *P. cubensis* infection when the presence of the isolated nucleic acid molecule and/or polypeptide in the sample is detected; and selecting a treatment regimen for the *P. cubensis* infection.

Additionally provided is a method of selecting a treatment regimen for a *P. cubensis* infection of a cucurbit plant, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the isolated nucleic acid molecule of the invention, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the presence of *P. cubensis* in the sample; and selecting a treatment regimen for the *P. cubensis* infection.

A further aspect of the invention provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or from an environmental sample with an oligonucleotide that hybridizes to an isolated nucleic acid molecule and/or an antibody of the invention; detecting whether the isolated nucleic acid molecule, and/or a fragment thereof, is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule, and/or a fragment thereof, is present by detecting binding between the antibody and the polypeptide, wherein the presence of the isolated nucleic acid molecule and/or the polypeptide identifies the presence of *P. cubensis*; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

A further aspect of the invention provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with a pair of oligonucleotides that hybridize to an isolated nucleic acid molecule of the invention, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis* in the sample; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

In a still further aspect, a method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a cucurbit plant or from an environmental sample with a first oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16** and/or a fragment thereof, but not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and/or a second oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but not to the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by

detecting binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof; and identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash in the sample.

Further provided is method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a cucurbit plant or from an environmental sample with a first antibody that hybridizes to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and not to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, or fragment thereof, and/or with a second antibody that hybridizes to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and not to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, or fragment thereof; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof is present by detecting binding between the second antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof; and identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the first antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the second antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In a still further aspect, a method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and/or whether the nucleotide sequence of

SEQ ID NO:52, and/or fragment thereof, is present by detecting binding between the amplification product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and not to the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof; and identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the amplification product and the first oligonucleotide probe, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the amplification product and the second oligonucleotide probe, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A-1H shows downy mildew symptoms in different cucurbits caused by *Pseudoperonospora cubensis*. **Fig. 1A**: early infection (<30% severity) on a cucumber leaf; **Fig. 1B**: advanced infection with characteristic angular lesions delimited by leaf veins; and **Fig. 1C**: profuse pathogen sporulation on the underside of the leaf. **Fig. 1D**: early infection on a cantaloupe leaf; **Fig. 1E**: advanced infection with irregular, necrotic lesions; and **Fig. 1F**: little pathogen sporulation on the underside of the leaf. **Fig. 1G**: infected watermelon leaf with rounded, necrotic lesions; and **Fig. 1H**: little pathogen sporulation on the underside of the leaf.

Fig. 2 provides a flow diagram outlining an approach of the present inventors for identifying candidate diagnostic markers for *Pseudoperonospora cubensis*.

Fig. 3 provides a Venn diagram describing results from HTSeq analysis. The number of expressed and not-expressed *Pseudoperonospora cubensis* exons in *P. cubensis* and *Pseudoperonospora humuli* isolates sequenced are shown in the Venn diagram.

Fig. 4 shows the use of next generation sequencing to identify species-specific regions in *Pseudoperonospora cubensis* utilizing comparative genomics with the closely related species *P. humuli*. Seven species-specific regions (c52.12e13, c572.6e19, c2183.6e2, c2555.2e1, c2555.3e7, c3155.4e9, c10851.1e0) are shown in the figure as columns with the gene functional annotation above each column and the candidate diagnostic code below each column. The *P. cubensis* genome panel shows predicted genes in the *P. cubensis* genome that are absent or have missing exons in the *P. humuli* genome. RNA-seq was performed in a diverse panel of *P. cubensis* (CA08A1, SC1982, NC2013C3, SC2013F2, NCA11, NCWAY2-1, SCD3) and *P. humuli* (OR501BA, NY482CA, VT503A3, JP490-5, JP498SA, WI510-1, OR502AA) isolates to detect transcripts always present in *P. cubensis* but not present in *P. humuli* by mapping reads to the *P. cubensis* genome using HTSeq. To confirm the absence of the exon or gene at the DNA level in *P. humuli* and develop a PCR-based assay, DNA-seq was performed on *P. humuli* (OR502AA). Primers were designed flanking each gene to detect a size difference in PCR products between *P. cubensis* and *P. humuli* due to the absence of exons or genes in *P. humuli*. This figure was generated using the CLC Genomics Workbench (CLCbio, Boston, MA).

Fig. 5 provides gel electrophoresis images showing seven *P. cubensis* diagnostic candidate PCR products (c52.12e13, c572.6e19, c2183.6e2, c2555.2e1, c2555.3e7, c3155.4e9, c10851.1e0) for diverse cucurbit downy mildew (C37, C12, C13, C35, C29, C21, C18, C38) and hop downy mildew samples (H3, H4, H12, H10, H24, H26, H31, H32). See **Table 2** for sample information and **Table 5** for primer information. Diagnostic candidate codes corresponding to each gel are indicated on the left hand side of each gel image.

Figs. 6A-6B provide the percentage of uniquely and multiple mapped reads when aligning to *Pseudoperonospora cubensis* and *Cucumis sativus* genomes using Bowtie2. **Fig. 6A** shows uniquely (dark grey) and multiple (light grey) mapped RNAseq reads to the *P. cubensis* genome.

Fig. 6B shows uniquely (dark grey) and multiple (light grey) mapped RNAseq reads to the *C. sativus* genome.

DETAILED DESCRIPTION

The present invention now will be described hereinafter with reference to the accompanying drawings and examples, in which embodiments of the invention are shown. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. Thus, the invention contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

The term "about," as used herein when referring to a measurable value such as a dosage or time period and the like refers to variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y" and phrases such as "from about X to Y" mean "from about X to about Y."

The term "comprise," "comprises" and "comprising" as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising."

As used herein, the terms "increase," "increasing," "increased," "enhance," "enhanced," "enhancing," and "enhancement" (and grammatical variations thereof) describe an elevation of at least about 5%, 10%, 15%, 20%, 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400%, 500% or more as compared to a control.

As used herein, the terms "reduce," "reduced," "reducing," "reduction," "diminish," and "decrease" (and grammatical variations thereof), describe, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% as compared to a control. In particular embodiments, the reduction can result in no or essentially no (*i.e.*, an insignificant amount, *e.g.*, less than about 10% or even 5%) detectable activity or amount.

As used herein, a "sample from a plant" and a "sample from a cucurbit plant" may be taken from any plant, plant part or portion thereof, including but not limited to a leaf, seed, flower, fruit, stem, and the like.

The term "cucurbit plant" as used herein, refers to any plant in the *Cucurbitaceae* family, including any wild genus or species, breeding line, and/or commercial line. Non-limiting examples of a cucurbit genus useful with this invention includes *Cucumis* spp., *Cucurbita* spp., and/or *Citrullus* spp. Exemplary cucurbit plants useful with this invention include, but are not limited to, cucumber, watermelon, squash, pumpkin and/or cantaloupe.

As used herein, an "environmental sample" includes but is not limited to a sample taken from the air (e.g., an air sampler, a spore trap, and any other method for collecting one or more spores for testing).

As used herein, the terms "express," "expresses," "expressed" or "expression," and the like, with respect to a nucleotide sequence (e.g., RNA or DNA) indicates that the nucleotide sequence is transcribed and, optionally, translated. Thus, a nucleotide sequence may express a polypeptide of interest or a functional untranslated RNA. A "functional" RNA includes any untranslated RNA that has a biological function in a cell, e.g., regulation of gene expression. Such functional RNAs include but are not limited to RNAi (e.g., siRNA, shRNA, antisense RNA), miRNA, ribozymes, RNA aptamers, and the like.

As used herein, "nucleic acid," "nucleotide sequence," and "polynucleotide" are used interchangeably and encompass both RNA and DNA, including cDNA, genomic DNA, mRNA, synthetic (e.g., chemically synthesized) DNA or RNA and chimeras of RNA and DNA. The term polynucleotide, nucleotide sequence, or nucleic acid refers to a chain of nucleotides without regard to length of the chain. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be a sense strand or an antisense strand. The nucleic acid can be synthesized using oligonucleotide analogs or derivatives (e.g., inosine or phosphorothioate nucleotides). Such oligonucleotides can be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases. The present invention further provides a nucleic acid that is the complement (which can be either a full complement or a partial complement) of a nucleic acid, nucleotide sequence, or polynucleotide of this invention.

As used herein, the term "gene" refers to a nucleic acid molecule capable of being used to produce mRNA, antisense RNA, miRNA, anti-microRNA antisense oligodeoxyribonucleotide (AMO) and the like. Genes may or may not be capable of being used to produce a functional protein or gene product. Genes can include both coding and non-coding regions (e.g., introns, regulatory elements, promoters, enhancers, termination sequences and/or 5' and 3' untranslated regions). A gene may be "isolated" by which is meant a nucleic acid that is substantially or essentially free from components normally found in association with the nucleic acid in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," in which only some of the nucleotides bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. Thus, a nucleic acid (e.g., polynucleotide, oligonucleotide, and the like) of the invention can be about 70% to about 100% complementary to a target nucleic acid (e.g., a polynucleotide having the nucleotide sequence of any of SEQ ID NOs:1-52; e.g., about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87,

88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein) and therefore hybridizes to that target nucleic acid. In particular embodiments, an oligonucleotide of the invention can be about 80 to about 100%, about 85% to about 100%, about 90% to about 100%, about 95% to about 100%, and the like, complementary to a target nucleic acid.

5 The term "oligonucleotide" refers to a nucleic acid sequence of at least about five nucleotides to about 500 nucleotides, for example, about 10 to 20, about 15 to 30, about 20 to 25, about 20 to 40, about 20 to 50, about 25 to 100, about 50 to 100, about 25 to 150, about 50 to 150, about 50 to 200, about 50 to 250, and the like nucleotides, which can be used, for example, as a primer in a PCR amplification and/or as a probe in a hybridization assay or in a microarray. In particular
10 embodiments, the oligonucleotides of the invention can be at least about five to about 500 consecutive nucleotides of a nucleotide sequence of **SEQ ID NOs: 1-52**. Oligonucleotides can be natural or synthetic, e.g., DNA, RNA, modified backbones, etc. Peptide nucleic acids (PNAs) can also be used as probes in the methods of this invention.

Thus, the present invention further provides fragments or oligonucleotides of the nucleic
15 acids of this invention (e.g., **SEQ ID NOs: 1-52**), which can be used, for example as primers and/or probes. Thus, in some embodiments, the present invention provides a fragment or oligonucleotide, which is a nucleotide sequence that comprises, consists essentially of and/or consists of at least, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 125, 135, 150, 160, 170, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 or 500
20 contiguous nucleotides of a nucleic acid of this invention (e.g., the nucleotide sequences of **SEQ ID NOs: 1-52**). Such fragments or oligonucleotides can be detectably labeled or modified, for example, to include and/or incorporate a restriction enzyme cleavage site when employed as a primer in an amplification (e.g., PCR) assay.

The terms "coding region" and "coding sequence" are used interchangeably and refer to a
25 polynucleotide region that encodes a polypeptide or functional RNA and, when placed under the control of appropriate regulatory sequences, expresses the encoded polypeptide or functional RNA. The boundaries of a coding region are generally determined by a translation start codon at its 5' end and a translation stop codon at its 3' end. A coding region can encode one or more polypeptides or functional RNAs. For instance, a coding region can encode a polypeptide or functional RNA that is
30 subsequently processed into two or more polypeptides or functional RNAs. A regulatory sequence or regulatory region is a nucleotide sequence that regulates expression of a coding region to which it is operably linked. Nonlimiting examples of regulatory sequences include promoters, transcription initiation sites, translation start sites, internal ribosome entry sites, translation stop sites, and terminators. "Operably linked" refers to a juxtaposition wherein the components so described are in
35 a relationship permitting them to function in their intended manner. A regulatory sequence is "operably linked" to a coding region when it is joined in such a way that expression of the coding region is achieved under conditions compatible with the regulatory sequence.

The term "fragment" or "portion," as applied to a polynucleotide, will be understood to mean a nucleotide sequence of reduced length relative to a reference nucleic acid or nucleotide sequence
40 and comprising, consisting essentially of, and/or consisting of a nucleotide sequence of contiguous

nucleotides identical (e.g., 100% identical) or almost identical (e.g., 90%, 92%, 95%, 98%, 99% identical) to the reference nucleic acid or nucleotide sequence. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of oligonucleotides having a length of at least about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 70, 75, 80, 85, 90, 95, 100, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 215, 220, 225, 230, 235, 240, 245, 250, or more consecutive nucleotides (up to nearly the full length) of a nucleic acid or nucleotide sequence according to the invention.

The term "fragment" or "portion," as applied to a polypeptide, will be understood to mean an amino acid sequence of reduced length relative to a reference polypeptide or amino acid sequence and comprising, consisting essentially of, and/or consisting of an amino acid sequence of contiguous amino acids identical or almost identical (e.g., 90%, 92%, 95%, 98%, 99% identical) to the reference polypeptide or amino acid sequence. Such a polypeptide fragment according to the invention may be, where appropriate, included in a larger polypeptide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of peptides having a length of at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, or more consecutive amino acids (up to nearly the full length) of a polypeptide or amino acid sequence according to the invention.

Polypeptides and fragments thereof of the invention (e.g., polypeptide, and fragments thereof, encoded by the nucleotide sequences of the invention, e.g., **SEQ ID NOs:1-52**) may be modified for use by the addition, at the amino- and/or carboxyl-terminal ends, of a blocking agent. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. For example, one or more non-naturally occurring amino acids, such as D-alanine, can be added to the termini. Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Additionally, the peptide terminus can be modified, e.g., by acetylation of the N-terminus and/or amidation of the C-terminus. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to use.

In particular embodiments, nucleic acids of the present invention may encode any suitable epitope tag, including, but not limited to, poly-Arg tags (e.g., RRRRR (**SEQ ID NO:53**) and RRRRRR (**SEQ ID NO:54**) and poly-His tags (e.g., HHHHHH (**SEQ ID NO:55**)). In some embodiments, the nucleic acid may comprise a nucleotide sequence encoding a poly-Arg tag, a poly-His tag, a FLAG tag (i.e., DYKDDDDK (**SEQ ID NO:56**)), a Strep-tag II™ (GE Healthcare, Pittsburgh, PA, USA) (i.e., WSHPQFEK (**SEQ ID NO:57**)), and/or a c-myc tag (i.e., EQKLISEEDL (**SEQ ID NO:58**)). Thus in some embodiments, the polynucleotides, nucleic acid molecules, and nucleotide sequences of the invention (e.g., **SEQ ID Nos:1-52**) may be modified to comprise an epitope tag.

Similarly, in some embodiments, proteins of the present invention may comprise any suitable epitope tag, including, but not limited to, poly-Arg tags (e.g., RRRRR (SEQ ID NO:53) and RRRRRR (SEQ ID NO:54) and poly-His tags (e.g., HHHHHH (SEQ ID NO:55)). In some embodiments, the polypeptide may comprise a poly-Arg tag, a poly-His tag, a FLAG tag (i.e., DYKDDDDK (SEQ ID NO:56)), a Strep-tag II™ (GE Healthcare, Pittsburgh, PA, USA) (i.e., WSHPQFEK (SEQ ID NO:57)), and/or a c-myc tag (i.e., EQKLISEEDL (SEQ ID NO:58)). Thus in some embodiments, the polypeptides and fragments thereof of the invention (e.g., polypeptide, and fragments thereof, encoded by the nucleotide sequences of the invention, e.g., SEQ ID NOs:1-52) may be modified to comprise an epitope tag.

The term "isolated" can refer to a nucleic acid, nucleotide sequence or polypeptide that is substantially free of cellular material, viral material, and/or culture medium (when produced by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Moreover, an "isolated fragment" is a fragment of a nucleic acid, nucleotide sequence or polypeptide that is not naturally occurring as a fragment and would not be found in the natural state. "Isolated" does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the polypeptide or nucleic acid in a form in which it can be used for the intended purpose.

In some embodiments, the recombinant nucleic acid construct, nucleotide sequences and polypeptides of the invention are "isolated." An "isolated" nucleic acid molecule, an "isolated" nucleotide sequence or an "isolated" polypeptide is a nucleic acid molecule, nucleotide sequence or polypeptide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule, nucleotide sequence or polypeptide may exist in a purified form that is at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. In representative embodiments, the isolated nucleic acid molecule, the isolated nucleotide sequence and/or the isolated polypeptide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more pure.

In other embodiments, the recombinant nucleic acid construct, nucleotide sequence or polypeptide may exist in a non-native environment such as, for example, a recombinant host cell. Thus, for example, with respect to nucleotide sequences, the term "isolated" means that it is separated from the chromosome and/or cell in which it naturally occurs. A polynucleotide is also isolated if it is separated from the chromosome and/or cell in which it naturally occurs in and is then inserted into a genetic context, a chromosome and/or a cell in which it does not naturally occur (e.g., a different host cell, different regulatory sequences, and/or different position in the genome than as found in nature). Accordingly, the recombinant nucleic acid molecules, nucleotide sequences and their encoded polypeptides are "isolated" in that, by the hand of man, they exist apart from their native environment and therefore are not products of nature, however, in some embodiments, they can be introduced into and exist in a recombinant host cell.

In some embodiments, the nucleotide sequences and/or recombinant nucleic acid molecules of the invention can be operatively associated with a variety of promoters for expression in plant cells. Thus, in representative embodiments, a recombinant nucleic acid of this invention can further comprise one or more promoters operably linked to one or more nucleotide sequences.

By “operably linked” or “operably associated” as used herein, it is meant that the indicated elements are functionally related to each other, and are also generally physically related. Thus, the term “operably linked” or “operably associated” as used herein, refers to nucleotide sequences on a single nucleic acid molecule that are functionally associated. Thus, a first nucleotide sequence that is operably linked to a second nucleotide sequence means a situation when the first nucleotide sequence is placed in a functional relationship with the second nucleotide sequence. For instance, a promoter is operably associated with a nucleotide sequence if the promoter effects the transcription or expression of said nucleotide sequence. Those skilled in the art will appreciate that the control sequences (e.g., promoter) need not be contiguous with the nucleotide sequence to which it is operably associated, as long as the control sequences function to direct the expression thereof. Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a nucleotide sequence, and the promoter can still be considered “operably linked” to the nucleotide sequence.

Different nucleic acids or proteins having homology are referred to herein as “homologues.” The term homologue includes homologous sequences from the same and other species and orthologous sequences from the same and other species. “Homology” refers to the level of similarity between two or more nucleic acid and/or amino acid sequences in terms of percent of positional identity (i.e., sequence similarity or identity). Homology also refers to the concept of similar functional properties among different nucleic acids or proteins. Thus, the compositions and methods of the invention further comprise homologues to the nucleotide sequences and polypeptide sequences of this invention. “Orthologous,” as used herein, refers to homologous nucleotide sequences and/ or amino acid sequences in different species that arose from a common ancestral gene during speciation. A homologue of a nucleotide sequence of this invention has a substantial sequence identity (e.g., at least about 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and/or 100%) to said nucleotide sequence of the invention.

As used herein “sequence identity” refers to the extent to which two optimally aligned polynucleotide or peptide sequences are invariant throughout a window of alignment of components, e.g., nucleotides or amino acids. “Identity” can be readily calculated by known methods including, but not limited to, those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology* (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribnikov, M. and Devereux, J., eds.) Stockton Press, New York (1991).

