Virus Bangledesh-1975). These regions were put through a BLAST search against the NCBI nr database to check for uniqueness and to display the closest sequence matches. 12 of the 14 regions had only 1 to 3 nucleotides that were different from the closest hit sequence, which was likely to be not enough difference to generate Variola-specific assays, while two regions (Regions 7 and 8 in Table 1) displayed a greater degree of differentiation from the nearest matches. These two regions were therefore used to create assays as described further below.

[00104] Table 1 shows Insignia results for Variola virus (major and minor included)-specific regions. Signature chain length was set to ≥35 nucleotides.

Table 1

Region	Starting Nucleotide Position	Stopping Nucleotide Position	Sequence
1	153149	153183	GCAAGGATATTCTCATGGAG ATATTAAAGCGAGCA
2	6262	6297	AGTATATGTTGGAGGACATT CGAGTTCATTGTTTTC
3	128198	128233	CGACTAAGTTCAAGTTCTCC TTGACAAGATCCATCT
4	74961	74996	CGGAATTGGAATGTTTTGGT CACTGGGGTAAAGTAA
5	2634	2669	ATTATGCGGTCCAGAGGGAA ATGGATATTGTTTTCA
6	34950	34986	TAACGTCTTTCCAAGTGGCA AATTCCAAATTTTTTC
7	970	1007	AGTGTATTGAGAGTGAGTGA GCATGAAAAAGATTTAGT
8	161598	161635	AACACTATTAGGAGAAAGCC AGGTCATATGGAAGATGT
9	184180	184218	TGCTAGATTGTCCAGTGTGC CCCCAGGACAAGGTAAGGA
10	23346	23384	ATCTAGGTTTCAAAAGACTT GGCATATTAACCCAAGCAG
11	14365	14403	ATACGGCAGCAACTAGTATT ATCTCTACATTGTTTACGG
12	48985	49023	CTGATGGCGACATAATTGTC CAAAACTGCCAATCTATAA
13	3808	3846	TACCCAATTAGAACACGTAT GCTTATTATCATCATTCGG
14	50495	50533	CCGACACCATCAAAGAATTT TTATGATATAAGGAAACAG

[00105] The first unique region of interest (Region 7 in Table 1) that was identified corresponds to nucleotides 970-1007 of Variola Major Virus Bangledesh-1975. Upon further analysis of the larger region surrounding this 38 nucleotide sequence, it was discovered that only

the 31 nucleotide stretch from nucleotides 977-1007 was conserved within all sequenced Variola virus stains. Within that 31 nucleotide region, 5 nucleotides did not match the sequence of the closest neighbor, cowpox virus. This difference was utilized in assay VAR-1 (*see* Table 7 for amplification primer and target probe sequences) by placing Primer 1 in this sequence (*see* Table 2). Primer 2 of this assay took advantage of 2 additional SNPs, while the probe has exact homology with several poxviruses.

[00106] Table 2 shows the 120 base pair area around Insignia-identified Region 7 (which corresponds to the 119 base pair region of 977-1095 of Variola Major Virus Bangledesh-1975). The conserved 31 nucleotides within Region 7 are underlined. Amplification primers P1 and P2 and the target probe from VAR-1 are indicated in bold italics. SNPs between Variola virus and other genomes are highlighted in black boxes. SNPs within Variola virus (in >1 genome sequences) are indicated in gray boxes – and were, and typically should be, avoided in Variola assay designs.

Table 2

1 ***TGAGAGTGAGTGAGCATGAAAAAGAT TTAGTATTTAGCAGTGCGGATATGATCCAAGAGG*** 60

61 GTGA***AATAGTCGTTCTCGTTCAGAATCTTTTG***CAGCATAAGTAGTATGTCGATATACTTA 120

[00107] The second unique region identified (Region 8 in Table 1) corresponds to nucleotides 161598-161635 of Variola Major Virus Bangledesh-1975. When this sequence was subjected to a BLAST search against the NCBI nr database, 3 SNPs were identified in this region. More interestingly, when a larger region surrounding this sequence was analyzed, it was discovered that genomic rearrangements are found both upstream and downstream of this sequence in the most closely related genomes, those of some cowpox strains (*see* Table 3 below). These rearrangements allowed for the design of assays with amplification primers or target probes spanning regions that are contiguous in Variola virus but non-contiguous (junction regions) in other genomes. Such assays can afford even greater specificity than those that rely solely on SNPs for differentiation.

