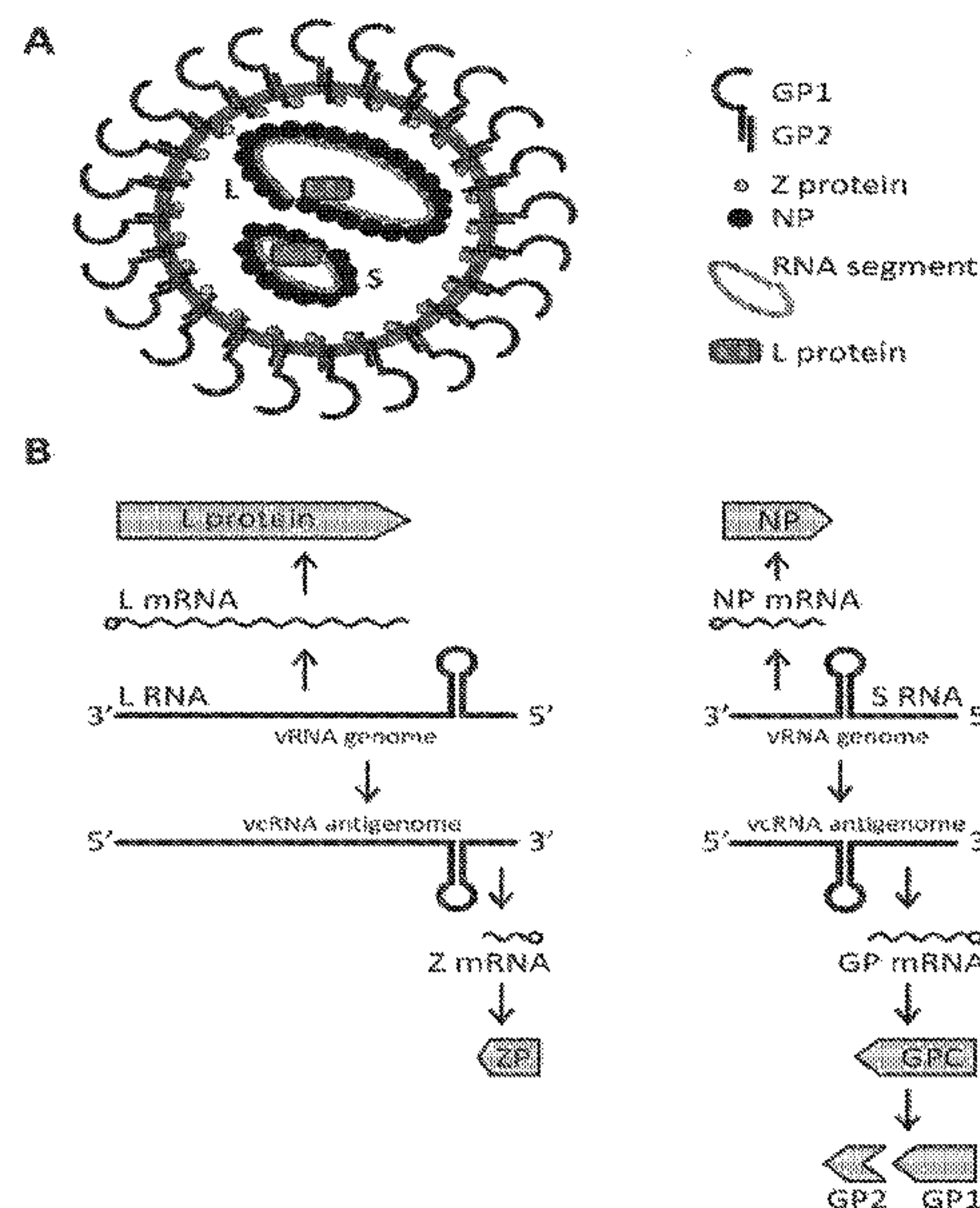




(86) Date de dépôt PCT/PCT Filing Date: 2012/01/06
(87) Date publication PCT/PCT Publication Date: 2012/07/12
(85) Entrée phase nationale/National Entry: 2013/07/04
(86) N° demande PCT/PCT Application No.: IB 2012/000293
(87) N° publication PCT/PCT Publication No.: 2012/093340
(30) Priorité/Priority: 2011/01/07 (US61/430,822)

(51) Cl.Int./Int.Cl. *C12Q 1/70* (2006.01),
G01N 33/569 (2006.01)
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FIGs. 1A-1B

(57) **Abrégé/Abstract:**

The present disclosure provides methods for determining whether a subject is infected with lymphocytic choriomeningitis virus (LCMV). These methods include obtaining a sample from a subject with increased susceptibility to LCMV infection, contacting the sample with one or more compositions for detecting LCMV, and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.



(10) International Publication Number
WO 2012/093340 A3

(57) Abstract: The present disclosure provides methods for determining whether a subject is infected with lymphocytic choriomeningitis virus (LCMV). These methods include obtaining a sample from a subject with increased susceptibility to LCMV infection, contacting the sample with one or more compositions for detecting LCMV, and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

Viral Diagnostics

RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 61/430,822 filed January 7, 2011, the entire content of which application is hereby
5 expressly incorporated by reference herein in its entirety.

TECHNICAL FIELD

The present disclosure relates to compositions and methods for detecting viruses in subjects.

BACKGROUND

10 Compositions and methods for reliable detection and diagnosis of certain viruses are limited. Techniques for discriminating between acute and chronic viral infections are also limited and can be unreliable.

SUMMARY

15 The present disclosure provides compositions and methods for detecting lymphocytic choriomeningitis virus (LCMV) in subjects and/or for discriminating between acute and chronic LCMV infections. Accordingly, the present disclosure can be used, e.g., to identify LCMV and develop personalized therapies for the treatment of LCMV infection (e.g., to select subjects for LCMV antiviral therapy and to monitor
20 and, if necessary, modify, LCMV antiviral therapy), reduce LCMV spread, reduce host-to-host LCMV transmission, reduce LCMV disease development and pathogenesis, and for evaluation of LCMV pathological states.

Accordingly, in one aspect, the disclosure provides a method for determining whether a subject is infected with lymphocytic choriomeningitis virus (LCMV), the
25 method comprising: selecting a subject with increased susceptibility to LCMV infection;
obtaining a sample from the subject; contacting the sample with one or more compositions for detecting LCMV; and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the

sample, wherein detection of an association indicates that that the subject is infected with LCMV.

In another aspect, the disclosure provides a method for determining whether a subject is infected with lymphocytic choriomeningitis virus (LCMV), the method comprising: selecting a subject suspected of being infected with LCMV; obtaining a sample from a subject; contacting the sample with one or more compositions for detecting LCMV; and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

In still another aspect, the disclosure provides a method for determining whether a subject is infected with lymphocytic choriomeningitis virus (LCMV), the method comprising: obtaining a sample from a subject; contacting the sample with at least two compositions selected from the group consisting of: one or more probes or primers that bind specifically to one or more LCMV nucleic acids or a portion of one or more LCMV nucleic acids; one or more LCMV proteins or fragments thereof; and one or more compositions for detecting one or more LCMV peptides or LCMV peptide fragments (e.g., one or more antibodies or antibody fragments); and determining whether the two or more compositions are associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

In yet another aspect, the disclosure provides a method for determining whether a subject is infected with lymphocytic choriomeningitis virus (LCMV), the method comprising: selecting a subject experiencing or at risk for hypoxia; obtaining a sample from the subject; contacting the sample with one or more compositions for detecting LCMV; and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

In some embodiments, the subject can be e.g., pregnant, immunocompromised, a transplant recipient, at risk for developing cancer, or having cancer, or any combination thereof. In some embodiments, the subject can be experiencing a hypoxic condition or at risk for a hypoxic condition.

In other embodiments, the one or more compositions for detecting LCMV can be one or more probes or primers that bind specifically to one or more LCMV nucleic acids or a portion of one or more LCMV nucleic acids.

5 In still other embodiments, the one or more compositions for detecting LCMV can be one or more LCMV proteins or fragments thereof.

In yet other embodiments, the one or more compositions for detecting LCMV can be one or more compositions for detecting one or more LCMV peptides or LCMV peptide fragments (e.g., one or more antibodies or antibody fragments).

10 In one aspect, the disclosure provides a composition comprising two or more compositions selected from the group consisting of: one or more probes or primers that bind specifically to one or more LCMV nucleic acids or a portion of one or more LCMV nucleic acids; one or more LCMV proteins or fragments thereof; and one or more compositions for detecting one or more LCMV peptides or LCMV peptide fragments (e.g., one or more antibodies or antibody fragments).

15 In another aspect, the disclosure provides a diagnostic kit, wherein the kit comprises: at least one isolated LCMV polypeptide or fragment thereof, e.g., an NP, GP, GPC, GP1, or ZP antigen, or fragment thereof. Alternatively or in addition, the kit can include at least one isolated antibody or antibody fragment that binds to an NP, GP, GPC, GP1, or ZP antigen, or antigen binding fragment thereof. Alternatively or
20 in addition, the kit can include at least one probe or primer that binds specifically to one or more LCMV nucleic acids or a portion thereof, e.g., nucleic acids that encode NP, GP, GPC, GP1, or ZP antigens. In some instances, the kit can include any combination thereof.

In still another aspect, the disclosure provides a plurality of isolated
25 polypeptides, wherein the plurality comprises or consists of at least two, e.g., three, four, or five, types of polypeptides, selected from the group consisting of isolated NP antigen or a fragment thereof, isolated GP antigen or a fragment thereof, isolated GPC antigen or a fragment thereof, isolated GP1 antigen or a fragment thereof, and isolated ZP antigen or a fragment thereof. In some instances, the disclosure provides a
30 diagnostic kit comprising such a plurality of polypeptides.

In yet another aspect, the disclosure provides a plurality of isolated antibodies, e.g., monoclonal or polyclonal antibodies, wherein the plurality comprises or consists

of antibodies that specifically bind to at least two, e.g., three, four, or five, types of polypeptides, selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof. In some instances, the disclosure provides a diagnostic kit comprising such a plurality of isolated antibodies.

In still another aspect, the disclosure provides a plurality of isolated nucleic acid probes or primers, wherein the plurality comprises or consists of probes or primers that specifically bind to nucleotide sequences that encode at least two, e.g., three, four, or five, types of polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof. In some instances, the disclosure provides a diagnostic kit comprising such a plurality of probes or primers.

In some embodiments, a diagnostic kit described herein can further include an agent, e.g., an antiviral agent, for treating LCMV or a symptom thereof in a subject.

In yet another aspect, the disclosure provides a method of treating a subject for LCMV infection, comprising: obtaining a biological sample from a subject having or at risk for infection with LCMV; screening the sample using a diagnostic kit described above; a plurality of polypeptides described above; a plurality of antibodies described above; or the plurality of probes or primers described above; or any combination thereof; to determine whether the subject is infected with LCMV; and administering to the subject an agent, e.g., an antiviral agent, that treats LCMV or a symptom thereof if the patient is infected with LCMV. In some instances, the subject having or at risk for LCMV infection has a condition involving hypoxia. In some instances, the subject having or at risk for LCMV infection is pregnant, immunocompromised, a transplant recipient, at risk for developing cancer, or has cancer, or any combination thereof.

In still another aspect, the disclosure provides a method of determining whether a subject is infected with LCMV, the method comprising: obtaining a biological sample from a subject having or at risk for LCMV; contacting the sample with a plurality of polypeptides described above, a plurality of antibodies described above, or a plurality of probes or primers described above, or any combination

thereof; determining whether the plurality of polypeptides, plurality of antibodies, plurality of probes or primers, or any combination thereof, associate with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

5 In another aspect, the disclosure provides a monoclonal antibody M59 that binds specifically to LCMV NP or antigen binding fragment thereof, e.g., a complementarity determining region (CDR), e.g., CDR3, thereof. In some instances, the disclosure provides a kit comprising such an antibody.

10 In another aspect, the disclosure provides a monoclonal antibody M87 that binds specifically to LCMV NP or antigen binding fragment thereof, e.g., a complementarity determining region (CDR), e.g., CDR3, thereof. In some instances, the disclosure provides a kit comprising such an antibody.

15 In another aspect, the disclosure provides a monoclonal antibody that binds specifically to LCMV GP1 or antigen binding fragment thereof, e.g., a complementarity determining region (CDR), e.g., CDR3, thereof. In some instances, the disclosure provides a kit comprising such an antibody.

20 In another aspect, the disclosure provides a monoclonal or polyclonal antibody that binds specifically to the amino acid sequence RSGWGWAGSDGKTT (SEQ ID NO:89), or an antigen binding fragment of such an antibody, e.g., a complementarity determining region (CDR), e.g., CDR3, thereof. In some instances, the disclosure provides a kit comprising such an antibody.

25 In another aspect, the disclosure provides a monoclonal antibody MJ3 that binds specifically to LCMV ZP or fragment thereof, e.g., a complementarity determining region (CDR), e.g., CDR3, thereof. In some instances, the disclosure provides a kit comprising such an antibody.

30 In some aspects, the disclosure provides methods of assessing (e.g., detecting, determining, evaluating, and/or monitoring) lymphocytic choriomeningitis virus (LCMV) infection or activity in a subject. Such methods can include selecting a subject for assessment, wherein candidate subjects have or are suspected of being exposed to LCMV or a LCMV infected person or animal. The methods also include obtaining or providing a sample from a selected subject, contacting the sample with one or more compositions for detecting LCMV, and determining whether the one or

more compositions for detecting LCMV are associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

5 In some aspects, the disclosure provides methods for assessing (e.g., detecting, determining, evaluating, and/or monitoring) lymphocytic choriomeningitis virus (LCMV), including levels (e.g., levels of LCMV nucleic acid, protein(s), and/or activity) in a subject, e.g., a subject infected with LCMV or that is suspected of being exposed to a source of LCMV infection, e.g., an LCMV infected human or animal. In some embodiments, such methods can include selecting a subject (e.g., a candidate
10 subject), obtaining or providing a sample from the subject, contacting the sample with one or more compositions for detecting LCMV, and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

15 In some aspects, the disclosure provides methods for assessing (e.g., detecting, determining, evaluating, and/or monitoring) lymphocytic choriomeningitis virus (LCMV), including levels (e.g., levels of LCMV nucleic acid, protein(s), and/or activity) in a subject, e.g., a subject infected with LCMV or that is suspected of being exposed to a source of LCMV infection, e.g., an LCMV infected human or animal. In
20 some embodiments, methods include obtaining or providing a sample from a subject (e.g., a suitable subject), contacting the sample with at least two compositions selected from the group consisting of: one or more probes or primers that bind specifically to one or more LCMV nucleic acids or a portion of one or more LCMV nucleic acids; one or more LCMV proteins or fragments thereof; and one or more compositions for
25 detecting one or more LCMV peptides or LCMV peptide fragments (e.g., one or more antibodies or antibody fragments); and determining whether the two or more compositions are associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

In some aspects, the disclosure provides methods for assessing (e.g., detecting,
30 determining, evaluating, and/or monitoring) lymphocytic choriomeningitis virus (LCMV), including levels (e.g., levels of LCMV nucleic acid, protein(s), and/or activity) in a subject, e.g., a subject infected with LCMV or that is suspected of being

exposed to a source of LCMV infection, e.g., an LCMV infected human or animal. In some embodiments, such methods can include, obtaining or providing a sample from the subject, contacting the sample with one or more compositions for detecting LCMV, and determining whether the one or more compositions for detecting LCMV is associated with a marker of LCMV from the sample, wherein detection of an association indicates that the subject is infected with LCMV.

In some embodiments, the methods of the disclosure can include selecting a subject that has or is at risk of a condition associated with an increased level of hypoxia and/or free radical formation. Such subjects include, for example, those that are pregnant, immunocompromised, transplant recipients, and/or that are at risk for developing cancer, or has cancer.

In some embodiments, the methods of the disclosure can include use of one or more compositions disclosed herein alone or in combination with any of the other compositions disclosed herein. For example, use of two or more compositions in combination is not limited to simultaneous use, but rather includes, for example, parallel use or subsequent use. In some embodiments, a result observed using one composition can be verified or confirmed using one or more of the other compositions disclosed herein.

In some embodiments, the methods of the disclosure can include use of one or more probes or primers that bind specifically to one or more LCMV nucleic acids or a portion of one or more LCMV nucleic acids. For example, such probes or primers can include nucleic acid probes or primers having 10 or more nucleic acids, wherein the 10 or more nucleic acids have at least at least 80% identity to one or more target regions within one or more of SEQ ID NOs:1-51, such that the one or more nucleic acid probes or primers bind specifically to the one or more target regions.

In some embodiments, the methods of the disclosure can include one or more probes or primers selected from the group consisting of one or more nucleic acid sequences with at least 80% identity to one or more of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73. In some embodiments, the methods of the

disclosure can include one or more probes or primers selected from the group consisting of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73.

5 In some embodiments, the methods of the disclosure can include use of one or more probes or primers that bind to a nucleic acid encoding LCMV NP. For example, such probes or primers can include, SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59; or SEQ ID NOs:66 and 67 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID
10 NOs:66 and 67.

 In some embodiments, the methods of the disclosure can include use of one or more probes or primers that bind to a nucleic acid encoding LCMV GP. For example, such probes or primers can include SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61; or SEQ ID NOs:68
15 and 69 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:68 and 69.

 In some embodiments, the methods of the disclosure can include use of one or more probes or primers that bind to a nucleic acid encoding LCMV ZP. For example, such probes or primers can include, SEQ ID NOs:62 and 63 or a pair of nucleic acid
20 sequences with at least 80% identity to SEQ ID NOs:62 and 63; or SEQ ID NOs: 72 and 73 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73.

 In some embodiments, the methods of the disclosure can include use of one or more probes or primers that bind to a nucleic acid encoding LCMV L. For example,
25 such probes or primers can include SEQ ID NOs: 70 and 71 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 70 and 71.

 In some embodiments, the methods of the disclosure can include use of nucleic acids encoding two or more of LCMV NP, LCMV GP, LCMV ZP, and LCMV L.

30 In some embodiments, the methods of the disclosure can include use of SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59, or SEQ ID NOs:66 and 67 or a pair of nucleic acid sequences

with at least 80% identity to SEQ ID NOs:66 and 67, wherein the primers bind to LCMV NP;

SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61, or SEQ ID NOs:68 and 69 or a pair of nucleic acid

5 sequences with at least 80% identity to SEQ ID NOs:68 and 69, wherein the primers bind to LCMV GP; SEQ ID NOs:62 and 63 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:62 and 63, or SEQ ID NOs: 72 and 73 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73, wherein the primers bind to LCMV ZP; or SEQ ID NOs: 70 and 71 or a pair of nucleic acid
10 sequences with at least 80% identity to SEQ ID NOs: 70 and 71, wherein the primers bind to LCMV L.

In some embodiments, the methods of the disclosure can include use of one or more LCMV proteins or fragments thereof.

In some embodiments, the methods of the disclosure can include use of one or
15 more antibodies or antibody fragments. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a
20 monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the
25 Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or
30 antibody fragments can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University,

Belgium, and a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a

5 monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

10 In some embodiments, such antibodies or antibody fragments can include CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, comprising no (e.g., zero) or at least 1 (e.g., 1, 2, 3, 4, 5, less than 10, less than 20, less than 30, less than 50, or less than 100) conservative amino acid substitutions, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal

15 antibody M87, comprising no (e.g., zero) or at least 1 (e.g., 1, 2, 3, 4, 5, less than 10, less than 20, less than 30, less than 50, or less than 100) conservative amino acid substitutions, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87, comprising no (e.g., zero) or at least 1 (e.g., 1, 2, 3, 4, 5, less than 10, less than 20, less than 30, less than 50, or less than 100) conservative

20 amino acid substitutions. In some embodiments, such antibodies or antibody fragments can include an antigen binding peptide (e.g., including an antibody and/or an antigen binding antibody fragment) with identity to SEQ ID NO: 74, wherein regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino

25 acid substitutions, regions the amino acid sequence that correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region in SEQ ID NO:74, and/or the antigen binding peptide binds to LCMV NP.

In some aspects, the present disclosure includes compositions comprising combinations (e.g., including 1, 2 or 3) of: one or more probes or primers that bind

30 specifically to one or more LCMV nucleic acids or a portion of one or more LCMV nucleic acids; one or more LCMV proteins or fragments thereof; and one or more antibodies or antibody fragments. In some embodiments, the one or more probes or

primers of the compositions herein can include one or more nucleic acid probes or primers having 10 or more nucleic acids, wherein the 10 or more nucleic acids have at least at least 80% identity to one or more target regions within one or more of SEQ ID NOs:1-51, such that the one or more nucleic acid probes or primers bind specifically to the one or more target regions. In some embodiments, the one or more probes or primers of the compositions herein are selected from the group consisting of one or more nucleic acid sequences with at least 80% identity to one or more of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73. In some embodiments, the one or more probes or primers of the compositions herein are selected from the group consisting of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73. In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers that bind to a nucleic acid encoding LCMV NP. In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers selected from SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59; or SEQ ID NOs:66 and 67 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:66 and 67.

In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers that bind to a nucleic acid encoding LCMV GP. For example, such probes or primers can include SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61; or SEQ ID NOs:68 and 69 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:68 and 69.