As used herein, the term "percent sequence identity" or "percent identity" refers to the percentage of identical nucleotides in a linear polynucleotide sequence of a reference ("query") polynucleotide molecule (or its complementary strand) as compared to a test ("subject") polynucleotide molecule (or its complementary strand) when the two sequences are optimally aligned. In some embodiments, "percent identity" can refer to the percentage of identical amino acids in an amino acid sequence.

As used herein, the phrase "substantially identical," in the context of two nucleic acid molecules, nucleotide sequences or protein sequences, refers to two or more sequences or subsequences that have at least about 80%, least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in reference sequence segment, *i.e.*, the entire reference sequence or a smaller defined part of the reference sequence. As used herein, the term "percent sequence identity" or "percent identity" refers to the percentage of identical nucleotides in a linear polynucleotide sequence of a reference ("query") polynucleotide molecule (or its complementary strand) as compared to a test ("subject") polynucleotide molecule (or its complementary strand) when the two sequences are optimally aligned (with appropriate nucleotide insertions, deletions, or gaps totaling less than 20 percent of the reference sequence over the window of comparison). In some embodiments, "percent identity" can refer to the percentage of identical amino acids in an amino acid sequence.

Optimal alignment of sequences for aligning a comparison window are well known to those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and optionally by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the GCG® Wisconsin Package® (Accelrys Inc., San Diego, CA). An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, *i.e.*, the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction multiplied by 100. The comparison of one or more polynucleotide sequences may be to a full-length polynucleotide sequence or a

portion thereof, or to a longer polynucleotide sequence. For purposes of this invention "percent identity" may also be determined using BLASTX version 2.0 for translated nucleotide sequences and BLASTN version 2.0 for polynucleotide sequences.

The percent of sequence identity can be determined using the "Best Fit" or "Gap" program of the Sequence Analysis Software Package™ (Version 10; Genetics Computer Group, Inc., Madison, Wis.). "Gap" utilizes the algorithm of Needleman and Wunsch (Needleman and Wunsch, *J Mol. Biol.* 48:443-453, 1970) to find the alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. "BestFit" performs an optimal alignment of the best segment of similarity between two sequences and inserts gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman (Smith and Waterman, *Adv. Appl. Math.* 2:482 (1981); Smith *et al.*, *Nucleic Acids Res.* 11:2205 (1983)).

Useful methods for determining sequence identity are also disclosed in Guide to Huge Computers (Martin J. Bishop, ed., Academic Press, San Diego (1994)), and Carillo, H., and Lipton, D., *Applied Math* 48:1073(1988)). More particularly, preferred computer programs for determining sequence identity include but are not limited to the Basic Local Alignment Search Tool (BLAST) programs which are publicly available from National Center Biotechnology Information (NCBI) at the National Library of Medicine, National Institute of Health, Bethesda, Md. 20894; see BLAST Manual, Altschul *et al.*, NCBI, NLM, NIH; (Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990)); version 2.0 or higher of BLAST programs allows the introduction of gaps (deletions and insertions) into alignments; for peptide sequence BLASTX can be used to determine sequence identity; and, for polynucleotide sequence BLASTN can be used to determine sequence identity.

Two nucleotide sequences can be considered to be substantially complementary when the two sequences hybridize to each other under stringent conditions. In representative embodiments, two nucleotide sequences considered to be substantially complementary hybridize to each other under highly stringent conditions.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes* part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" Elsevier, New York (1993). Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH.

The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleotide sequences which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.1 5M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC

wash at 65°C for 15 minutes (see, Sambrook, *infra*, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of a medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example of a low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleotide sequences that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This can occur, for example, when a copy of a nucleotide sequence is created using the maximum codon degeneracy permitted by the genetic code.

The following are examples of sets of hybridization/wash conditions that may be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the invention or may be used for hybridization conditions for probes and/or primers in amplification assays. In one embodiment, a reference nucleotide sequence hybridizes to the "test" nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C. In another embodiment, the reference nucleotide sequence hybridizes to the "test" nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C or in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C. In still further embodiments, the reference nucleotide sequence hybridizes to the "test" nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, or in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

A "promoter" is a nucleotide sequence that controls or regulates the transcription of a nucleotide sequence (*i.e.*, a coding sequence) that is operably associated with the promoter. The coding sequence may encode a polypeptide and/or a functional RNA. Typically, a "promoter" refers to a nucleotide sequence that contains a binding site for RNA polymerase II and directs the initiation of transcription. In general, promoters are found 5', or upstream, relative to the start of the coding region of the corresponding coding sequence. The promoter region may comprise other elements that act as regulators of gene expression. These include a TATA box consensus sequence, and often a CAAT box consensus sequence (Breathnach and Chambon, (1981) *Annu. Rev. Biochem.* 50:349). Any promoter useful for initiation of transcription in a cell of a plant can be used in the expression cassettes of the present invention. Promoters can include, for example, constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and/or tissue-specific promoters for use in the preparation of recombinant nucleic acid molecules, *i.e.*, "chimeric genes" or "chimeric polynucleotides." In particular aspects, a "promoter" useful with

the invention is a promoter capable of initiating transcription of a nucleotide sequence in a cell of interest. The choice of promoter will vary depending on the temporal and spatial requirements for expression, and also depending on the host cell to be transformed. A promoter can be identified in and isolated from the organism to be transformed and then inserted into the nucleic acid construct to be used in transformation of the organism.

Non-limiting examples of a promoter include the promoter of the RubisCo small subunit gene 1 (PrbcS1), the promoter of the actin gene (Pactin), the promoter of the nitrate reductase gene (Pnr) and the promoter of duplicated carbonic anhydrase gene 1 (Pdca1) (See, Walker et al. *Plant Cell Rep.* 23:727-735 (2005); Li et al. *Gene* 403:132-142 (2007); Li et al. *Mol Biol. Rep.* 37:1143-1154 (2010)). PrbcS1 and Pactin are constitutive promoters and Pnr and Pdca1 are inducible promoters. Pnr is induced by nitrate and repressed by ammonium (Li et al. *Gene* 403:132-142 (2007)) and Pdca1 is induced by salt (Li et al. *Mol Biol. Rep.* 37:1143-1154 (2010)).

Examples of constitutive promoters useful for plants include, but are not limited to, cestrum virus promoter (cmp) (U.S. Patent No. 7,166,770), the rice actin 1 promoter (Wang et al. (1992) *Mol. Cell. Biol.* 12:3399-3406; as well as US Patent No. 5,641,876), CaMV 35S promoter (Odell et al. (1985) *Nature* 313:810-812), CaMV 19S promoter (Lawton et al. (1987) *Plant Mol. Biol.* 9:315-324), nos promoter (Ebert et al. (1987) *Proc. Natl. Acad. Sci USA* 84:5745-5749), Adh promoter (Walker et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:6624-6629), sucrose synthase promoter (Yang & Russell (1990) *Proc. Natl. Acad. Sci. USA* 87:4144-4148), the 35S promoter and the ubiquitin promoter. The constitutive promoter derived from ubiquitin accumulates in many cell types. Ubiquitin promoters have been cloned from several plant species for use in transgenic plants, for example, sunflower (Binet et al., 1991. *Plant Science* 79: 87-94), maize (Christensen et al., 1989. *Plant Molec. Biol.* 12: 619-632), and arabidopsis (Norris et al. 1993. *Plant Molec. Biol.* 21:895-906). The maize ubiquitin promoter (*UbiP*) has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926. The ubiquitin promoter is suitable for the expression of the nucleotide sequences of the invention in transgenic plants, especially monocotyledons. Further, the promoter expression cassettes described by McElroy et al. (*Mol. Gen. Genet.* 231: 150-160 (1991)) can be easily modified for the expression of the nucleotide sequences of the invention and are particularly suitable for use in monocotyledonous hosts.

In some embodiments, a recombinant nucleic acid construct of the invention can be an "expression cassette" or can be comprised within an expression cassette. As used herein, "expression cassette" means a recombinant nucleic acid molecule comprising a nucleotide sequence of interest (e.g., a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof, a nucleotide sequence having at least about 80% identity to of any of **SEQ ID NOs:1-52**, or a complement thereof; a fragment thereof; or any combination thereof), wherein said nucleotide sequence is operably associated with at least one control sequence (e.g., a promoter). Thus, some embodiments of the invention provide expression cassettes designed to express the nucleotide sequences of the invention in a cell.

An expression cassette comprising a nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. An expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

5 An expression cassette also can optionally include a transcriptional and/or translational termination region (*i.e.*, termination region) that is functional in the cell in which the nucleotide sequence of interest is to be expressed. A variety of transcriptional terminators are available for use in expression cassettes and are responsible for the termination of transcription beyond the heterologous nucleotide sequence of interest and correct mRNA polyadenylation. The termination
10 region may be native to the transcriptional initiation region, may be native to the operably linked nucleotide sequence of interest, may be native to the host organism, or may be derived from another source (*i.e.*, foreign or heterologous to the promoter, the nucleotide sequence of interest, the host organism, or any combination thereof). In addition, in some embodiments, a coding sequence's native transcription terminator can be used.

15 An expression cassette of the invention also can include a nucleotide sequence for a selectable marker, which can be used to select a transformed organism and/or cell. As used herein, "selectable marker" means a nucleotide sequence that when expressed imparts a distinct phenotype to the transformed organism or cell expressing the marker and thus allows such transformed organisms or cells to be distinguished from those that do not have the marker. Such a nucleotide
20 sequence may encode either a selectable or screenable marker, depending on whether the marker confers a trait that can be selected for by chemical means, such as by using a selective agent (*e.g.*, an antibiotic, herbicide, or the like), or on whether the marker is simply a trait that one can identify through observation or testing, such as by screening. Of course, many examples of suitable selectable markers useful in various organisms are known in the art and can be used in the
25 expression cassettes described herein.

In addition to expression cassettes, the nucleic acid molecules and nucleotide sequences described herein can be used in connection with vectors. The term "vector" refers to a composition for transferring, delivering or introducing a nucleic acid (or nucleic acids) into a cell. A vector comprises a nucleic acid molecule comprising the nucleotide sequence(s) to be transferred,
30 delivered or introduced. Vectors for use in transformation of plants and other organisms are well known in the art. Non-limiting examples of general classes of vectors include a viral vector including but not limited to a plasmid vector, a phage vector, a phagemid vector, a cosmid vector, a fosmid vector, a bacteriophage, an artificial chromosome, or an *Agrobacterium* binary vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable. A
35 vector as defined herein can transform prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (*e.g.* autonomous replicating plasmid with an origin of replication). Additionally included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different host organisms, which may be selected from prokaryotic and eukaryotic organisms. In some representative embodiments, the nucleic acid in the
40 vector is under the control of, and operably linked to, an appropriate promoter or other regulatory

elements for transcription in a host cell such as a microbial, e.g. bacterial, or a plant cell. The vector may be a bi-functional expression vector which functions in multiple hosts. In the case of genomic DNA, this may contain its own promoter or other regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell.

The inventors have discovered nucleotide sequences and the polypeptides encoded by these nucleotide sequences that may be useful for diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, and for distinguishing *P. cubensis* from *Pseudoperonospora humuli*, as well as for distinguishing between different types of *P. cubensis*. As understood in the art, downy mildew pathogens including *P. cubensis* can be spread by seed and other contaminated plant material. Further, downy mildew pathogens can spread for miles by wind. Thus, when an outbreak of *P. cubensis* occurs in a first region, a second region that is downwind may be at risk for an outbreak too. Environmental and plant samples in the second region may be tested to evaluate the risk that an outbreak will occur in that region. Typically, the samples are evaluated by manual counting of the spores. The present invention provides a substantial advantage over the prior art by providing more sensitive and efficient methods for detecting *P. cubensis*, and for distinguishing between *P. cubensis* and *P. humuli* infections and between types of *P. cubensis* that infect cucumber and cantaloupe and types that infect pumpkin, watermelon, and squash.

Accordingly, the present invention provides an isolated nucleic acid molecule is provided comprising: (a) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; (b) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of (a); (c) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any of (a) and/or (b), or a complement thereof; (d) a nucleotide sequence that differs from the nucleotide sequence of any of (a) to (c) above due to the degeneracy of the genetic code; and (e) a fragment of a nucleotide sequence of any of (a) to (d) above. In some embodiments, the invention provides a recombinant nucleic acid construct comprising at least one isolated nucleic acid molecule of the invention. In some embodiments, the isolated nucleic acid molecule may be operatively linked to a heterologous promoter. These isolated nucleic acids may be used as diagnostic markers for the presence of *P. cubensis*, and for distinguishing between *P. cubensis* and *P. humuli* infections and between types of *P. cubensis* that infect cucumber and cantaloupe and types that infect pumpkin, watermelon, and squash.

In some embodiments, an isolated polypeptide is provided comprising: (a) an amino acid sequence encoded by a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; and (b) an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of (a); and a fragment of any of (a) and/or (b). In some embodiments, a polyclonal and/or monoclonal antibody is provided that is specifically reactive with an isolated polypeptide of the invention. Methods for making polyclonal or monoclonal antibodies are well-known in the art. Similarly to the isolated nucleic acids of the invention, the polypeptides encoded by the isolated nucleic acids of the invention and the polyclonal and/or monoclonal antibodies that are specifically reactive with an isolated polypeptide of the invention may be used as diagnostic markers for the

presence of *P. cubensis*, and for distinguishing between *P. cubensis* and *P. humuli* infections and between the type of *P. cubensis* that infect cucumber and cantaloupe and the type that infect pumpkin, watermelon, and squash.

Accordingly, in some embodiments, the invention provides a method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising detecting at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, in a sample from the cucurbit plant, thereby diagnosing a *P. cubensis* infection.

In some embodiments, a method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant is provided, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to a polynucleotide comprising, consisting essentially of or consisting of any one of the nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof; and detecting binding between the oligonucleotide and the polynucleotide, thereby diagnosing a *P. cubensis* infection.

A further embodiment provides a method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to a polynucleotide comprising, consisting essentially of or consisting of any one of the nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis*, thereby diagnosing the cucurbit plant as having a *P. cubensis* infection. In some embodiments, detecting whether the polynucleotide, or fragment thereof, is present comprises detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the polynucleotide, or fragment thereof.

In some embodiments, a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, thereby diagnosing an infection by the type of *P. cubensis* that infects cucumber and cantaloupe.

In some embodiments, a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment

thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, thereby diagnosing an infection by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

5 In some embodiments, a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the
10 nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, thereby
15 diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis* that infects cucumber and cantaloupe.

In some embodiments, the invention provides a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that
20 hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the
25 nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, thereby diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis* that infects cucumber and cantaloupe.

In some embodiments, a method of diagnosing an infection in a cucurbit plant by a type of
30 *Pseudoperonospora cubensis* that infects cucumber and cantaloupe (type 2) or by a type of *P. cubensis* that infects pumpkin, watermelon, and squash (type 1) is provided, comprising: contacting a sample from a cucurbit plant with a first oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and a second oligonucleotide that hybridizes to the
35 nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof is present by detecting binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is
40 present by detecting binding between the second oligonucleotide and the nucleotide sequence of

SEQ ID NO:52, and/or fragment thereof, wherein the presence of binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, thereby diagnosing an infection by the type of *P. cubensis* that infects cucumber and cantaloupe, and the presence of binding between
5 the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash, thereby diagnosing an infection by a type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In further embodiments, a method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe or by a type of *P. cubensis* that
10 infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a cucurbit plant suspected of being infected with *P. cubensis* with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the
15 nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification
20 product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the first oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, thereby diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis*
25 that infects cucumber and cantaloupe, and the presence of binding between the second oligonucleotide probe and the amplification product identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash, thereby diagnosing an infection by a type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In some embodiments, a method for determining a risk of a *Pseudoperonospora cubensis*
30 outbreak is provided, comprising: detecting at least one polynucleotide comprising, consisting essentially of or consisting of any one of the nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, in a sample from a plant or in an environmental sample, wherein the presence of the at least one polynucleotide indicates the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak

A further embodiment of the invention provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to a polynucleotide comprising,
35 consisting essentially of or consisting of any one of the nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof; and detecting binding between the oligonucleotide and the polynucleotide,

wherein the presence of the polynucleotide or fragment thereof, indicates the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak.

Some embodiments of the invention provide a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising:

5 contacting a sample from a cucurbit plant or an environmental sample with a pair of oligonucleotides that hybridize to a polynucleotide comprising, consisting essentially of or consisting of any one of the nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting the amplification product, wherein the presence of the amplification product identifies
10 the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak. In some embodiments, detecting whether the polynucleotide and/or fragment thereof is present comprises detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the polynucleotide, and/or fragment thereof.

 In some embodiments, a method for determining a risk of a *Pseudoperonospora cubensis*
15 outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or the fragment thereof is present by detecting binding
20 between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, indicates the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe

25 In some embodiments, a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and detecting
30 whether the nucleotide sequence of **SEQ ID NO:52** and/or the fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and the risk of a *P. cubensis* outbreak
35 by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

 In some embodiments, a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions
40 whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of binding
 5 between the oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe.

In some embodiments, a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided,
 10 comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an
 15 oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that
 20 infects pumpkin, watermelon, and squash.

A further embodiment provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe or by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a plant or an environmental sample with a first oligonucleotide that hybridizes to the nucleotide
 25 sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and a second oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof is present by detecting binding
 30 between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, indicates
 35 the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and a risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe, and the presence of binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and a risk of a *P. cubensis* outbreak by the type of *P. cubensis*
 40 that infects pumpkin, watermelon, and squash.

A further embodiment provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe or by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the first oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and a risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe, and the presence of binding between the second oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and a risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In some embodiments, the sample may be taken from a region suspected of being at risk for an outbreak of infection by *P. cubensis*, or from a neighboring region. A region may be determined to be at risk for an outbreak by the detection of at least one of the nucleotide sequences of **SEQ ID NOs:1-52** or a fragment thereof.

In additional embodiments, a method of selecting a treatment regimen for a *P. cubensis* infection is provided, comprising: detecting in a sample from the plant at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof, thereby identifying a *P. cubensis* infection; and selecting a treatment regimen for the *P. cubensis* infection.

A further embodiment provides a method of selecting a treatment regimen for a *P. cubensis* infection, comprising: contacting a sample from the plant with an oligonucleotide that hybridizes to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof; detecting binding between the oligonucleotide and the nucleotide sequence, thereby identifying a *P. cubensis* infection; and selecting a treatment regimen for the *P. cubensis* infection.

In some embodiments, a method of selecting a treatment regimen for a *P. cubensis* infection is provided, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur,

thereby producing an amplification product; and detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis*, thereby identifying a *P. cubensis* infection; and selecting a treatment regimen for the *P. cubensis* infection. In some embodiments, detecting whether the polynucleotide and/or fragment thereof is present comprises
5 detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the polynucleotide, and/or fragment thereof.

In some embodiments, a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide
10 sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment
15 thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe; and selecting a treatment regimen effective against the type of *P. cubensis* that infects cucumber and cantaloupe.

In some embodiments, a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the
20 nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment
25 thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and selecting a treatment regimen effective against the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In some embodiments, a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the
30 nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide
35 sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe; and selecting a treatment regimen effective against the type of *P. cubensis* that infects cucumber and cantaloupe.

In some embodiments, a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects pumpkin, watermelon, and squash; and selecting a treatment regimen effective against the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In further embodiments, a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe or for a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a cucurbit plant suspected of being infected with *P. cubensis* with a first oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and a second oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, and the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and selecting a treatment regimen effective against the type of *P. cubensis* identified.