[00108] Table 3 shows the 300 base pair area around Insignia-identified Region 8. Region 8 is underlined. SNPs between Variola virus and other genomes are highlighted in black boxes. SNPs within Variola virus (in >1 genome sequences) are indicated in gray boxes - these were, and should be, avoided in Variola assay designs. The sequence indicated by # symbols underneath the sequence is duplicated in the nearest neighbor genomes (some cowpox genomes) and forms the

upstream junction. The downstream junction (also present in the cowpox genomes) falls between the three nucleotides indicated by ^ symbols underneath the sequence.

Table 3

```
161487    GTATCGGGGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT  161546

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
                                              ##############

161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTGGATAAACGT  161666

161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG  161726
                  ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
```

[00109] Three assays were designed to take advantage of these junction regions: VAR-2, VAR-3, and VAR-4 (again, *see* Table 7 for amplification primer and target probe sequences).

[00110] In the VAR-2 assay (Table 4), the P1 amplification primer spans the junction region just upstream of Insignia-identified Region 8, and the P2 amplification primer spans the junction region downstream of this region. The probe lies within the Insignia-identified Region 8 itself and takes advantage of the 3 SNPs in this region. Table 4 shows relative location variola-specific assay VAR-2. The amplification primer sequences P1 and P2 are indicated by asterisks underneath the sequences while the probe region is indicated by plus symbols underneath the sequence. Other formats are as in Table 3, except that the black-highlighted SNPs have been omitted for clarity.

Table 4

```
161487    GTATCGGGGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT  161546

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
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161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTGGATAAACGT  161666
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161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG  161726
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161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
```

[00111] The VAR-3 assay (Table 5) has the probe spanning the upstream junction region, with the P1 and P2 amplification primers taking advantage of 1 SNP each as well as being on different sides of the junction (approximately 300 base pairs apart in the cowpox genomes, with the sequences inverted in those genomes). Table 5 shows variola-specific assay VAR-3. The P1 and P2 amplification primers are indicated by asterisks underneath the sequences while the probe region is indicated by plus symbols underneath the sequence. Other formats are as in Table 3, except that the black-highlighted SNPs have been omitted for clarity.

Table 5

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161487    GTATCGGCGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT  161546
                                                      **************

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
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161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTCGATAAACGT  161666
          ++++++++++          ******************************

161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG  161726
                    ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
```

[00112] The sequence region amplified by the amplification primers (120 base pairs) in the VAR-4 assay (Table 6) spans the downstream junction, with P1 overlapping (in the opposite orientation) P2 from the VAR-3 region (118 bp), taking advantage of 1 SNP, the probe spanning the downstream junction, and P2 taking advantage of 1 SNP and being on the other side of the junction (about 7 kb away and inverted in near neighbors). Table 6 shows variola-specific assay VAR-4. P1 and P2 are indicated by asterisks underneath the sequences while the probe region is indicated by plus marks underneath the sequence. Other formats are as in Table 3, except that the black-highlighted SNPs have been omitted for clarity.

Table 6

```
161487    GTATCGGGGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT  161546

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
                                            ##############