In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers that bind to a nucleic acid encoding LCMV ZP. For example, such probes or primers can include SEQ ID NOs:62 and 63 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:62 and

63; or SEQ ID NOs: 72 and 73 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73.

In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers that bind to a nucleic acid encoding LCMV L. For example, such probes or primers can include SEQ ID NOs: 70 and 71 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 70 and 71.

In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers that bind to a nucleic acid encoding LCMV GP. For example, such probes or primers can include one or more probes or primers bind to nucleic acids encoding two or more of LCMV NP, LCMV GP, LCMV ZP, and/or LCMV L.

In some embodiments, the one or more probes or primers of the compositions herein include one or more probes or primers that bind to a nucleic acid encoding LCMV GP. For example, such probes or primers can include one or more probes or primers comprise: SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59, or SEQ ID NOs:66 and 67 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:66 and 67, wherein the primers bind to LCMV NP; SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61, or SEQ ID NOs:68 and 69 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:68 and 69, wherein the primers bind to LCMV GP; SEQ ID NOs:62 and 63 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:62 and 63, or SEQ ID NOs: 72 and 73 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73, wherein the primers bind to LCMV ZP; or SEQ ID NOs: 70 and 71 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 70 and 71, wherein the primers bind to LCMV L.

In some aspects, the present disclosure includes compositions comprising one or more antibodies. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some

embodiments, such antibodies or antibody fragments can include a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, such antibodies or antibody fragments can include CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

In some embodiments, antibodies or antibody fragments included in the compositions of the disclosure can include CDR3 (SEQ ID NO:78) of the heavy chain

variable region of monoclonal antibody M87, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87.

5 In some embodiments, antibodies or antibody fragments included in the compositions of the disclosure can include an antigen binding peptide (e.g., including an antibody and/or antigen binding antibody fragment) with identity to SEQ ID NO: 74, wherein: regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino acid substitutions; regions the amino acid sequence that
10 correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region in SEQ ID NO:74; and the antigen binding peptide binds to LCMV NP.

In some aspects, the present disclosure includes diagnostic kits. In some embodiments, such diagnostic kits can include, at least one isolated LCMV
15 polypeptide or fragment thereof, wherein the LCMV polypeptide is an NP, GP, or ZP antigen, or fragment thereof; one or more isolated antibodies or antibody fragments that bind to an NP, GP, or ZP antigen, or antigen binding fragment thereof; and/or one or more probes or primers that bind specifically to one or more LCMV nucleic acids or a portion thereof; and/or combinations thereof.

20 In some embodiments, diagnostic kits of the present disclosure can include one or more probes or primers comprise one or more nucleic acid probes or primers having 10 or more nucleic acids, wherein the 10 or more nucleic acids have at least at least 80% identity to one or more target regions within one or more of SEQ ID NOs:1-51, such that the one or more nucleic acid probes or primers bind specifically
25 to the one or more target regions. In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include probes or primers selected from the group consisting of one or more nucleic acid sequences with at least 80% identity to one or more of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73. In
30 some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include probes or primers selected from the group consisting of SEQ ID

NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73.

5 In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include probes or primers that bind to a nucleic acid encoding LCMV NP. In some embodiments, such probes or primers can include, for example, SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59; or SEQ ID NOs:66 and 67 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:66 and 67.

10 In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include probes or primers that bind to a nucleic acid encoding LCMV GP. In some embodiments, such probes or primers can include, for example, SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61; or SEQ ID NOs:68 and 69 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:68 and 69.

15 In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include probes or primers that bind to a nucleic acid encoding LCMV ZP. In some embodiments, such probes or primers can include, for example, SEQ ID NOs:62 and 63 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:62 and 63; or SEQ ID NOs: 72 and 73 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73.

20 In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include probes or primers that bind to a nucleic acid encoding LCMV L. In some embodiments, such probes or primers can include, for example, SEQ ID NOs: 70 and 71 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 70 and 71.

In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include one or more probes or primers that bind to nucleic acids encoding two or more of LCMV NP, LCMV GP, LCMV ZP, and LCMV L.

30 In some embodiments, probes or primers contained in the diagnostic kits of the present disclosure include one or more of SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59, or SEQ

ID NOs:66 and 67 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:66 and 67, wherein the primers bind to LCMV NP; SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61, or SEQ ID NOs:68 and 69 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:68 and 69, wherein the primers bind to LCMV GP; SEQ ID NOs:62 and 63 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:62 and 63, or SEQ ID NOs: 72 and 73 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73, wherein the primers bind to LCMV ZP; and/or SEQ ID NOs: 70 and 71 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 70 and 71, wherein the primers bind to LCMV L.

In some embodiments, diagnostic kits of the present disclosure include one or more isolated antibodies or antibody fragments. In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include a monoclonal antibody having the same epitope specificity as hybridoma

MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include

a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87.

In some embodiments, isolated antibodies or antibody fragments included in the diagnostic kits of the present disclosure can include an antigen binding peptide (e.g., an antibody or antigen binding antibody fragment) with identity to SEQ ID NO: 74, wherein: regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino acid substitutions; regions the amino acid sequence that

correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region in SEQ ID NO:74; and/or the antigen binding peptide binds to LCMV NP.

In some aspects, the present disclosure include pluralities of isolated polypeptides, wherein the plurality comprises at least two polypeptides selected from the group consisting of isolated NP antigen or a fragment thereof, isolated GP antigen or a fragment thereof, isolated GPC antigen or a fragment thereof, isolated GP1 antigen or a fragment thereof, and isolated ZP antigen or a fragment thereof. In some embodiments, pluralities of the disclosure can include: at least three polypeptides selected from the group consisting of isolated NP antigen or a fragment thereof, isolated GP antigen or a fragment thereof, isolated GPC antigen or a fragment thereof, isolated GP1 antigen or a fragment thereof, and isolated ZP antigen or a fragment thereof, at least four polypeptides selected from the group consisting of isolated NP antigen or a fragment thereof, isolated GP antigen or a fragment thereof, isolated GPC antigen or a fragment thereof, isolated GP1 antigen or a fragment thereof, and isolated ZP antigen or a fragment thereof, isolated NP antigen or a fragment thereof, isolated GP antigen or a fragment thereof, isolated GPC antigen or a fragment thereof, isolated GP1 antigen or a fragment thereof, and isolated ZP antigen or a fragment thereof. In some embodiments, such pluralities can be provided as or contained within a kit.

In some aspects, the present disclosure include pluralities of isolated antibodies or antibody fragments, wherein the plurality comprises antibodies or fragments that specifically bind to at least two polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof.

In some aspects, the present disclosure include pluralities of isolated antibodies or antibody fragments, wherein the plurality comprises antibodies or fragments that specifically bind to at least three polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof.

In some aspects, the present disclosure include pluralities of isolated antibodies or antibody fragments, wherein the plurality comprises antibodies or fragments that specifically bind to at least four polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof.

In some aspects, the present disclosure include pluralities of isolated antibodies or antibody fragments, wherein the plurality comprises antibodies or fragments that specifically bind to NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof.

In some embodiments, pluralities of the present disclosure can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, pluralities of the present disclosure can include a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, pluralities of the present disclosure can include a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, pluralities of the present disclosure can include a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB. In some embodiments, pluralities of the present disclosure can include a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium. In some embodiments, pluralities of the present disclosure can include a monoclonal antibody produced by hybridoma MJ3, LMBP accession number

9217CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

5 In some embodiments, pluralities of the present disclosure can include an antigen binding peptide with identity to SEQ ID NO: 74, wherein: regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino acid substitutions; regions
10 the amino acid sequence that correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region in SEQ ID NO:74; and/or the antigen binding peptide binds to LCMV NP.

In some aspects, the pluralities of the present disclosure can be provided as (e.g., sold, offered for sale, marketed, shipped, stored, and/or packaged) or contained within a diagnostic kit.

15 In some aspects, pluralities of the present disclosure can include probes or primers that specifically bind to nucleotide sequences that encode at least two polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof. In some embodiments,
20 pluralities of the present disclosure can include probes or primers that specifically bind to nucleotide sequences that encode at least three polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof. In some embodiments, pluralities of the present
25 disclosure can include probes or primers that specifically bind to nucleotide sequences that encode at least four polypeptides selected from the group consisting of NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof. In some embodiments, pluralities of the present disclosure can include
30 probes or primers that specifically bind to nucleotide sequences that encode NP antigen or a fragment thereof, GP antigen or a fragment thereof, GPC antigen or a

fragment thereof, GP1 antigen or a fragment thereof, and ZP antigen or a fragment thereof.

In some aspects, the pluralities of the present disclosure can be provided as (e.g., sold, offered for sale, marketed, shipped, stored, and/or packaged) or contained
5 within a diagnostic kit. In some embodiments, such diagnostic kits can further include at least one (e.g., 1, 2, 3, 4, 5 or more) agent (e.g., a pharmaceutical) for treating LCMV or a symptom thereof in a subject. In some embodiments, at least one such agent is an antiviral agent.

In some embodiments, the present disclosure provides methods of treating a
10 subjects for LCMV infection, comprising: obtaining a biological sample from a subject having or at risk for infection with LCMV; screening the sample using the method of claim 1 to determine whether the subject is infected with LCMV; and administering to the subject an agent that treats LCMV or a symptom thereof if the patient is infected with LCMV. In some embodiments, treatment methods of the
15 present disclosure can include selecting a subject with or at risk for LCMV infection has a condition involving hypoxia. Such subjects can include, for example, those that are pregnant, immunocompromised, transplant recipients, and those at risk for developing cancer, or with cancer.

In some aspects, the present disclosure provides a monoclonal antibody M59
20 that binds specifically to LCMV NP or fragment thereof.

In some aspects, the present disclosure provides a monoclonal antibody M87 that binds specifically to LCMV NP or fragment thereof.

In some aspects, the present disclosure provides a monoclonal antibody that binds specifically to LCMV GP1 or fragment thereof.

25 In some aspects, the present disclosure provides a monoclonal antibody that binds specifically to the amino acid sequence RSGWGWAGSDGKTT (SEQ ID NO:89).

In some aspects, the present disclosure provides a monoclonal antibody MJ3 that binds specifically to LCMV ZP or fragment thereof.

30 In some aspects, the present disclosure provides an antigen binding fragment of one or more of the antibodies disclosed herein.

In some aspects, the present disclosure provides a complementarity determining region (CDR) of one or more of the antibodies disclosed herein. In some embodiments, the CDR is CDR3.

5 In some aspects, one or more of the antibodies or antibody binding fragments disclosed herein can be provided as (e.g., sold, offered for sale, marketed, shipped, stored, and/or packaged) or contained within a kit.

In some aspects, the present disclosure provides a monoclonal antibody having the same epitope specificity as hybridoma MJ3, LMBP accession number 9217CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent
10 University, Belgium.

In some aspects, the present disclosure provides a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

15 In some aspects, the present disclosure provides a cell of hybridoma MJ3, LMBP accession number 9217CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

In some aspects, the present disclosure provides a monoclonal antibody having the same epitope specificity as hybridoma M166, LMBP accession number 9216CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent
20 University, Belgium.

In some aspects, the present disclosure provides a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.
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In some aspects, the present disclosure provides a cell of hybridoma M166, LMBP accession number 9216CB, deposited with Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

In some aspects, the present disclosure provides an isolated antibody or
30 antibody fragment comprising CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of

monoclonal antibody M87, comprising one or more conservative amino acid substitutions, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

5 In some aspects, the present disclosure provides an isolated antibody or antibody fragment comprising CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, and/or CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, and/or CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87.

10 In some aspects, the present disclosure provides an antigen binding peptide (e.g., an antibody or antibody fragment) with identity to SEQ ID NO: 74, wherein: regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino acid substitutions; regions the amino acid sequence that correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region
15 in SEQ ID NO:74; and the antigen binding peptide binds to LCMV NP.

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. Methods and materials are described herein for use in
20 the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will
25 control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1A is an illustration showing the structure of LCMV, including the outer
30 trans-membrane glycoproteins 1 and 2 (GP1 and GP2), Z protein, NP, RNA, and L protein of LCMV virion and virus replication strategy.

FIG. 1B is a schematic illustrating the LCMV life cycle.

FIG. 2 (i.e., FIG. 2i-2vi) shows alignments of nucleotide sequences for the NP genomic region on S segment of selected LCMV strains/isolates.

FIG. 3 (i.e., FIG. 3i-3vi) shows alignments of nucleotide sequences for the GP (i.e. GPC, encompassing GP1 and GP2) genomic region on S segment of selected LCMV strains/isolates.

FIG. 4 shows alignments of nucleotide sequences for the ZP genomic region on L segment of selected LCMV strains/isolates.

FIG. 5 (i.e., FIGs. 5i-5ii) shows alignment of amino acid sequences for NP antigens of selected LCMV strains/isolates.

FIG. 6 (i.e., FIGs. 6i-6ii) shows alignment of amino acid sequences for GP antigens of selected LCMV strains/isolates.

FIG. 7 shows alignment of amino acid sequences for ZP antigens of selected LCMV strains/isolates.

FIGs. 8A-8D are illustrations showing exemplary antibody based assays for use in the methods disclosed herein.

FIG. 9 is an illustration showing an exemplary RNA based assay for use in the methods disclosed herein.

FIG. 10A is a histogram showing that hypoxia up-regulates LCMV gene expression. Fold induction was determined in comparison with the normoxic control. Values represent means of three separate experiments done in quadruplicates. Error bars denote the standard deviations. *P<0.02, *** P< 0.001 (hypoxic (HY) vs. normoxic (NO)).

FIG. 10B is a histogram showing that DMOG treatment up-regulates LCMV gene expression. Values represent means of triplicate determinations in one representative experiment out of two, with error bars denoting standard deviations. *P<0.05, *** P< 0.001 (DMOG vs. no DMOG).

FIG. 10C is an image of an immunoblot showing that hypoxia up-regulates LCMV protein expression. Actin is shown as a control for loading and transfer efficiency. Detection of HIF-1alpha served as a control for the induction of cellular response to hypoxia. One representative of at least three independent experiments with similar results is shown.

FIG. 10D is a bar graph showing relative expression levels of various LCMV genes under normoxic and hypoxic conditions as assed by RLM-RACE.

FIG. 11A is an image of gel confirming the presence of LCMV genome in the medium from HeLa-MX cells cultured under normoxia (NO) and in the medium from
5 cells cultured under hypoxia (HY) as detected by RT-PCR.

FIG. 11B is an image of a gel showing LCMV gene expression in cells infected using the medium from HeLa-MX cells at passage (P)1,P3, P5, and P10, as assessed by RT-PCR.

FIG. 11C is an image of cells infected using the medium from HeLa-MX
10 cells. Cells were stained for LCMV. A nuclear stain was also used. Cells were stained at P1, P3, and P10. 20-times magnification was used. Data is representative of two independent experiments.

FIG. 12 A is a diagram illustrating a protocol used to characterize the sequence of the variable regions of antibody heavy chains.

FIG. 12B shows the amino acid sequence of the variable region of the heavy
15 chain of MAb M87. Yellow shading indicates a signal peptide sequence. Green shading indicates hypervariable regions.

FIG. 12C illustrates structure of the heavy chain of MAb M87.

FIG. 13 is an image of an immunoblot confirming the presence of of anti-NP
20 antibodies in sera of women who had spontaneous abortion. Anti-NP antibodies in human sera were detected by immunoprecipitation.

FIG. 14 is an image showing immunohistochemical detection of viral NP in kidney tumor (A) and a negative control (B).

FIG. 14C is an image of an immunoblot showing immunodetection of LCMV
25 NP in tissue from human RCC subjects by immunoprecipitation and subsequent immunoblotting with NP-specific monoclonal antibody. HT= healthy tissue, TT= tumor tissue, HMX= HeLa/MX (positive control), H= HeLa (negative control).

FIGs. 15A and 15B are graphs illustrating the results of competitive binding studies between M87 and M59 antibodies.

FIG. 16 is a line graph showing epitope binding of NP-specific antibodies to
30 NP fragments.

5 **FIGs. 18A-18B** are images of agarose gels showing electrophoresis of LCMV
PCR products.

FIG. 20 is a line graph showing real-time detection of LCMV MX NP and GP genes in duplex format.

FIG. 22 is a line graph showing real-time detection of LCMV ARM NP and GP genes in duplex format.

FIG. 24 is a bar graph showing LCMV MX NP and GP gene expression under hypoxic and normoxic conditions as assessed by real-time PCR.

The present disclosure is based, *inter alia*, on the surprising discovery LCMV in persistence can be reactivated by hypoxia. Such viral reactivation can manifest clinically, e.g., in vulnerable subjects that include, but are not limited to, e.g., pregnant subjects, immunocompromised subjects, transplant recipients, and subjects at risk for developing or with cancer. Accordingly, the present disclosure provides compositions and methods for reliably detecting LCMV, e.g., reactivated LCMV.

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peripheral glycoprotein 1 (GP1) and transmembrane glycoprotein 2 (GP2) (Southern et al, Virology, 157(1):145-55 (1987)). The L RNA segment encodes an RNA-dependent RNA polymerase (L) and a regulatory ring finger Z protein (ZP) (Buchmeier Curr Top Microbiol Immunol. 262:159-73 (2002); Salvato, Virology 173, 1-10 (1989)).

Virus replication starts with the L polymerase-driven transcription of the 3' RNA genome arms of negative polarity and produces mRNAs that are subsequently translated to NP and L polymerase. These viral proteins assist in the transcription of the RNA genome to virus cRNA, serving as a template for the synthesis of the new genomic RNA molecules as well as for the subgenomic mRNAs translated to GPC and ZP. This two-stage replication strategy facilitates establishment of virus persistence, which can be sustained by the virus ribonucleoprotein composed of NP, the RNA genome, and L polymerase in the absence of mature virion production caused by absent or limited expression of glycoproteins (van der Zeijst et al, J Virol 48:249-61 (1983); Buchmeier, 2003). LCMV can easily set up persistent infection in a wide variety of cell types derived from various species, where it does not perturb vital cell functions but modulates nonessential phenotypic features (Oldstone, Curr Top Microbiol Immunol. 263:83-117 (2002); Peters et al, Supra).

LCMV is distributed worldwide due to its association with rodents of the species *Mus musculus*. Humans are generally infected through the respiratory tract after direct or indirect contact with infected rodents or pets (via inhalation of virus-contaminated aerosols of animal saliva, urine, and feces). In immunocompetent individuals, LCMV causes illnesses varying from mild flu-like symptoms to rare severe encephalitis (Jahrling and Peters, 1992, Buchmeier et al, 2007). Infection with this virus during pregnancy has been linked to spontaneous abortions and malformations (Jamieson et al, 2006, Meritet et al, 2009). More strikingly, fatal cases of LCMV infections transmitted via transplanted organs from infected donors to immunosuppressed recipients were recently reported and call for more attention to this seemingly innocent virus (Fischer et al, 2006, Amman et al, 2007).

Acute infections involve production of mature GP1 and GP2, whereas chronic/persistent infection exhibits production of immature GPC, but mature forms of glycoproteins are missing or reduced, NP is produced both in acute and

chronic/persistent situations, similarly ZP is produced both in acute and chronic/persistent situations, but its expression increases upon reactivation by hypoxia (Buchmeier, 2003, Tomaskova et al, unpublished data). This fact was overlooked in previous attempts to detect LCMV, but was recalled recently by our research data showing relative increase of GP, NP and ZP production and formation of infectious virions in response to hypoxia, which is associated with many physiological and pathological situations, including embryonic development, heart and brain ischemia, cancer etc. Moreover, LCMV “reactivation” is known to occur due to immunosuppression (for transplantation purposes, due to chemotherapy and in other situations) and is also likely to be associated with increased expression of viral GP, NP and ZP.

So far, no reliable method for LCMV detection, screening and diagnosis of LCMV acute and/or chronic infection is available for routine use in clinical laboratories, no target (risk) populations to be screened/diagnosed have been determined, since comprehensive epidemiological data is missing. Existing assays (including, for example, immunofluorescence, ELISA, complement-fixation, and RT PCR assays) do not show sufficient sensitivity and reproducibility, do not discriminate between acute and chronic/persistent infection, and are applied only occasionally in outbreak situations or when other viruses cannot be detected. Furthermore, existing assays generally do not combine NP, GP, ZP (or alternatively GCP and GP1) and ZP-derived oligonucleotides or polypeptides to detect virus and/or virus-specific antibodies and thus most probably fail to detect many cases of LCMV infections even when the infection is proven otherwise. Therefore, availability of an accurate, sensitive and reproducible routine assay is highly desirable.

Compositions for Detecting LCMV

Compositions encompassed by the present disclosure include biological and synthetic materials that can specifically detect one or more markers of LCMV in a biological sample.