In a further embodiment, a method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe or for a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a cucurbit plant suspected of being infected with *P. cubensis* with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide

sequence of **SEQ ID NO:52**, and/or a fragment thereof, , and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the first oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, and the presence of binding between the second oligonucleotide probe and the amplification product identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash, selecting a treatment regimen effective against the type of *P. cubensis* identified.

In some embodiments, the invention provides a method for reducing the risk of a *P. cubensis* outbreak is provided, comprising: detecting in a sample from a cucurbit plant or in an environmental sample at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52** and/or a fragment thereof, thereby identifying the presence of *P. cubensis* in the sample; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

An additional embodiment provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with an oligonucleotide that hybridizes to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof; detecting binding between the oligonucleotide and the polynucleotide and/or fragment thereof, thereby identifying the presence of *P. cubensis* in the sample; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

A further embodiment provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with a pair of oligonucleotides that hybridize to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis*; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

In some embodiments, detecting whether the polynucleotide and/or fragment thereof is present comprises detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the polynucleotide, and/or fragment thereof.

In some embodiments, a method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**

and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe; and implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe, thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe.

5 In some embodiments, a method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting
10 whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and implementing a treatment regimen against the type of *P.*
15 *cubensis* that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects pumpkin, watermelon, and squash.

In some embodiments, a method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide
20 sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ**
25 **ID NO:52**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe; and implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe.

30 In some embodiments, a method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the
35 nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that
40 infects pumpkin, watermelon, and squash; and implementing a treatment regimen against the type of

P. cubensis that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects pumpkin, watermelon, and squash.

Another embodiment of the invention provides a method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe or by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a plant or an environmental sample with a first oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and a second oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, wherein the presence of binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, and the presence of binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe or against the type of *P. cubensis* that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe and/or by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In another embodiment, a method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe, or by a *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, wherein the presence of binding between the first oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, and the presence of binding between the second oligonucleotide probe and the amplification product

identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe or against the type of *P. cubensis* that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe and/or by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In representative embodiments, the polynucleotide that is selected for diagnosing a *P. cubensis* infection, determining or reducing the risk of a *P. cubensis* outbreak, and/or selecting a treatment regimen for a *P. cubensis* infection may be the nucleotide sequence of **SEQ ID NO:2, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:39, SEQ ID NO:43, SEQ ID NO:52**, or a fragment thereof, or any combination thereof.

A further embodiment of the invention provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or from an environmental sample with an oligonucleotide that hybridizes to an isolated nucleic acid molecule and/or with an antibody of the invention; and detecting whether the isolated nucleic acid molecule, or fragment thereof, is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, or fragment thereof, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule, or fragment thereof, is present by detecting binding between the antibody and the polypeptide, wherein the presence of the isolated nucleic acid molecule and/or the polypeptide indicates the presence of *P. cubensis* in the sample and the risk of a *P. cubensis* outbreak.

In another embodiment, the invention provides a method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with a pair oligonucleotides that hybridize to an isolated nucleic acid molecule of the invention, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak.

A further embodiment provides a method of selecting a treatment regimen for a *P. cubensis* infection of a cucurbit plant, comprising: contacting a sample from a cucurbit plant suspected of being infected with *P. cubensis* with an oligonucleotide that hybridizes to an isolated nucleic acid molecule and/or with an antibody of the invention;

detecting whether the isolated nucleic acid molecule and/or a fragment thereof is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule and/or a fragment thereof is present by detecting binding between the antibody and the polypeptide, thereby identifying a *P. cubensis* infection when the presence of the isolated nucleic acid molecule and/or polypeptide is detected; and selecting a treatment regimen for the *P. cubensis* infection.

Additionally provided is a method of selecting a treatment regimen for a *P. cubensis* infection of a cucurbit plant, comprising: contacting a sample from a cucurbit plant suspected of being infected with *P. cubensis* with a pair oligonucleotides that hybridize to an isolated nucleic acid

molecule of the invention, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the presence of *P. cubensis* in the sample; and selecting a treatment regimen for the *P. cubensis* infection.

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A further embodiment of the invention provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or from an environmental sample with an oligonucleotide that hybridizes to an isolated nucleic acid molecule and/or with an antibody of the invention; detecting whether the isolated nucleic acid molecule, and/or a fragment thereof, is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule, and/or a fragment thereof, is present by detecting binding between the antibody and the polypeptide, wherein the presence of the isolated nucleic acid molecule and/or the polypeptide identifies the presence of *P. cubensis*; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

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A further embodiment of the invention provides a method for reducing the risk of a *P. cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with a pair oligonucleotides that hybridize to an isolated nucleic acid molecule of the invention, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis* in the sample; and implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

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In a still further embodiment, a method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising: contacting a sample from a cucurbit plant or from an environmental sample with a first oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16** and/or a fragment thereof, but not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and/or a second oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but not to the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof; and identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash in the sample.

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In a still further embodiment, a method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a cucurbit plant or from an environmental sample with a first antibody that hybridizes to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and not to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, or fragment thereof, and/or with a second antibody that hybridizes to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and not to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, or fragment thereof; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof is present by detecting binding between the second antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof; and identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the first antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the second antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In an additional embodiment, a method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash is provided, comprising: contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the amplification product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and not to the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof; and identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the amplification product and the first oligonucleotide probe, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the amplification product and the second oligonucleotide probe, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

In some embodiments, "detecting" may comprise hybridizing an oligonucleotide (e.g., an oligonucleotide probe, oligonucleotide primer, nucleic acid probe, or nucleic acid primer) to a nucleotide sequence of any one of **SEQ ID NOs:1-52**, wherein the oligonucleotide may comprise, consist essentially of, or consist of at least about 10 consecutive nucleotides of the nucleotide sequence selected from the group of nucleotide sequences of **SEQ ID NOs:1-52** (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 70, 75, 80, 85, 90, 95, 100, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200 consecutive nucleotides, or more, or any range or value therein), and the presence of *P. cubensis* is detected when the oligonucleotide hybridizes to the at least one nucleotide sequence in the sample. In some embodiments, the oligonucleotide may comprise, consist essentially of, or consist of about 10 to about 2000 consecutive nucleotides of any one of the nucleotide sequences of **SEQ ID NOs:1-52** (e.g., about 10 to about 30, about 10 to about 50, about 10 to about 80, about 10 to about 100, about 10 to about 150, about 10 to about 200, about 10 to about 250, about 10 to about 300, about 10 to about 350, about 10 to about 400, about 10 to about 450, about 10 to about 500, about 10 to about 550, about 10 to about 600, about 10 to about 650, about 10 to about 700, about 10 to about 750, about 10 to about 800, about 10 to about 850, about 10 to about 900, about 10 to about 950, about 10 to about 1000, about 10 to about 1100, about 10 to about 1200, about 10 to about 1300, about 10 to about 1400, about 10 to about 1500, about 10 to about 1600, about 10 to about 1700, about 10 to about 1800, about 10 to about 1900, about 10 to about 2000, about 100 to about 200, about 100 to about 250, about 100 to about 300, about 100 to about 350, about 100 to about 400, about 100 to about 450, about 100 to about 500, about 100 to about 550, about 100 to about 600, about 100 to about 650, about 100 to about 700, about 100 to about 750, about 100 to about 800, about 100 to about 850, about 100 to about 900, about 100 to about 950, about 100 to about 1000, about 100 to about 1500, about 100 to about 2000, about 200 to about 400, about 200 to about 500, about 200 to about 600, about 200 to about 800, about 200 to about 1000, about 200 to about 2000, about 500 to about 600, about 500 to about 800, about 500 to about 1000, about 500 to about 2000, or any range or value therein, of consecutive nucleotides of any one of the nucleotide sequences of **SEQ ID NOs:1-52**). In some embodiments, the percent identity between the oligonucleotide and the reference nucleotide can be between 70% to 100% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein).

Thus, an oligonucleotide useful with this invention may comprise, consist essentially of, or consist of about 50% to about 100% (e.g., about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein) complementarity to a nucleotide sequence of **SEQ ID NOs:1-52**. As understood in the art, the percent complementarity between an oligonucleotide and reference polynucleotide may depend on the length of the oligonucleotide. Thus, in some embodiments, an oligonucleotide comprising, consisting essentially of, or consisting of at least about at least about 10 nucleotides can be about 80% to about 100%, about 85% to about 100%, about 90% to about 100%, about 95% to about 100% (e.g., at least about

80, 81, 82, 83, 84, 85 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein) complementary to a portion of consecutive nucleotides of a reference polynucleotide comprising, consisting essentially of, consisting of the nucleotide sequence of any one of **SEQ ID NOs:1-52**. In some embodiments, an oligonucleotide comprising, consisting essentially of, or

5 consisting of at least about 10 to about 30 nucleotides, or about 10 to about 50 nucleotides, can be about 90% to about 100% (e.g., at least about 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein) complementary to a portion of consecutive nucleotides of a nucleotide sequence of any one of **SEQ ID NOs:1-52**. In some embodiments, an oligonucleotide comprising, consisting essentially of, or consisting of at least about 10 to about 100, about 30 to about 100

10 nucleotides, or about 50 to about 100 nucleotides can be about 80% to about 100%, about 85% to about 100%, about 90% to about 100%, about 95% to about 100% (e.g., about 80, 81, 82, 83, 84, 85 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein) complementary to a portion of consecutive nucleotides of a nucleotide sequence of any one of **SEQ ID NOs:1-52**. In further embodiments, an oligonucleotide comprising, consisting essentially of, or

15 consisting of at least about 100 to about 500 nucleotides can be about 70% to about 100% complementary to a portion of consecutive nucleotides of a nucleotide sequence of any one of **SEQ ID NOs:1-52** (e.g., about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein). In still further

20 embodiments, an oligonucleotide comprising, consisting essentially of, or consisting of at least about 100 to about 1000 nucleotides can be about 70% to about 100% complementary to a portion of consecutive nucleotides of a nucleotide sequence of any one of **SEQ ID NOs:1-52**. In some

embodiments, an oligonucleotide comprising, consisting essentially of, or consisting of at least about 1000 to about 2000 nucleotides can be about 50% to about 100% complementary to a portion of consecutive nucleotides of a nucleotide sequence of any one of **SEQ ID NOs:1-52** (e.g., about 50,

25 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, or any range or value therein).

A pair of oligonucleotides as used herein means two oligonucleotides that may be used, for example, as primers to carry out an amplification reaction (e.g., PCR). Those of skill in the art are

30 aware of the requirements for amplification primers and can develop primers to amplify any region of a known nucleotide sequence.

Detecting may comprise any method known or later developed to detect the presence of a nucleic acid or a polypeptide. A nucleic acid and/or polypeptide may be also be distinguished from another nucleic acid and/or polypeptide by analysis of the length of the nucleic acid or polypeptide or

35 by sequencing. Methods for detecting the presence of a nucleic acid or a polypeptide, or determining its length or sequence are known and include, but are not limited to, Southern analysis, PCR amplification for detection of a polynucleotide, Northern blots, RNase protection, primer-extension, RT-PCR amplification for detecting RNA transcripts, enzymatic assays for detecting enzyme or ribozyme activity of polypeptides and polynucleotides, and protein gel electrophoresis,

40 Western blots, immunoprecipitation, and enzyme-linked immunoassays to detect polypeptides.

Other techniques such as *in situ* hybridization, enzyme staining, and immunostaining also can be used to detect the presence or expression of polypeptides and/or polynucleotides. Methods for performing all of the referenced techniques are known.

In some embodiments, detecting a nucleotide sequence of the invention (e.g., **SEQ ID NOs:1-52**, or a fragment thereof) in a sample includes but is not limited to an amplification reaction, an amplification reaction with a single base extension, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS), sequencing, hybridization, restriction endonuclease digestion analysis, electrophoresis, or any combination thereof.

In some embodiments, more than one nucleotide sequence of **SEQ ID NOs:1-52** (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) may be detected by contacting a sample with more than one oligonucleotide probe or more than one pair of oligonucleotide primers.

In some embodiments, the detecting may comprise amplifying a fragment comprising at least about 25 consecutive nucleotides of a nucleotide sequence of **SEQ ID NOs:1-52**, or any combination thereof, detecting the presence of the fragment, wherein the presence of the fragment indicates the presence of *P. cubensis* in the sample. In some embodiments, the fragment can be about 15 consecutive nucleotides to the full length of the nucleotide sequence (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500 consecutive nucleotides, or any range or value therein).

In some embodiments, the fragment can be about 15 consecutive nucleotides to 50 consecutive nucleotides, about 25 consecutive nucleotides to 50 consecutive nucleotides, about 25 consecutive nucleotides to 75 consecutive nucleotides, about 25 consecutive nucleotides to 100 consecutive nucleotides, about 50 consecutive nucleotides to 100 consecutive nucleotides, about 50 consecutive nucleotides to 150 consecutive nucleotides, about 50 consecutive nucleotides to 200 consecutive nucleotides, about 100 consecutive nucleotides to 150 consecutive nucleotides, about 100 consecutive nucleotides to 200 consecutive nucleotides, about 100 consecutive nucleotides to 250 consecutive nucleotides, about 100 consecutive nucleotides to 350 consecutive nucleotides, about 100 consecutive nucleotides to 500 consecutive nucleotides, about 200 consecutive nucleotides to 250 consecutive nucleotides, about 200 consecutive nucleotides to 350 consecutive nucleotides, about 200 consecutive nucleotides to 450 consecutive nucleotides, about 200 consecutive nucleotides to 500 consecutive nucleotides, about 200 consecutive nucleotides to 600 consecutive nucleotides, about 300 consecutive nucleotides to 500 consecutive nucleotides, about 400 consecutive nucleotides to 500 consecutive nucleotides, about 400 consecutive nucleotides to 600 consecutive nucleotides, about 400 consecutive nucleotides to 700 consecutive nucleotides, about 400 consecutive nucleotides to 800 consecutive nucleotides, about 500 consecutive nucleotides to 1000 consecutive nucleotides, about 500 consecutive nucleotides to 1200 consecutive nucleotides, about 500 consecutive nucleotides to 1500 consecutive nucleotides, about 750 consecutive

nucleotides to 1000 consecutive nucleotides, about 750 consecutive nucleotides to 1500 consecutive nucleotides, about 1000 consecutive nucleotides to 1500 consecutive nucleotides, about 1000 consecutive nucleotides to 2000 consecutive nucleotides, about 1000 consecutive nucleotides to 2500 consecutive nucleotides, and any value or range therein). In further

embodiments, a method of amplifying can comprise amplifying the full length of an nucleotide sequence of any one or more of **SEQ ID NOs:1-52**.

Once *P. cubensis* is identified in a sample or the type of *P. cubensis* is identified in a sample (e.g., whether the *P. cubensis* is identified as a type that infects cucumber and cantaloupe or a type that infects pumpkin, watermelon, and squash) a treatment regimen may be selected and/or implemented. Any effective treatment(s) known or later developed for treating a *P. cubensis* infection may be used with the methods of the invention. Non-limiting examples of compositions that may be used in a treatment regimen for a *P. cubensis* infection of a cucurbit plant includes, but is not limited to, oxathiapiprolin, cyazofamid, propamocarb, famoxadone and cymoxanil, ametoctradin and dimethomorph, mancozeb, mancozeb + zoxamide, and/or chlorothalonil. In some embodiments, a treatment for a type of *P. cubensis* that infects cucumber and cantaloupe may differ from a treatment for a type of *P. cubensis* that infects pumpkin, watermelon, and squash by the timing of the treatment. For example cucumber and cantaloupe typically become infected earlier in the growing season, thus, the treatments begin earlier and more sprays are required in contrast to the other crops, which typically become infected later in the growing season

By the terms "treat," "treating," or "treatment of" (and grammatical variations thereof) it is meant that the severity of a plant's condition is reduced, at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one symptom is achieved and/or there is a delay in the progression of the disease or disorder. The terms "treat," "treating," or "treatment of" are also meant to refer to the prevention or reduction in the severity of a *P. cubensis* infection or outbreak, which may be measured by a reduction in the number of plants infected and/or the severity of the condition of the plants, which condition may be at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one symptom is achieved and/or there is a delay in the progression of the disease or disorder. An "effective treatment" as used herein is a treatment that is sufficient to reduce the severity of a plant's condition due to infection by *P. cubensis* (e.g., at least partially improve or ameliorate said condition), to alleviate, mitigate or decrease at least one symptom due to a *P. cubensis* infection and/or results in a delay in the progression of the disease or disorder. An effective treatment further refers to a treatment that alleviates, mitigates or reduces a *P. cubensis* outbreak or its severity; that is the treatment mitigates or reduces the number of plants that become infected. Those skilled in the art will appreciate that for a treatment to be effective it need not be complete or curative, as long as some benefit is provided to the crop, the plant and/or plant part.

In addition, to methods for identifying *P. cubensis* and diagnosing *P. cubensis* infections, the present invention further provides methods for selecting genes from cucurbits that may be useful for conferring resistance to *P. cubensis*. Thus, in some embodiments, a method for selecting a cucurbit plant comprising at least one gene that confers resistance to *P. cubensis* is provided, comprising

introducing into the cucurbit plant at least one of (a) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; (b) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of any one of (a); (c) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any one of (a) to (b), or a complement thereof; and (d) a nucleotide sequence that differs from the nucleotide sequences of any one of (a) to (c) above due to the degeneracy of the genetic code, wherein the expression of the at least one nucleotide sequence produces a hypersensitive response in a cucurbit plant comprising a gene that confers resistance to *P. cubensis*, thereby identifying the cucurbit plant as comprising a gene that confers resistance to *P. cubensis*; and selecting the plant having the hypersensitive response and identified as comprising the gene that confers resistance to *P. cubensis*. In some embodiments, the at least one nucleotide sequence of **SEQ ID NOs:1-52**, or fragment thereof, may be comprised in recombinant nucleic acid construct and/or an expression vector.

In some embodiments, a method of producing a plant having increased resistance to *P. cubensis* is provided, comprising: (a) introducing into the cucurbit plant at least one of (i) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; (ii) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of any one of (i); (iii) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any one of (i) to (ii), or a complement thereof; and (iv) a nucleotide sequence that differs from the nucleotide sequences of any one of (i) to (iii) above due to the degeneracy of the genetic code, wherein the expression of the at least one nucleotide sequence produces a hypersensitive response in a cucurbit plant comprising a gene that confers resistance to *P. cubensis*, thereby identifying the cucurbit plant as comprising a gene that confers resistance to *P. cubensis*; selecting a plant having the hypersensitive response and identified as comprising the gene that confers resistance to *P. cubensis*; (b) crossing the selected plant with a second cucurbit plant; and (c) selecting progeny comprising increased resistance to *P. cubensis*, thereby producing a plant having increased resistance to *P. cubensis*.

In representative embodiments, the at least one nucleotide sequence of **SEQ ID NOs:1-52**, or a fragment thereof, that is introduced may be the nucleotide sequence of **SEQ ID NO:2**, **SEQ ID NO:13**, **SEQ ID NO:16**, **SEQ ID NO:17**, **SEQ ID NO:24**, **SEQ ID NO:39**, **SEQ ID NO:43**, **SEQ ID NO:52**, or a fragment thereof, or any combination thereof.