161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTGGATAAACGT  161666
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161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGCATTATTG  161726
          ++++++++++++++++++++                          **************
                    ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
          ************
```

Table 7

Assay Name	Amplicon size	Primer 1 (P1)	P1 length	Primer 2 (P2)	P2 length	Probe	Probe length
VAR-1	92	TGA GAG TGA GTG AGC ATG AAA AAG	24	CAA AAG ATT CTG AAC GAG AAC GAC TAT T	28	TTA GTA TTT AGC AGT GCG GAT ATG ATC CAA GAG G	34
VAR-2	110	ACA TAT AGC GAA TAT AAG GAG ATC CAA	27	GCT CCG ACA GCC TGT TCA C	19	TCT TCC ATA TGA CCT GGC TTT CTC CTA ATA GTG	34
VAR-3	118	ACC ACA ATG CAT TGG CAT CA	20	TGA TAT TAA CAG CTT GAA CAT CTT CCA	27	CGAATATAAGGAGA TCCAACACTATTAG GAGAAAGCC	37
VAR-4	120	GGTCATATGGAAG ATGTTCAAGCT	24	CAT TCT ATC ATG CAA TAA TCC CAA CA	26	CTG TGG CTC CGA CAG CCT GTT CAC	25

Table 7 shows primer/probe sequences and characteristics of the Variola virus-specific ssays.

[00113] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above can be used in various combinations. All publications, patents, patent applications, and/or other documents cited in this

application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually and separately indicated to be incorporated by reference for all purposes.

Virus Bangledesh-1975). These regions were put through a BLAST search against the NCBI nr database to check for uniqueness and to display the closest sequence matches. 12 of the 14 regions had only 1 to 3 nucleotides that were different from the closest hit sequence, which was likely to be not enough difference to generate Variola-specific assays, while two regions (Regions 7 and 8 in Table 1) displayed a greater degree of differentiation from the nearest matches. These two regions were therefore used to create assays as described further below.

[00104] Table 1 shows Insignia results for Variola virus (major and minor included)-specific regions. Signature chain length was set to ≥35 nucleotides.

Table 1

Region	SEQ ID NO:	Starting Nucleotide Position	Stopping Nucleotide Position	Sequence
1	1	153149	153183	GCAAGGATATTCTCATGGAG ATATTAAAGCGAGCA
2	2	6262	6297	AGTATATGTTGGAGGACATT CGAGTTCATTGTTTTC
3	3	128198	128233	CGACTAAGTTCAAGTTCTCC TTGACAAGATCCATCT
4	4	74961	74996	CGGAATTGGAATGTTTTGGT CACTGGGGTAAAGTAA
5	5	2634	2669	ATTATGCGGTCCAGAGGGAA ATGGATATTGTTTTCA
6	6	34950	34986	TAACGTCTTTCCAAGTGGCA AATTCCAAATTTTTTTC
7	7	970	1007	AGTGTATTGAGAGTGAGTGA GCATGAAAAAGATTTAGT
8	8	161598	161635	AACACTATTAGGAGAAAGCC AGGTCATATGGAAGATGT
9	9	184180	184218	TGCTAGATTGTCCAGTGTGC CCCCAGGACAAGGTAAGGA
10	10	23346	23384	ATCTAGGTTTCAAAAGACTT GGCATATTAACCCAAGCAG
11	11	14365	14403	ATACGGCAGCAACTAGTATT ATCTCTACATTGTTTACGG
12	12	48985	49023	CTGATGGCGACATAATTGTC CAAAACTGCCAATCTATAA
13	13	3808	3846	TACCCAATTAGAACACGTAT GCTTATTATCATCATTCGG
14	14	50495	50533	CCGACACCATCAAAGAATTT TTATGATATAAGGAAACAG

[00105] The first unique region of interest (Region 7 in Table 1) that was identified corresponds to nucleotides 970-1007 of Variola Major Virus Bangledesh-1975. Upon further analysis of the larger region surrounding this 38 nucleotide sequence, it was discovered that only

the 31 nucleotide stretch from nucleotides 977-1007 was conserved within all sequenced Variola virus stains. Within that 31 nucleotide region, 5 nucleotides did not match the sequence of the closest neighbor, cowpox virus. This difference was utilized in assay VAR-1 (*see* Table 7 for amplification primer and target probe sequences) by placing Primer 1 in this sequence (*see* Table 2). Primer 2 of this assay took advantage of 2 additional SNPs, while the probe has exact homology with several poxviruses.

[00106] Table 2 shows the 120 base pair area around Insignia-identified Region 7 (SEQ ID NO 15, which corresponds to the 119 base pair region of 977-1095 of Variola Major Virus Bangledesh-1975). The conserved 31 nucleotides within Region 7 are underlined. Amplification primers P1 and P2 and the target probe from VAR-1 are indicated in bold italics. SNPs between Variola virus and other genomes are highlighted in black boxes. SNPs within Variola virus (in >1 genome sequences) are indicated in gray boxes – and were, and typically should be, avoided in Variola assay designs.

Table 2