Markers of LCMV can include, for example, one or more or at least one (e.g., 1, 2, 3, 4, 5 or more, including combinations of 2, 3, 4, or 5) LCMV nucleic acids (e.g., LCMV mRNA and/or LCMV genomic DNA/RNA such as LCMV encoding one

or more LCMV peptides (e.g., LCMV GP (1 and/or 2), Z protein, NP, and/or L protein)) and/or LCMV proteins or peptides (e.g., e.g., 1, 2, 3, 4, 5 or more, including combinations of 2, 3, 4, or 5 of LCMV GP (1 and/or 2), Z protein, NP, and/or L protein). For example, in some instances, markers of LCMV can include LCMV GP and/or LCMP NP nucleic acid and/or protein. In some instances, markers of LCMV can include or can be detected by targeting a portion of the marker. In some instances, portions of LCMV markers can include, for example, regions of nucleic acids that are conserved between one or more LCMV strains or isolates. For example, suitable portions for detection can include those regions identified as being conserved in FIGs. 2-4 (e.g., in one or more of SEQ ID NOs:1-57). Alternatively or in addition, suitable portions can include a region within a LCMV nucleic acid that has at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to a region in one or more distinct LCMV strains (e.g., in one or more of SEQ ID NOs:1-57). In some instances, suitable portions can include a region within a LCMV protein that has at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to a region in a protein encoded by one or more of SEQ ID NOs:1-51. In some instances, suitable portions can include a region within a LCMV protein that has at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to a region in one or more of SEQ ID NOs:52-57.

Compositions suitable for specifically detecting such one or more or at least one LCMV nucleic acids or portions thereof can include, but are not limited to nucleic acid probes or primers. Methods for designing and synthesizing suitable probes or primers are known in the art. In some instances, nucleic acid probes or primers that can be used to detect one or more or at least one marker of LCMV can include nucleic acid probes or primers containing, for example, 10 or more nucleic acids (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more than 1000 nucleic acids), e.g., wherein the 10 or more nucleic acids has at least at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to a target region within one or more LCMV markers (e.g., within one or more of SEQ ID NOs:1-51), such that the nucleic acid probe or primer binds specifically to the target region (e.g., within one or more of SEQ ID NOs:1-51). In

some instances, the probe or primer can bind (e.g., bind specifically) to the target region under stringent binding conditions (e.g., low stringency, medium stringency, or high stringency). Hybridization conditions that qualify as low, medium, and high stringency hybridization conditions are known in the art. It is understood in the art that a complementary nucleic acid sequence need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. A complementary nucleic acid sequence of the invention is specifically hybridizable when binding of the sequence or a portion thereof, to the target sequence occurs such that amplification or the target portion can occur. In some instances, the multiple probes or primers can be used to detect the same LCMV nucleic acid in a sample (the term “sample” or “biological sample” refers to a sample of tissue or body fluid obtained from a subject (human or animal), including but not limited to blood, serum, plasma, tissue biopsies and surgical specimens, saliva, urine, cerebrospinal fluid etc. Biological sample also includes in vitro cultured cells and culture media. The samples may be treated prior to analysis by heating, centrifugation, precipitation etc.). In such instances, the probes can detect overlapping portions of the same LCMV nucleic acid or they can detect non-overlapping portions of the same LCMV nucleic acid. Alternatively or in addition, the multiple probes or primers can be used to detect distinct LCMV nucleic acids in a sample. In some instances, nucleic acid probes or primers that can be used to detect one or more or at least one marker of LCMV can include, for example, one or more or at least one of SEQ ID NO:58, SEQ ID NO:59 (e.g., a combination of SEQ ID NO:58 and 59, which detect LCMV NP), SEQ ID NO:60, SEQ ID NO:61 (e.g., a combination of SEQ ID NO:60-61, which detect LCMV GP), SEQ ID NO:62, SEQ ID NO:63 (e.g., a combination of SEQ ID NO:62-63, which detect LCMV ZP), SEQ ID NO:66, SEQ ID NO:67 (e.g., a combination of SEQ ID NO:66-67, which detect LCMV NP), SEQ ID NO:68, SEQ ID NO:69 (e.g., a combination of SEQ ID NO:68-69, which detect LCMV GP), SEQ ID NO:70, SEQ ID NO:71 (e.g., a combination of SEQ ID NO:70-71, which detect LCMV L), SEQ ID NO:72, SEQ ID NO:73 (e.g., a combination of SEQ ID NO:72-73, which detect LCMV Z). In some instances, nucleic acid probes or primers that can be used to detect one or more or at least one marker of LCMV can include nucleic acid probes or primers with at least 50% (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, and 100%) sequence

homology or identity to one or more of SEQ ID NO:58, SEQ ID NO:59 (e.g., a combination of SEQ ID NO:58 and 59, which detect LCMV NP), SEQ ID NO:60, SEQ ID NO:61 (e.g., a combination of SEQ ID NO:60-61, which detect LCMV GP), SEQ ID NO:62, SEQ ID NO:63 (e.g., a combination of SEQ ID NO:62-63, which
5 detect LCMV ZP), SEQ ID NO:66, SEQ ID NO:67 (e.g., a combination of SEQ ID NO:66-67, which detect LCMV NP), SEQ ID NO:68, SEQ ID NO:69 (e.g., a combination of SEQ ID NO:68-69, which detect LCMV GP), SEQ ID NO:70, SEQ ID NO:71 (e.g., a combination of SEQ ID NO:70-71, which detect LCMV L), SEQ ID NO:72, SEQ ID NO:73 (e.g., a combination of SEQ ID NO:72-73, which detect
10 LCMV Z). In some instances, nucleic acid probes or primers that can be used to detect one or more or at least one marker of LCMV can include one or more of the probes or primers described in Example 21 herein.

In some instances, methods suitable for detecting one or more or at least one LCMV nucleic acids or portions thereof can include, for example, RT-PCR and/or
15 RLM-RACE (e.g., as described in Example 2 herein).

Alternatively or in addition, markers of LCMV can include one or more LCMV peptides (e.g., including polypeptides or proteins), e.g., and methods for detecting LCMV can include, for example, detection of one or more LCMV peptides.

The terms “polypeptide”, and “protein” refer to a polymer or oligomer of
20 amino acid residues, including full-length proteins, fragments, peptides, oligopeptides, multimers and the like. The term also includes posttranslational modifications (glycosylation, phosphorylation, acetylation etc.), as well as deletions, additions, substitutions, mutations to the native sequence (natural mutations and variations), e.g., as long as the product maintains the desired activity), e.g., one or
25 more LCMV peptides disclosed herein.

In some instances, the LCMV peptides can be a full length LCMV peptide or a fragment of a LCMV peptide, e.g., a fragment of a LCMV peptide disclosed herein (see, e.g., peptides or peptide fragments encoded by one or more of SEQ ID NOs:1-51 and/or one or more of SEQ ID NOs:52-58 or fragments/portions of one or more of
30 SEQ ID NOs:52-58). Suitable fragments can include regions of amino acids that are conserved between one or more LCMV strains or isolates. For example, suitable fragments can include those regions identified as being conserved in FIGs. 5-7 or

SEQ ID NOs:1-58 (including for example, peptides encoded by one or more of SEQ ID NOs:1-51). Alternatively or in addition, suitable fragments can include a region within an LCMV amino acid that has at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to a region in one or more distinct LCMV strains. In some instances, suitable fragments can include at least 3 amino acids (e.g., 3-10 amino acids). Alternatively or in addition, suitable fragments can include 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more amino acids. In some instances, suitable fragments can include an antigen or epitope. The term “antigen” refers to various LCMV polypeptides and their fragments (native, recombinant or synthetic), which contain one or more epitopes that bind LCMV antibodies and are derived from any of the isolates/strains of LCMV. Furthermore, the antigen may be a fusion protein between the reference LCMV antigen molecule (full-length or fragment thereof) and another antigen/protein/ peptide that does not disrupt the reactivity of LCMV antigen. The antigen may be a component of an immunogenic composition, which refers to a sample (including but not limited to infected cell, whole cell lysate, protein extract), that may not or may be substantially purified (comprising more than 50% of sample in which in resides), or may be isolated (in separated and discrete form).

The term NP antigen refers to an antigen derived from the nucleocapsid protein of LCMV (e.g., including any LCMV strain and isolate). The nucleotide and corresponding amino acid sequences for various NP antigens of LCMV are known (FIGs. 2 and 5, SEQ ID NOS: 1-11 and 31-40). Additional sequences have been deposited with Genbank as specified in Prior publications (see below). A representative immunoreactive NP antigen useful in the present assays is a fusion protein derived from MX strain of LCMV. It includes several epitopes, and antibodies binding this antigen are cross-reactive with NP antigen from Armstrong strain.

The term ribonucleoprotein or RNP refers to a complex of virus genomic RNA segments (S and/or L) covered by NP antigen. Such RNPs are formed during the LCMV infection within the infected cells and can be also released to extracellular space.

The term GP antigen refers to an antigen derived from the glycoprotein of LCMV (including any LCMV strain and isolate). The nucleotide and corresponding

amino acid sequences for various GP antigens of LCMV are known (see, e.g., FIGs. 3 and 6, SEQ ID NOS: 12-23 and 41-51). Additional sequences have been deposited with Genbank as specified in Prior publications (see below). A representative immunoreactive GP antigen useful in the present assays is a fusion protein derived from MX strain of LCMV. It includes several epitopes, and antibodies binding this antigen are cross-reactive with GP antigen from Armstrong strain.

The term GPC antigen is a precursor, immature form of a GP antigen and is typical for persistent or aberrant infection. It includes the epitope spanning the region (fragment) of GPC cleavage to GP1 and GP2, which is relevant for specific recognition of GPC only. The sequences for various GPC antigens of LCMV are known (see FIGs. 3 and 6, SEQ ID NOS: 12-23 and 41-51).

The term GP1 antigen means a mature external subunit of envelope antigen that is typical for acute infection. It includes the epitope of the C-terminal region (fragment) of GP1, which is not exposed in GPC. The amino acid sequences for various GP1 antigens of LCMV are known.

The term ZP antigen refers to an antigen derived from the Z protein of LCMV. The nucleotide and corresponding amino acid sequences for various ZP antigens of LCMV are known. (see FIGs. 4 and 7, SEQ ID NOS: 24-30 and 52-57). Additional sequences have been deposited with Genbank as specified in Prior publications (see below). A representative immunoreactive ZP antigen useful in the present assays is a fusion protein derived from MX strain of LCMV. It includes several epitopes, and antibodies binding this antigen are cross-reactive with ZP antigen from Armstrong strain.

The term epitope means a site on an antigen, to which specific B cells and/or T cells respond, and which reacts with LCMV antibodies present in a biological sample and which stimulates antibody production. The term is used interchangeably with “antigenic determinant or antigenic site. An epitope can comprise 3 to 10 or more amino acids orchestrated in a unique conformational or linear manner.

The term immunogenic composition refers to at least one immunogenic polypeptide (e.g. NP, GP, ZP and/or ribonucleoprotein (RNP)).

In some embodiments, peptide markers of LCMV can be used as diagnostics, e.g., to detect antibodies directed against LCMV in a biological sample.

The antigens may be also used to produce polyclonal and monoclonal antibodies for use in diagnostics. Polyclonal antibodies can be produced by administering the LCMV antigens, either isolated, or substantially purified, or as part of immunogenic compositions (i.e. in the form of infected cells) to a mammal, such as a mouse, rat, rabbit, goat, sheep, lama, horse etc. Serum from the immunized antigen can be collected and the antibodies can be further purified. Techniques for producing and processing polyclonal antibodies are known in the art.

The term antibody is used in the broadest sense and specifically covers, for example, single monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies, de-immunized, murine, chimeric or humanized antibodies), antibody compositions with polyepitopic specificity, single-chain antibodies, diabodies, triabodies, immuno-conjugates and antibody fragments.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma (murine or human) method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No.4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991), for example.

An “antibody fragment” or “antigen binding fragment” comprises a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include less than full length antibodies, Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; single-chain antibodies, single domain antibody molecules, fusion proteins, recombinant proteins and multispecific antibodies formed from antibody fragment(s).

An antibody “which binds” an antigen of interest, e.g. an LCMV antigen or marker, is one capable of binding that antigen with sufficient affinity such that the antibody is useful as a therapeutic or diagnostic agent in targeting a cell expressing the antigen. Where the antibody is one which binds an LCMV antigen or marker, it will usually preferentially bind the LCMV antigen or marker as opposed to other antigens, and does not include incidental binding such as non-specific Fc contact, or binding to post-translational modifications common to other antigens and may be one which does not significantly cross-react with other proteins. Methods, for the detection of an antibody that binds an antigen of interest, are well known in the art and can include but are not limited to assays such as FACS, cell ELISA and Western blot.

“Humanized” and/or “chimeric” forms of non-human (e.g. murine) immunoglobulins refer to antibodies which contain specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which results in the decrease of a human anti-mouse antibody (HAMA), human anti-chimeric antibody (HACA) or a human anti-human antibody (HAHA) response, compared to the original antibody, and contain the requisite portions (e.g. CDR(s), antigen binding region(s), variable domain(s) and so on) derived from said non-human immunoglobulin, necessary to reproduce the desired effect, while simultaneously retaining binding characteristics which are comparable to said non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from the complementarity determining regions (CDRs) of the recipient antibody are replaced by residues from the CDRs of a non-human species (donor antibody) such as mouse, rat or rabbit

having the desired specificity, affinity and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human FR residues. Furthermore, the humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or FR sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR residues are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some instances, antibodies disclosed herein can be humanized.

Throughout the application, hybridoma cell lines, as well as the monoclonal antibodies which are produced therefrom, are referred to by their internal designation, e.g., M59, M87, M166, and MJ3 or their Depository Designation, LMBP 9216CB (M166) and LMBP 9217CB (MJ3).

As used herein, an "immuno-conjugate" means any molecule, such as an antibody or antibody fragment, chemically or biologically linked to reporter moieties. The antibody may be linked to the reporter moiety at any location along the molecule so long as it is able to bind its target.

Monoclonal antibodies can be generated following immunization with the LCMV antigens or their fragments as described above. Spleen of immunized animal containing normal B cells can be fused to myeloma cells essentially by a procedure developed by Kohler and Milstein (1975) and generated hybridomas can be screened for production of specific antibodies using various immunodetection methods. Specific monoclonal antibodies can be obtained in the form of hybridoma medium or purified by affinity chromatography on Protein A/G Sepharose. Antibody molecule fragments, e.g. F(ab')₂, Fv and sFv molecules can be produced using known techniques. Alternatively, a phage-display system can be used to identify and expand monoclonal antibody molecule populations in vitro and/or improve the immunological properties of the antibodies.

Compositions suitable for specifically detecting one or more LCMV peptides or LCMV peptide fragments can include, but are not limited to antibodies and/or antibody fragments. In some instances, the term “antibody” refers to a molecule, its fragments (Fab’2, Fab, Fv, sFv, minibodies and any other functional fragments), its
5 hybrid (chimeric) or bispecific variants, which specifically bind to an antigen and/or epitope of interest. The term includes antibodies obtained both from polyclonal and monoclonal preparations. In some instances, the antibody or antibody fragment can be humanized.

In some instances, compositions for detecting one or more LCMV peptides or
10 fragments can include (e.g., can consist, consist essentially of, or can comprise), for example, the heavy and/or light chain variable regions, or portions thereof, of one or more antibodies disclosed herein, e.g., one or more of M87, M59, M166, and/or MJ3. For example, compositions can include the heavy and light chain variable regions, or portions thereof, of M87, M59, M166, and MJ3. Alternatively, compositions can
15 include the heavy chain variable region, or a portion thereof, of M87, M59, M166, or MJ3 and the light chain variable region, or a portion thereof, of M87, M59, M166, or MJ3, wherein the heavy chain variable region and the light chain variable are not derived from the same antibody. In some instances, compositions can include one or more complementarity determining regions (CDRs) of one or more of M87, M59,
20 M166, and/or MJ3 (e.g., one or more of CDR1, CDR2, and or CDR3). For example, CDRs from different antibodies can be combined.

In some instances, compositions for detecting one or more LCMV peptides or fragments can include (e.g., can consist, consist essentially of, or can comprise), for example, the heavy chain variable region of antibody M87 (i.e., SEQ ID NO:74) or an
25 amino acid sequence with at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to SEQ ID NO:74. In some instances, compositions for detecting one or more LCMV peptides or fragments can include (e.g., can consist, consist essentially of, or can comprise), for example, the heavy chain variable region of antibody M87 (i.e., SEQ ID NO:74) containing at least one
30 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, less than 30, less than 40, less than 50, or less than 100) conservative amino acid substitutions, as described below. For example, in some instances, compositions for

detecting one or more LCMV peptides or fragments can include (e.g., can consist, consist essentially of, or can comprise), for example, the heavy chain variable region of antibody M87 (i.e., SEQ ID NO:74) wherein regions corresponding to CDR1, CDR2, and/or CDR3 can contain at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, less than 30, less than 40, less than 50, or less than 100) conservative amino acid substitution, and regions outside those corresponding to CDR1, CDR2, and/or CDR3 (e.g., the framework regions (i.e., FR1, FR2, and/or FR3)) have at least 50% identity (e.g., 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% identity) to the corresponding regions in SEQ ID NO:74.

In some instances, compositions for detecting one or more LCMV peptides or fragments can include (e.g., can consist, consist essentially of, or can comprise): CDR3 of the heavy chain variable region of M87 (e.g., SEQ ID NO:78), and/or CDR2 of the heavy chain variable region of M87 (e.g., SEQ ID NO:77), and/or CDR1 of the heavy chain variable region of M87 (e.g., SEQ ID NO:76), wherein any of CDR3, 2, and 1 can optionally include at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, less than 30, less than 40, less than 50, or less than 100) conservative amino acid substitutions.

In some instances, compositions for detecting one or more LCMV peptides or fragments can include (e.g., can consist, consist essentially of, or can comprise) LMBP 9216CB (M166) and/or LMBP 9217CB (MJ3).

As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably, and all such designations include progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. It will be clear from the context where distinct designations are intended.

Other compositions suitable for specifically detecting one or more LCMV peptides or LCMV peptide fragments can include antigen binding peptides. Such peptides bind specifically to one or more LCMV peptides or LCMV peptide fragments. In some instances, such peptides can include a complementarity determining region (CDR) of an antibody disclosed herein (e.g., one or more of CDR1, CDR2, CDR3).

In some instances, the one or more of the antibodies or antigen binding fragments thereof can be modified by insertion of one or more conservative amino acid substitutions.

5 A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of a polypeptide (without abolishing or substantially altering its activity. An "essential" amino acid residue is a residue that, when altered from the wild-type sequence of the polypeptide, results in abolishing or substantially abolishing the polypeptide activity.

10 A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar
15 side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

In some embodiments, the term "essential" amino acid residue as used herein,
20 includes conservative substitutions of the essential amino acid. Generally, the "essential" amino acid residues are found at the interacting face of the alpha helix.

The "interacting face" of the alpha helix includes those amino acid residues which interact with other amino acid residues. In this case, the interacting face includes those amino acids that interact with LCMV. Methods for identifying the
25 interactive face of a peptide are known in the art (see, e.g., Broglia et al., Protein sci., 14(10):2668-81, 2005; Hammond et al., J. Pharm. Sci., 98(1):4589-603, 2009; Ng and Yang, J. Phys. Chem. B., 111(50):13886-93, 2007; and Bird et al., PNAS USA, 197:14093, 2010). In some embodiments, the amino acid sequence of any peptide disclosed herein can be varied as long as the residues of the interacting face are
30 identical to those of SAH-p53-8 or are conservative substitutions thereof.

In some embodiments, compositions suitable for specifically detecting one or more LCMV peptides or LCMV peptide fragments can be modified to include, for

example, amino and/or carboxyl terminal labels or moieties (e.g., detectable moieties). Exemplary moieties can include, but are not limited to, fluorescent moieties (e.g., a fluorescent probe (e.g. fluorescein or rhodamine)), a metal chelating group, a radioisotope, or moieties that can chelate a radioisotope (e.g., mercaptoacetyltriglycine or 1, 4, 7, 10-tetraazacyclododecane-N,N',N'', N'''-tetraacetic acid (DOTA)) chelated to a radioactive isotope of Re, In or Y), a targeting moiety, a biotin moiety, a tat protein, an affinity label, a fatty acid-derived acyl group, and any other detectable moiety that is not otherwise present in the peptide and/or in any other peptide present (e.g., a naturally occurring peptide) or to be used in the methods disclosed herein (e.g., a LCMV peptide or fragment thereof or a peptide for detection of an LCMV peptide or fragment thereof) . Methods for preparing peptides with amino and/or carboxyl terminal moieties are routine and are known in the art. The term "label" refers to a molecule capable of detection, such as radioisotope, fluorescent dyes, chemiluminescent dyes, chromophores, metal ions, metal salts, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, adaptors (biotin, avidin, streptavidin, digoxigenin) etc.

Those of skill in the art readily understand how to determine the identity of two nucleic acids or amino acids. For example, identity can be calculated after aligning the two sequences so that the identity is at its highest level. For example, nucleic acid identity can be determined using the algorithms disclosed in Zuker, Science 244:48-52 (1989); Jaeger et al., Proc. Natl. Acad. Sci. USA 86:7706-10 (1989); and Jaeger et al., Methods Enzymol. 183:281-306 (1989), which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if the required level of identity is found with at least one of these methods, the sequences would be said to have the stated identity and to be disclosed herein.

The peptides of this invention can be made by chemical synthesis methods, which are well known to the ordinarily skilled artisan. See, for example, Fields et al., Chapter 3 in Synthetic Peptides: A User's Guide, ed. Grant, W. H. Freeman & Co., New York, N.Y., 1992, p. 77. Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α -NH₂ protected by

either t-Boc or Fmoc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431.