"Introducing," in the context of a polynucleotide of interest (e.g., a nucleotide sequence encoding an amino acid sequence having at least about 80% identity to any of **SEQ ID NOs:1-52**, a nucleotide sequence having at least about 80% identity to any of **SEQ ID NOs:1-52**, and/or functional fragments thereof), means presenting the polynucleotide of interest to the cell of an organism in such a manner that the nucleotide sequence gains access to the interior of the cell. The methods of the invention do not depend on a particular method for introducing one or more nucleotide sequences into an organism, only that they gain access to the interior of at least one cell of the organism. Where more than one nucleotide sequence is to be introduced, these nucleotide sequences can be assembled as part of a single polynucleotide or nucleic acid construct, or as separate polynucleotide or nucleic acid constructs, and can be located on the same or different

expression constructs or transformation vectors. Accordingly, these polynucleotides may be introduced into cells in a single transformation event, in separate transformation events, or, for example, they may be incorporated into an organism as part of a breeding protocol.

The term "transformation" as used herein refers to the introduction of a heterologous nucleic acid into a cell. Transformation of a cell may be stable or transient. Thus, in some embodiments, a cell of the invention may be stably transformed with a nucleotide sequence of the invention. In other embodiments, a cell may be transiently transformed with a nucleotide sequence of the invention. In particular embodiments, the plant leaf is infiltrated with a vector comprising a nucleotide sequence of the invention and the cells are transiently transformed.

"Transient transformation" in the context of a polynucleotide means that a polynucleotide is introduced into the cell and does not integrate into the genome of the cell.

By "stably introducing" or "stably introduced" in the context of a polynucleotide introduced into a cell is intended that the introduced polynucleotide is stably incorporated into the genome of the cell, and thus the cell is stably transformed with the polynucleotide.

"Stable transformation" or "stably transformed" as used herein means that a polynucleotide is introduced into a cell and integrates into the genome of the cell. As such, the integrated polynucleotide is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations. "Genome" as used herein also includes the nuclear, mitochondrial, and plastid genome, and therefore includes integration of the nucleic acid into, for example, the chloroplast or mitochondrial genome. Stable transformation as used herein can also refer to a transgene that is maintained extrachromasomally, for example, as a minichromosome.

Transient transformation may be detected by, for example, an enzyme-linked immunosorbent assay (ELISA) or Western blot, which can detect the presence of a peptide or polypeptide encoded by one or more transgene introduced into an organism. Stable transformation of a cell can be detected by, for example, a Southern blot hybridization assay of genomic DNA of the cell with nucleic acid sequences which specifically hybridize with a nucleotide sequence of a transgene introduced into an organism (e.g., a plant). Stable transformation of a cell can be detected by, for example, a Northern blot hybridization assay of RNA of the cell with nucleic acid sequences, which specifically hybridize with a nucleotide sequence of a transgene introduced into an organism. Stable transformation of a cell can also be detected by, e.g., a polymerase chain reaction (PCR) or other amplification reactions as are well known in the art, employing specific primer sequences that hybridize with target sequence(s) of a transgene, resulting in amplification of the transgene sequence, which can be detected according to standard methods. Transformation can also be detected by direct sequencing and/or hybridization protocols well known in the art.

The term "hypersensitive response" as used herein refers to the plant response characterized by rapid cell death in a limited region surrounding an infection. A hypersensitive response may serve to restrict the growth and spread of pathogens to other parts of the plant.

Any cucurbit now known or later identified may be useful with any of the methods of this invention. Thus, in some embodiments, a cucurbit plant may be a wild cucurbit, a cucurbit breeding line, or a commercial cucurbit line. In some embodiments, the cucurbit plant may be from the

Cucurbitaceae plant family. In some embodiments, a cucurbit plant may be from a genus in the *Cucurbitaceae* plant family as set forth in **Table 1**, or any cultivar, variety or line therein. In representative embodiments, the cucurbit plant may be in the genus of *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp., *Lagenaria* spp., or *Luffa* spp., or any cultivar, variety or line therein. In some

5 embodiments, the cucurbit plant may be a cucumber plant, a watermelon plant, a squash plant, a pumpkin plant, and/or a cantaloupe plant, or any cultivar, variety or line thereof. In some aspects, a cucurbit plant, as used herein, can be one that is suspected of being infected with *P. cubensis*.

The invention will now be described with reference to the following examples. It should be appreciated that these examples are not intended to limit the scope of the claims to the invention,

10 but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the invention.

EXAMPLES

Example 1. Sample preparation for sequencing and candidate validation.

Isolates of *P. cubensis* and *P. humuli* used in this study for genome and transcriptome

15 sequencing are listed in **Table 1**. Isolates were propagated on detached leaves to minimize contamination from other pathogens and insects and to obtain high quality RNA and DNA for sequencing. *P. cubensis* and *P. humuli* isolates were propagated on the host that they were originally isolated from. Detached leaves were placed upside down on moist sterile paper towels inside clear acrylic boxes, spray inoculated with a sporangial suspension of 1 to 3×10^4

20 sporangia/ml for each isolate, and incubated at 25°C with an 12 h light/dark cycle in a precision plant growth chamber (Thermo Fisher Scientific, Waltham, MA). Sporangia were dislodged from leaves using a Preval® sprayer (Preval, Coal City, IL) with sterile water, collected into a tube, and pelleted by centrifugation. Sporangia intended for use in RNA extractions were then suspended in

25 RNALater® (ThermoFisher Scientific, Waltham, MA) at a sporangial concentration of $1 \text{ mg}/\mu\text{l}$, while sporangia for DNA extraction were suspended in sterile water. Additional samples used for the diagnostic candidate validation included infected lesions with sporulation or no sporulation collected from field samples (**Table 2**), mycelia from other oomycetes (**Table 2**), and uninfected plant tissue grown in the laboratory (**Table 3**). Samples were stored in microcentrifuge tubes in liquid nitrogen for long-term preservation.

Example 2. RNA and DNA extraction, library preparation, and sequencing.

In preparation for RNA and DNA extraction, $50 \mu\text{l}$ of sporangial suspension from each isolate was collected in a microcentrifuge tube with $500 \mu\text{m}$ glass beads (Sigma, St. Louis, Missouri) and 1 mg polyvinyl polypyrrolidone (PVPP). Infected lesions, uninfected plant tissue, and other oomycete

35 tissues were each collected into microcentrifuge tubes with three 2.3 mm Zircon beads (Biospec, Bartlesville, Oklahoma), 50 mg $500 \mu\text{m}$ glass beads (Sigma), and 1 mg PVPP. All tissues for RNA and DNA extraction were lysed using an OMNI International Bead Ruptor (OMNI International, Tulsa, Oklahoma). RNA was extracted using the Qiagen Plant RNeasy® Kit (Qiagen, Valencia, California) with an on-column DNase digestion using the Qiagen DNase Digest (Qiagen), both

according to manufacturer's instructions. RNA was eluted with 30 to 100µl DEPC-treated water and re-precipitated using 1/10 volume of 3M sodium acetate and two volumes of 96% ethanol, washed with 500µl 80% ethanol and re-suspended with 30-100µl DEPC-treated water. DNA extractions were completed using 500µl SDS extraction buffer (200 mM Tris-HCl pH8, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) followed by one 500µl phenol and two 500µl chloroform extractions. DNA was precipitated with 0.5 volume of 100% isopropanol, washed with 500µl 80% ethanol, suspended in 100µl sterile distilled water, then re-precipitated with sodium acetate and ethanol as described above, and re-suspended in sterile 30 to 100µl distilled water. RNA samples for sequencing were quantified using the Qubit® RNA HS assay (Life Technologies, Grand Island, New York), DNA samples for sequencing using the Qubit® DNA BR assay (Life Technologies), and DNA samples for the diagnostic screen were quantified using a Nanodrop 1000 (Thermo Fisher Scientific, Waltham, Massachusetts). Total RNA was checked for integrity and quality using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, California). DNA quality assessment was based on gel electrophoresis on a 1% agarose gel containing 0.2µg/ml ethidium bromide, followed by detection with a Biorad Geldoc™ Imager using Quantity One® software (Biorad, Hercules, California).

High quality total RNA and DNA were submitted to the Michigan State University Research Technology Support Facility (MSU-RTSF) for Illumina Truseq® RNA (350bp insert size) and DNA (500bp insert size) library preparation (Illumina, San Diego, California). RNA and DNA libraries were barcoded and multiplexed for 50bp single-end RNA-seq and 100bp paired-end DNA-seq, respectively. All samples were run on an Illumina HiSeq® 2500 (Illumina, San Diego, California) and base calling and quality values were determined using the Illumina software as part of the service provided by the MSU-RTSF.

Example 3. Identification of candidate diagnostic markers and primer design

RNA-seq and DNA-seq reads were analyzed for quality using FastQC (v. 0.10.1) (Andrews, 2012). Cutadapt (v. 1.8.1) (Martin, 2012) and FASTX-Toolkit (v. 0.0.13) (Hannon, 2010) were used to remove adaptors and trim low quality sequences from the dataset, respectively. Due to the obligate nature of *P. cubensis* and *P. humuli*, one may find plant contamination in the RNA and DNA samples. Thus, to determine the level of plant contamination in the samples RNA-seq reads were aligned with the *Cucumis sativus* (cucumber) genome (Huang et al., 2009) using Bowtie2 short read aligner (v. 2.1.0) (Langmead et al., 2009). To identify diagnostic candidates, RNA-seq reads from *P. cubensis* and *P. humuli* were aligned to the *P. cubensis* draft genome (Savory et al., 2012a; Savory et al., 2012b) using Bowtie2 short read aligner (v.2.1.0). The number of aligned reads in each exon of the *P. cubensis* draft genome was quantified using *htseq-count* from HTSeq (v. 0.6.1) (Anders et al., 2015) with default parameters. Exons in the *P. cubensis* draft genome with at least two mapped reads from *P. cubensis* RNA-seq samples and less than two mapped reads from *P. humuli* RNA-seq samples were identified and selected using Perl and Bash programming. Exons present in all *P. cubensis* RNA-seq samples (≥ 2 mapped reads) but absent in all *P. humuli* RNA-seq samples (< 2 mapped reads) were matched to specific genes using the *P. cubensis* draft genome GFF3 file coordinates (Savory et al., 2012a). To further reduce the number of diagnostic candidates to

validate with PCR, DNA-seq data was aligned in single end mode to the *P. cubensis* draft genome using Bowtie2 (v. 2.1.0) (Langmead et al., 2009) and mapped reads were quantified using *htseq-count* from HTSeq (v. 0.6.1) (Anders et al., 2015). Candidates were eliminated if two or more DNA-seq reads from *P. humuli* were present in the candidate exon. Approximately 50% of the remaining candidates were validated by PCR against a larger selection of lesions infected with *P. cubensis* or *P. humuli*, other oomycete isolates (Table 2), and host plants (Table 3) to eliminate candidates that occur infrequently, or that are present in more distantly related oomycetes, or in the host (Figure 2). To determine if candidate diagnostic markers were in putative single copy genes, a self-BLASTP (Altschul et al., 1990) analysis of the *P. cubensis* predicted proteome (Savory et al., 2012a) using an E-value cutoff of $1e^{-10}$ was performed. Candidates were considered single copy if protein sequences had only one match to the proteome. Since several candidate diagnostic markers were annotated as genes of unknown function, a BLASTX analysis against *Phytophthora infestans*, *Phytophthora capsici* and *Hyaloperonospora arabidopsis* proteins was performed to improve gene functional annotation. The collection of protein sequences were downloaded from NCBI on 3 June 2015 and an E-value cutoff of $1e^{-05}$ was used to define homologous sequences. To further determine that candidates were specific to *P. cubensis*, gene sequences of the diagnostic candidates were used to perform a BLASTN (Altschul et al., 1990) search against the NCBI nucleotide collection (nr/nt) (non-redundant nucleotide) database using default parameters to retain candidates with no significant sequence similarity to other organisms for laboratory validation.

Example 4. Validation of diagnostic candidates

Fasta sequences of genes containing candidate diagnostic exons and the 200 bp region flanking each gene were selected using *extracseq* from EMBOSS (v. 6.5.7). Primers were designed for *P. cubensis* diagnostic candidates selected for validation within 200 bp 5' and 3' end of the candidate gene using CLC Genomics Workbench (CLCbio, Boston, Massachusetts) and IDT Oligo Analyzer (Integrated DNA Technologies, Coralville, Iowa). The *P. cubensis* draft genome and the RNA-seq and DNA-seq read alignments were imported into *RNA-seq tools* of the CLC Genomics Workbench to identify primers flanking diagnostic candidates. Primers were designed to produce product sizes between 200 and 1,200bp with an annealing temperature of 55 to 57°C, and were ordered through IDT (Integrated DNA Technologies). The PCR reactions, completed according to manufacturer's guidelines, contained 1X Promega GoTaq® Green Master Mix (Promega, Durham, North Carolina), 10µM of forward and reverse primers, and 10µM DNA, and were amplified with a program starting with 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, with a final elongation step of 72°C for 5 minutes. Products were analyzed by gel electrophoresis on a 2% agarose gel containing 0.2µg/ml ethidium bromide, followed by detection with a Biorad Geldoc™ Imager using Quantity One® software (Bio-Rad, Hercules, California). Product sizes were estimated by comparison to the Life Technologies 100bp DNA ladder. Diagnostic candidates with product sizes similar in *P. cubensis* and *P. humuli* samples, as well as any that were amplified in any of the plant hosts or other oomycetes screened were

eliminated. Candidates remaining after at least three validation iterations were considered highly specific diagnostic molecular markers for *P. cubensis* (e.g., **SEQ ID NOs:1-52**).

Example 5. Identification of diagnostic markers

The approach used in our study successfully identified seven diagnostic markers for *P. cubensis* by using comparative genomics with the closely related species *P. humuli*, a previously published *P. cubensis* draft genome assembly (Savory et al., 2012b), minimal additional genomic data, and validation of diagnostic candidates with diverse samples (Figure 2).

A range of 11,942,671 to 29,304,711 million of RNA-seq and 36,599,100 million of DNA-seq reads were obtained and checked for quality (Table 4). In general, only 0.21 to 0.49% of reads were removed from RNA-seq samples and 40% from DNA-seq samples due to low quality and plant contamination (Table 4). High quality RNA-seq and DNA-seq reads aligned to the *P. cubensis* draft genome confirmed that the majority of sequences were from the pathogens (Table 4). The highest and lowest percentages of total aligned RNA-seq reads for *P. cubensis* samples to the *P. cubensis* genome were 95% and 79.9%, respectively. For *P. humuli* samples, the highest and lowest percentage of total aligned RNA-seq reads to the *P. cubensis* draft genome was 92.3% and 66.9%, respectively, and 97.5% and 96% was the highest and lowest total alignment percentage for DNA-seq reads. A small percentage of RNA-seq reads of *P. cubensis* (0.5% to 6.7%) and *P. humuli* (0.5% to 7.5%) isolates aligned to the *C. sativus* reference genome. Similarly, a low percentage (12 to 13%) of DNA-seq reads from a single *P. humuli* isolate aligned to the *C. sativus* reference genome (Table 4). The total *P. cubensis* and *P. humuli* RNA-seq and DNA-seq reads aligned to the *P. cubensis* draft genome and the *C. sativus* reference genome for each sample were sorted in uniquely mapped reads and multiple mapped reads to further assess read quality. A range of 92% to 96% of uniquely mapped reads was observed, confirming the good quality of the RNA-seq reads (Fig. 6).

An analysis of exon expression performed on mapped RNA-seq reads indicated that 2,696 *P. cubensis* exons were expressed in all *P. cubensis* isolates, 649 *P. cubensis* exons were expressed in all *P. humuli* isolates, and 19,275 *P. cubensis* exons were expressed in all sequenced isolates of both species. Moreover, 762 and 1,134 *P. cubensis* exons were found to be not expressed in any *P. cubensis* and *P. humuli* isolates, respectively, and 13,612 *P. cubensis* exons were not expressed in isolates of either species. A total of 70 *P. cubensis* exons were identified from 51 genes that were expressed in all *P. cubensis* isolates and not in any *P. humuli* isolates sequenced (Fig. 3, Table 6). Self-BLASTP analyses revealed that six of the 51 candidate diagnostic genes were putative single copy genes (Table 6). Of the 51 identified genes, 29 were annotated as genes or proteins of unknown function. The remaining 22 genes had functional annotations related to virulence (elicitors, elicitors, NPP1 and CRN proteins) and signaling, among others. BLAST® searches performed against a protein database from *P. infestans*, *P. capsici* and *H. arabidopsidis* allowed annotation of three more genes. These three genes were putatively identified as involved in functions related to virulence (c27478.0e0) (**SEQ ID NO:20**), glycolysis (c30239.0e0), and chromatin remodeling (c2555.4e5) (**SEQ ID NO:18**) (Table 6). Candidate c2163.6e5 (**SEQ ID NO:12**) had 78%

identity (38% query cover and $5e^{-87}$ E value) with a *Phytophthora sojae* hypothetical protein (XM_009528096.1), c31609.0e0 (**SEQ ID NO:25**) had 93% identity (17% query cover and $2e^{-06}$ E value) with an *Aphanomyces invadans* protein kinase (XM_008871021.1), and c6944.1e0-1 (**SEQ ID NO:46**) had 89% identity (24% query cover and $8e^{-65}$ E value) with a *Phytophthora parasitica* hypothetical protein (XM_008912304.1).

Validation of diagnostic candidates

An additional filtering step using HTSeq and DNA-seq data from a single *P. humuli* isolate allowed us to eliminate 29 *P. cubensis* exons in 21 genes that were not present in the RNA-seq data of *P. humuli* but were present at the DNA level (**Table 6**). Of the remaining 30 genes determined to contain 41 exons unique to *P. cubensis* after bioinformatics analyses, a subset of 16 genes containing 26 exons (about 50% of candidates) were chosen for PCR validation with 96 diverse isolates (**Table 2**). An initial screening with 15 *P. cubensis* and nine *P. humuli* samples eliminated 15 candidate exons in eight genes that had a product in *P. humuli* samples indistinguishable in size from the PCR product obtained in *P. cubensis* samples. The final screening for the remaining 11 exons in eight genes included 49 *P. cubensis*, 34 *P. humuli*, five *P. belbahrii*, three *P. obducens*, six *Phytophthora capsici* and 8 *Phytophthora infestans* samples or isolates (**Table 2**). Eight plant hosts were also included in the validation due to the obligate nature of *P. cubensis* and comprised cucumber, cantaloupe, butternut squash, acorn squash, pumpkin, watermelon, basil, and hop (**Table 3**). This final screening eliminated 1 exon in 1 gene that had a product in *P. obducens* samples indistinguishable in size from the PCR product obtained in *P. cubensis* samples (**Table 6**). No other candidates presented amplification of a product in other samples that could not be distinguished from *P. cubensis*.

These remaining 10 exons in 7 genes (c10851.1e0, c2555.2e1, c2555.3e7, c3155.4e9, c52.12e13, c572.6e19, and c2183.6e2; **SEQ ID NOs:2, 17, 16, 24, 39, 43, and 13**, respectively) are considered highly specific diagnostic molecular markers for *P. cubensis* that can be used to distinguish it from its sister species *P. humuli* (**Table 5, Fig. 4**). Two (c2555.2e1 (**SEQ ID NO:17**) and c2555.3e7 (**SEQ ID NO:16**)) of these genes were found to be putative single copy genes through self-BLASTP analyses (**Table 5, Table 6**). PCR assays with the designed primers and gel electrophoresis of the seven final diagnostic candidates revealed that three candidates (c2555.2e1 (**SEQ ID NO:17**), c2555.3e7 (**SEQ ID NO:16**), and c10851.1e0 (**SEQ ID NO:2**)) did not produce a PCR product in any *P. humuli* samples tested, while four candidates (c52.12e13 (**SEQ ID NO:39**), c572.6e19 (**SEQ ID NO:43**), c2183.6e2 (**SEQ ID NO:13**), and c3155.4e9 (**SEQ ID NO:24**)) produced a PCR product of different size in some or all of the *P. humuli* samples tested. All diagnostic candidates successfully detected *P. cubensis* in hosts such as watermelon, cantaloupe, and buffalo gourd that typically have lower pathogen sporulation (**Table 2, Fig. 5**). Interestingly, one diagnostic candidate (c2555.3e7) (**SEQ ID NO:16**) with unknown function had polymorphism among *P. cubensis* samples tested depending on the host. Samples from infected watermelon, pumpkin, and butternut squash had a larger PCR product than those from cucumber, cantaloupe, and buffalo gourd.