```
1    TGAGAGTGAGTGAGCATGAAAAAGATTTAGTATTTAGCAGTGCGGATATGATCCAAGAGG   60

61   GTGAAATAGTCGTTCTCGTTCAGAATCTTTTGCAGCATAAGTAGTATGTCGATATACTTA   120
```

[00107] The second unique region identified (Region 8 in Table 1) corresponds to nucleotides 161598-161635 of Variola Major Virus Bangledesh-1975. When this sequence was subjected to a BLAST search against the NCBI nr database, 3 SNPs were identified in this region. More interestingly, when a larger region surrounding this sequence was analyzed, it was discovered that genomic rearrangements are found both upstream and downstream of this sequence in the most closely related genomes, those of some cowpox strains (*see* Table 3 below). These rearrangements allowed for the design of assays with amplification primers or target probes spanning regions that are contiguous in Variola virus but non-contiguous (junction regions) in other genomes. Such assays can afford even greater specificity than those that rely solely on SNPs for differentiation.

[00108] Table 3 shows the 300 base pair area around Insignia-identified Region 8 (SEQ ID NO 16). Region 8 is underlined. SNPs between Variola virus and other genomes are highlighted in black boxes. SNPs within Variola virus (in >1 genome sequences) are indicated in gray boxes - these were, and should be, avoided in Variola assay designs. The sequence indicated by # symbols underneath the sequence is duplicated in the nearest neighbor genomes (some cowpox genomes)

and forms the upstream junction. The downstream junction (also present in the cowpox genomes) falls between the three nucleotides indicated by ^ symbols underneath the sequence.

Table 3

```
161487    GTATCGGCGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT  161546

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
                                              ###############

161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTCGATAAACGT  161666

161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG  161726
                    ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
```

[00109] Three assays were designed to take advantage of these junction regions: VAR-2, VAR-3, and VAR-4 (again, *see* Table 7 for amplification primer and target probe sequences).

[00110] In the VAR-2 assay (Table 4, area listed as SEQ ID NO 16), the P1 amplification primer spans the junction region just upstream of Insignia-identified Region 8, and the P2 amplification primer spans the junction region downstream of this region. The probe lies within the Insignia-identified Region 8 itself and takes advantage of the 3 SNPs in this region. Table 4 shows relative location variola-specific assay VAR-2. The amplification primer sequences P1 and P2 are indicated by asterisks underneath the sequences while the probe region is indicated by plus symbols underneath the sequence. Other formats are as in Table 3, except that the black-highlighted SNPs have been omitted for clarity.

Table 4

```
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161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
                                          ****************************+++++++
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161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTCGATAAACGT  161666
          ++++++++++++++++++++++++++                                    **

161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG  161726
          ****************
                    ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
```

[00111] The VAR-3 assay (Table 5, area listed as SEQ ID NO 16) has the probe spanning the upstream junction region, with the P1 and P2 amplification primers taking advantage of 1 SNP each as well as being on different sides of the junction (approximately 300 base pairs apart in the cowpox genomes, with the sequences inverted in those genomes). Table 5 shows variola-specific assay VAR-3. The P1 and P2 amplification primers are indicated by asterisks underneath the sequences while the probe region is indicated by plus symbols underneath the sequence. Other formats are as in Table 3, except that the black-highlighted SNPs have been omitted for clarity.

Table 5

```
161487    GTATCGGCGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT   161546
                                                          ************

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT   161606
          *******                              ++++++++++++++++++++++++++
                                                 ###############