One manner of making of the peptides described herein is using solid phase peptide synthesis (SPPS). The C-terminal amino acid is attached to a cross-linked polystyrene resin via an acid labile bond with a linker molecule. This resin is insoluble in the solvents used for synthesis, making it relatively simple and fast to wash away excess reagents and by-products. The N-terminus is protected with the Fmoc group, which is stable in acid, but removable by base. Any side chain functional groups are protected with base stable, acid labile groups.

Longer peptides could be made by conjoining individual synthetic peptides using native chemical ligation. Alternatively, the longer synthetic peptides can be synthesized by well-known recombinant DNA techniques. Such techniques are provided in well-known standard manuals with detailed protocols. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated to obtain a nucleic acid sequence encoding the amino acid sequence, preferably with codons that are optimum for the organism in which the gene is to be expressed. Next, a synthetic gene is made, typically by synthesizing oligonucleotides which encode the peptide and any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and transfected into a host cell. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

The peptides can be made in a high-throughput, combinatorial fashion, e.g., using a high-throughput multiple channel combinatorial synthesizer available from Advanced Chemtech.

Peptides can also be expressed (e.g., recombinantly expressed) in a wide variety of systems and host cells, including insect, mammalian, bacterial, viral and yeast expression systems and cells, all of which are well known in the art.

A number of appropriate host cells for use with the above systems are also known. For example, mammalian cell lines include immortalized cell lines available from cell culture collections, such as, but not limited to CHO, HeLa, COS, MDCK, etc.

Following preparation, the peptides can be assayed, e.g., to confirm their sequence, binding affinities, and stability (in vitro and in vivo) using routine methods.

In some embodiments, one or more of the compositions disclosed herein can be mounted onto a solid support. The term “solid support” refers to a solid surface to which a macromolecule, e.g. protein, polypeptide, peptide, polynucleotide can be attached, including but not limited to microplate well, sepharose/agarose matrix, magnetic beads, glass slide, nylon, polyacrylamide, nitrocellulose membrane, silica plate, etc. Furthermore, solid support can be represented by infected cells in culture or in tissue specimens that contain LCMV antigens either exposed on cell surface or present within the cytoplasm.

The term “immune complex” refers to the molecular composite formed via binding of antibody to an antigen in the assay settings. It also refers to naturally occurring multimolecular composite containing viral ribonucleoprotein and immunoglobulins in biological samples.

Diagnostic Methods

The present disclosure also features methods for detecting LCMV in a sample, e.g., using one or more of the compositions for detecting LCMV disclosed herein (e.g., one or more probes or primers and/or one or more LCMV peptides or LCMV peptide fragments and/or one or more compositions for detecting one or more LCMV peptides or LCMV peptide fragments (e.g., one or more antibodies or antibody fragments)).

Antibodies against LCMV antigens/epitopes may be used for detection of the presence of LCMV proteins or ribonucleoproteins in a biological sample using, for example, different immunoassays or immunoassays combined with molecular methods. Immunoassays may use one or more antibodies and protocols may have different formats, including direct reaction, sandwich, competition, immunoprecipitation, immunoblotting etc. They can be also combined with amplification procedures in immuno-PCR formats, where amplification templates are represented either by virus RNA or by oligonucleotides linked to antibody/antibodies or detectors. Such procedures are known in the art.

Diagnostic assays may detect either virus antigens or antiviral antibodies. The assays may include immunoprecipitation (IP), immunoblotting (IB), IP combined with IB, enzyme-labeled immunoassays, biotin/avidin type assays, PCR, immuno-PCR and the like. The detection generally includes revealing labels such as
5 fluorescent, chemiluminescent, radioactive, enzymatic label or dye molecules, or oligonucleotides for amplification to generate labeled product.

The assays generally involve separation of unbound antibody or antigen in a liquid phase from a solid support (either without or with capture) to which antigen-antibody complexes are bound. Then the antigen-antibody complexes are detected
10 with detector interacting or conjugated with label.

Typically, a solid support is first reacted with capture. Then any non-immobilized components are removed by washing, and the solid support-bound component is then contacted with a biological sample under suitable conditions. After washing to remove any unbound material, a secondary binder moiety, i.e.
15 detector, can be added after suitable binding conditions. The presence of the detector can then be detected using techniques well known in the art. Alternatively, detector can be added simultaneously with the labeled competitor molecule and extent of competition can reveal the amount of the detector present in the sample.

Several different variations of the assay can be performed as illustrated on
20 FIGs. 8 and 9. FIG. 8 shows compositions of exemplary immunodetection tests that employ either LCMV antigens or antibodies.

In A series of the assays, individual virus antigens or their mixtures serve as capture to which anti/LCMV antibodies from biological sample are bound and then detected with various detectors or their combinations. In A1 variant, detector is
25 represented by Protein A/G directly conjugated with label. In A2 variant, detector is represented by secondary anti-human (or anti-mouse or other animal species-specific) IgG or IgM directly conjugated with label. In A3 variant, detector antibody is conjugated with biotin, which is further bound with avidin/streptavidin and then revealed with label-conjugated biotin. In A4 variant, secondary antibody is linked
30 with oligonucleotide, which can be amplified by corresponding primers in presence of labeled trinucleotides. In A5 variant, secondary antibody is conjugated with biotin, which is then reacted with avidin/streptavidin and with biotin linked with

oligonucleotide, which can be amplified by corresponding primers in presence of labeled trinucleotides.

In B series of the assays, antibodies specific for NP, GP (e.g. GPC, GP1) and/or ZP are attached to solid phase and serve as capture to which LCMV antigens from biological sample are bound and then detected with non-competing antibody or antibody/associated detectors. In B1 variant, detector is represented by LCMV antigen-specific antibody directly conjugated with label. In B2 variant, detector is represented by anti-human (or anti-mouse or other animal species-specific) IgG or IgM directly conjugated with label. In B3 variant, detector antibody is conjugated with biotin, which is further bound with avidin/streptavidin and then revealed with label-conjugated biotin. In B4 variant, secondary antibody is linked with oligonucleotide, which can be amplified by corresponding primers in presence of labeled trinucleotides. In B5 variant, secondary antibody is conjugated with biotin, which is then reacted with avidin/stretavidin and the with biotin linked with oligonucleotide, which can be amplified by corresponding primers in presence of labeled trinucleotides.

In some embodiments, the methods include selecting a subject (the term “subject” is used throughout the specification to describe an animal, human or non-human. Both human and veterinary applications are contemplated. The term can include, for example, mammals, e.g., humans, other primates, pigs, rodents such as mice and rats, rabbits, guinea pigs, hamsters, cows, horses, cats, dogs, sheep and goats). For example, subjects at risk for LCMV infection or subjects suspected of having LCMV can be selected. Alternatively or in addition, subjects with a condition or disease that renders the subject more susceptible to damage caused by LCMV can be selected. Such subjects can include subjects that are planning to become pregnant or that are pregnant, immunocompromised subjects, transplant recipients, and subjects at risk for developing or having cancer. In some embodiments, a subject is selected if the subject has a condition known to manifest hypoxia in the subject.

In some embodiments, following selection, a sample can be obtained from the subject. The sample can then be contacted with one or more of the compositions for detecting LCMV disclosed herein (e.g., one or more probes or primers and/or one or more LCMV peptides or LCMV peptide fragments and/or one or more compositions

for detecting one or more LCMV peptides or LCMV peptide fragments (e.g., one or more antibodies or antibody fragments). In some instances, the methods can include treating or recommending the subject for treatment for LCMV infection if LCMV is detected in the subject.

5 The methods can also include monitoring or evaluating the subject during and after treatment to determine the efficacy of the treatment, and, if necessary, adjusting treatment to improve efficacy of the treatment.

Kits

10 The present disclosure also features kits comprising one or more of the compositions for detecting LCMV disclosed herein. The kits can also include informational material relevant to the compositions and methods of using the compositions. The informational material can be descriptive, instructional, marketing or other material that relates to the compositions and methods described herein.

15 The informational material of the kits is not limited in its form. In many cases, the informational material (e.g., instructions) is provided in printed matter, such as in a printed text, drawing, and/or photograph, such as a label or printed sheet. However, the informational material can also be provided in other formats, such as Braille, computer readable material, video recording, or audio recording. Of course,
20 the informational material can also be provided in any combination of formats.

 In addition to the compound, the composition of the kit can include other ingredients, such as a solvent or buffer, a stabilizer, a preservative, and/or a second agent for treating a condition or disorder described herein. Alternatively, the other ingredients can be included in the kit, but in different compositions or containers than
25 the compound. In such embodiments, the kit can include instructions for admixing the agent and the other ingredients, or for using one or more compounds together with the other ingredients.

 The sequences disclosed herein are publically available, e.g., online at the National Center for Biotechnology Information (NCBI) website (see
30 ncbi.nlm.nih.gov) and in the literature, as follows:

 LCMV strain MX GPC gene, NCBI Accession no. EU195888 (EU195888.1) and Tomaskova, J et al., Virus Genes, 37:31-38 (2008);

LCMV strain MX NP gene, NCBI Accession no. Y16308 (Y16308.1) and Reiserova, L. et al., *Virology* 257:73-83 (1999);

LCMV strain MX Z gene, NCBI accession no. AJ131281 (AJ131281.1) and Gibadulinova, A. et al., *Acta Virol.*, 42:369-374 (1998);

5 LCMV strain Armstrong 53b – S segment, NCBI accession no. M20869 (M20869.1) and Salvato, M. et al., *Virology*, 164:517-522 (1988);

LCMV strain Armstrong 53b – LS segment, NCBI accession no. AY847351 (AY847351.1) and Grande-Perez, A. et al., *J. Virol.*, 79:10451-10459 (2005);

10 LCMV strain CH-5692 – S segment, NCBI accession no. AF325214 (AF325214.1);

LCMV strain CH-5692 – L segment, NCBI accession no. DQ868484 (DQ868484.1);

LCMV strain CH-5871 - S segment, NCBI accession no. AF325215 (AF325215.1) and Asper, M. et al., *Virology*, 284:203-213 (2001);

15 LCMV strain Traub – S segment, NCBI accession no. DQ868487 (DQ868487.1);

LCMV strain Traub – L segment, NCBI accession no. DQ868488 (DQ868488.1) and Emonet, S. et al., Genetic comparisons and evolution of 6 LCMV strains;

20 LCMV strain LE GPC gene, NCBI accession no. EF164923 (EF164923.1) and Meritet, J.F. et al., Human Fetal Lymphocytic Choriomeningitis Virus Infection with a New Genomic Variant;

LCMV strain M1 – S segment, NCBI accession no. AB261991 (AB261991.1);

25 LCMV strain M2 – S segment, NCBI accession no. AB261990 (AB261990.1) and Ike, F. et al., *Comp. Med.* 57:272-281 (2007);

LCMV isolate Marseille #12 – S segment, NCBI accession no. DQ286931 (DQ286931.1);

LCMV isolate Marseille #12 – L segment, NCBI accession no. DQ286932 (DQ286932.1) and Emonet, S. et al., *Emerging Infect. Dis.*, 13:472-475 (2007);

30 LCMV strain WE – S segment, NCBI accession no. M22138 (M22138.1) and Romanowski, V. et al., *Virus Res.*, 3:101-114 (1985);

LCMV strain WE – S segment, NCBI accession no. AF004519 (AF004519.1)

and

Djavani, M. et al., Virus Genes, 17:151-155 (1998);

LCMV strain Bulgaria – S segment, NCBI accession no. GQ862982

5 (GQ862982.1);

LCMV strain Bulgaria – S segment, NCBI accession no. GQ862981

(GQ862981.1) and Palacios, G. et al., Genetic diversity of Lymphocytic

choriomeningitis viruses;

LCMV strain Y – S segment, NCBI accession no. DQ118959 (DQ118959.1)

10 and Compton, S.R., Lymphocytic choriomeningitis virus strain Y; and

NCBI accession nos. FJ607019-FJ607038, 13-JUL-2010, Albarino, C.G. et al.,

Emerging Infect. Dis., 16:1093-1100 (2010).

EXAMPLES

15 The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1: Regions of Sequence Conservation Exist Between Diverse Strains/Isolates of LCMV

20 Recent genomic analysis of 29 LCMV strains collected from a variety of geographic and temporal sources showed that these viruses are diverse. Several distinct lineages exist, but there is little correlation with time or place of isolation (Albarino et al, 2010). The S and L segment sequences of all known LCMV isolates were distributed in 3 (L segment) or 4 (S segment) different genetic groups or

25 lineages. Up to 25% nucleotide divergence was observed between the S segment lineages, and 28% divergence between the L segment lineages. This nucleotide divergence translates to 18%, 13%, 10%, and 6% divergence in the amino acid sequences of the Z, L, GPC, and NP proteins, respectively (Albarino et al, 2010). However, regions of considerable sequence identity exist among different

30 strains/isolates (*see* FIGs. 2-7) and derived antigens show cross-reactivity with LCMV-specific antibodies.

These observations support that multiple LCMV strains and/or isolates could be detected using suitable molecular and immunodetection approaches.

Example 2: Hypoxia Reactivates LCMV from Persistent Infection

HeLa cells persistently infected with LCMV MX strain (HeLa-MX) were incubated at normoxic (21% O₂) or hypoxic (2% O₂) conditions for 48 h. A separate population of HeLa-MX cells were also treated with 1mM of DMOG (a hypoxia mimicking agent) for 24 hours under normoxic conditions.

RT-PCR

Total cellular RNA was extracted with InstaPure reagent according to the manufacturer's instructions. Reverse transcription was performed with M-MuLV reverse transcriptase using random heptameric primers. Levels of viral gene expression were analyzed by quantitative real-time PCR on a StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA.) using POWER SYBR® Green PCR Master Mix and the following gene-specific primers:

NP (246 base pair (bp) PCR product):

Forward: 5'- GATCAGAAACAGTTCAAACAGGACT-3' (SEQ ID NO:58)

Reverse: 5'- GTCCCACACTTTGTCTTCATACTCT-3' (SEQ ID NO:59)

GP (251bp PCR product):

Forward: 5'- AACCAGTGCAGAACTTTTAGAGGTA-3' (SEQ ID NO:60)

Reverse: 5'- GCAAGTCTTCTAGTGAGGAACTTTG-3' (SEQ ID NO:61)

ZP (272bp PCR product):

Forward: 5'- CCTGTGAGAGTACAGAGACAAACCT-3' (SEQ ID NO:62)

Reverse: 5'- GATATCTTCAGCTTGGTTGGTAATG-3' (SEQ ID NO:63)

β-actin (236bp PCR product):

Forward: 5'- CCAACCGCGAGAAGATGA-3' (SEQ ID NO:64)

Reverse: 5'- GATCTTCATGAGGTAGTCAGT-3' (SEQ ID NO:65)

For each gene, fold induction was determined in comparison with value from normoxic control using β-actin as an endogenous control.

As shown in FIGs. 10A and 10B, hypoxia and DMOG similarly increased expression of mRNA encoding all LCMV proteins tested (i.e., NP, ZP, and GP)

relative to normoxia, as assessed by RT-PCR. No change was observed for the control (β -actin).

In order to prove that hypoxia influences virus genes at the transcriptional level, RNA ligase-mediated rapid amplification of 5' cDNA ends (RLM-RACE) was performed using the GeneRacer method, which allows for selective amplification of the 5' capped transcripts and eliminates non-capped genomic/antigenomic LCMV RNA templates.

Selective amplification of 5' capped transcripts of MX LCMV was carried out using the GeneRacerTM kit according to instructions of the manufacturer (Invitrogen, Life Technologies). LCMV-MX gene-specific primers employed in RLM-RACE on RNA isolated from normoxic and hypoxic HeLa-MX cells are listed below:

NP gene

5' RACE reverse primer: CAAGGTCGGCAGCGAGAGACATCA (SEQ ID NO:66)

5' RACE nested reverse primer: AGAAGGCTAGTTGCGTCCTTGATG (SEQ ID NO:67)

GP gene

5' RACE reverse primer: GGCTGAACATGCATTGGGCATTGT (SEQ ID NO:68)

5' RACE nested reverse primer: TAGGAGAAGGAAGCTGACCAATGC (SEQ ID NO:69)

L gene

5' RACE reverse primer: TCCTGGACACACAACCTCCGGACTCTA (SEQ ID NO:70)

5' RACE nested reverse primer: ACAGCCACTTTTGTCTGCACTGTC (SEQ ID NO:71)

Z gene

5' RACE reverse primer: CTTCGTAGGGAGGTGGTGGGCTTG (SEQ ID NO:72)

5' RACE nested reverse primer: AGTTCAGTGGACCGAGATAGGTGGT (SEQ ID NO:73)

β -actin was employed as internal standard and control of RLM quality using the primers included in the kit. Resulting PCR fragments were run on 1.5% agarose gels and their specificity was verified by sequencing and by reamplification with independent gene-specific primers. The intensity of bands corresponding to individual PCR products was evaluated with GeneTools Software from Syngene. Amount of gene-specific PCR products was semi-quantitatively expressed as the ratio of the intensity of each LCMV-specific band to the intensity of the corresponding β -actin internal standard. Commercial HeLa total RNA included in the kit was used for β -actin amplification as a control for activity of CIP and TAP enzymes.

As shown in FIG. 10D, the semi-quantitatively evaluated results of the RLM-RACE carried out on total RNA isolated from hypoxic (2% O₂) versus normoxic HeLa-MX cells were consistent with the above described RT PCR data (*see* FIG. 10A) suggesting that hypoxia affects the virus transcription.

Immunoblotting

One million HeLa-MX were plated into Petri dishes, left to attach overnight and then incubated for 48 h under normoxic or hypoxic conditions. Cells were disrupted in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate and 1x Complete protease inhibitor cocktail [Roche, Mannheim, Germany] in PBS) and total protein concentrations were determined by BCA assay (Pierce, Rockford, IL, USA) according to manufacturer's instructions. Total protein extracts (100 μ g/lane) were separated by SDS/PAGE under reducing conditions, blotted onto PVDF membrane (ImmobilonTM, Millipore, Billerica, MA, USA), and detected by using specific antibodies against NP (mouse monoclonal antibody M59), Z protein (mouse monoclonal antibody MJ3), GP1 (anti-peptide polyclonal antibody), HIF-1 α or α -actin followed by appropriate secondary antibody conjugated with horseradish peroxidase. All immunoblots were developed with the ECL detection system.

As shown in FIG. 10C, hypoxia increased expression levels of all LCMV proteins tested (i.e., NP, ZP, and GP) relative to normoxia. No change was observed for the control (actin).

Example 3: LCMV Reactivated by Hypoxia are Infectious

Filtered medium from HeLa-MX cultured under normoxia or hypoxia for 48 h was used to infect non-infected HeLa cells. These cells were then cultivated in normoxic conditions, passaged, and then assessed for viral replication.

5 The presence of LCMV genome in the medium from HeLa-MX cells cultured under normoxia (NO) and under hypoxia (HY) was confirmed by RT-PCR method (see FIG. 11A). In addition, the spread of infection in the HeLa populations infected with indicated medium was followed by RT-PCR analysis in the first 5 passages and then in tenth passage (see FIG. 11B). The progress of infection was monitored also
10 by immunofluorescence detection of viral nucleoprotein in recipient cells under 20x magnification (see FIG. 11C). The experiment was repeated twice, each time showing similar results. As shown in FIGs. 11A-11C, hypoxia increases the infectivity of LCMV.

The data shown in Examples 2 and 3 demonstrate that hypoxia can increase
15 expression of viral NP, Z and GP (including appearance of GP1) genes and proteins in culture (Example 1) and can trigger formation of infectious virus particles (Example 2).

These data support a rationale for diagnostic detection of LCMV in subjects at risk for hypoxia with hypoxia virus genes, antigens or antibodies against these
20 antigens and their combinations

Example 4: Generation of Antibodies that bind Specifically to LCMV NP

Mouse monoclonal antibody M59, M166 and M87 are specific for the LCMV NP. These antibodies were prepared using the hybridoma technique (Kohler and
25 Milstein 1975).

BALB/c mice were immunized with three doses of 5×10^6 HeLa-MX cells and their splenocytes were fused with NS-0 myeloma cells. Hybridomas were selected in DMEM-HAT medium containing hypoxanthine, aminopterin and thymidine, and screened for the specific reactivity towards NP by differential ELISA using cell
30 extract of HeLa and HeLa/MX cells as an antigen. Positive hybridoma cultures were cloned by limiting dilution, expanded and used for MAbs production.

All of M59, M166, and M87 bound NP from LCMV MX and cross-reacted with NPs of other LCMV strains.

Example 4A: Deposit of M166

5 The hybridoma cell line expressing mouse monoclonal antibody M166 was deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium (Universiteit Gent, Vakgroep Moleculaire Biologie-Plasmidicollectie (BCCM™/LMBP), under accession number LMBP 9216CB.