Example 6

Next Generation Sequencing allows for development of sequence-based, culture-independent diagnostics of pathogens (Pallen et al., 2010). A recent study used genomics approaches to develop diagnostics for obligate pathogens of humans such as *Parachlamydia acanthamoebae* (Greub et al., 2009). In that study, a draft genome sequence of the emergent pathogen *P. acanthamoebae* was obtained and used in combination with proteomics to identify immunogenic proteins and develop a serological diagnostic assay (Greub et al., 2009). However, there have been few examples of genomics-enabled diagnostics development for plant pathogens (Studholme et al., 2011; De Boer and Lopez, 2012), and most of these studies have been applied to either bacterial (Lang et al., 2010) or viral pathogens (Adams et al., 2009; Studholme et al., 2011). In this study, we used a comparative genomics strategy to develop species-specific molecular diagnostics for the obligate oomycete *P. cubensis*, a devastating downy mildew pathogen of cucurbit crops (Savory et al., 2011).

A draft *P. cubensis* genome was available for this study; however, this genome is not of finished quality (N50 contig size of 4.0 Kbp) (Savory et al., 2012a; Savory et al., 2012b). Nonetheless, our study highlights the usefulness of genomic data even in unfinished format for marker development, especially for obligate pathogens where genomic resources are scarce and obtaining adequate amounts of DNA for laboratory testing may be difficult (Baxter et al., 2010; Links et al., 2011). The RNA-seq data allowed us to identify diagnostic candidates with differential expression between *P. cubensis* and *P. humuli*, but conserved within each species, and thus, robust across isolates of these two frequently indistinguishable pathogens. The DNA-seq data allowed confirmation of presence or absence of an exon or a gene at the DNA level in *P. humuli*, since no genomic data is available for this pathogen. Prior knowledge of the genetic structure of *P. cubensis* and *P. humuli* (Mitchell et al., 2011; Quesada-Ocampo et al., 2012) informed our selection of diverse samples for sequencing and likely improved the efficient elimination of unsuitable candidates and increased validation success. The close relatedness between *P. cubensis* and *P. humuli* allowed for rapid identification of lineage-specific regions in *P. cubensis* that can be used for diagnostics, highlighting the importance of resolving phylogenetic relationships for development of robust diagnostics (Choi et al., 2005; Mitchell et al., 2011). Interestingly, several of the candidates identified had no significant sequence similarity to other organisms or informative functional annotation, while other candidates had gene functions that suggest that they may be related to stress (virulence), as has been observed for lineage-specific genes in plants (Campbell et al., 2007; Lin et al., 2010).

Since the diagnostic candidates were selected based on absence of an exon or a complete gene in *P. humuli*, we show that diagnostic assays can be implemented as conventional PCR-based diagnostics that detect presence of product, product size differences, and or potentially with serological assays. In this study, we pursued validation of diagnostic candidates through PCR methods since such assays can be readily adopted by Plant Disease Clinics as well as extension personnel, while serological methods frequently require adoption and commercialization (De Boer and Lopez, 2012). Nonetheless, since some of the diagnostic candidates identified in this study are

present in *P. cubensis* but completely absent in *P. humuli* at the DNA level and potentially the protein level, they could be used for future development of serological-based diagnostics once monoclonal antibodies are developed for the *P. cubensis*-specific protein. A clear advantage of serological diagnostics is that it can be incorporated in field-friendly formats that can be used directly by growers, extension agents, and agricultural consultants, as is the case with the Phytophthora ImmunoStrip® (Adgia, Elkhart, Indiana) (De Boer and Lopez, 2012).

Accurate and timely identification of plant pathogens is the first step towards successful mitigation of crop diseases (De Boer and Lopez, 2012). In cucumber, downy mildew symptoms have very characteristic angular leaf spots delimited by leaf veins and profuse sporulation on the underside of the leaf, making diagnosis simple by visual means once the disease has >30% of severity on a leaf (Holmes et al., 2015) (see also, **Fig. 1**). However, diagnosis during early stages of infection when fungicide applications would be most effective, or in hosts such as watermelon where symptoms are nondescript, can be difficult and delay disease mitigation efforts (Holmes et al., 2015). Recently, *P. cubensis* was also reported to be seed borne as is the case with other downy mildew pathogens such as *P. belbahrii* (Cohen et al., 2014). The contribution of seed borne inoculum to yearly cucurbit downy mildew epidemics remains unclear but movement of infected plant material likely plays a role in *P. cubensis* population changes in the US (Cohen et al., 2014; Holmes et al., 2015). Molecular diagnostics could be used for detection of early infections or infected seed in combination with appropriate sampling strategies, which would prevent pathogen introduction into new regions (De Boer and Lopez, 2012). PCR assays with the seven nuclear genes identified in our study successfully detected *P. cubensis* in infected tissue of all plant hosts tested, including watermelon, which typically yields low quantities of pathogen DNA due to small lesions and poor sporulation of the pathogen on this host (**Fig. 5**).

Another common method for detection of oomycetes is the use of spore traps, which is especially useful for monitoring airborne inoculum of downy mildew pathogens since many species are spread via airborne sporangia (Gent et al., 2009; Granke et al., 2013; Klosterman et al., 2014). In spore traps, pathogen sporangia are impacted on adhesive tape or grease covered rods on hourly, weekly, or daily time intervals that can later be stained and counted under a microscope (Jackson and Bayliss, 2011). In Michigan, a defined threshold of sporangia per week is used to determine when it is prudent to begin fungicide sprays for *P. cubensis* since sporangia concentrations are associated with the timing of downy mildew occurrence (Granke et al., 2014). A drawback of volumetric spore traps that has limited their deployment is the time and labor needed to enumerate sporangia (Granke and Hausbeck, 2011), and often morphological characters such as spore type and size overlap between closely related organisms (Runge et al., 2012). Limitations of tape-based spore traps have been addressed in other downy mildew pathogens by combining spore trapping with molecular detection and quantification of the pathogen (Gent et al., 2009; Klosterman et al., 2014). Due to its usefulness in identification of fungi and oomycetes, internal transcribed spacer (ITS) and other ribosomal DNA sequences have become common markers for diagnostics (Schoch et al., 2012), including for many of the downy mildew pathogens (Belbahri et al., 2005; Valsesia et al., 2005; Hukkanen et al., 2006; Gent et al., 2009; Zipper et al., 2009; Montes-Borrego

et al., 2010; Mota et al., 2011; loos et al., 2012; Testen et al., 2013; Feng et al., 2014; Klosterman et al., 2014). However, in closely related species there may be insufficient variability in the ITS (Gent et al., 2009; Klosterman et al., 2014), or copy number variation (Martin, 1990; Belbahri et al., 2008) for useful delineation. Other assays utilizing locked nucleic acid probes and high resolution melt curve analysis can partially overcome this limitation, but add expense to the diagnostics (Summers et al., 2015). Two of the seven diagnostic markers identified in our study are putative single-copy markers (c2555.2e1 and c2555.3e7), making them attractive candidates for developing real-time PCR assays for *P. cubensis* inoculum monitoring efforts. Candidates c2555.2e1 and c2555.3e7 were also two of the three candidates that had no amplification in any *P. humuli* samples tested under the PCR conditions and using the primers designed in this study (Fig. 5).

Beyond the traditional use of plant disease diagnostics for agricultural decision-making, the need for rapid diagnostics has dramatically increased due to globalization of agricultural products (De Boer and Lopez, 2012). Losses due to invasive plant pathogens could be reduced if improved disease detection methods were available (De Boer and Lopez, 2012). For obligate parasites, such as downy mildew pathogens, development and validation of molecular assays may be complicated by lack of available genetic material, minimal sequence data to identify diagnostic markers, and unresolved phylogenetic status within the different genera (Thines et al., 2009). NGS and bioinformatics approaches allowed us to develop diagnostic markers that can differentiate *P. cubensis* from *P. humuli*. The approach detailed in this study also may also be amenable to identifying strains or pathotypes of *P. cubensis*. Similar approaches can also be applied to other plant pathogens with scarce genomic resources for rapid development of diagnostic markers.

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Table 1: *Pseudoperonospora cubensis* and *Pseudoperonospora humuli* isolates used for Illumina 50bp single-end RNAseq and 100bp paired-end DNAseq.

Pathogen species	Isolate	Host	Location	Year collected	Source
<i>Pseudoperonospora cubensis</i>	CA08A1	<i>Cucumis sativus</i> (Cucumber)	California	2008	Ojiambo
<i>P. cubensis</i>	SC1982	<i>Cucumis melo</i> (Cantaloupe)	South Carolina	1982	Ojiambo
<i>P. cubensis</i>	NC2013C3	<i>Cucurbita maxima</i> (Pumpkin)	North Carolina	2013	Ojiambo
<i>P. cubensis</i>	SC2013F2	<i>Cucurbita pepo</i> (Acorn squash)	South Carolina	2013	Ojiambo
<i>P. cubensis</i>	NCA11	Cucumber	North Carolina	2012	Ojiambo
<i>P. cubensis</i>	SCD3	<i>Cucurbita moschata</i> (Butternut squash)	South Carolina	2012	Ojiambo
<i>P. cubensis</i>	NCWAY2-1	Cucumber	North Carolina	2013	Quesada
<i>Pseudoperonospora humuli</i>	NY482CA	<i>Humulus lupulus</i> (Hop)	New York	2011	Gent
<i>P. humuli</i>	JP490-5	<i>Humulus japonicus</i> (Japanese hop)	Aomori, Japan	2012	Gent
<i>P. humuli</i>	JP490	Japanese hop	Aomori, Japan	2012	Gent
<i>P. humuli</i>	JP498SA	Japanese hop	Ehoro, Japan	2012	Gent
<i>P. humuli</i>	OR501BA	Hop	Oregon	2013	Gent
<i>P. humuli</i>	OR502AA*	Hop	Oregon	2013	Gent
<i>P. humuli</i>	VT503A3	Hop	Virginia	2013	Gent
<i>P. humuli</i>	WI510-1	Hop	Wisconsin	2013	Gent

* Isolate also used for DNAseq

Table 2: *Peronospora belbahrii*, *Pseudoperonospora cubensis*, *Pseudoperonospora humuli*, *Plasmopara obducens*, *Phytophthora capsici*, and *Phytophthora infestans* isolates used for validation of *P. cubensis*-specific diagnostic candidates.

Pathogen species or sample	Isolate	Host	Location	Year collected	Source
<i>Peronospora belbahrii</i>	B1	<i>Ocimum basilicum</i> (Basil, sweet)	Clayton, North Carolina	2014	Quesada
<i>P. belbahrii</i>	B2	Basil (Sweet)	Chapel Hill, North Carolina	2014	Quesada
<i>P. belbahrii</i>	B3	Basil (Genovese)	Roxboro, North Carolina	2014	Quesada
<i>P. belbahrii</i>	B4	Basil (Saim queen thai)	Chapel Hill, North Carolina	2014	Quesada
<i>P. belbahrii</i>	B5	Basil (Genovese)	Chapel Hill, North Carolina	2014	Quesada
<i>Pseudoperonospora cubensis</i>	C1	<i>Cucurbita moschata</i> (Butternut squash)	South Carolina	2012	Quesada

<i>P. cubensis</i>	C2	<i>Cucumis sativus</i> (Cucumber)	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C3	Cucumber	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C4	Cucumber	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C5 (L)	<i>Cucumis melo</i> (Cantaloupe)	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C6	<i>Cucurbita pepo</i> (Acorn squash)	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C7	<i>Cucurbita maxima</i> (Pumpkin)	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C8	Butternut squash	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C9 (L)	<i>Citrullus lanatus</i> (Watermelon)	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C10	Butternut squash	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C11	Pumpkin	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C12	Cucumber	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C13 (L)	Cantaloupe	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C14	Cucumber	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C15	Cucumber	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C16	Pumpkin	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C17	Acorn squash	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C18	<i>Cucurbita foetidissima</i> (Buffalo gourd)	Waynesville, North Carolina	2014	Quesada
<i>P. cubensis</i>	C19	Buffalo gourd	Waynesville, North Carolina	2014	Quesada
<i>P. cubensis</i>	C20	Butternut squash	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C21	Butternut squash	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C22	Butternut squash	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C23 (L)	Cantaloupe	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C24 (L)	Cantaloupe	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C25 (L)	Cantaloupe	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C26	Cucumber	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C27	Cucumber	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C28	Cucumber	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C29	Cucumber	Michigan	2013	Hausbeck

<i>P. cubensis</i>	C30	Cucumber	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C31	Cucumber	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C32	Cucumber	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C33	Cucumber	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C34	Pumpkin	Kinston, North Carolina	2013	Quesada
<i>P. cubensis</i>	C35	Pumpkin	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	C36	Pumpkin	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C37 (L)	Watermelon	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C38 (L)	Watermelon	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C39 (L)	Watermelon	Cleveland, North Carolina	2013	Quesada
<i>P. cubensis</i>	C40 (L)	Watermelon	Waynesville, North Carolina	2013	Quesada
<i>P. cubensis</i>	1938 (L)	Cucumber	New Jersey	2007	Hausbeck
<i>P. cubensis</i>	1933 (L)	Butternut squash	Puerto Rico	2010	Hausbeck
<i>P. cubensis</i>	1155 (L)	Butternut squash	Italy	2003	Hausbeck
<i>P. cubensis</i>	1923 (L)	Cucumber	Italy	2009	Hausbeck
<i>P. cubensis</i>	1841 (L)	Cucumber	Norway	2009	Hausbeck
<i>P. cubensis</i>	1921 (L)	Cucumber	Germany	2007	Hausbeck
<i>P. cubensis</i>	1924 (L)	Cucumber	Netherlands	2001	Hausbeck
<i>P. cubensis</i>	1925 (L)	Cucumber	Spain	2001	Hausbeck
<i>P. cubensis</i>	1927 (L)	Butternut squash	Spain	2003	Hausbeck
<i>Pseudoperonospora humuli</i>	H1	<i>Humulus lupulus</i> (Hop)	Mills River, North Carolina	2014	Quesada
<i>P. humuli</i>	H2	Hop	Mills River, North Carolina	2014	Quesada
<i>P. humuli</i>	H3	Hop	Mills River, North Carolina	2014	Quesada
<i>P. humuli</i>	H4	Hop	Oregon	2012	Gent
<i>P. humuli</i>	H5	Hop	New York	2011	Gent
<i>P. humuli</i>	H6	Hop	New York	2011	Gent
<i>P. humuli</i>	H7	Hop	Virginia	2013	Gent
<i>P. humuli</i>	H8	Hop	Oregon	2013	Gent
<i>P. humuli</i>	H9	Hop	Oregon	2013	Gent

<i>P. humuli</i>	H10	Hop		Japan	2012	Gent
<i>P. humuli</i>	H11	Hop		Japan	2012	Gent
<i>P. humuli</i>	H12	Hop		Mills River, North Carolina	2014	Quesada
<i>P. humuli</i>	H13	Hop		Oregon	2006	Gent
<i>P. humuli</i>	H14	Hop		Oregon	2008	Gent
<i>P. humuli</i>	H15	Hop		Washington	2008	Gent
<i>P. humuli</i>	H16	Hop		Oregon	2008	Gent
<i>P. humuli</i>	H17	Hop		New York	2013	Gent
<i>P. humuli</i>	H18	Hop		New York	2013	Gent
<i>P. humuli</i>	H19	Hop		Virginia	2013	Gent
<i>P. humuli</i>	H20	Hop		Czech Republic	2012	Gent
<i>P. humuli</i>	H21	Hop		New York	2011	Gent
<i>P. humuli</i>	H22	Hop		Oregon	2011	Gent
<i>P. humuli</i>	H23	Hop		Mills River, North Carolina	2014	Quesada
<i>P. humuli</i>	H24	Hop		Mills River, North Carolina	2014	Quesada
<i>P. humuli</i>	H25	Hop		New York	2011	Gent
<i>P. humuli</i>	H26	Hop		Oregon	2013	Gent
<i>P. humuli</i>	H27	Hop		Aomori, Japan	2012	Gent
<i>P. humuli</i>	H28	Hop		Oregon	2013	Gent
<i>P. humuli</i>	H29	Hop		Oregon	2007	Gent
<i>P. humuli</i>	H30	Hop		Oregon	2008	Gent
<i>P. humuli</i>	H31	Hop		Washington	2008	Gent
<i>P. humuli</i>	H32	Hop		New York	2013	Gent
<i>P. humuli</i>	H33	Hop		Washington	2006	Gent
<i>P. humuli</i>	H34	Hop		Oregon	2008	Gent
<i>Plasmopara obducens</i>	I1	<i>Impatiens walleriana</i> (Impatiens)		Michigan	2014	Hausbeck
<i>P. obducens</i>	I2	Impatiens		Michigan	2014	Hausbeck
<i>P. obducens</i>	I3	Impatiens		Michigan	2014	Hausbeck
<i>Phytophthora capsici</i>	P1 (M)	Watermelon		North Carolina	2014	Quesada

<i>P. capsici</i>	P2 (M)	<i>Capsicum annuum</i> (Pepper)	NA	NA	Ristaino
<i>P. capsici</i>	P3 (M)	<i>Cucurbita pepo</i> (Zucchini)	South Carolina	NA	Ristaino
<i>P. capsici</i>	P4 (M)	Watermelon	South Carolina	NA	Ristaino
<i>P. capsici</i>	P5 (M)	Pepper	Michigan	NA	Hausbeck
<i>P. capsici</i>	P6 (M)	Pumpkin	Michigan	NA	Hausbeck
<i>Phytophthora infestans</i>	Pi1 (M)	<i>Solanum tuberosum</i> (Potato)	Pennsylvania	2009	Ristaino
<i>P. infestans</i>	Pi2 (M)	Potato	North Carolina	1994	Ristaino
<i>P. infestans</i>	Pi3 (M)	<i>Solanum lycopersicum</i> (Tomato)	California	1998	Ristaino
<i>P. infestans</i>	Pi4 (M)	Tomato	Pennsylvania	2009	Ristaino
<i>P. infestans</i>	Pi5 (M)	Tomato	North Carolina	2009	Ristaino
<i>P. infestans</i>	Pi6 (M)	Tomato	Virginia	2009	Ristaino
<i>P. infestans</i>	Pi7 (M)	Potato	North Dakota	2010	Ristaino
<i>P. infestans</i>	Pi8 (M)	Potato	North Dakota	2009	Ristaino

(L) Infected lesion sample with low or no sporulation
(M) Mycelia sample, other samples are lesions

Table 3: Plant hosts used for validation of *Pseudoperonospora cubensis*-specific diagnostic candidates.