161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTCGATAAACGT   161666
          ++++++++++          **************************

161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG   161726
                    ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA   161786
```

[00112] The sequence region amplified by the amplification primers (120 base pairs) in the VAR-4 assay (Table 6, area listed as SEQ ID NO 16) spans the downstream junction, with P1 overlapping (in the opposite orientation) P2 from the VAR-3 region (118 bp), taking advantage of 1 SNP, the probe spanning the downstream junction, and P2 taking advantage of 1 SNP and being on the other side of the junction (about 7 kb away and inverted in near neighbors). Table 6 shows variola-specific assay VAR-4. P1 and P2 are indicated by asterisks underneath the sequences while the probe region is indicated by plus marks underneath the sequence. Other formats are as in Table 3, except that the black-highlighted SNPs have been omitted for clarity.

Table 6

```
161487    GTATCGGCGTACGGAGATCATCAAGTTCATCATCCGATATTATTGTAACCACAATGCATT  161546

161547    GGCATCAAAAGAAGATAGATGAACTAACATATAGCGAATATAAGGAGATCCAACACTATT  161606
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161607    AGGAGAAAGCCAGGTCATATGGAAGATGTTCAAGCTGTTAATATCAATCTCGATAAACGT  161666
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161667    GAACAGGCTGTCGGAGCCACAGTTTCGAGACGAGGAGATTTAGAAATGTTGGGATTATTG  161726
          ++++++++++++++++++++++                          **************
                    ^^^