Example 4B: Characterization of M87 Variable Region Sequence

The amino acid sequence of the M87 heavy chain was determined, as follows and as depicted in FIG. 12A. RNA was isolated from 1 million M87 hybridoma cells and subjected to reverse transcription using random hexameric primers. The heavy chain variable region was amplified from the resulting cDNA using a degenerated
15 forward primer designed to be complementary to the signal peptide/leader sequence (see FIG. 12) and a reverse primer complementary to the CH1 region of the constant domain (see FIG. 12). Amplification was done using a high fidelity polymerase and PCR product of the expected size (cca 400 bp) was separated by electrophoresis and isolated from the gel. A linear PCR amplicon was ligated into pJet1.2 vector, which
20 was then transformed competent *Escherica coli*. Resulting transformed cells were screened and selected colonies were verified by restriction enzyme cleavage and sequencing. The sequence of the M87 heavy chain variable region was determined to be:

mdsrlnlvflvlilkgvqcdrvqlvesggglvqpqgsrklscasgftfssfgmhwvrqapekglewvayis
25 sgsstlhyadtvkgrftisrdnpkntlfqlmkplslygllgsrnlshrllsqndtpirlsigpwklgi (SEQ ID NO: 74)

Within the M87 heavy chain variable region (SEQ ID NO: 74), mdsrlnlvflvlilkgvqc (SEQ ID NO:75) is a signal peptide sequence; gftfssfgmhwv (SEQ ID NO:76) is CDR1; issgsstlhyadtvkgrft (SEQ ID NO:77) is CDR2; and
30 hrllsqndtpirlsigp (SEQ ID NO:78) is CDR3. Annotated versions of SEQ ID NO:74) are provided in FIGs. 12B-12C.

Example 5: Generation of Antibodies that bind Specifically to LCMV GP1

Polyclonal antibody against LCMV MX GP1 were raised, as follows.

Potential B-cell epitopes were identified using the complete sequence of the GPC LCMV MX using several available programs. Peptide RSGWGWAGSDGKTT (aa
5 205-218 of SEQ ID NO: 41) (SEQ ID NO:89) mapping within a region of the GP1 was chosen for production of GP1-specific polyclonal antibodies. Affinity purified rabbit polyclonal antibody was tested by immunoprecipitation and Western blotting.

Example 6: Generation of Antibodies that bind Specifically to LCMV ZP

10 Mouse monoclonal antibody MJ3 binds specifically to LCMV ZP. Antibodies were generated using the hybridoma technique (Kohler and Milstein, *supra*). Briefly, BALB/c mice were immunized with two doses of 5×10^6 HeLa-MX cells and boosted with 100 μ g GST-Z protein bound to Glutathione Sepharose 4B. Fusion of spleen cells with Sp2/0 myeloma cells was carried out 3 days later. Hybridomas were
15 selected in DMEM-HAT medium and monoclonal antibodies produced by the hybridomas were screened for the specific reactivity towards Z protein in GST-Z vs GST and HeLa-MX vs. noninfected HeLa cells by ELISA and immunoblotting. The hybridoma culture (MJ3) was subcloned by limiting dilution, expanded and used for the MAb production.

20 MAb MJ3 was shown to react with Z protein using different immunodetection methods.

Example 6A: Deposit of MJ3

The hybridoma cell line expressing mouse monoclonal antibody MJ3 was
25 deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium (Universiteit Gent, Vakgroep Moleculaire Biologie-Plasmidicollectie (BCCMTM/LMBP), under accession number LMBP 9217CB.

Example 7: Detection of Anti-LCMV NP Antibody in Human Subjects

30 Serum samples were obtained from (i) women with spontaneous abortions without diagnosed cause; (ii) women with full-term gravidity; and (iii) oncology patients with renal cell carcinoma (RCC) tumors. Anti-LCMV antibodies in human

sera were analyzed by immunoprecipitation and subsequent immunoblotting with NP-specific monoclonal antibody.

The approach employed in this example is very sensitive and enables detection of NP-specific antibodies produced during both acute and chronic/persistent
5 infections. Briefly, protein G-Sepharose (50% slurry) was washed with PBS and mixed with whole cell lysate from HeLa-MX cells to preclear non-specifically binding proteins. 10 μ l of human serum in 190 μ l PBS containing 10% FCS was added to 100 μ l precleared cell extract. Immunocomplexes were then allowed to form at 4°C overnight. The resulting immunocomplexes were precipitated by adding 30 μ l
10 of washed Protein G-Sepharose slurry for one hour at 4°C. Beads were then washed five times in PBS, subjected to SDS-PAGE, and transferred onto a polyvinylidene difluoride membrane. Membranes were probed with purified MAb conjugated with horseradish peroxidase. After an additional washing step, immunoblots were visualized using the ECL detection system.

15 As shown in Tables 1A-1B, seroprevalence of NP antibodies in women with abortions was by 19.4% higher compared to control.

Table 1A: Detection of Anti-LCMV NP Antibodies in Human Female Subjects that Experienced Spontaneous Abortion

	<i>Women with abortion</i>		<i>women without abortion</i>	
	number	%	number	%
positive sera	34	75,6	18	56,2
negative sera	11	24,4	14	43,8
total	45	100	32	100

5 Detected antibodies are also shown as immunoblot in FIG. 13.

As shown in Table 4, very high seroprevalence of NP antibodies was also observed for patients with RCC tumors.

10 **Table 1B: Detection of Anti-LCMV NP Antibodies in Subjects with RCC Tumors**

	<i>Oncology patients</i>	
	number	%
Positive sera	25	89,3
negative sera	3	10,7
Total	28	100

Example 7B: Detection of NP-Specific Antibodies in Human Sera by Tandem Mass Spectrometry

One human serum shown to be positive for NP by immunoprecipitation and subsequent immunoblotting with NP-specific monoclonal antibody was immunoprecipitated as described in Example 7. After washing, immunocomplexes were eluted with citrate buffer (pH 3). The final pH of eluate was adjusted by adding of 1M Tris pH 8.5. Subsequently, eluates containing immunocomplexes were subjected to tandem mass spectrometry. Briefly, samples were reduced (10 mM dithiotreitol), alkylated (55 mM iodoacetamide) and digested by 20 µg/ml trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate overnight at 37°C. Resulting peptides were transferred to microplate and dried by lyophilization. The extracted peptide mixture was dissolved in 20 µl of 2% acetonitrile in water with addition of 0.1% of formic acid and separated by a nanoAcquity UPLC system (Waters, Milford, MA) as described previously (Henrychová et al., 2008). The data

acquisition was performed in data-dependent manner for the time of the separation collecting up to 3 MS/MS events at the same time. Data were processed by ProteinLynx Global Server v. 2.4 (Waters) that provided background subtraction (polynomial order 5 and threshold 35%), smoothing (Savitzky Golay, twice, over three channels) and centroiding (top, 80%, minimal peak width at half height 4). Resulting data were searched against UniProt_LCMV database under the following criteria: fixed carbamidomethylation of Cys, variable Met oxidation, tryptic fragments with 1 miss cleavage, peptide mass tolerance 50 ppm, and fragment mass tolerance 0.05 Da. Results were validated by the identification of three or more consecutive fragment ions from the same series. The protein assignments with at least two matching peptides to the theoretical sequences were considered as positive identification.

As shown in Table 2, in analyzed samples was identified LCMV NP.

Table 2: Identification of NP in Immunocomplexes by UPLC-MS

Sample	Accession	Description	mW (Da)	pI (pH)	PLGS Score	Peptides
MSE3	C3VVN3	Nucleoprotein OS Lymphocytic choriomeningitis virus	62399	8,6614	65,510	4
2. processing	Q9YPM1	Glycoprotein 1 Fragment OS Lymphocytic choriomeningitis virus strain WE	15535	8,4294	282,26	1
	C3VVN3	Nucleoprotein OS Lymphocytic choriomeningitis virus	62399	8,6614	66,551	3
MSE5	Q86867	S RNA product protein Fragment OS Lymphocytic choriomeningitis virus	1596	6,2774	250,47	1

This result clearly proved that tested human serum contains NP-specific antibodies.

Example 8: Immunodetection of LCMV Antigens in Tissue from Human RCC Subjects

LCMV antigens were detected in RCC specimens by immunochemistry. Briefly, dissected tissues were fixed in 4% neutral buffered formalin and embedded in

paraffin according to the standard histological procedures. Four- μ m sections were placed on polylysine-coated slides, de-waxed and rehydrated. The slides were first subjected to tissue pretreatment procedure at 125°C for 5 min in target retrieval solution (Pascal pressure chamber, DakoCytomation, Carpinteria, CA). The rest of the immunostaining procedure was performed using the Dako Cytomation EnVision(r)+ System-HRP (DAB) according to the manufacturer's instructions: a) peroxidase and protein block (10 min each); b) incubation for 1 h with primary antibody M59 specific for the NP (undiluted hybridoma medium) or PBS (negative control); c) incubation for 30 min with peroxidase-conjugated goat anti-mouse antibody diluted 1:1000 in antibody diluent (DakoCytomation). Staining was visualized with DAB solution for 1 min with 3,3'-diaminobenzidine as a chromogenic substrate. The slides were washed in PBS with 0.1% Tween-20 for 10 min after the step a, 2 times for 10 min after steps b and c, and three times in distilled water after visualisation with DAB. All incubations and washings were carried out at room temperature. Finally, the sections were counterstained with Mayer's hematoxylin, washed for 5 min and mounted in DePeX (Serva, Heidelberg, Germany). The stained sections were examined with Leica DM4500B microscope and photographed with Leica DFC480 camera.

As shown in FIG. 14A-B, LCMV antigens were detected in tissue from RCC subjects.

In addition, LCMV antigens were detected in RCC specimens by immunoprecipitation and subsequent immunoblotting with NP-specific antibody M87. Briefly, 100 mg of frozen tissue specimens were homogenized in ice-cold lysis buffer (1% Triton X-100; 150 mM NaCl; 50 mM Tris, pH 7.5; 0.5% Nonidet P-40; 50mM NaF) containing inhibitors of proteases (Roche Applied Science, Mannheim, Germany). The insoluble material was removed by centrifugation for 15 min at 12,000 g at 4 °C. MAb M87 (1,5ml cultivated medium) was bound to 30 μ l 50% suspension of protein G-Sepharose in PBS for 2 hrs at RT. Tissue protein extract (200 μ l) was pre-cleared with 20 μ l of 50% suspension protein G-Sepharose and added to bound MAb. Immunocomplexes were then allowed to form at 4°C overnight. Beads were then washed six times in PBS, subjected to SDS-PAGE, and transferred onto a polyvinylidene difluoride membrane. Membranes were probed with

purified MAb M87 conjugated with horseradish peroxidase. After an additional washing step, immunoblots were visualized using the ECL detection system.

As shown in FIG. 14C, LCMV NP was detected in tissue from RCC subjects using MAb M87.

5

Example 9: Analysis of NP-specific monoclonal antibodies

Mouse monoclonal antibodies M59, M166, and M87, specific for the NP LCMV were prepared by the hybridoma technique (Kohler and Milstein 1975). Briefly, BALB/c mice were immunized with three doses of $5 \cdot 10^6$ HeLa-MX cells and their splenocytes were fused with NS-0 myeloma cells. Hybridomas were selected in DMEM-HAT medium containing hypoxanthine, aminopterin and thymidine, and screened for the specific reactivity towards NP by differential ELISA using cell extract of HeLa and HeLa/MX cells as an antigen. Positive hybridoma cultures were cloned by limiting dilution, expanded and used for MAbs production. Each of MAbs M59, M166, and M87 were shown to cross-react with NPs of other LCMV strains.

MAb isotypes were determined by ELISA using affinity purified rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgM and IgA antibodies (Mouse Monoclonal Antibody Isotyping Reagents, Sigma) according to the instructions of the manufacturer. M59 and M166 MAbs were found to be of IgG2a isotype and M87 MAb was of IgG1 isotype.

The antibodies were further tested in different immunodetection methods. M59 was found to react with NP in ELISA, immunoprecipitation, and immunohistochemistry; M166 was found to react with NP in ELISA, IFA, and immunoprecipitation; and M87 was found to bind to NP in ELISA, immunoprecipitation, IFA, and immunoblotting. These data suggest that the antibodies recognize different epitopes of NP molecule, with M59 and M166 being directed to a conformational epitope and M87 binding to linear epitope. Differential epitope specificity of these antibodies was proven by competitive binding assay.

Briefly, MAb was first purified by affinity chromatography on Protein A/G sepharose and labeled with NHS-LC-Biotin (Pierce) according to the instructions of the manufacturer. Extract from HeLa-MX cells was adsorbed on microplate wells at a concentration corresponding to 50% of maximal binding of labelled MAbs. Coated

plates were washed and saturated with 10% FCS in PBS. Serial fivefold dilutions of purified MAbs in 30 μ l and a constant amount of biotinylated MAb in 30 μ l were added and incubated overnight at 4 °C. The plates were washed and peroxidase-labelled streptavidin (Pierce) was used as a detector.

5 Results are illustrated in Figs. 15A and 15B. These are graphs illustrating an examination of the competitive binding between M87 and M59 MAbs. Biotin-labelled purified antibodies (*) were allowed to bind in the presence of increasing amounts of non-labelled competitive antibodies. The extent of binding of the labelled antibody in the presence of the non-labelled competitor was expressed as percentage
10 of binding in the absence of the competitor. Dilution 0 corresponds to 10 μ g/well of non-labeled competing antibody. The results show only homologous competition, but no heterologous hindrance of binding of the labeled competitor was observed, suggesting that the MAb bind to non-overlapping epitopes.

15 **Example 10: Virus response to hypoxia: LCMV arenavirus as a paradigm**

Physiological context of the virus-infected cells can markedly affect multiplication and spread of the virus progeny. Mainly during persistent infection, when the virus strongly depends on host cell and usually does not disturb its vital functions, microenvironmental stresses such as hypoxia can uncouple the intimate
20 virus-host relation and escalate the virus pathogenesis. Accumulating evidence suggests that hypoxia-induced molecular responses governed by HIF transcription factor modulate gene expression of viruses that pass through a DNA stage, contain HRE in their promoters and replicate in the nucleus. We could show for the first time, that hypoxia can also influence the outcome of persistent cytoplasmic RNA virus
25 infection. As a model, we used lymphocytic choriomeningitis virus (LCMV) which can persist in different cell types without perturbing their integrity and causes mostly inapparent infections. It is therefore considered innocent, although LCMV-associated abortions and fatal LCMV infections in transplant recipients warn that it can be dangerous. MX strain of LCMV replicates in a persistent mode in human HeLa cells
30 and spreads in a cell-to-cell manner in absence of extracellular infectious virions. Exposure of MX-infected HeLa cells to chronic hypoxia led to increased virus RNA transcription and higher levels of the viral proteins via a HIF-1 α -dependent

mechanism. Hypoxia also enhanced formation of infectious virions capable to transmit LCMV infection via cell-free medium. This hypoxia-induced LCMV “reactivation” might have health-compromising consequences, e.g. for developing fetus or receiver of transplant from asymptomatic donor.

5

Example 11: Cloning and Expression of Recombinant LCMV-NP-Fragments

Three overlapping fragments of LCMV (MX)-NP cDNA were cloned by PCR using the plasmid pBluescript-NP as a template, numbers in parentheses show positions with respect to published NP sequence of MX strain (GenBank accession number Y16308, Reiserova et al. 2001).

10

Fragment I containing amino acids 1-205 was amplified using the primers designated NPMXF1S 5'-CCGAATTCATGTCTCTGTCCAAGGAAGTCA-3' (46-67) (SEQ ID NO:79) and NPMXF1A 5'-GGCTCGAGGTAAAGCAGACCAAGGTCTGTG-3' (660-639) (SEQ ID NO:80);

15

Fragment II with amino acids 198-391 was amplified with the primers NPMXF2S 5'-GGAATTCCTCACAGACCTTGGTCTGCTTT-3' (637-658) (SEQ ID NO:81) and NPMXF2A 5'-CCCTCGAGCACTGGATCATTGAACCTACCC-3' (1218-1197) (SEQ ID NO:82); and

20

Fragment III containing the amino acids 384-558 was obtained by amplification with the primers NPMXF3S 5'-CCGAATTCGAGGGTAGGTTCAATGATCCAG-3' (1195-1226) (SEQ ID NO:83) and NPMXF3A 5'-CCTCGAGTAGAGTGTCACAACATTTGGTC-3' (1722-1700) (SEQ ID NO:84). All the primers were designed with EcoRI and Xho I restriction sites (underlined), respectively. PCR reactions were performed using the primers listed above and EXT DNA polymerase (Finnzymes, Oy, Finland). Following an initial denaturation at 94 °C for 3 min, the amplification program was set as follows: denaturation at 94°C for 30 s, annealing at 60 °C for 40 s, and extension at 72 °C during 1 min 20 s for a total of 35 cycles, and finally 7 min at 72 °C. PCR products were purified on a 1.2% agarose gel using the Wizard® SV Gel & PCR clean-Up System (Promega, USA) and subcloned into either pBluescript SK(+) (Stratagene, USA) linearised with EcoRV and tailed with dT for T-A cloning or into pGEM®-T vector (Promega, USA). Next, all three fragments were cloned in-frame with

25

30

glutathione S-transferase into pGEX-4T-1 (Amersham Pharmacia Biotech AB, Sweden) using EcoRI and XhoI restriction enzymes. To produce GST-fusion proteins, verified plasmid constructs (designated pGEX-4T1-NPI, pGEX-4T1-NPII, pGEX-4T1-NPIII) were transformed into *E. coli* BL21-CodonPlus (DE3)-RIPL (Stratagene, USA) competent cells, and induced with 0.2 mM IPTG (Sigma-Aldrich, USA, USA) for 3 hours.

Example 12: Purification of GST-Tagged Fusion Proteins

Induced cultures of *E. coli* were pelleted by centrifugation, resuspended in ice-cold lysis buffer STE (10 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA in 1x PBS), pH 8 and incubated on ice for 15 min with lysozyme (Serva, Germany) in the final concentration of 0.4 mg / ml. Before sonication of bacterial cells (2 x 30 s) 10% Sarcosyl (Sigma-Aldrich, USA, USA) in STE to final concentration of 1.5% and 1M DTT (Sigma-Aldrich, USA, USA) to final concentration of 5 mM was added to cell suspension. The insoluble material was removed by centrifugation for 15 min at 12,000 g at 4 °C. The appropriate volume of the 50 % slurry of Glutathione Sepharose 4B (Amersham Pharmacia Biotech) equilibrated with STE, pH 8 was added to bacterial lysate and incubated overnight with gentle agitation at 4 °C. Next day, the fusion proteins bound on Glutathione Sepharose 4B were extensively washed with ice-cold STE, pH 8 and eluted with 15 mM reduced glutathione (Merck) in 50 mM Tris-HCl, pH 8.0 at room temperature. The yield of fusion proteins in purified samples was determined by SDS-PAGE and visual comparison to defined concentration of BSA.

Example 13: NP-IgG ELISA-1

Microplate wells were coated overnight at 37°C with the purified fusion proteins GST- NPI, GST-NPII, GST-NPIII and GST (50 ng/well) diluted in 0.05M sodium carbonate-bicarbonate buffer (pH 9,6). After blocking with 10% skimmed milk in PBS+ 0.1% Tween 20, the coated wells were incubated with serum samples (50 ml aliquots), which were diluted in two-fold steps starting with 1:20 in blocking solution and incubated 1 hours at room temperature. Plates were washed four times with PBS- 0,1% Tween 20 and incubated with peroxidase-conjugated goat anti-

human IgG (Sigma) diluted 1:35000 in blocking solution for 45 minutes at room temperature. After washing, substrate solution (10 ml of Mc Ilweine buffer pH 5,5 (100 mM Na₂HPO₄, 40 mM citric acid), 10 mg o-phenylenediamine (Sigma), 10 µl of 30 % H₂O₂) was added into each well and incubating for 5-10 min in a dark place. Reaction was stopped by adding of 2M H₂SO₄ and optical density was measured for absorbance at 492 nm. The adjusted OD was calculated by subtracting the OD of the negative antigen-coated wells from that of corresponding wells.

Example 14: Epitope Mapping on Recombinantly Expressed GST-, NPI, GST-NPII, and GST-NPIII by Positive Human Sera

To determine epitopes present on NP recognized by NP-specific serum antibodies, ELISA according to the protocol described in Example 13 was performed. 15 serum samples that were verified to be positive by immunoprecipitation method were used.

As shown in Fig.16 , anti-NP serum antibodies preferentially recognized epitopes located on the fragment III of NP (containing the amino acids 384-558), while small fraction of antibodies reacted also with epitope located on the fragment II (amino acids 198-391).

Example 15: NP-IgG ELISA- 2

Detection of nucleoprotein (NP)-specific serum Abs was carried out by following method. 96-well polystyrene plates were coated with NP-specific monoclonal antibody M87 at concentration 6 µg/ml diluted in a 0,05M sodium carbonate-bicarbonate buffer (pH 9,6) at 4°C overnight. Plates were blocked for 1,5 h with 10% milk (200 µl per well) in PBS with 0,1% Tween 20 (PBS-T) and afterward were incubated for 1 h with the cell lysate (HeLa cells persistently infected with LCMV MX and uninfected HeLa cells) diluted 1:300 in blocking solution. On a parallel plate human serum samples were diluted 1:20 and 1:60 in blocking solution. A total of 50 µl per well of diluted serum samples was transferred to NP-saturated plates, followed by incubation for 1 hour. Finally, the plates were incubated for 45 min with HRP conjugated goat anti-human IgG (Fc-specific) Ab (SIGMA) diluted 1:35000 in blocking solution. HRP was detected by OPD color reaction, which was

stopped by adding 50 μ l of 2M H₂SO₄. Optical density was measured for absorbance at 492 nm. All steps were carried out at room temperature. Between each step the plates were washed four times with PBS-T. The adjusted OD was calculated by subtracting the OD of the negative antigen-coated wells from that of corresponding wells.