Host species	Common name	Cultivar
<i>Cucumis sativus</i>	Cucumber	Straight 8
<i>Cucumis melo</i>	Cantaloupe	Hale's Best Jumbo
<i>Cucurbita pepo</i>	Acorn squash	Table Queen
<i>Cucurbita maxima</i>	Pumpkin	Big Max
<i>Cucurbita moschata</i>	Butternut squash	Waltham Butternut
<i>Citrullus lanatus</i>	Watermelon	Mickey Lee
<i>Ocimum basilicum</i>	Basil	Sweet Basil
<i>Humulus lupulus</i>	Hop	Pacific Gem

Table 4: Summary of quality control analysis for *Pseudoperonospora cubensis* and *Pseudoperonospora humuli* samples used for Illumina 50bp single-end RNAseq and 100bp paired-end DNAseq.

Pathogen species	Isolate	Molecule	Sequencing mode	Number of total reads	Number of high quality reads	Reads mapped to <i>Pseudoperonospora cubensis</i> (%)	Reads mapped to <i>Cucumis sativus</i> (%)
<i>Pseudoperonospora cubensis</i>	CA08A1	RNA	SE	23,691,206	23,576,348	92.96	0.49
<i>P. cubensis</i>	SC1982	RNA	SE	22,117,725	22,049,442	93.6	6.24
<i>P. cubensis</i>	NC2013C3	RNA	SE	28,035,867	27,888,243	87.98	0.62
<i>P. cubensis</i>	SC2013F2	RNA	SE	16,956,357	16,886,934	79.89	5.8
<i>P. cubensis</i>	NCA11	RNA	SE	28,101,878	27,962,061	88.33	0.62
<i>P. cubensis</i>	SCD3	RNA	SE	29,304,711	29,218,196	95.08	4.26
<i>P. cubensis</i>	NCWAY2-1	RNA	SE	21,038,800	20,974,369	94.58	6.7
<i>Pseudoperonospora humuli</i>	NY482CA	RNA	SE	15,906,769	15,860,532	91.45	7.54
<i>P. humuli</i>	JP490-5	RNA	SE	20,211,068	20,149,698	92.12	4.58
<i>P. humuli</i>	JP490	RNA	SE	11,942,671	11,913,949	66.91	6.45
<i>P. humuli</i>	JP498SA	RNA	SE	26,977,356	26,866,989	90.01	0.54
<i>P. humuli</i>	OR501BA	RNA	SE	14,429,953	14,399,612	88.53	5.55
<i>P. humuli</i>	OR502AA*	RNA	SE	26,193,148	26,086,672	92.28	0.51
<i>P. humuli</i>	VT503A3	RNA	SE	14,576,962	14,543,704	91.75	5.69
<i>P. humuli</i>	WI510-1	RNA	SE	29,264,282	29,150,378	88.78	0.68
<i>P. humuli</i>	OR502AA_R1*	DNA	PE	36,599,100	21,778,230	97.46	12.02
<i>P. humuli</i>	OR502AA_R2*	DNA	PE	36,599,100	21,778,230	95.99	13.2

* Isolate also used for DNAseq paired-end sequencing but reads were aligned to the *P. cubensis* genome as single-end reads (R1 and R2).**Table 5:** Candidate code, gene identifier, gene functional annotation, primers, and expected product sizes for final *Pseudoperonospora cubensis*-specific diagnostic candidates identified in this study.

Candidate code ^a	Gene identifier	Functional annotation	Forward primer	Reverse primer	Product size
c52.12e13	maker-pcu_contig_52-snap-gene-0.12-	Crinkler CRN family	ATTTCACCTTAGAGTC	CGGCCATCAACTCGT	486

	mRNA-1:exon:13	protein	ACGGC	ATCTC	
c572.6e19	maker-pcu_contig_572-snap-gene-0.6-mRNA-1:exon:19	Gene of unknown function	GGAGACACGACAACA AATGCAG	TGCGCGGCAAGAAT TTAGG	854
c2183.6e2	snap_masked-pcu_contig_2183-abinit-gene-0.6-mRNA-1:exon:2	Conserved gene of unknown function	ATGCAGCGGGTATCC TTCC	CACGAAGAAGCAGG AAATGC	448
c2555.2e1 (S)	maker-pcu_contig_2555-snap-gene-0.2-mRNA-1:exon:1	Gene of unknown function	CGCGCAACCTTGAAT ACCTAC	CACCCGCTCGCAGT AATGAC	831
c2555.3e7 (S)	maker-pcu_contig_2555-snap-gene-0.3-mRNA-1:exon:7	Gene of unknown function	GACGCAACCCAGCAT AACAAG	TGCGCTCGAGTATCT TCTACC	787
c3155.4e9	maker-pcu_contig_3155-snap-gene-0.4-mRNA-1:exon:9	Gene of unknown function	TCATCACCATATCCGC TTCCTG	TGCGTGAATGCTGTG CGAC	595
c10851.1e0	maker-pcu_contig_10851-snap-gene-0.1-mRNA-1:exon:0	NPP1	CAGTTCTCGATTTCCC TCGTC	CTCTACTCACAAGAT CTGCCAC	867

^a (S) Putative single-copy gene according to self-BLASTP analyses

Table 6: Candidate code, gene identifier, exon content, gene functional annotation, validation results, primers, and expected product sizes of genes containing exons expressed in *Pseudoperonospora cubensis* isolates but not in *Pseudoperonospora humuli* isolates.

Candidate code	Gene identifier	Exon	Functional annotation	Functional annotation using an oomycete protein database (D)	Validation results	Forward primer	Reverse primer	Product size
c52.12e13	maker-pcu_contig_52-snap-gene-0.12-mRNA-1:exon:13	13	Crinkler CRN family protein	crinkler (CRN) family protein, partial [Phytophthora infestans T30-4]	Final diagnostic candidate	ATTTCACC TTAGAGTC ACGGC	CGGCCAT CAACTCG TATCTC	486
c572.6e19	maker-pcu_contig_572-snap-gene-0.6-mRNA-1:exon:19	19	Gene of unknown function	Expressed gene of unknown function	Final diagnostic candidate	GGAGACA CGACAACA AATGCAG	TGCGCGG CAAGAAT TTAGG	854
c572.6e20	maker-pcu_contig_572-snap-gene-0.6-mRNA-1:exon:20	20*	Gene of unknown function	Expressed gene of unknown function	*			
c572.6e21	maker-pcu_contig_572-	21*	Gene of unknown function	Expressed gene of unknown function	*			

[illegible]

c11460.0e0 SEQ ID NO:6	maker- pcu_contig_114 60-snap-gene- 0.0-mRNA- 1:exon:0	0	NPP1	NPP1-like protein [Phytophthora infestans T30-4]	Similar product in <i>P. humuli</i>	GGGTGACA AGCAACCA AGAG	AAAAGCT GCCACTC GAGC	858
c12435.0e1 SEQ ID NO:7	maker- pcu_contig_124 35-snap-gene- 0.0-mRNA- 1:exon:1	1	Folate-Biopterin Transporter FBT Family	Folate-Biopterin Transporter (FBT) Family [Phytophthora infestans T30-4]	Similar product in <i>P. humuli</i>	ACTGCGAC GGCATAAA TCG	AACCTAG TGCAACC ACCGC	697
c23803.0e0 SEQ ID NO:14	maker- pcu_contig_238 03-snap-gene- 0.0-mRNA- 1:exon:0	0	Polyprotein	pol protein [Phytophthora infestans]	Similar product in <i>P. humuli</i>	TACGCTTT GTTCCGGA CGC	ACACTTT TCGGTAC CCTTCGC	427
c2555.4e5 SEQ ID NO:18	maker- pcu_contig_255 5-snap-gene- 0.4-mRNA- 1:exon:5	5	Gene of unknown function	chromodomain protein, putative [Phytophthora infestans T30-4]	Similar product in <i>P. humuli</i>	AGATGGTC GAGAAAGAT ACGTGG	ATCCGGG TTGCTAT TGCTTG	783
c2982.0e0 SEQ ID NO:22	maker- pcu_contig_298 2-snap-gene- 0.0-mRNA- 1:exon:0	0	Glycoprotein elicitor	transglutaminase elicitor M81C [Phytophthora infestans]	Similar product in <i>P. humuli</i>	AGGTGCAA TGGTCGAA GTCG	TTCCTTG TCTGACG CCCGTG	618
c2982.0e1 SEQ ID NO:22	maker- pcu_contig_298 2-snap-gene- 0.0-mRNA- 1:exon:1	1*	Glycoprotein elicitor	transglutaminase elicitor M81C [Phytophthora infestans]	*			
c2982.0e2 SEQ ID NO:22	maker- pcu_contig_298 2-snap-gene- 0.0-mRNA- 1:exon:2	2*	Glycoprotein elicitor	transglutaminase elicitor M81C [Phytophthora infestans]	*			
c364.4e2 SEQ ID NO:30	maker- pcu_contig_364- snap-gene-0.4-	2	Hypothetical polyprotein	Expressed gene of unknown function	Similar product in <i>P. humuli</i>	GGGCTTG GCGATTGT GC	CTGAAAC CCCCTC CACCAC	940

	mRNA-1:exon:2										
c5000.0e0 SEQ ID NO:35	maker-pcu_contig_500 0-snap-gene- 0.0-mRNA- 1:exon:0	0(S)	Gene of unknown function	Expressed gene of unknown function	Similar product in <i>P. humuli</i>	TCCACACC AGCTCGAT ACACG	ATTGACA GCAC TTC GGCGG				1160
c5000.0e3 SEQ ID NO:35	maker-pcu_contig_500 0-snap-gene- 0.0-mRNA- 1:exon:3	3*	Gene of unknown function	Expressed gene of unknown function	*						
c5000.0e4 SEQ ID NO:35	maker-pcu_contig_500 0-snap-gene- 0.0-mRNA- 1:exon:4	4*	Gene of unknown function	Expressed gene of unknown function	*						
c5000.0e5 SEQ ID NO:35	maker-pcu_contig_500 0-snap-gene- 0.0-mRNA- 1:exon:5	5*	Gene of unknown function	Expressed gene of unknown function	*						
c8214.1e1 SEQ ID NO:49	maker-pcu_contig_821 4-snap-gene- 0.1-mRNA- 1:exon:1	1	Gene of unknown function	Expressed gene of unknown function	Similar product in <i>P. humuli</i>	ATGCGTCT AAGGGTG CTACG	CAGCAAC GCCAAGC CAAC				584
c8214.1e2 SEQ ID NO:49	maker-pcu_contig_821 4-snap-gene- 0.1-mRNA- 1:exon:2	2*	Gene of unknown function	Expressed gene of unknown function	*						
c8214.1e3 SEQ ID NO:49	maker-pcu_contig_821 4-snap-gene- 0.1-mRNA- 1:exon:3	3*	Gene of unknown function	Expressed gene of unknown function	*						
c10864.0e2	maker-pcu_contig_108	2	Gene of unknown function	Expressed gene of unknown function	In <i>P. humuli</i> DNaseq						

SEQ ID NO:4	64-snap-gene-0.0-mRNA-1:exon:2 maker-pcu_contig_108 64-snap-gene-0.1-mRNA-1:exon:5	5	Gene of unknown function	Expressed gene of unknown function	In <i>P. humuli</i> DNaseq				
SEQ ID NO:3	c10864.1e5								
SEQ ID NO:5	c11281.1e0	0	Multiple inositol polyphosphate phosphatase 1	multiple inositol polyphosphate phosphatase 1, putative [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNaseq				
SEQ ID NO:9	c13498.0e0	0	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNaseq				
SEQ ID NO:10	c13981.0e1	1	Transmembrane protein	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNaseq				
SEQ ID NO:15	c2539.0e0	0	Gene of unknown function	Expressed gene of unknown function	In <i>P. humuli</i> DNaseq				
SEQ ID NO:15	c2539.0e1	1*	Gene of unknown function	Expressed gene of unknown function	*				
SEQ ID NO:19	c26701.0e1	1	Gene of unknown function	Expressed gene of unknown function	In <i>P. humuli</i> DNaseq				

c2955.2e0 SEQ ID NO:21	maker- pcu_contig_295 5-snap-gene- 0.2-mRNA- 1:exon:0	0	Gene of unknown function	putative polyprotein [Phytophthora infestans]	In <i>P. humuli</i> DNAseq			
c2955.2e1 SEQ ID NO:21	maker- pcu_contig_295 5-snap-gene- 0.2-mRNA- 1:exon:1	1*	Gene of unknown function	putative polyprotein [Phytophthora infestans]	*			
c3294.1e2 SEQ ID NO:26	maker- pcu_contig_329 4-snap-gene- 0.1-mRNA- 1:exon:2	2 (S)	Dumpy CG33196-PB	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq			
c34577.0e0 SEQ ID NO:29	maker- pcu_contig_345 77-snap-gene- 0.0-mRNA- 1:exon:0	0	Polyprotein	putative polyprotein [Phytophthora infestans]	In <i>P. humuli</i> DNAseq			
c5000.1e6 SEQ ID NO:34	maker- pcu_contig_500 0-snap-gene- 0.1-mRNA- 1:exon:6	6	Cleavage induced predicted protein	cleavage induced predicted protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq			
c5001.0e0 SEQ ID NO:36	maker- pcu_contig_500 1-snap-gene- 0.0-mRNA- 1:exon:0	0	Gene of unknown function	Expressed gene of unknown function	In <i>P. humuli</i> DNAseq			
c5058.0e0 SEQ ID NO:38	maker- pcu_contig_505 8-snap-gene- 0.0-mRNA- 1:exon:0	0	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq			
c5058.0e1 SEQ ID NO:38	maker- pcu_contig_505 8-snap-gene-	1*	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	*			

	c5058.0e2	0.0-mRNA- 1:exon:1 maker- pcu_contig_505 8-snap-gene- 0.0-mRNA- 1:exon:2	2*	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	*				
	SEQ ID NO:38									
	c5058.1e3	maker- pcu_contig_505 8-snap-gene- 0.1-mRNA- 1:exon:3	3	Gene of unknown function	Expressed gene of unknown function	In <i>P. humuli</i> DNaseq				
	SEQ ID NO:37									
	c5058.1e4	maker- pcu_contig_505 8-snap-gene- 0.1-mRNA- 1:exon:4	4*	Gene of unknown function	Expressed gene of unknown function	*				
	SEQ ID NO:37									
	c5058.1e5	maker- pcu_contig_505 8-snap-gene- 0.1-mRNA- 1:exon:5	5*	Gene of unknown function	Expressed gene of unknown function	*				
	SEQ ID NO:37									
	c5504.2e1	maker- pcu_contig_550 4-snap-gene- 0.2-mRNA- 1:exon:1	1 (S)	Multidrug FOligosaccharidy l-lipid Fpolysaccharide MOP Flippase Superfamily	Multidrug/Oligosaccharid yl-lipid/Polysaccharide (MOP) Flippase Superfamily [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNaseq				
	SEQ ID NO:41									
	c5799.1e2	maker- pcu_contig_579 9-snap-gene- 0.1-mRNA- 1:exon:2	2	Ubiquitin- conjugating enzyme E2	ubiquitin-conjugating enzyme E2, putative [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNaseq				
	SEQ ID NO:44									
	c6122.2e0	maker- pcu_contig_612 2-snap-gene- 0.2-mRNA- 1:exon:0	0	Polygalacturonas e	polygalacturonase [Phytophthora capsici]	In <i>P. humuli</i> DNaseq				
	SEQ ID NO:45									

c6944.1e0 SEQ ID NO:46	maker- pcu_contig_694 4-snap-gene- 0.1-mRNA- 1:exon:0	0	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq		
c6944.1e1 SEQ ID NO:46	maker- pcu_contig_694 4-snap-gene- 0.1-mRNA- 1:exon:1	1*	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	*		
c7215.0e0 SEQ ID NO:47	maker- pcu_contig_721 5-snap-gene- 0.0-mRNA- 1:exon:0	0	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq		
c7215.1e2 SEQ ID NO:48	maker- pcu_contig_721 5-snap-gene- 0.1-mRNA- 1:exon:2	2	Retrotransposon protein unclassified	conserved hypothetical protein [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq		
c7215.1e5 SEQ ID NO:48	maker- pcu_contig_721 5-snap-gene- 0.1-mRNA- 1:exon:5	5*	Retrotransposon protein unclassified	conserved hypothetical protein [Phytophthora infestans T30-4]	*		
c962.4e3 SEQ ID NO:51	maker- pcu_contig_962- snap-gene-0.4- mRNA-1:exon:3	3	Conserved gene of unknown function	hypothetical protein PITG_14255 [Phytophthora infestans T30-4]	In <i>P. humuli</i> DNAseq		
c10522.0e0 SEQ ID NO:1	maker- pcu_contig_105 22-fgenes- gene-0.0- mRNA-1:exon:0	0	Elicitor RAL13E	putative GPI-anchored serine rich elicitor SOL13E-like protein [Phytophthora infestans T30-4]	NA		
c12575.0e0 SEQ ID NO:8	maker- pcu_contig_125 75-snap-gene- 0.0-mRNA-	0	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	NA		

c12575.0e1	1:exon:0	maker- pcu_contig_125 75-snap-gene- 0.0-mRNA- 1:exon:1	1*	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	*				
SEQ ID NO:8										
c2129.2e6	maker- pcu_contig_212 9-snap-gene- 0.2-mRNA- 1:exon:6	6	Conserved gene of unknown function	conserved hypothetical protein [Phytophthora infestans T30-4]	NA					
SEQ ID NO:11										
c2163.6e5	maker- pcu_contig_216 3-snap-gene- 0.6-mRNA- 1:exon:5	5	Gamma- butyrobetaine dioxxygenase	trimethyllysine dioxxygenase, putative [Phytophthora infestans T30-4]	NA					
SEQ ID NO:12										
c27478.0e0	maker- pcu_contig_274 78-snap-gene- 0.0-mRNA- 1:exon:0	0	Gene of unknown function	RxLR effector candidate protein, partial [Hyaloperonospora arabidopsidis Emoy2]	NA					
SEQ ID NO:20										
c30239.0e0	maker- pcu_contig_302 39-snap-gene- 0.0-mRNA- 1:exon:0	0	Gene of unknown function	aldose 1-epimerase, putative [Phytophthora infestans T30-4]	NA					
SEQ ID NO:23										
c31609.0e0	maker- pcu_contig_316 09-snap-gene- 0.0-mRNA- 1:exon:0	0	Protein kinase	protein kinase [Phytophthora infestans T30-4]	NA					
SEQ ID NO:25										
c3294.1e3	maker- pcu_contig_329 8-snap-gene- 0.1-mRNA- 1:exon:3	3	Gene of unknown function	Expressed gene of unknown function	NA					
SEQ ID NO:26										
c33204.0e0	maker- pcu_contig_332	0	Gene of unknown function	Expressed gene of unknown function	NA					

[illegible]

* Redundant exon in the same gene

(D) *Phytophthora capsici* and *Hyaloperonospora arabidopsidis* protein sequences

(S) Putative single-copy gene according to self-BLASTP analyses

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising:

detecting at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, in a sample from the cucurbit plant, thereby diagnosing a *P. cubensis* infection.

2. A method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising:

contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof; and

detecting binding between the oligonucleotide and the polynucleotide, thereby diagnosing a *P. cubensis* infection.

3. A method of diagnosing a *Pseudoperonospora cubensis* infection in a cucurbit plant, comprising:

contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis*, thereby diagnosing the cucurbit plant as having a *P. cubensis* infection.

4. A method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof; and

detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, thereby diagnosing an infection by the type of *P. cubensis* that infects cucumber and cantaloupe.

