

CLAIMS:

1. A positive amplification control plasmid (PAC) comprising a unique artificial plasmid-specific sequence (UAPS), and a target amplification polynucleotide sequence (TAP), wherein said TAP comprises a bacterial or viral nucleotide sequence, wherein the PAC
5 further comprises a nucleic acid extraction control (NEC) nucleotide sequence.
2. The PAC of claim 1, wherein said UAPS does not recognize or anneal to any natural parvovirus.
3. The PAC of claim 2, wherein said UAPS comprises SEQ ID NO. 10.
4. The PAC of claim 1, wherein said TAP comprises an NS-1 nucleotide sequence.
- 10 5. The PAC of claim 1, wherein said NEC comprises an M13 bacteriophage nucleotide sequence.
6. The PAC of claim 5, wherein said M13 bacteriophage nucleotide sequence is a M13K07 nucleotide sequence.
7. The PAC of claim 6, wherein said M13K07 nucleotide sequence comprises SEQ ID NO. 8.
- 15 8. The PAC of claim 1, further comprising one or more oligonucleotide primers.
9. The PAC of claim 8, wherein said one or more oligonucleotide primers is selected from the group consisting of a forward oligonucleotide primer, a reverse oligonucleotide primer, and a combination thereof.
10. The PAC of claim 8, wherein said one or more oligonucleotide primers are rodent parvovirus
20 oligonucleotide primers.
11. The PAC of claim 10, wherein said rodent parvovirus oligonucleotide primers is selected from the group consisting of a forward oligonucleotide primer, a reverse oligonucleotide primer, and a combination thereof.

12. The PAC of claim 1, further comprising M13 oligonucleotide primers.
13. The PAC of claim 12, wherein said M13 oligonucleotide primers is selected from the group consisting of a forward oligonucleotide primer, a reverse oligonucleotide primer, and a combination thereof.
- 5 14. A composition comprising a unique detection probe (UDP), wherein said UDP comprises an artificial nucleotide sequence, and wherein said artificial nucleotide sequence comprises SEQ ID NO: 3.
15. The composition of claim 14, wherein said UDP further comprise at least one fluorophore and at least one quencher.
- 10 16. The composition of claim 15, wherein said fluorophore is selected from the group of fluorophores having an excitation wavelength anywhere between 495 nm to 680 nm, inclusively, and an emission wavelength anywhere between 515 nm to 710 nm, inclusively.
17. The composition of claim 16, wherein said fluorophore has an excitation wavelength of 495 nm and an emission wavelength of 520 nm.
- 15 18. The composition of claim 17, wherein said fluorophore is FAM.
19. The composition of claim 18, wherein said quencher is selected from the group of dyes having an absorbance peak anywhere between 430 nm to 672 nm, inclusively.
20. The composition of claim 19, wherein said quencher is BHQ-1.
21. The composition of claim 19, wherein said fluorophore is attached to the 5-prime end of said UDP and said quencher is attached to the 3-prime end of said UDP.
- 20 22. The composition of claim 3, wherein said NS-1 sequence is selected from the group consisting of minute virus of mice prototype strain (MVMp), minute virus of mouse immunosuppressive strain (MVMi), minute virus of mouse Cutter strain (MVMc); mouse

parvovirus 1b (MPV-1b), mouse parvovirus 1a (MPV-1a), mouse parvovirus 1c (MPV-1c), hamster parvovirus (HaPV), Toolan's parvovirus (H-1), Kilham rat virus (KRV), rat parvovirus 1a, rat minute virus, and Umass strain of Rat virus L (RV-Umass).

23. A method of detecting the presence or absence of a biological contaminant comprising the steps of:

a. combining (i) a positive amplification control plasmid (PAC) sample, wherein the PAC comprises a unique artificial plasmid-specific sequence (UAPS), a target amplification polynucleotide sequence (TAP), and a nucleic acid extraction control (NEC) nucleotide sequence, (ii) a unique detection probe (UDP), (iii) a target amplification polynucleotide sequence (TAP) forward oligonucleotide primer, (iv) a TAP reverse oligonucleotide primer, (v) a TAP detection probe and (vi) a DNA polymerase, wherein the TAP detection probe binds TAP in the PAC, wherein the UDP binds the UAPS in the PAC;

b. combining (i) a test sample, (ii) a UDP, (iii) a TAP forward oligonucleotide primer, (iv) a TAP reverse oligonucleotide primer, (v) a TAP detection probe and (vi) a DNA polymerase, wherein the TAP detection probe binds TAP in the PAC sample, wherein the UDP binds the UAPS in a PAC;

c. subjecting the combination of step (a)-(b) to polymerase chain reaction (PCR);

d. monitoring said PCR in real time for production of a TAP signal resulting from binding of the TAP detection probe to TAP and for the production of a UDP signal resulting from binding of UDP to UAPS; wherein,

i. if no TAP signal is observed for the test sample but a TAP signal is observed for the PAC sample, then the test sample is free of a biological contaminant; or,

ii. if a TAP signal and a UDP signal are observed for the test sample, then the test sample is cross-contaminated with PAC, and the TAP signal is a false positive; or,

iii. if a TAP signal but no UDP signal are observed for the test sample, then the test sample has a biological contaminant that is not due to cross-contamination from PAC, thereby signifying a true positive.

24. The method of claim 23, wherein said UDP further comprises at least one fluorophore and at least one quencher.

25. The method of claim 24, wherein said fluorophore is selected from the group of fluorophores having an excitation wavelength anywhere between 495 nm to 680 nm, inclusively, and an emission wavelength anywhere between 515 nm to 710 nm, inclusively.

26. The method of claim 23, wherein said TAP detection probe further comprises at least one fluorophore and at least one quencher.

27. The method of claim 26, wherein the production of TAP is monitored at about 533 nm to about 580 nm.

28. The method of claim 23, wherein steps (a)-(b) further comprise: (vii) a NEC specific forward oligonucleotide primer, (viii) a NEC specific reverse oligonucleotide primer, and (ix) a NEC specific oligonucleotide detection probe.

29. The method of claim 28, wherein said NEC is a M13 bacteriophage.

30. The method of claim 28, wherein said NEC specific oligonucleotide detection probe comprises a cyanine dye.

31. The method of claim 30, wherein said cyanine dye is Cy5 having an absorbance maximum of about 650 nm and an emission maximum of about 670 nm.

32. The method of claim 23, further comprising combining (i) a buffer sample, (ii) a UDP, (iii) a TAP forward oligonucleotide primer, (iv) a TAP reverse oligonucleotide primer, (v) a TAP detection probe and (vi) a DNA polymerase;

a. subjecting the combination to polymerase chain reaction (PCR);

5 b. monitoring said PCR in real time for production of a TAP signal resulting from binding of the TAP detection probe to TAP and for the production of a UDP signal resulting from binding of UDP to UAPS; wherein,

i. if a TAP signal and a UDP signal are observed, then the PCR reagents are contaminated with the PAC plasmid; or,

10 ii. if a TAP signal and no UDP signal are observed then the PCR reagents are contaminated with the biological contaminant; or,

iii. if no TAP signal and no UDP signal are observed in the buffer sample and a TAP and UDP signal are observed in the PAC sample, and a TAP signal and no UDP signal are observed in the test sample, then the test sample has a biological contaminant.

15 33. The method of claim 31, wherein a signal from the NEC specific probe is indicative of oligonucleotide extraction from a reaction vessel.