161727    CATGATAGAATGGTTCAGTGGCAAACTTCCATGGAAAAACAAAAGTAGTATAGCAATACA  161786
          ************
```

Table 7

Assay Name	Ampl icon size	Primer 1 (P1)	P1 length	Primer 2 (P2)	P2 length	Probe	Probe length
VAR-1	92	TGA GAG TGA GTG AGC ATG AAA AAG (SEQ ID NO:17)	24	CAA AAG ATT CTG AAC GAG AAC GAC TAT T (SEQ ID NO:18)	28	TTA GTA TTT AGC AGT GCG GAT ATG ATC CAA GAG G (SEQ ID NO:19)	34
VAR-2	110	ACA TAT AGC GAA TAT AAG GAG ATC CAA (SEQ ID NO:20)	27	GCT CCG ACA GCC TGT TCA C (SEQ ID NO:21)	19	TCT TCC ATA TGA CCT GGC TTT CTC CTA ATA GTG (SEQ ID NO:22)	34
VAR-3	118	ACC ACA ATG CAT TGG CAT CA (SEQ ID NO:23)	20	TGA TAT TAA CAG CTT GAA CAT CTT CCA (SEQ ID NO:24)	27	CGAATATAAGGAGA TCCAACACTATTAG GAGAAAGCC (SEQ ID NO:25)	37
VAR-4	120	GGTCATATGGAAG ATGTTCAAGCT (SEQ ID NO:26)	24	CAT TCT ATC ATG CAA TAA TCC CAA CA (SEQ ID NO:27)	26	CTG TGG CTC CGA CAG CCT GTT CAC (SEQ ID NO:28)	25

Table 7 shows primer/probe sequences and characteristics of the Variola virus-specific assays.

[00113] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above can be used in various combinations. All publications, patents, patent applications, and/or other documents cited in this

application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually and separately indicated to be incorporated by reference for all purposes.

SEQUENCE LISTING

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What is claimed is:

1. A method of detecting the presence of at least a first target nucleic acid sequence in a sample, comprising:

subjecting the sample to an amplification reaction capable of amplifying the target nucleic acid sequence in the presence of at least a first set of nucleic acid probes, the first set of nucleic acid probes comprising:

a capture probe comprising a fluorophore attached thereto;

a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe; and

detecting fluorescence from the sample following one or more cycles of the polymerase chain reaction, an increase in fluorescence being indicative of the presence of the target nucleic acid sequence.

2. The method of claim 1, wherein the capture probe is attached to a surface of a substrate.

3. The method of claim 1, wherein the amplification reaction comprises a PCR reaction.

4. The method of clam 3, wherein the PCR reaction employs a polymerase enzyme having exonuclease activity.

5. The method of claim 4, wherein the PCR reaction is capable of amplifying a plurality of different target nucleic acid sequences and is carried out in the presence of a plurality of different sets of nucleic acid probes, each different set of probes comprising a diferent capture probe having a fluorophore attached thereto and a different target specific probe, each target specific probe being complementary to a different capture probe and a different one of the plurality of target nucleic acid

sequences, and having a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when each target specific probe is hybridized to a complementary capture probe.

6. The method of claim 5, wherein each of the capture probes in the plurality of different sets of probes is immobilized upon a substrate in a detectably distinct location.

7. The method of claim 1, wherein the at least first target nucleic acid sequence comprises a variola virus sequence.

8. The method of claim 7, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe, VAR-2 probe, VAR-3 probe, or VAR-4 probe.

9. The method of claim 1, wherein the target nucleic acid sequence is amplified through use of one or more of: VAR-1 primer 1, VAR-1 primer 2, VAR-2 primer 1, VAR-2 primer 2; VAR-3 primer 1, VAR-3 primer 2, VAR-4 primer 1, or VAR-4 primer 2.

10. A reaction mixture, comprising:

a sample containing a target nucleic acid of interest;

amplification reagents for amplifying the target nucleic acid sequence of interest; and

at least a first probe set comprising a capture probe comprising a fluorophore attached thereto, and a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe

11. The mixture of claim 10, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe, VAR-2 probe, VAR-3 probe, or VAR-4 probe.

12. The mixture of claim 11, wherein the mixture further comprises one or more amplification primers chosen from the group consisting of: VAR-1 primer 1, VAR-1 primer 2,

VAR-2 primer 1, VAR-2 primer 2; VAR-3 primer 1, VAR-3 primer 2, VAR-4 primer 1, or VAR-4 primer 2.

13. A reaction chamber, comprising:

a reaction region having disposed therein:

a sample containing a target nucleic acid of interest;

amplification reagents for amplifying the target nucleic acid sequence of interest; and

at least a first probe set comprising a capture probe comprising a fluorophore attached thereto, and a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe.

14. The reaction chamber of claim 13, wherein the capture probe is immobilized upon an interior surface of the reaction chamber.

15. The reaction chamber of claim 13, wherein a plurality of different capture probes are immobilized in an array on an interior surface of the reaction chamber, each different capture probe being immobilized in a discrete location.

16. A method of detecting the presence of at least a first target nucleic acid sequence in a sample, comprising:

subjecting the sample to an amplification reaction capable of amplifying the target nucleic acid sequence in the presence of at least a first set of nucleic acid probes, the first set of nucleic acid probes comprising:

a target specific nucleic acid probe having a fluorescent label associated therewith complementary to at least a portion of the target nucleic acid sequence, a capture probe associated with a solid support and complementary to at least a portion of the target specific nucleic acid probe; and

detecting fluorescence from the solid support following one or more cycles of the polymerase chain reaction, a decrease in fluorescence being indicative of the presence of the target nucleic acid sequence.