Alternatively, was as antigen used purified recombinant LCMV NP at concentration 4 μ g/ml.

To find suitable internal positive and negative controls, human sera were analyzed by immunoprecipitation and subsequent immunoblotting with NP-specific monoclonal antibody (*see* Example 7). Later, one of the sera was chosen as a negative control and another as was selected as a positive control for subsequent testing. 25 sera which have been proved negative with the immunoprecipitation test, were subsequently analyzed in NP ELISA and the adjusted OD values obtained were then expressed as percent positivity (PP) of the internal positive control by finding the average adjusted OD value of two replicates divided by the median value of the adjusted OD values of the four replicates of the positive control multiplied by 100. The ELISA cut-off value, which would serve as a threshold between the positive and negative sera samples was determined as the mean PP value obtained with this 25 samples plus two standard deviations.

The cut-off value was determined to be 4.2 % using this method and was used for subsequent calculation.

Example 16: Detection of Anti-LCMV NP Antibody in Human Samples by NP-IgG ELISA-2

Serum samples were obtained from (i) women with spontaneous abortions without diagnosed cause; (ii) women with full-term gravidity; and (iii) oncology patients with renal cell carcinoma (RCC) tumors. Anti-LCMV antibodies in human sera were tested under the conditions described in Example 15 and PP values were determined and are shown in Table 3.

Table 3: Detection of Anti-LCMV NP Antibodies in Human Female Subjects that Experienced Spontaneous Abortion

	<i>Women with abortion</i>		<i>Women without abortion</i>	
	number	%	number	%
positive sera	11	23,9	13	11,8
negative sera	35	76,1	97	88,2
total	46	100	110	100

As shown in Table 3, seroprevalence of NP antibodies in women with abortions was by 12,1% higher compared to control. As shown in Table 4, very high

5 seroprevalence of NP antibodies was also observed for patients with RCC tumors.

Table 4: Detection of Anti-LCMV NP Antibodies in Subjects with RCC Tumors

	<i>Oncology patients</i>	
	number	%
positive sera	23	37
negative sera	39	63
total	62	100

5 Example 17: Generation of Recombinant Baculovirus that Expressed His-LCMV-NP

The recombinant baculovirus that expressed His-LCMV-NP was generated by using Bac-to-Bac(R) Baculovirus Expression System (Invitrogen). In order to construct the recombinant donor vector, a cDNA from HeLa/MX cells was used. A complete NP gene with the initiation and stop codons was amplified by PCR using the primers NPMXF1S 5'-CCGAATTCATGTCTCTGTCCAAGGAAGTCA-3' (46-67) (SEQ ID NO:85) and NPMXF3A 5'-CCTCGAGTTAGAGTGTCAACAACATTTGGTC-3' (1722-1700) (SEQ ID NO:86). The primers were designed with EcoRI and Xho I restriction sites (underlined), respectively. Numbers in parentheses show positions with respect to published NP sequence of MX strain (GenBank accession number Y16308, Reiserova et al. 2001).

The PCR reaction was performed with Phusion High Fidelity PCR Master MIX (Thermo Scientific) using gene-specific primers. The PCR protocol consisted of 98°C for 2 min followed by 35 cycles of: denaturation at 98°C for 30 sec, annealing at 58°C for 40 sec, and extension at 72°C for 2 min, followed by final extension at 72°C for 7 min. The amplification product was digested with EcoRI and XhoI and cloned into pFastBac HT A vector. The inserted LCMV-NP DNA was sequenced and confirmed to be in proper orientation downstream the promoter and identical to the original sequence. Verified recombinant donor plasmid (pFastBac HT-LCMV-NP) was transformed to E. coli DH10Bac competent cells. Successful transposition to the recombinant bacmid DNA was verified by PCR using a combination of the pUC/M13 and gene-specific primers. Recombinant bacmid DNA containing the gene of the interest was used for transfection of SF9 insect cells. Finally, recombinant

baculovirus clones overexpressing His-LCMV-NP were obtained after three successive plaque purifications.

Example 18: Expression and Purification of His-LCMV-NP

5 SF9 cells infected with the recombinant baculovirus expressing His-LCMV-NP were incubated at 26°C for 96 h. The cells were then harvested and washed three time with PBS. The cells were resuspended in 1% NP40 in PBS, allowed to stand on ice for 15 min, and centrifuged at 10,000 rpm for 10 min. The pellet was serially treated with urea solutions at different concentrations. First, the pellet was suspended
10 in 1 M urea in 1% NP40 in PBS, sonicated, and centrifuged at 8,000 rpm for 5 min. Then, the pellet was washed in PBS and suspended in 2 M urea in PBS. After the suspension was sonicated and centrifuged, the pellet was washed in PBS and suspended in 8 M urea in PBS. The suspension was sonicated and centrifuged, and the supernatant was used as LCMV-NP antigen. The control antigen was produced
15 from SF9 cells infected with baculovirus that do not contain LCMV-NP gene. The protein concentration of antigens was determined by using a Bradford protein assay (Bio-Rad Laboratories). The expression and purification efficiency of His-LCMV-NP was analyzed on 10% SDS-PAGE gel after staining with Coomassie blue (*see* FIG. 17).

20

Example 19: Cloning and Expression of Recombinant LCMV-GP1

A sequence corresponding to the GP1 sequence (amino acids 1 to 265) according to published GPC sequence of MX strain (GenBank accession number EU195888, Tomaskova et al. 2008) was amplified by PCR using the primers
25 GPSBamHI 5'-TTGGATCCTGTCAAACCTTTGTCCCA CACAAAG-3' (54-77) (SEQ ID NO:87) and GP1AEcoRI 5'-
AGAATTCTCATCATCTAGTGAGGAACTTTGTCTTT TC-3' (863-840) (SEQ ID NO:88). In this way, BamHI and EcoRI restriction sites (underlined) were introduced. PCR reactions were performed using the primers listed above and
30 GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA). Following an initial denaturation at 95°C for 2 min, the amplification program was set as follows: denaturation at 95°C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C

during 45 s for a total of 35 cycles, and finally 7 min at 72 °C. The PCR product was purified on a 1% agarose gel using NucleoSpin Extract II kit (Macherey-Nagel) and cloned in-frame with glutathione S-transferase into pGEX-4T-1 using BamHI and EcoRI restriction enzymes. To produce GST-fusion protein, verified plasmid
5 construct (designated pGEX-4T1-GP1) was transformed into *E. coli* DH5 α competent cells and induced with 0.75 mM IPTG (Sigma-Aldrich, USA, USA) for 3 hours in 37°C. Induced cultures of *E. coli* were pelleted by centrifugation, resuspended in ice-cold lysis buffer STE (10 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA in 1xPBS), pH 8 and incubated on ice for 15 minutes with lysosyme (Serva, Germany) in the final
10 concentration of 0,4 mg/ml. Before sonication of bacterial cells (5 x 15s) 10% Sarcosyl (Sigma-Aldrich, USA) in STE to final concentration of 1,7% and 1 M DTT (Sigma-Aldrich, USA) to final concentration of 0,5 mM was added to cell suspension. After sonication 10% Triton X-100 (AppliChem) in STE was added to final concentration 2,5% and incubated on ice for 15 minutes. Then the insoluble material
15 was removed by centrifugation for 15 minutes at 10 000 rpm at 4°C. The yield of fusion protein in induced samples was determined by SDS-PAGE and visual comparison to defined concentration of BSA.

Example 20: GP1-IgG ELISA

20 96-well polystyrene plates were coated overnight at 37°C with lysate containing approximately 4 μ g of recombinant GST-GP1 and/or GST/ml. Thereafter, plates were blocked for 1.5 h with 10% milk (200 μ l per well) in PBS with 0,1% Tween 20 (PBS-T). On a parallel plate serum samples were prediluted 1:20 in blocking solution and a twofold dilution series was performed. A total of 50 μ l per
25 well of diluted serum samples was transferred to GP1- and GST-saturated, plates, followed by incubation for 1 hour. Finally, plates were incubated for 45 min with HRP conjugated goat anti-human IgG (Fc-specific) Ab (SIGMA) diluted 1:35000 in blocking solution. HRP was detected by OPD color reaction, which was stopped by adding 50 μ l of 2M H₂SO₄. Optical density was measured for absorbance at 492 nm.
30 All steps were carried out at room temperature. Between each step the plates were washed four times with PBS-T. The adjusted OD was calculated by subtracting the OD of the negative antigen-coated wells from that of corresponding wells.

Example 21: Assays for LCMV Detection

The following assays were performed to demonstrate detection of LCMV.

LCMV Detection using MX Strain

For LCMV detection real-time PCR was used with dual labeled oligonucleotide probe (TaqMan) based on fluorescent detection system. Two specific regions of the LCMV genome were amplified: a fragment of the nucleoprotein encoding gene (NP) and a fragment of the glycoprotein encoding gene (GP). Amplifications were carried out both in singleplex formats (NP or GP) and in a duplex format (NP+GP). cDNA reverse transcribed from RNA of uninfected HeLa cells and molecular grade water were included as negative controls. For each target, the presence of only one PCR product on 1% agarose gels was confirmed (see FIG. 18).

As shown in FIGs. 19A-19B, LCMV was detected via singleplex format. As demonstrated in FIG. 20, LCMV was also detected via the duplex format. Each reaction contained two sets of PCR primers for unique NP and GP nucleotide sequences and two TagMan probes, each specific for one of the two amplification products and labeled with a differently colored fluorophore. Fluorescent signals from the HEX-labeled TaqMan (NP specific), and from the FAM-labeled TaqMan, are plotted in green, and grey, respectively.

LCMV Detection using ARM Strain

The feasibility of the assay for detection of different LCMV strains was tested using the LCMV ARM strain. As shown in FIGs. 21 and 22, amplification products were successfully detected with both TaqMan probes in both singleplex (NP or GP) and duplex formats (NP+GP). The robustness of the assay was confirmed as the same TaqMan probes were suitable for amplicon detection even in the presence of mismatched oligonucleotides under the regions covered by the used probes. The primer and probe sequences were designed to perfectly match the sequence of the MX strain. Sequence differences between the MX and ARM strains resulted in 4 mismatched oligonucleotides in the NP probe/target region and in 2 mismatched

nucleotides in the GP probe/target region. Despite this fact efficient amplification signal was generated by both TaqMan probe. cDNA reverse transcribed from RNA of uninfected HeLa cells and molecular grade water were included as negative controls.

5 **Example 22: Sensitivity of Assays for LCMV Detection**

 The analytical sensitivity of qPCR was assessed by testing of serial dilution of the standard virus strain MX. Blood from healthy individual was spiked with serial dilution of LCMV infected cells (10⁵, 10³, 10, 1, 0.5, 0.25 infected cell) to standardize RNA extraction and to detect the analytical sensitivity of the PCR. Serial
10 dilutions were prepared in a Dulbecco modified Eagle growth medium. Healthy blood samples were spiked with each of these dilutions. Blood from healthy individuals and molecular grade water were included as negative controls.

 Dilution assays showed a reproducible detection limit of 0.25 LCMV infected cell (*see* FIG. 23A-23D).

15

Example 23: Hypoxia-Induced Upregulation of Expression LCMV MX NP and GP Genes

 Using real-time PCR hypoxia-induced changes in the expression of LCMV NP and GP genes were measured. Total RNAs were prepared from cells incubated in
20 normoxic or hypoxic conditions (2% O₂) for 24 h for reverse transcription. Expression of LCMV genes was determined by quantitative real-time PCR. Differences in gene expression, expressed as fold-change, were calculated using the 2Ct method using ACTB (β -actin) as internal control. For each gene, the fold induction was determined in comparison with the normoxic control.

25 As shown in FIG. 24, hypoxia significantly increased an expression of NP and GP genes in hypoxic HeLa-MX cells compared to the normoxic controls of the same strain (*see* FIGs. 21-22).

30

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the
5 scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of assessing lymphocytic choriomeningitis virus (LCMV) infection status or activity in a subject, the method comprising:

5 selecting a subject for assessment, wherein the subject has been exposed to LCMV and/or is at risk of increased LCMV activity;
obtaining a sample from the subject:
contacting the sample with at least one composition for detecting LCMV; and
determining whether the at least one composition for detecting LCMV is
10 associated with a marker of LCMV from the sample, wherein detection of an association indicates that that the subject is infected with and/or has active LCMV.

2. The method of claim 1, wherein determining comprises reporting that a subject is infected with and/or has active LCMV to the subject and/or a medical practitioner associated with the subject.

15

3. The method of claim 1, wherein determining further comprises detecting the level and/or activity of LCMV in a subject that is infected with and/or has active LCMV.

20

4. The method of claim 3, further comprising reporting the level and/or activity of LCMV detected to the subject and/or a medical practitioner associated with the subject.

25

5. The method of claim 1, wherein a subject at risk of increased LCMV activity is at risk of a condition associated with hypoxia.

30

6. The method of claim 5, wherein the subject is pregnant, immunocompromised, a transplant recipient, at risk for developing cancer, or has cancer.

7. The method of claim 1, wherein the composition for detecting LCMV comprises at least one primer that binds specifically to one or more LCMV nucleic acids.

5 8. The method of claim 7, wherein the at least one primer comprises 10 or more nucleic acids, wherein the 10 or more nucleic acids have at least at least 80% identity to a target region within any one of SEQ ID NOs:1-51, such that the primer binds specifically to the target region.

10 9. The method of any one of claims 7 or 8, wherein the primer is selected from the group consisting of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, and SEQ ID NO:73.

15 10. The method of claim 9, wherein the primer binds to a nucleic acid encoding LCMV NP.

20 11. The method of claim 10, wherein the primer comprises a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:58 and 59 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:66 and 67.

25 12. The method of claim 9, wherein the primer binds to a nucleic acid encoding LCMV GP.

 13. The method of claim 12, wherein the primer comprises a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:60 and 61 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:68 and 69.

30 14. The method of claim 9, wherein the primer binds to a nucleic acid encoding LCMV ZP.

15. The method of claim 14, wherein the primer comprises a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs:62 and 63 or a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 72 and 73.

5 16. The method of claim 9, wherein the primer binds to a nucleic acid encoding LCMV L.

17. The method of claim 16, wherein the primer comprises a pair of nucleic acid sequences with at least 80% identity to SEQ ID NOs: 70 and 71.

10

18. The method of claim 9, wherein the primer binds to nucleic acids encoding two or more of LCMV NP, LCMV GP, LCMV ZP, and LCMV L.

19. The method of claim 1, wherein the composition for detecting LCMV
15 comprises at least one isolated LCMV protein or a fragment thereof.

20. The method of claim 1, wherein the composition for detecting LCMV comprises at least one isolated monoclonal antibody or antibody fragment.

20 21. The method of claim 20, wherein the antibody or fragment comprises an antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

25 22. The method of claim 20, wherein the antibody or fragment comprises an antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

30 23. The method of claim 20, wherein the antibody or fragment comprises an antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent

University, Belgium, and an antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

5 24. The method of claim 20, wherein the antibody or fragment comprises CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

10 25. The method of claim 24, wherein the antibody or fragment comprises CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

15 26. The method of claim 25, wherein the antibody or fragment comprises CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

20 27. The method of claim 20, wherein the antibody or fragment comprises CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87.

 28. The method of claim 27, wherein the antibody or fragment comprises CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87.

25 29. The method of claim 28, wherein the antibody or fragment comprises CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87.

30 30. The method of claim 20, wherein the antibody or fragment comprises an antigen binding peptide with identity to SEQ ID NO: 74, wherein:

regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino acid substitutions;

5 regions the amino acid sequence that correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region in SEQ ID NO:74; and

the antigen binding peptide binds to LCMV NP.

10 31. The method of claim 30, wherein the antigen binding peptide is an antibody or an antibody fragment.

32. A diagnostic kit for assessing lymphocytic choriomeningitis virus (LCMV) infection status or activity in a sample, wherein the kit comprises:
15 at least one primer that binds specifically to one or more LCMV nucleic acids;

at least one LCMV protein or fragment thereof;

at least one isolated LCMV binding antibody or antibody fragment; or
any combination thereof.

20 33. The kit of claim 32, wherein the kit comprises at least one primer that binds specifically to one or more LCMV nucleic acids.

34. The kit of claim 33, wherein the primer comprises at least one nucleic acid primer having 10 or more nucleic acids, wherein the 10 or more nucleic acids have at
25 least at least 80% identity to one or more target regions within one or more of SEQ ID NOs:1-51, such that the primer binds specifically to the one or more target regions.

35. The kit of claim 33, wherein the primer is selected from the group consisting of SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ
30 ID NO:62, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, and SEQ ID NO:73.

36. The kit of claim 33, wherein the primer binds to a nucleic acid encoding LCMV NP.

5 37. The kit of claim 33, wherein the primer binds to a nucleic acid encoding LCMV GP.

38. The kit of claim 33, wherein the primer binds to a nucleic acid encoding LCMV ZP.

10 39. The kit of claim 33, wherein the primer binds to a nucleic acid encoding LCMV L.

40. The kit of claim 33, wherein the primer binds to nucleic acids encoding two or more of LCMV NP, LCMV GP, LCMV ZP, and LCMV L.

15 41. The kit of claim 32, wherein the kit comprises at least one isolated LCMV binding antibody or antibody fragment.

20 42. The kit of claim 41, wherein the antibody or fragment comprises a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

25 43. The kit of claim 41, wherein the antibody or fragment comprises a monoclonal antibody produced by hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

30 44. The kit of claim 41, wherein the antibody or fragment comprises a monoclonal antibody produced by hybridoma MJ3, LMBP accession number 9217CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium, and a monoclonal antibody produced by

hybridoma M166, LMBP accession number 9216CB, deposited with the Belgian Coordinated Collections of Microorganisms (BCCM) at Ghent University, Belgium.

5 45. The kit of claim 41, wherein the antibody or fragment comprises CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

10 46. The kit of claim 45, wherein the antibody or fragment comprises CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

15 47. The kit of claim 46, wherein the antibody or fragment comprises CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87, comprising one or more conservative amino acid substitutions.

48. The kit of claim 41, wherein the antibody or fragment comprises CDR3 (SEQ ID NO:78) of the heavy chain variable region of monoclonal antibody M87.

20 49. The kit of claim 48, wherein the antibody or fragment comprises CDR2 (SEQ ID NO:77) of the heavy chain variable region of monoclonal antibody M87.

50. The kit of claim 49, wherein the antibody or fragment comprises CDR1 (SEQ ID NO:76) of the heavy chain variable region of monoclonal antibody M87.

25 51. The kit of claim 41, wherein the antibody or fragment comprises an antigen binding peptide with identity to SEQ ID NO: 74, wherein:

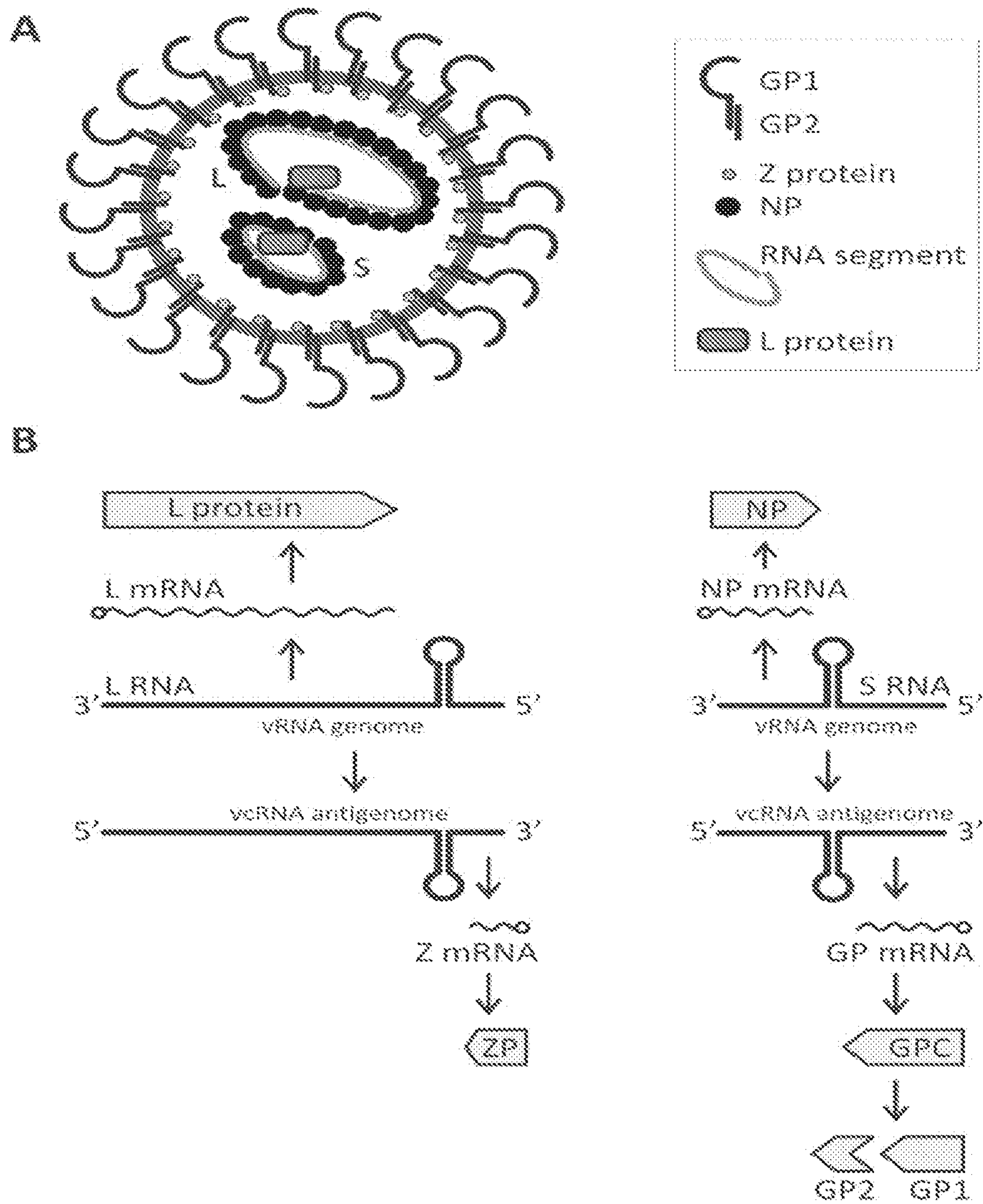
regions within the amino acid sequence that correspond to a complementarity determining region within SEQ ID NO:74 comprise one or more conservative amino acid substitutions;

30 regions the amino acid sequence that correspond to a framework region within SEQ ID NO:74 have at least 80% identity to the corresponding region in SEQ ID NO:74; and

the antigen binding peptide binds to LCMV NP.