5. A method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and

detecting whether the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe, thereby diagnosing an infection by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

6. A method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof,

wherein the presence of the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, thereby diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis* that infects cucumber and cantaloupe.

7. A method of diagnosing an infection in a cucurbit plant by a type of *Pseudoperonospora cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof,

wherein the presence of the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe, thereby diagnosing the infection of the cucurbit plant as being by a type of *P. cubensis* that infects cucumber and cantaloupe.

8. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising:
detecting at least one polynucleotide selected from the group of nucleotide sequences of
SEQ ID NOs:1-52, or a fragment thereof, in a sample from a plant or in an environmental
5 sample, wherein the presence of the at least one polynucleotide indicates the presence of *P.*
cubensis and the risk of a *P. cubensis* outbreak.

9. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising:
contacting a sample from a plant or an environmental sample with an oligonucleotide that
10 hybridizes to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID**
NOs:1-52, or a fragment thereof; and
detecting binding between the oligonucleotide and the polynucleotide, wherein the
presence of the polynucleotide or fragment thereof, indicates the presence of *P. cubensis* and
the risk of a *P. cubensis* outbreak.

10. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising:
contacting a sample from a cucurbit plant or an environmental sample with a pair of
oligonucleotides that hybridize to a polynucleotide selected from the group of nucleotide
sequences of **SEQ ID NOs:1-52**, or a fragment thereof, under conditions whereby nucleic acid
20 amplification can occur, thereby producing an amplification product; and
detecting the amplification product, wherein the presence of the amplification product
identifies the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak.

11. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of
25 *P. cubensis* that infects cucumber and cantaloupe, comprising:
contacting a sample from a plant or an environmental sample with an oligonucleotide that
hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not
hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and
detecting whether the nucleotide sequence of **SEQ ID NO:16** and/or the fragment thereof
30 is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ**
ID NO:16, and/or fragment thereof,
wherein the presence of binding between the oligonucleotide and the nucleotide
sequence of **SEQ ID NO:16**, and/or fragment thereof, indicates the presence of the type of *P.*
cubensis that infects cucumber and cantaloupe and the risk of a *P. cubensis* outbreak by the
35 type of *P. cubensis* that infects cucumber and cantaloupe.

12. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of
P. cubensis that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and

detecting whether the nucleotide sequence of **SEQ ID NO:52** and/or the fragment thereof is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and squash and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

13. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof,

wherein the presence of binding between the oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects cucumber and cantaloupe and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects cucumber and cantaloupe.

14. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof,

wherein the presence of binding between the oligonucleotide probe and the amplification product indicates the presence of the type of *P. cubensis* that infects pumpkin, watermelon, and

squash and the risk of a *P. cubensis* outbreak by the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

15. A method of selecting a treatment regimen for a *P. cubensis* infection in a cucurbit plant, comprising:

detecting in a sample from the plant at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof, thereby identifying a *P. cubensis* infection; and

selecting a treatment regimen for the *P. cubensis* infection.

16. A method of selecting a treatment regimen for a *P. cubensis* infection in a cucurbit plant, comprising:

contacting a sample from the plant with an oligonucleotide that hybridizes to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof;

detecting binding between the oligonucleotide and the nucleotide sequence, thereby identifying a *P. cubensis* infection; and

selecting a treatment regimen for the *P. cubensis* infection.

17. A method of selecting a treatment regimen for a *P. cubensis* infection in a cucurbit plant, comprising:

contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis*, thereby identifying a *P. cubensis* infection; and

selecting a treatment regimen for the *P. cubensis* infection.

18. A method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**; and

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe; and

selecting a treatment regimen effective against the type of *P. cubensis* that infects cucumber and cantaloupe.

19. A method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**; and

detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and

selecting a treatment regimen effective against the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

20. A method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from the cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof,

wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe; and

selecting a treatment regimen effective against the type of *P. cubensis* that infects cucumber and cantaloupe.

21. A method of selecting a treatment regimen for an infection in a cucurbit plant by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a cucurbit plant with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product; and

detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof,

wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects pumpkin, watermelon, and squash; and

selecting a treatment regimen effective against the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

22. A method for reducing the risk of a *P. cubensis* outbreak, comprising:

detecting in a sample from a cucurbit plant or in an environmental sample at least one polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52** and/or a fragment thereof, thereby identifying the presence of *P. cubensis* in the sample; and

implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

23. A method for reducing the risk of a *P. cubensis* outbreak, comprising:

contacting a sample from a cucurbit plant or an environmental sample with an oligonucleotide that hybridizes to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof;

detecting binding between the oligonucleotide and the polynucleotide and/or fragment thereof, thereby identifying the presence of *P. cubensis* in the sample; and

implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

24. A method for reducing the risk of a *P. cubensis* outbreak, comprising:

contacting a sample from a cucurbit plant or an environmental sample with a pair of oligonucleotides that hybridize to a polynucleotide selected from the group of nucleotide sequences of **SEQ ID NOs:1-52**, or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis*; and

implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

25. A method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof;

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof, identifies the *P. cubensis* as a type that infects cucumber and cantaloupe; and

implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe, thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe.

26. A method for reducing the risk of a *Pseudoperonospora cubensis* outbreak by a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a plant or an environmental sample with an oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof; and

detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof,

wherein the presence of binding between the oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, identifies the *P. cubensis* as a type that infects pumpkin, watermelon, and squash; and

implementing a treatment regimen against the type of *P. cubensis* that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects pumpkin, watermelon, and squash.

27. A method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* that infects cucumber and cantaloupe, comprising:

contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that

hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof,

wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects cucumber and cantaloupe; and

implementing a treatment regimen against the type of *P. cubensis* that infects cucumber and cantaloupe thereby reducing the risk of an outbreak by *P. cubensis* that infects cucumber and cantaloupe.

28. A method for reducing the risk of a *P. cubensis* outbreak by a *P. cubensis* infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

detecting whether the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, is present by detecting binding between the amplification product and an oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but does not hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof,

wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the *P. cubensis* as the type that infects pumpkin, watermelon, and squash; and

implementing a treatment regimen against the type of *P. cubensis* that infects pumpkin, watermelon, and squash, thereby reducing the risk of an outbreak by *P. cubensis* that infects pumpkin, watermelon, and squash.

29. The method of any one of claims 1 to 21, further comprising: implementing a treatment regimen for a *P. cubensis* infection or outbreak.

30. The method of any one of claims 1 to 3, 8 to 10, 15 to 17, 22 or 24 wherein the at least one polynucleotide or the polynucleotide is selected from the group of nucleotide sequences of **SEQ ID NO:2**, **SEQ ID NO:13**, **SEQ ID NO:16**, **SEQ ID NO:17**, **SEQ ID NO:24**, **SEQ ID NO:39**, **SEQ ID NO:43**, or a fragment thereof.

31. A method of selecting a cucurbit plant comprising at least one gene that confers resistance to *P. cubensis*, comprising:

introducing into the cucurbit plant at least one nucleotide sequence selected from the group of

(a) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof;

(b) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of (a);

(c) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any of (a) and/or (b), or a complement thereof; and

5 (d) a nucleotide sequence that differs from the nucleotide sequence of any of (a) to (c) above due to the degeneracy of the genetic code, wherein the expression of the at least one nucleotide sequence produces a hypersensitive response in a cucurbit plant comprising a gene that confers resistance to *P. cubensis*, thereby identifying the cucurbit plant as comprising a gene that confers resistance to *P. cubensis*; and

10 selecting the plant having the hypersensitive response and identified as comprising the gene that confers resistance to *P. cubensis*.

32. The method of claim 31, wherein the at least one nucleotide sequence is in an expression vector.

15 33. A method of producing a plant having increased resistance to *P. cubensis*, comprising:

(a) selecting a cucurbit plant according to claim 31 or 32;

(b) crossing the selected plant with a second cucurbit plant; and

20 (c) selecting progeny having increased resistance to *P. cubensis*, thereby producing a plant having increased resistance to *P. cubensis*.

34. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof;

25 (b) a nucleotide sequence having at least about 80% sequence identity to the nucleotide sequence of (a);

(c) a nucleotide sequence which anneals under stringent hybridization conditions to the nucleotide sequence of any of (a) and/or (b), or a complement thereof;

30 (d) a nucleotide sequence that differs from the nucleotide sequence of any of (a) to (c) above due to the degeneracy of the genetic code; and

(e) a fragment of a nucleotide sequence of any of (a) to (d) above.

35 35. A recombinant nucleic acid construct comprising at least one nucleotide sequence of claim 34.

36. The recombinant nucleic acid construct of claim 35, wherein the at least one nucleotide sequence is operatively linked to a heterologous promoter.

37. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence encoded by a nucleotide sequence of any one of **SEQ ID NOs:1-52**, or a complement thereof; and

(b) an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of (a); and a fragment of any of (a) and/or (b).

38. A polyclonal or monoclonal antibody specifically reactive with the isolated polypeptide of claim 35.

39. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or from an environmental sample with an oligonucleotide that hybridizes to the isolated nucleic acid molecule of claim 34, and/or with the antibody of claim 38; and

detecting whether the isolated nucleic acid molecule, or fragment thereof, is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, or fragment thereof, and/or

detecting whether a polypeptide encoded by the isolated nucleic acid molecule, or fragment thereof, is present by detecting binding between the antibody and the polypeptide, wherein the presence of the isolated nucleic acid molecule and/or the polypeptide indicates the presence of *P. cubensis* in the sample and the risk of a *P. cubensis* outbreak.

40. A method for determining a risk of a *Pseudoperonospora cubensis* outbreak, comprising: contacting a sample from a cucurbit plant or an environmental sample with a pair of oligonucleotides that hybridize to the isolated nucleic acid molecule of claim 34, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

detecting the amplification product, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the presence of *P. cubensis* and the risk of a *P. cubensis* outbreak.

41. A method of selecting a treatment regimen for a *P. cubensis* infection of a cucurbit plant, comprising:

contacting a sample from the cucurbit plant with an oligonucleotide that hybridizes to the isolated nucleic acid molecule of claim 34, and/or with the antibody of claim 38;

detecting whether the isolated nucleic acid molecule and/or a fragment thereof is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule and/or a fragment thereof is present by detecting binding between the antibody and the polypeptide, thereby

identifying a *P. cubensis* infection when the presence of the isolated nucleic acid molecule and/or polypeptide is detected; and

selecting a treatment regimen for the *P. cubensis* infection in the cucurbit plant.

5 42. A method of selecting a treatment regimen for a *P. cubensis* infection of a cucurbit plant, comprising:

contacting a sample from the cucurbit plant with a pair oligonucleotides that hybridize to the isolated nucleic acid molecule of claim 34, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

10 detecting the amplification product, wherein the presence of binding between the oligonucleotide probe and the amplification product identifies the presence of *P. cubensis* in the sample; and

selecting a treatment regimen for the *P. cubensis* infection.

15 43. A method for reducing the risk of a *P. cubensis* outbreak, comprising:

contacting a sample from a cucurbit plant or from an environmental sample with an oligonucleotide that hybridizes to the isolated nucleic acid molecule of claim 34 and/or with the antibody of claim 38;

20 detecting whether the isolated nucleic acid molecule, and/or a fragment thereof, is present by detecting binding between the oligonucleotide and the isolated nucleic acid molecule, and/or detecting whether a polypeptide encoded by the isolated nucleic acid molecule, and/or a fragment thereof, is present by detecting binding between the antibody and the polypeptide, wherein the presence of the isolated nucleic acid molecule and/or the polypeptide identifies the presence of *P. cubensis*; and

25 implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

44. A method for reducing the risk of a *P. cubensis* outbreak, comprising:

30 contacting a sample from a cucurbit plant or an environmental sample with a pair oligonucleotides that hybridize to the isolated nucleic acid molecule of claim 34, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

detecting the amplification product, wherein the presence of the amplification product identifies the presence of *P. cubensis* in the sample; and

35 implementing a treatment regimen for a *P. cubensis* infection, thereby reducing the risk of a *P. cubensis* outbreak.

45. A method of distinguishing between a type of *P. cubensis* that infects cucumber and cantaloupe and a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a cucurbit plant or from an environmental sample with a first oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:16** and/or a fragment thereof, but not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and/or a second oligonucleotide that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, but not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof;

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the second

oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof; and

identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the first oligonucleotide and the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the second oligonucleotide and the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash in the sample.

46. A method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

contacting a sample from a cucurbit plant or from an environmental sample with a first antibody that hybridizes to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and not to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, or fragment thereof, and/or with a second antibody that hybridizes to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, and not to a polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, or fragment thereof;

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the first antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof is present by detecting binding between the second antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52** and/or fragment thereof; and

identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the first antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the second antibody and the polypeptide encoded by the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof,

thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

47. A method of distinguishing a type of *P. cubensis* that infects cucumber and cantaloupe from a type of *P. cubensis* that infects pumpkin, watermelon, and squash, comprising:

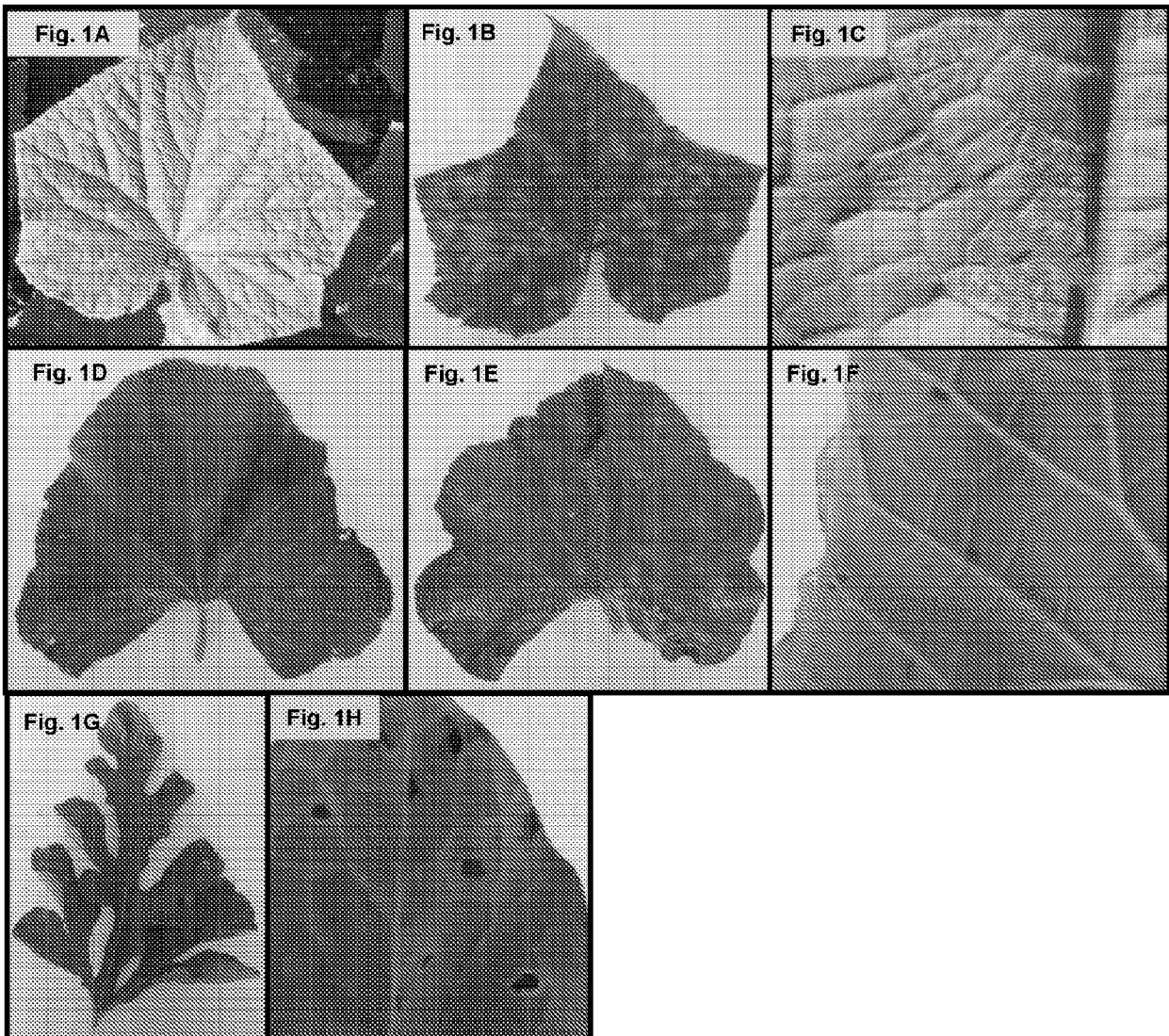
contacting a sample from a plant or an environmental sample with a pair of oligonucleotides that hybridize to the nucleotide sequence of **SEQ ID NO:16**, and/or a fragment thereof, and to the nucleotide sequence of **SEQ ID NO:52**, and/or a fragment thereof, under conditions whereby nucleic acid amplification can occur, thereby producing an amplification product;

detecting whether the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, is present by detecting binding between the amplification product and a first oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:16**, and/or fragment thereof, and not to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and/or whether the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, is present by detecting binding between the amplification product and a second oligonucleotide probe that hybridizes to the nucleotide sequence of **SEQ ID NO:52**, and/or fragment thereof, and not to the nucleotide sequence of **SEQ ID NO:16** and/or fragment thereof; and

identifying a type of *P. cubensis* that infects cucumber and cantaloupe when binding is detected between the amplification product and the first oligonucleotide probe, and identifying a type of *P. cubensis* that infects pumpkin, watermelon, and squash when binding is detected between the amplification product and the second oligonucleotide probe, thereby distinguishing the type of *P. cubensis* that infects cucumber and cantaloupe from the type of *P. cubensis* that infects pumpkin, watermelon, and squash.

48. The method of any one of claims 1-33, or 39-47, wherein the cucurbit plant is a wild cucurbit, a cucurbit breeding line, or a commercial cucurbit line.

49. The method of any one of claims 1-33, or 39-47, wherein the cucurbit plant is a *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp., *Lagenaria* spp., or *Luffa* spp., optionally a cucumber plant, a watermelon plant, a squash plant, a pumpkin plant, and/or a cantaloupe plant.