17. The method of claim 16, wherein the at least first target nucleic acid sequence comprises a variola virus sequence.

18. The method of claim 17, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe, VAR-2 probe, VAR-3 probe, or VAR-4 probe.

19. The method of claim 16, wherein the target nucleic acid sequence is amplified through use of one or more of: VAR-1 primer 1, VAR-1 primer 2, VAR-2 primer 1, VAR-2 primer 2; VAR-3 primer 1, VAR-3 primer 2, VAR-4 primer 1, or VAR-4 primer 2.

What is claimed is:

1. A method of detecting the presence of at least a first target nucleic acid sequence in a sample, comprising:

subjecting the sample to an amplification reaction capable of amplifying the target nucleic acid sequence in the presence of at least a first set of nucleic acid probes, the first set of nucleic acid probes comprising:

a capture probe comprising a fluorophore attached thereto;

a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe; and

detecting fluorescence from the sample following one or more cycles of the polymerase chain reaction, an increase in fluorescence being indicative of the presence of the target nucleic acid sequence.

2. The method of claim 1, wherein the capture probe is attached to a surface of a substrate.

3. The method of claim 1, wherein the amplification reaction comprises a PCR reaction.

4. The method of clam 3, wherein the PCR reaction employs a polymerase enzyme having exonuclease activity.

5. The method of claim 4, wherein the PCR reaction is capable of amplifying a plurality of different target nucleic acid sequences and is carried out in the presence of a plurality of different sets of nucleic acid probes, each different set of probes comprising a diferent capture probe having a fluorophore attached thereto and a different target specific probe, each target specific probe being complementary to a different capture probe and a different one of the plurality of target nucleic acid

sequences, and having a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when each target specific probe is hybridized to a complementary capture probe.

6. The method of claim 5, wherein each of the capture probes in the plurality of different sets of probes is immobilized upon a substrate in a detectably distinct location.

7. The method of claim 1, wherein the at least first target nucleic acid sequence comprises a variola virus sequence.

8. The method of claim 7, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO:28).

9. The method of claim 1, wherein the target nucleic acid sequence is amplified through use of one or more of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).

10. A reaction mixture, comprising:

a sample containing a target nucleic acid of interest;

amplification reagents for amplifying the target nucleic acid sequence of interest; and

at least a first probe set comprising a capture probe comprising a fluorophore attached thereto, and a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe

11. The mixture of claim 10, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO: 28).

12. The mixture of claim 11, wherein the mixture further comprises one or more amplification primers chosen from the group consisting of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).

13. A reaction chamber, comprising:

a reaction region having disposed therein:

a sample containing a target nucleic acid of interest;

amplification reagents for amplifying the target nucleic acid sequence of interest; and

at least a first probe set comprising a capture probe comprising a fluorophore attached thereto, and a target specific nucleic acid probe complementary to at least a portion of the capture probe and the target nucleic acid sequence, and comprising a quencher attached thereto, such that the quencher quenches fluorescence from the fluorophore when the target specific probe is hybridized to the capture probe.

14. The reaction chamber of claim 13, wherein the capture probe is immobilized upon an interior surface of the reaction chamber.

15. The reaction chamber of claim 13, wherein a plurality of different capture probes are immobilized in an array on an interior surface of the reaction chamber, each different capture probe being immobilized in a discrete location.

16. A method of detecting the presence of at least a first target nucleic acid sequence in a sample, comprising:

subjecting the sample to an amplification reaction capable of amplifying the target nucleic acid sequence in the presence of at least a first set of nucleic acid probes, the first set of nucleic acid probes comprising:

a target specific nucleic acid probe having a fluorescent label associated therewith complementary to at least a portion of the target nucleic acid sequence, a capture probe associated

with a solid support and complementary to at least a portion of the target specific nucleic acid probe; and

detecting fluorescence from the solid support following one or more cycles of the polymerase chain reaction, a decrease in fluorescence being indicative of the presence of the target nucleic acid sequence.

17. The method of claim 16, wherein the at least first target nucleic acid sequence comprises a variola virus sequence.

18. The method of claim 17, wherein the target specific nucleic acid probe comprises one or more of: VAR-1 probe (SEQ ID NO: 19), VAR-2 probe (SEQ ID NO: 22), VAR-3 probe (SEQ ID NO: 25), or VAR-4 probe (SEQ ID NO: 28).

19. The method of claim 16, wherein the target nucleic acid sequence is amplified through use of one or more of: VAR-1 primer 1 (SEQ ID NO: 17), VAR-1 primer 2 (SEQ ID NO: 18), VAR-2 primer 1 (SEQ ID NO: 20), VAR-2 primer 2 (SEQ ID NO: 21); VAR-3 primer 1 (SEQ ID NO: 23), VAR-3 primer 2 (SEQ ID NO: 24), VAR-4 primer 1 (SEQ ID NO: 26), or VAR-4 primer 2 (SEQ ID NO: 27).