52. The kit of claim 51, wherein the antigen binding peptide is an antibody or an antibody fragment.

5



FIGs. 1A-1B

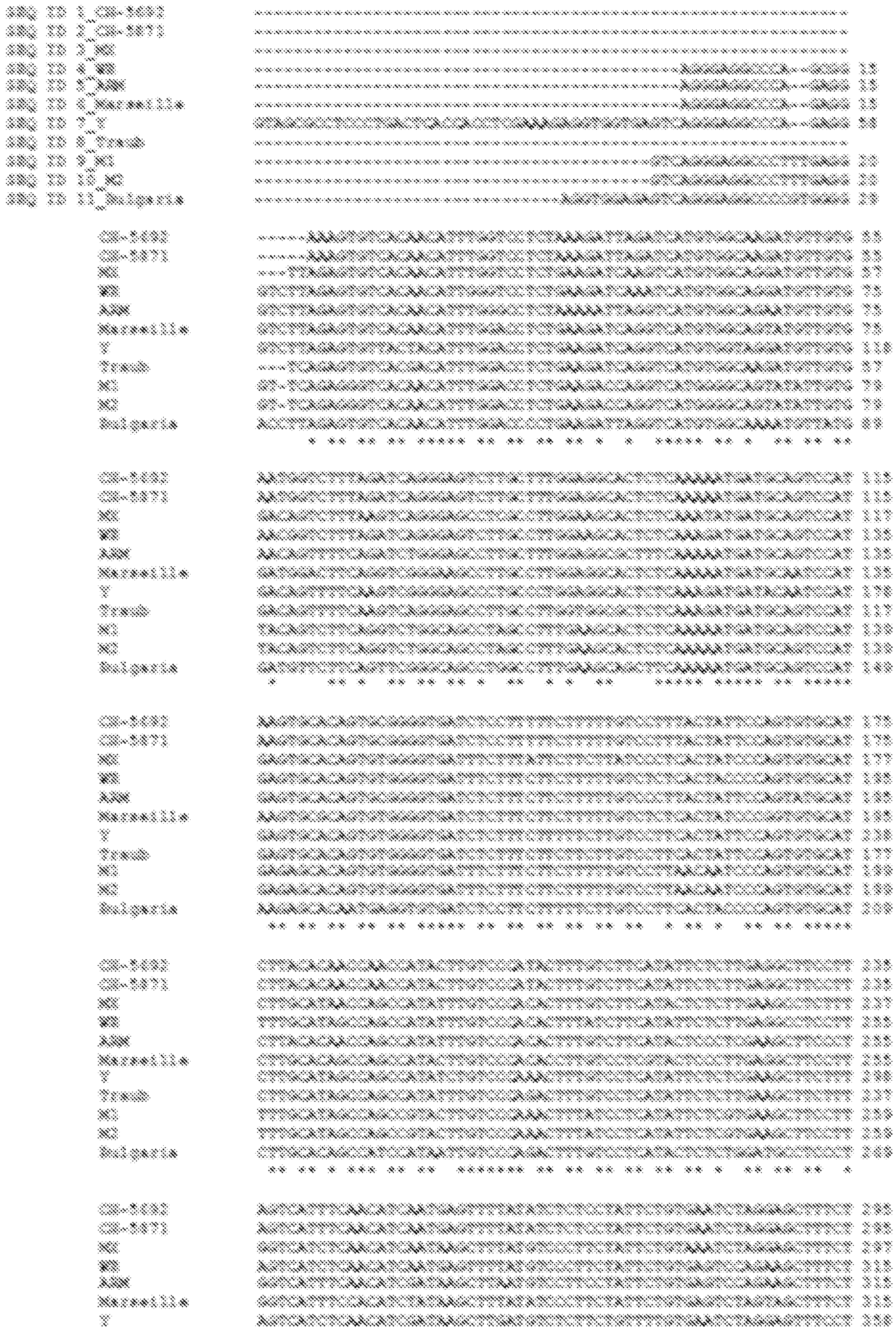


FIG. 2i

FIG. 2ii

Y	*****	718
Yeshu	*****	837
W3	*****	878
W2	*****	879
Belgaria	*****	888
	* * * * *	
CG-1882	*****	718
CG-1871	*****	718
W3	*****	717
W2	*****	738
W3W	*****	738
W3W311e	*****	738
Y	*****	778
Yeshu	*****	717
W3	*****	738
W2	*****	738
Belgaria	*****	748
	* ** * * * *	
CG-1882	*****	778
CG-1871	*****	778
W3	*****	777
W2	*****	798
W3W	*****	738
W3W311e	*****	788
Y	*****	838
Yeshu	*****	777
W3	*****	788
W2	*****	788
Belgaria	*****	888
	** ** * * * * *	
CG-1882	*****	818
CG-1871	*****	818
W3	*****	837
W2	*****	838
W3W	*****	838
W3W311e	*****	818
Y	*****	888
Yeshu	*****	837
W3	*****	838
W2	*****	838
Belgaria	*****	888
	** ** * * * * *	
CG-1882	*****	888
CG-1871	*****	888
W3	*****	887
W2	*****	818
W3W	*****	818
W3W311e	*****	818
Y	*****	838
Yeshu	*****	887
W3	*****	818
W2	*****	818
Belgaria	*****	888
	** ** * * * * *	
CG-1882	*****	888
CG-1871	*****	888
W3	*****	887
W2	*****	878
W3W	*****	878
W3W311e	*****	878
Y	*****	1018
Yeshu	*****	887
W3	*****	878
W2	*****	878
Belgaria	*****	888
	*** ** * * * *	
CG-1882	*****	1018
CG-1871	*****	1018
W3	*****	1017
W2	*****	1038
W3W	*****	1038

FIG. 2iii

*****		1075
F		1076
French		1077
NO		1078
NO		1079
Bulgaria		1080
** ***** ** ** * ** ** ** **		
CG-1492		1075
CG-1471		1076
NO		1077
NO		1078
NO		1079
*****		1080
F		1135
French		1077
NO		1078
NO		1079
Bulgaria		1136
***** * ** ** ** * ** ** ** *		
CG-1492		1135
CG-1471		1136
NO		1137
NO		1138
NO		1139
*****		1140
*****		1141
F		1142
French		1137
NO		1138
NO		1139
Bulgaria		1140
***** ** ** ** **		
CG-1492		1145
CG-1471		1146
NO		1147
NO		1148
NO		1149
*****		1150
*****		1151
F		1152
French		1147
NO		1148
NO		1149
Bulgaria		1150
*** ** * ** ***** ** ** ** * ** *****		
CG-1492		1255
CG-1471		1256
NO		1257
NO		1258
NO		1259
*****		1260
*****		1261
F		1262
French		1257
NO		1258
NO		1259
Bulgaria		1260
*** ** ** ** **		
CG-1492		1315
CG-1471		1316
NO		1317
NO		1318
NO		1319
*****		1320
*****		1321
F		1322
French		1317
NO		1318
NO		1319
Bulgaria		1320
** ***** ** * ** ** ** **		
CG-1492		1375
CG-1471		1376
NO		1377
NO		1378

FIG. 2iv

FIG. 2v

[illegible]

Country	Year	Value
Algeria	1990	1.00
Algeria	1991	1.00
Algeria	1992	1.00
Algeria	1993	1.00
Algeria	1994	1.00
Algeria	1995	1.00
Algeria	1996	1.00
Algeria	1997	1.00
Algeria	1998	1.00
Algeria	1999	1.00
Algeria	2000	1.00
Algeria	2001	1.00
Algeria	2002	1.00
Algeria	2003	1.00
Algeria	2004	1.00
Algeria	2005	1.00
Algeria	2006	1.00
Algeria	2007	1.00
Algeria	2008	1.00
Algeria	2009	1.00
Algeria	2010	1.00
Algeria	2011	1.00
Algeria	2012	1.00
Algeria	2013	1.00
Algeria	2014	1.00
Algeria	2015	1.00
Algeria	2016	1.00
Algeria	2017	1.00
Algeria	2018	1.00
Algeria	2019	1.00
Algeria	2020	1.00
Algeria	2021	1.00
Algeria	2022	1.00
Algeria	2023	1.00
Algeria	2024	1.00
Algeria	2025	1.00
Algeria	2026	1.00
Algeria	2027	1.00
Algeria	2028	1.00
Algeria	2029	1.00
Algeria	2030	1.00
Algeria	2031	1.00
Algeria	2032	1.00
Algeria	2033	1.00
Algeria	2034	1.00
Algeria	2035	1.00
Algeria	2036	1.00
Algeria	2037	1.00
Algeria	2038	1.00
Algeria	2039	1.00
Algeria	2040	1.00
Algeria	2041	1.00
Algeria	2042	1.00
Algeria	2043	1.00
Algeria	2044	1.00
Algeria	2045	1.00
Algeria	2046	1.00
Algeria	2047	1.00
Algeria	2048	1.00
Algeria	2049	1.00
Algeria	2050	1.00
Algeria	2051	1.00
Algeria	2052	1.00
Algeria	2053	1.00
Algeria	2054	1.00
Algeria	2055	1.00
Algeria	2056	1.00
Algeria	2057	1.00
Algeria	2058	1.00
Algeria	2059	1.00
Algeria	2060	1.00
Algeria	2061	1.00
Algeria	2062	1.00
Algeria	2063	1.00
Algeria	2064	1.00
Algeria	2065	1.00
Algeria	2066	1.00
Algeria	2067	1.00
Algeria	2068	1.00
Algeria	2069	1.00
Algeria	2070	1.00
Algeria	2071	1.00
Algeria	2072	1.00
Algeria	2073	1.00
Algeria	2074	1.00
Algeria	2075	1.00
Algeria	2076	1.00
Algeria	2077	1.00
Algeria	2078	1.00
Algeria	2079	1.00
Algeria	2080	1.00
Algeria	2081	1.00
Algeria	2082	1.00
Algeria	2083	1.00
Algeria	2084	1.00
Algeria	2085	1.00
Algeria	2086	1.00
Algeria	2087	1.00
Algeria	2088	1.00
Algeria	2089	1.00
Algeria	2090	1.00
Algeria	2091	1.00
Algeria	2092	1.00
Algeria	2093	1.00
Algeria	2094	1.00
Algeria	2095	1.00
Algeria	2096	1.00
Algeria	2097	1.00
Algeria	2098	1.00
Algeria	2099	1.00
Algeria	2100	1.00
Algeria	2101	1.00
Algeria	2102	

[illegible]