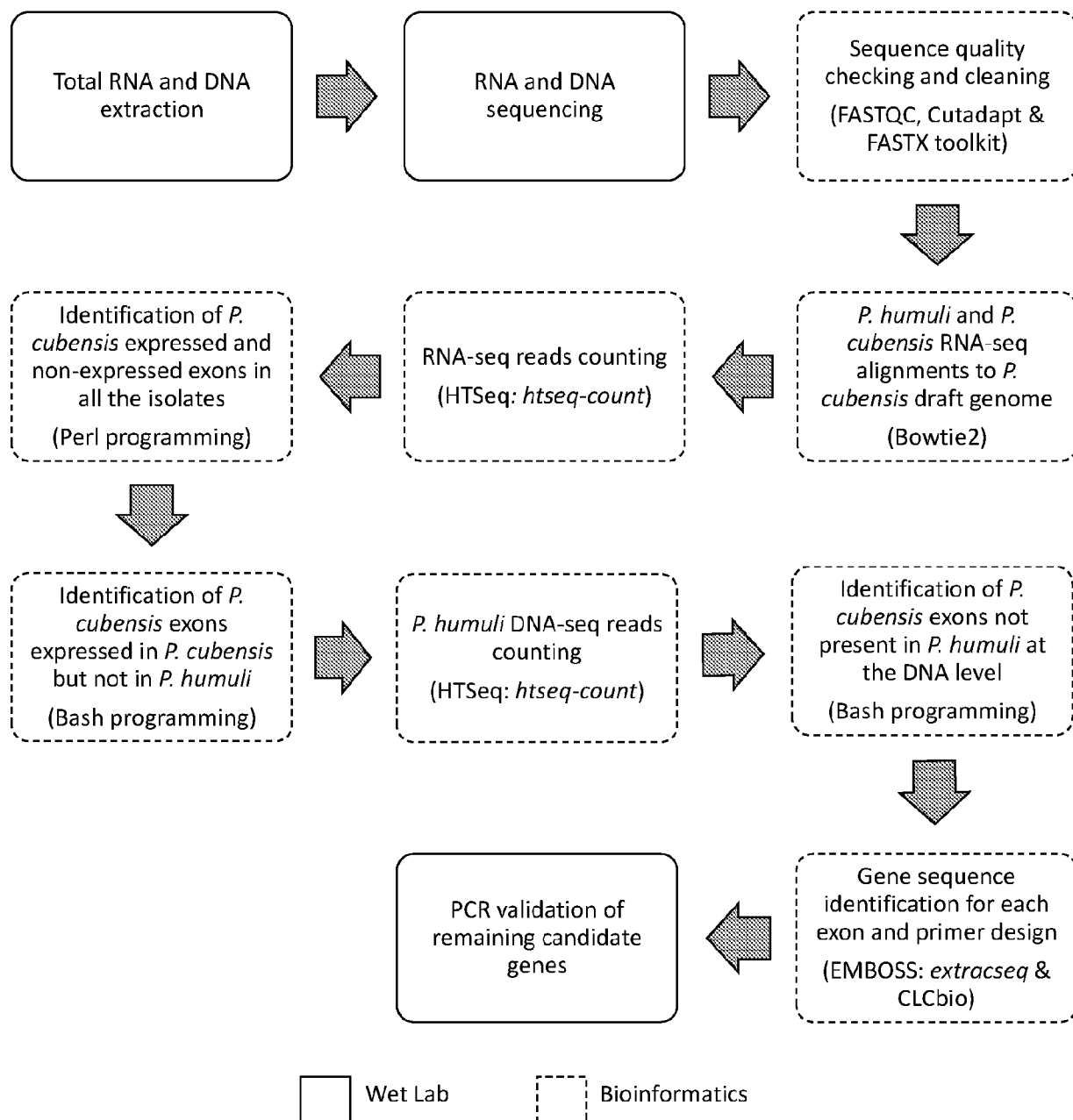


Fig. 2

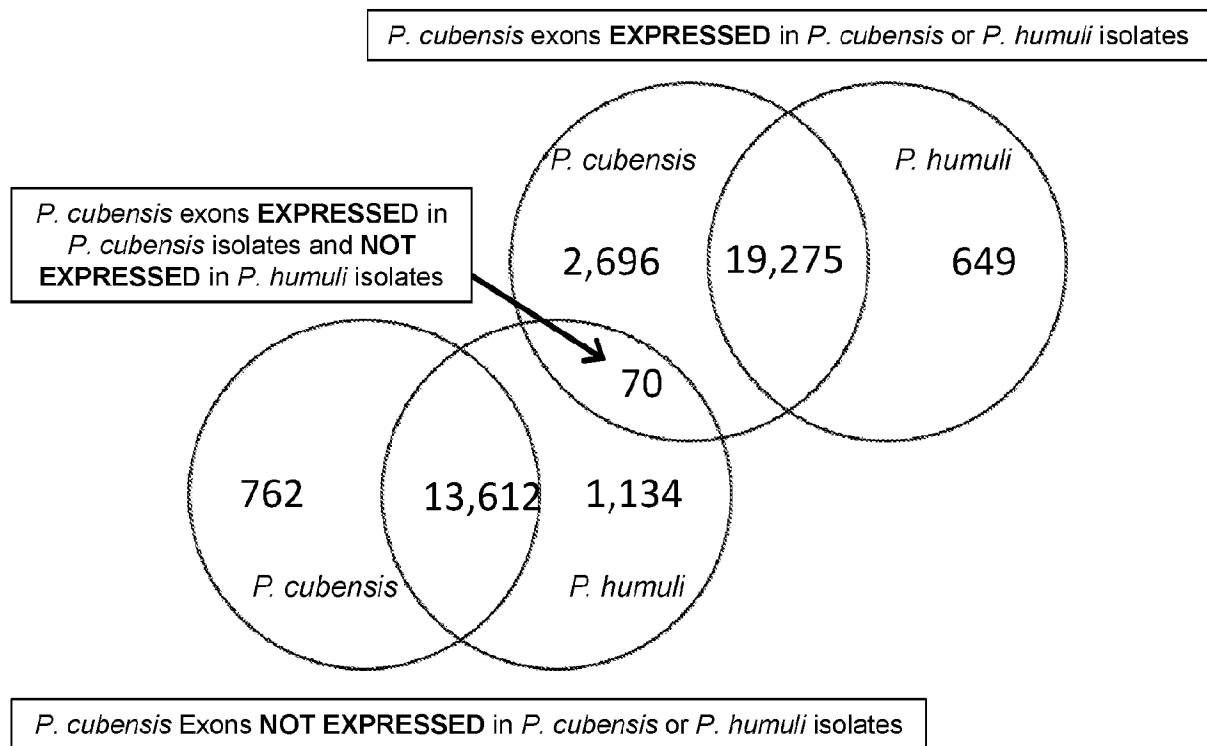


Fig. 3

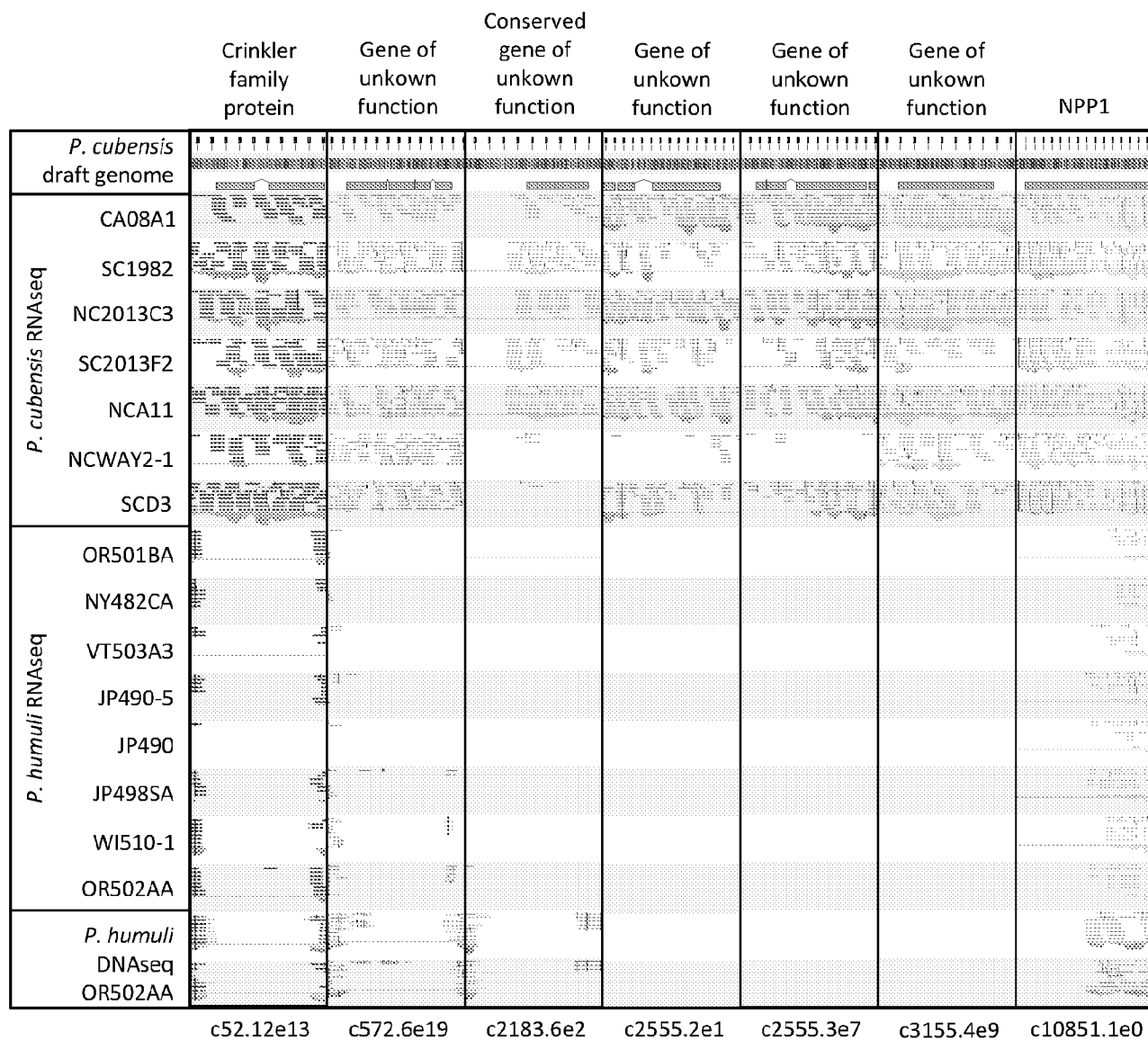


Fig. 4

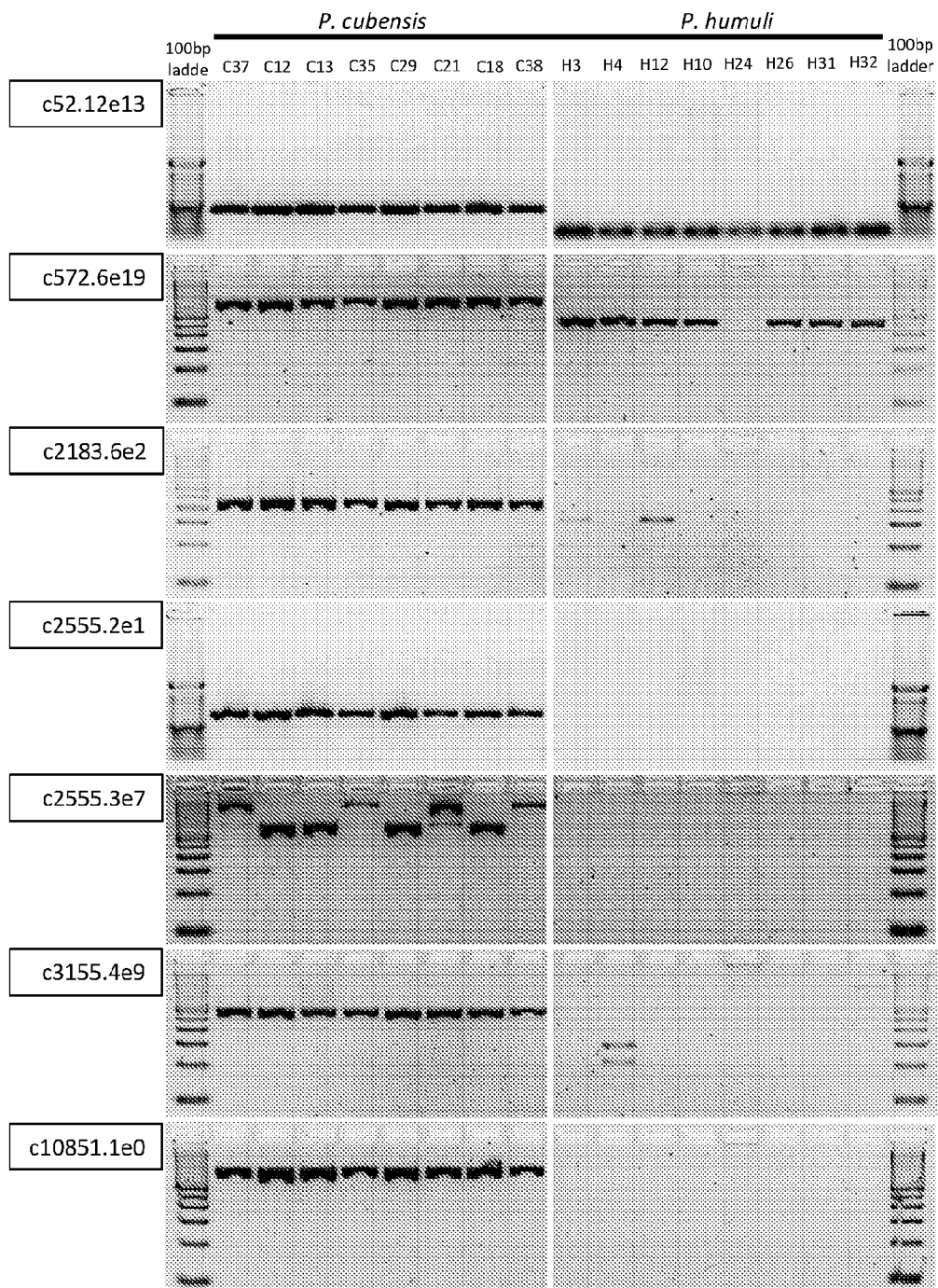


Fig. 5

Fig. 6A

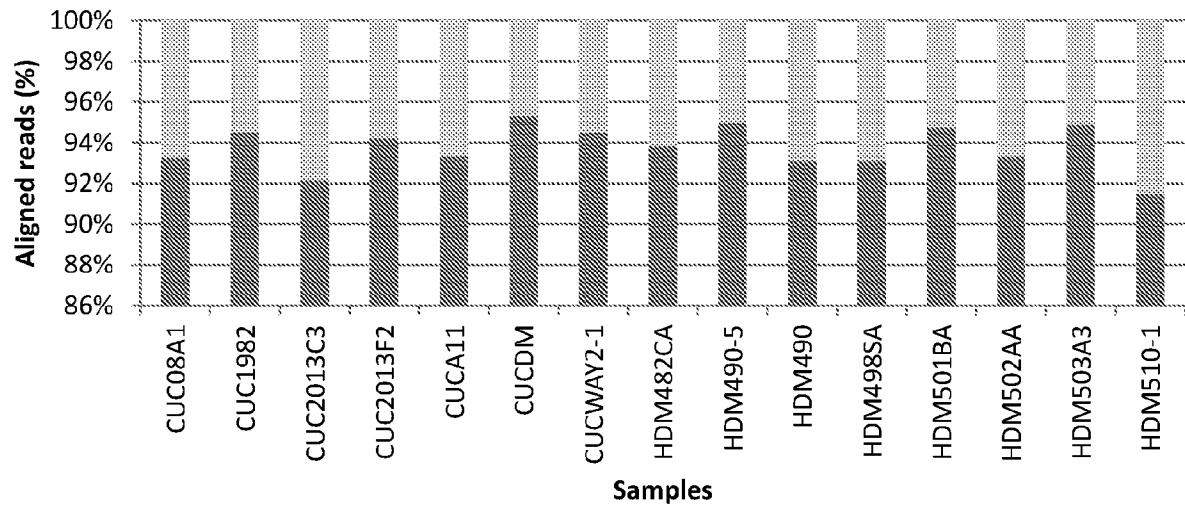
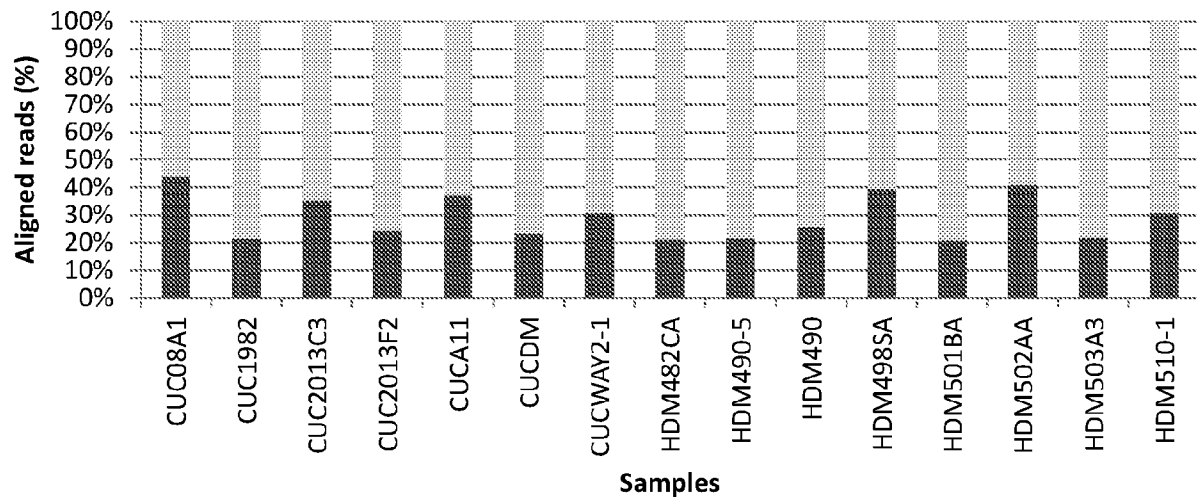


Fig. 6B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2017/050059

A. CLASSIFICATION OF SUBJECT MATTER		<i>C12Q 1/68 (2006.01)</i> <i>C07H 21/04 (2006.01)</i> <i>C07K 14/37 (2006.01)</i> <i>C07K 16/14 (2006.01)</i>													
According to International Patent Classification (IPC) or to both national classification and IPC															
B. FIELDS SEARCHED															
Minimum documentation searched (classification system followed by classification symbols)															
C12Q 1/68, C07H 21/04, C07K 14/37, 16/14															
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched															
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)															
PAJ, Esp@cent, PCT online, USPTODB, WIPO, RUPTO, EAPATIS, PatSearch															
C. DOCUMENTS CONSIDERED TO BE RELEVANT															
Category*	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.												
A	YIGAL COHEN et al. Seed Transmission of <i>Pseudoperonospora cubensis</i> , PLOS ONE, 2014, Vol. 9, Issue 10, e109766, doi:10.1371/journal.pone.0109766, pp.1-12, especially abstract, pp.1-3		1-29, 39-47												
X	DATABASE MBL-EBI: CF862060, 07.02.2011		34, 35, 37												
Y			36, 38												
A	WILLIAM L. HOLDSWORTH et al. Development of Downy Mildew-resistant Cucumbers for Late-season Production in the Northeastern United States, HortScience, 2014, Vol. 49, no. 1, pp. 10-17, especially abstract, p.11-13		31-33												
X	DATABASE: EMBL-EBI: FG054005, 29.04.2008		34, 35, 37												
Y			36, 38												
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.															
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier document but published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>				* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier document but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention														
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone														
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art														
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family														
"O" document referring to an oral disclosure, use, exhibition or other means															
"P" document published prior to the international filing date but later than the priority date claimed															
Date of the actual completion of the international search		Date of mailing of the international search report													
04 December 2017 (04.12.2017)		14 December 2017 (14.12.2017)													
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37		Authorized officer E.Tereshkina Telephone No. 495 531 65 15													

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2017/050059

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DATABASE EMBL-EBI: BE583118, 16.05.2010	34, 35, 37 36, 38
Y	EP 2444496 A1 (DEL MONTE FRESH PRODUCE COMPANY CORAL GABLES) 25.04.2012, abstract	36
Y	HONG Tsai-Hsia et al. The production of polyclonal and monoclonal antibodies in mice using novel immunization methods. J Immunol Methods, 1989, Vol.120, no.2, pp.151-157	38

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2017/050059

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 30, 48-49
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.