

CLAIMS

1. A positive amplification control plasmid (PAC) comprising: a unique artificial plasmid-specific sequence (UAPS) comprising a sequence of SEQ ID NO: 10, wherein the UAPS comprises a sequence of 17 to 20 nucleotides, and no more than 7 to 10 consecutive internal nucleotides and no more than 6 consecutive 3' nucleotides are identical to any parvovirus sequence, and

a target amplification polynucleotide sequence (TAP), wherein said TAP comprises all or part of a parvovirus NS-1 sequence sufficient to produce a Q-PCR amplicon, wherein the parvovirus NS-1 sequence is at least 88% identical to any one of SEQ ID NOs: 12-37 or 97% identical to SEQ ID NO: 9, and

a nucleic acid extraction control (NEC) nucleotide sequence, wherein said NEC comprises an M13 bacteriophage nucleotide sequence comprising a sequence of SEQ ID NO: 8.

~~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.~~

24. The PAC of claim 1, wherein the parvovirus NS-1 sequence comprises SEQ ID NO: 9 or any one of SEQ ID NOs: 12-37.

35. The PAC of claim 1, wherein the parvovirus NS-1 sequence comprises SEQ ID NO: 37.

~~6. The PAC of claim 1, 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.~~

48. The PAC of claim 1, further comprising one or more oligonucleotide primers.

59. The PAC of claim 48, wherein

- (a) 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; or
- (b) wherein said one or more oligonucleotide primers are rodent parvovirus oligonucleotide primers, preferably 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.

610. The PAC of claim 1, further comprising M13 oligonucleotide primers, preferably wherein said M13 oligonucleotide primers is selected from the group consisting of a forward oligonucleotide primer, a reverse oligonucleotide primer, and a combination thereof.

711. The PAC of claim 1, 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).

812. 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) comprising a sequence of SEQ ID NO: 10, a target amplification polynucleotide sequence (TAP) wherein said TAP comprises all or part of a parvovirus NS-1 sequence sufficient to produce a Q-PCR amplicon, wherein the parvovirus NS-1 sequence is at least 88% identical to any one of SEQ ID NOs: 12-37 or 97% identical to SEQ ID NO: 9, and a nucleic acid extraction control (NEC) nucleotide sequence comprising a sequence of an M13 bacteriophage used as a nucleic acid extraction control, (ii) a unique detection probe (UDP) complementary to the UAPS, (iii) a TAP forward oligonucleotide primer, (iv) a TAP reverse oligonucleotide primer, (v) a TAP detection probe and (vi) a DNA polymerase;
- (b) combining (i) a test sample, (ii) a UDP complementary to the UAPS, (iii) a TAP forward oligonucleotide primer, (iv) a TAP reverse oligonucleotide primer, (v) a TAP detection probe and (vi) a DNA polymerase;
- (c) subjecting the combined sample of step (a) and the combined sample of step (b) to polymerase chain reaction (PCR); and
- (d) monitoring said PCR in real time for production of a TAP signal resulting from binding of the TAP detection probe to the TAP sequence in the PAC or a TAP sequence in the biological contaminant, and for the production of a UDP signal resulting from binding of the UDP to the UAPS in the PAC; and
- (e) detecting the presence or absence of a biological contaminant in the test sample, 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;
 - ii. if a TAP signal and a UDP signal are observed for the test sample, then the test sample is cross-contaminated with a portion of the PAC sample; or,
 - iii. if a TAP signal but no UDP signal is observed for the test sample, then the TAP signal is not due to cross-contamination from PAC and the test sample has a biological contaminant.

913. The method of claim 812, wherein said UDP further comprises at least one fluorophore and at least one quencher.

1014. The method of claim 913, 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.

1115. The method of claim 812, wherein said TAP detection probe further comprises at least one fluorophore and at least one quencher.

1216. The method of claim 1115, wherein production of TAP is monitored at ~~about~~ 533 nm to ~~about~~ 580 nm.

1317. The method of claim 812, further comprising detecting oligonucleotide extraction from a reaction wherein steps (a)-(b) further comprise combining: (vii) a NEC specific forward oligonucleotide primer, (viii) a NEC specific reverse oligonucleotide primer, and (ix) a NEC specific oligonucleotide detection probe, wherein the NEC specific oligonucleotide detection probe binds a NEC sequence in the PAC sample or the test sample;

(d) further comprises monitoring said PCR in real time for production of a NEC signal resulting from binding of the NEC specific oligonucleotide detection probe to the NEC nucleotide sequence in the PAC sample or the test sample; and the method further comprises:

(f) detecting oligonucleotide extraction from a reaction vessel, wherein

- i. if a NEC signal is observed for the test sample and for the PAC sample, but no UDP signal is observed for the test sample, then the NEC signal is indicative of oligonucleotide extraction from a reaction vessel; or,
- ii. if a NEC signal and a UDP signal are observed for the test sample, then the test sample is cross-contaminated with a portion of the PAC sample.

1418. The method of claim 1317, wherein said:

~~(a) — NEC is a M13 bacteriophage; or~~

~~(b) —~~ NEC specific oligonucleotide detection probe comprises a cyanine dye, preferably wherein said cyanine dye is Cy5 having an absorbance maximum of ~~about~~ 650 nm and an emission maximum of ~~about~~ 670 nm.

1519. The method of claim 812, further comprising detecting the presence or absence of the biological contaminant or a portion of the PAC sample in a PCR reagent, comprising:

- (g) 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;
- (h) subjecting the combined buffer sample to polymerase chain reaction (PCR);
- (i) monitoring said PCR in real time for production of a TAP signal resulting from binding of the TAP detection probe to a TAP sequence in the PAC or the biological contaminant, and for the production of a UDP signal resulting from binding of the UDP to the UAPS in the PAC;
- (j) detecting the presence or absence of the biological contaminant or the PAC in a PCR reagent, wherein
 - i. if no TAP signal and no UDP signal is observed in the buffer sample, then no PCR reagent is contaminated with the biological contaminant or the PAC;
 - ii. if a TAP signal and a UDP signal are observed in the buffer sample, then a PCR reagent is contaminated with a portion of the PAC sample; or,
 - iii. if a TAP signal and no UDP signal are observed in the buffer sample, then a PCR reagent is contaminated with the biological contaminant.