FIG. 31

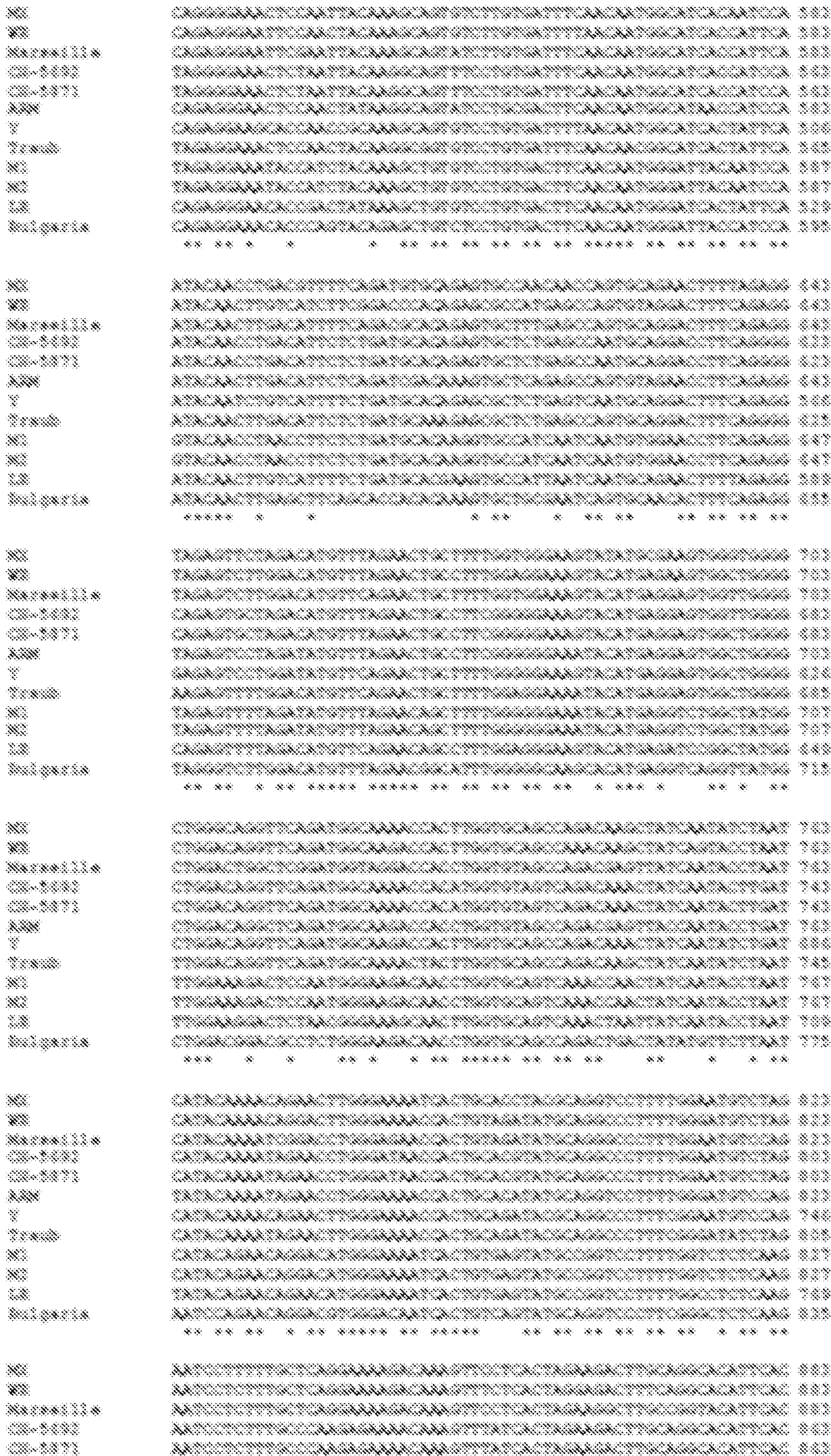


FIG. 3iii

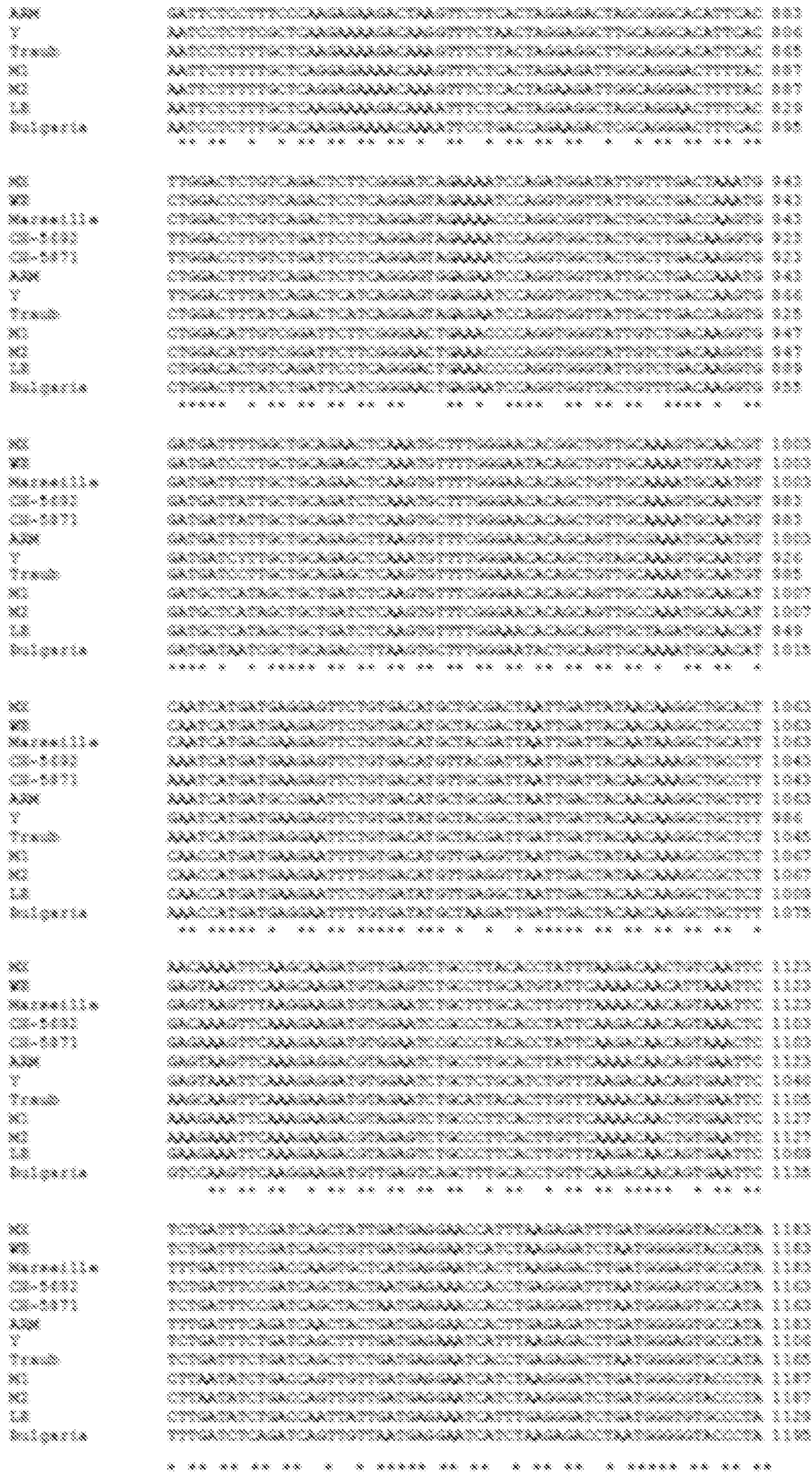


FIG. 3iv

FIG. 3v

FIG. 3vi

FIG. 4

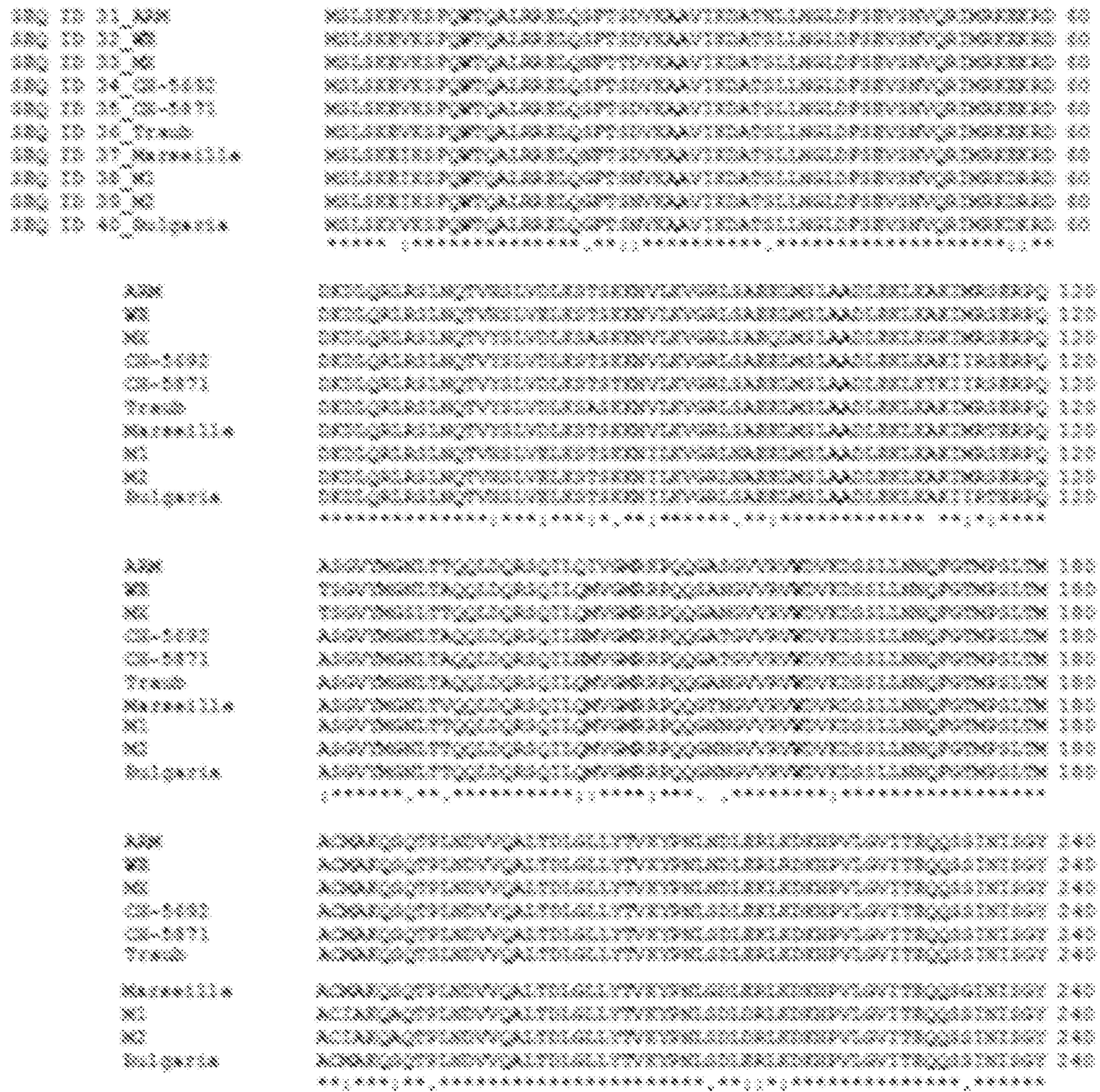


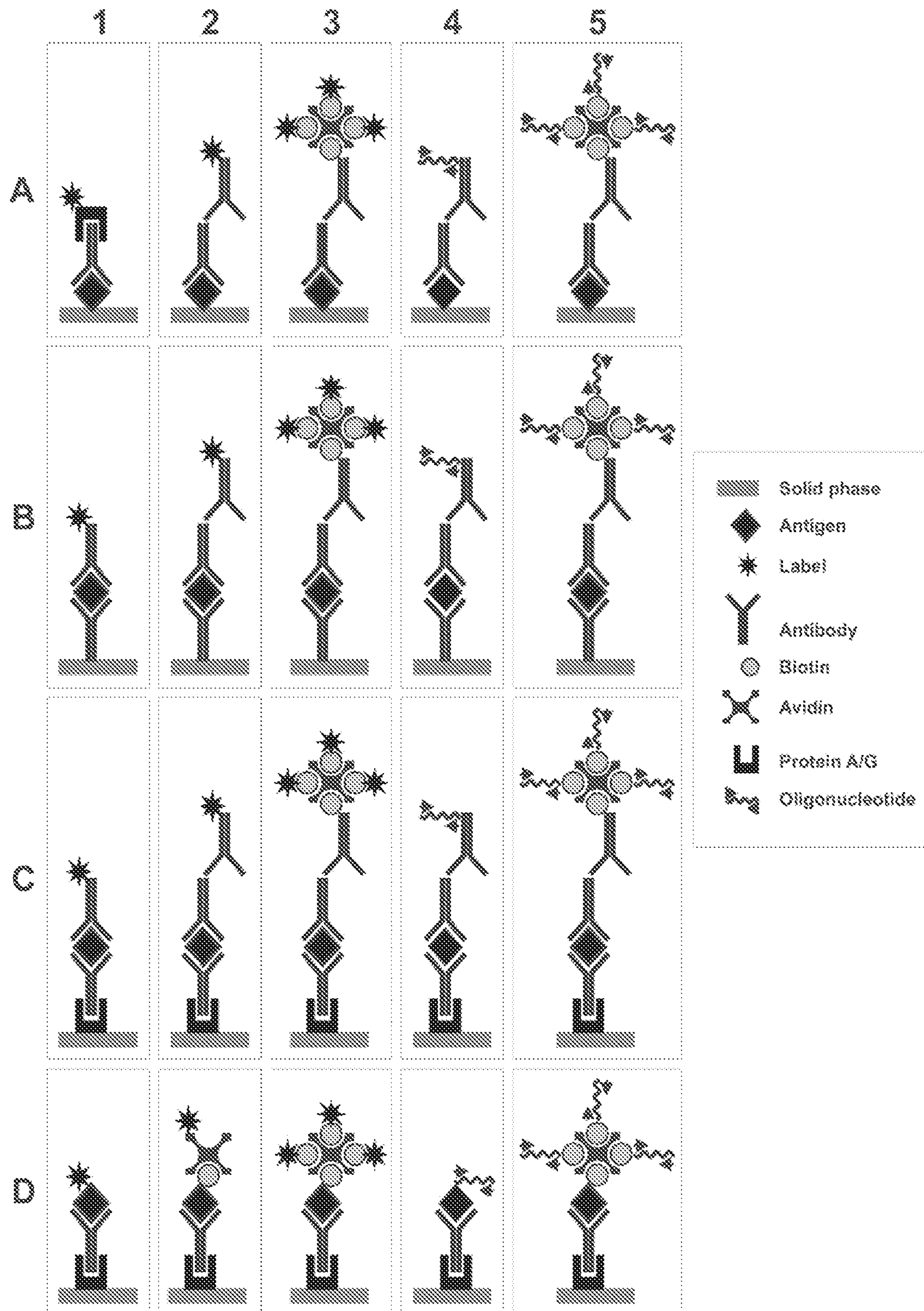
FIG. 5i

FIG. 6i

FIG. 6iii

[illegible]

FIG. 7



FIGs. 8A-8D

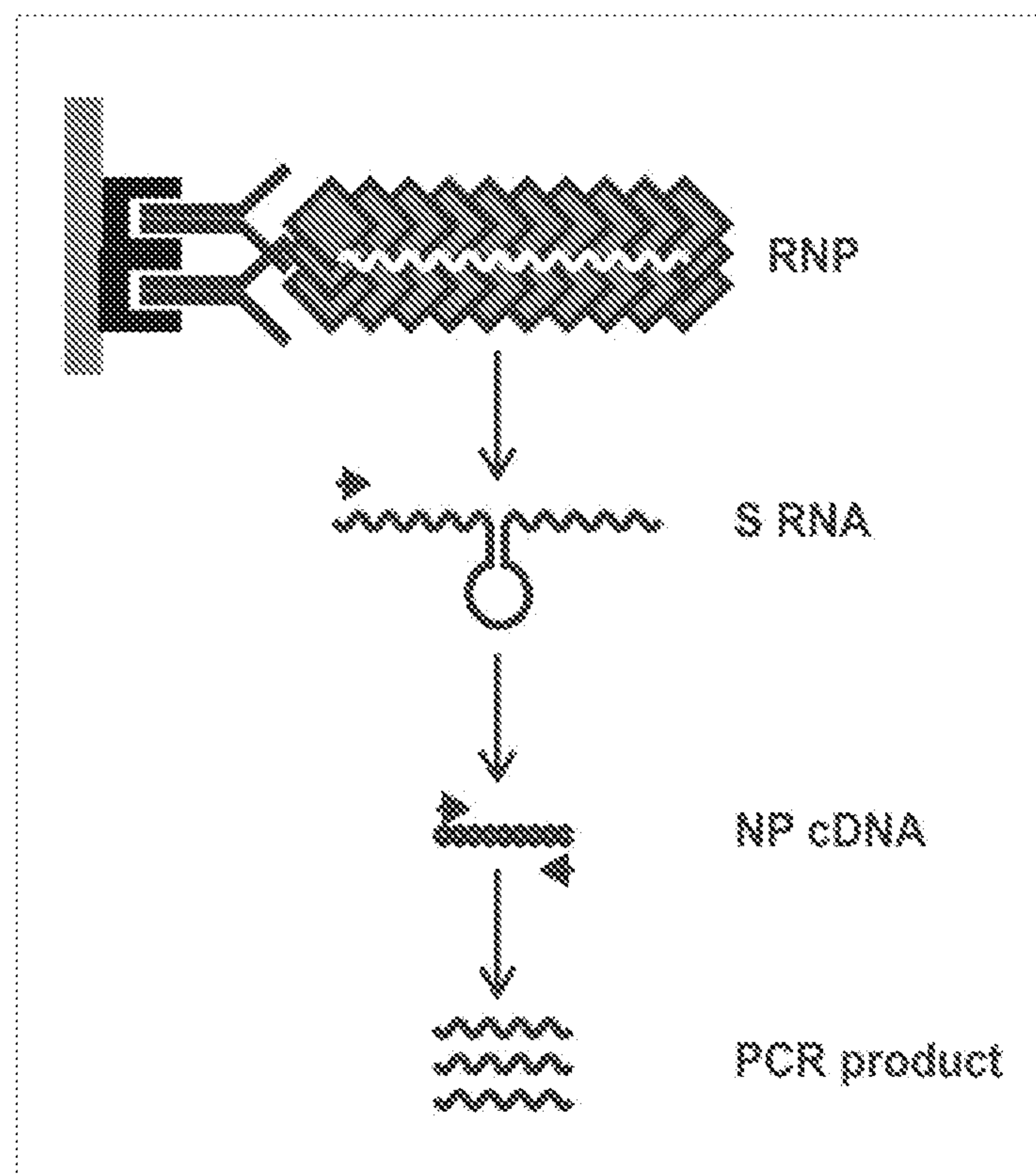
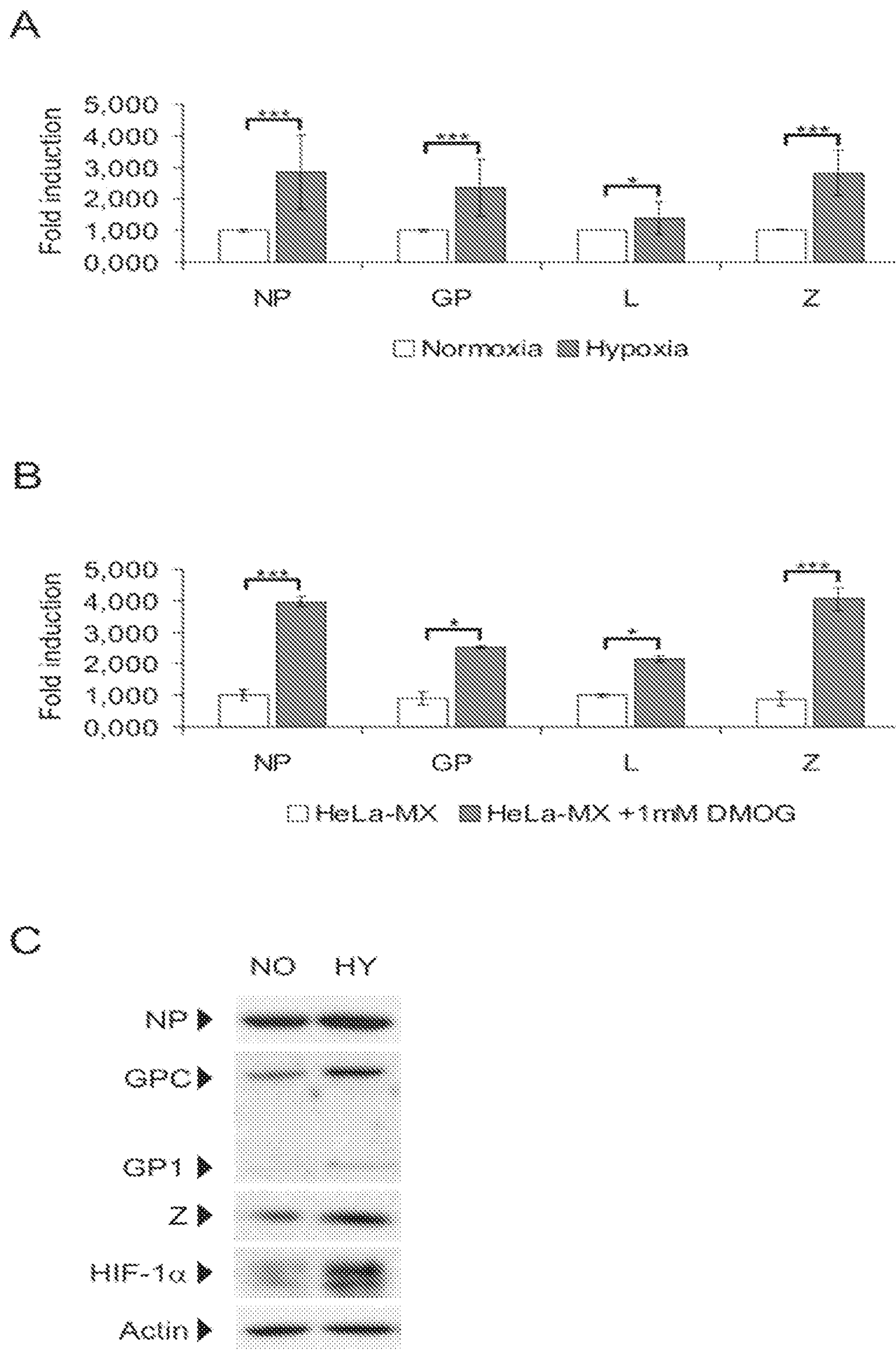
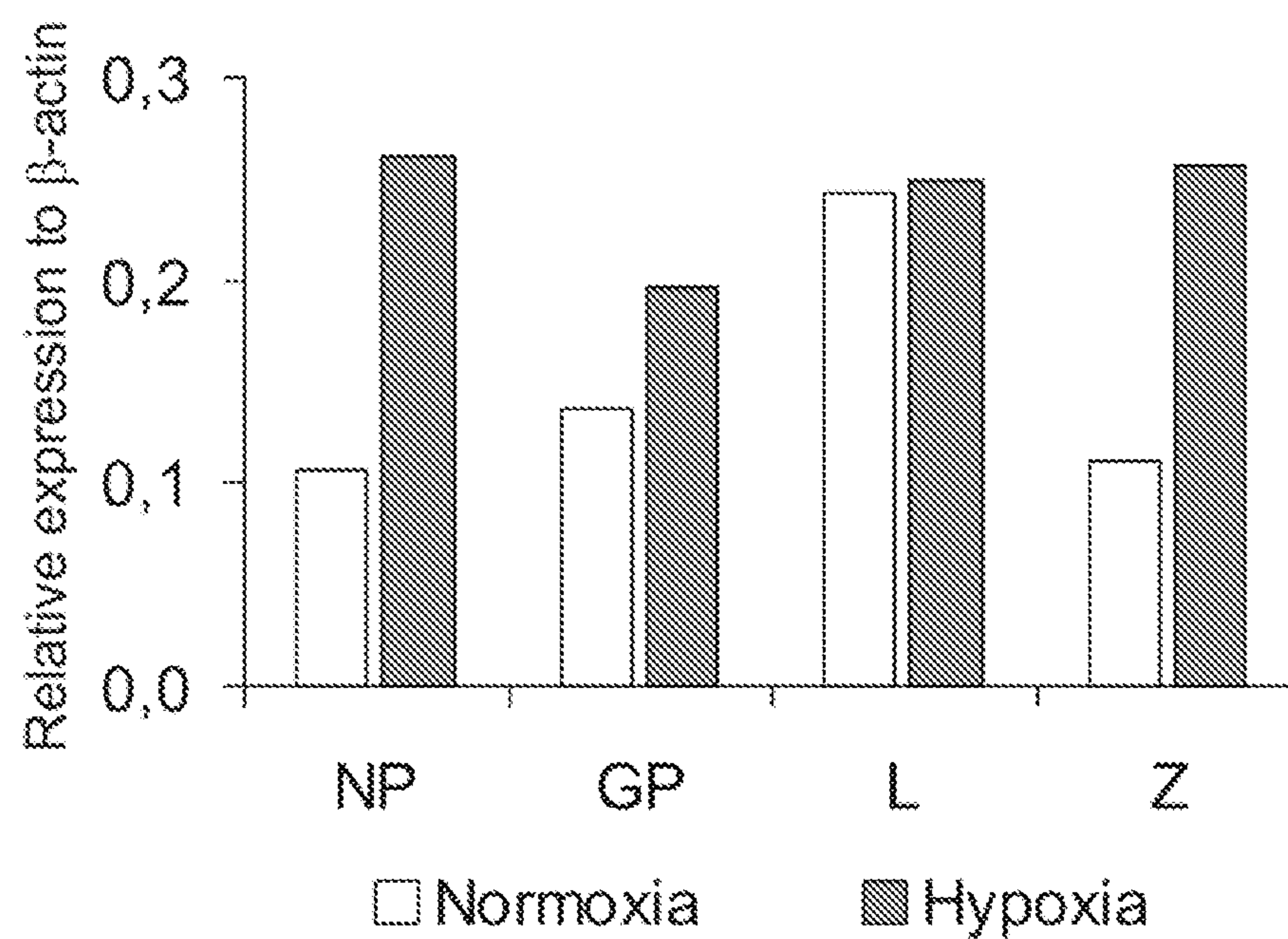


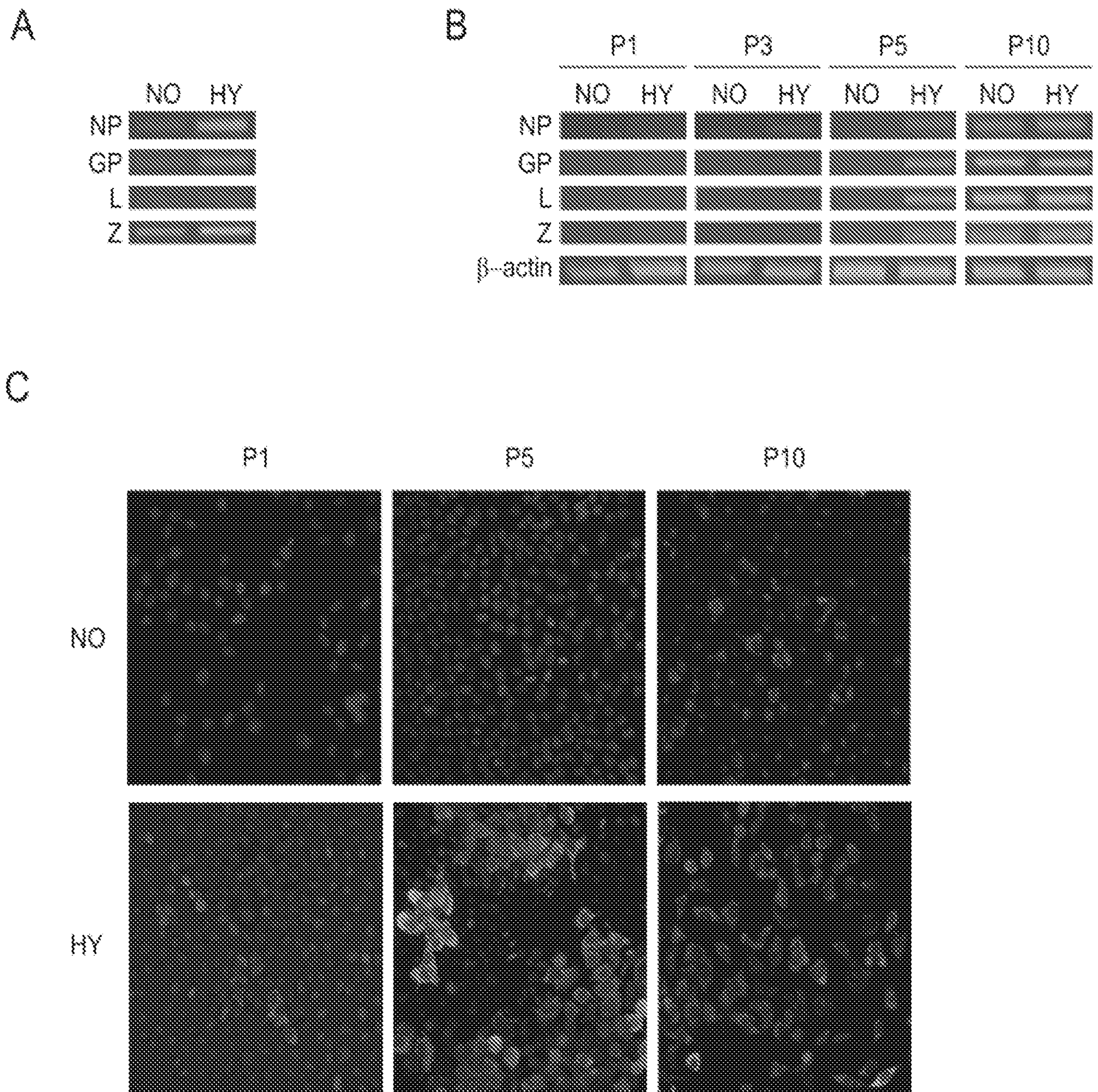
FIG. 9



FIGs. 10A-10C



FIGs 10D



FIGs. 11A-11C

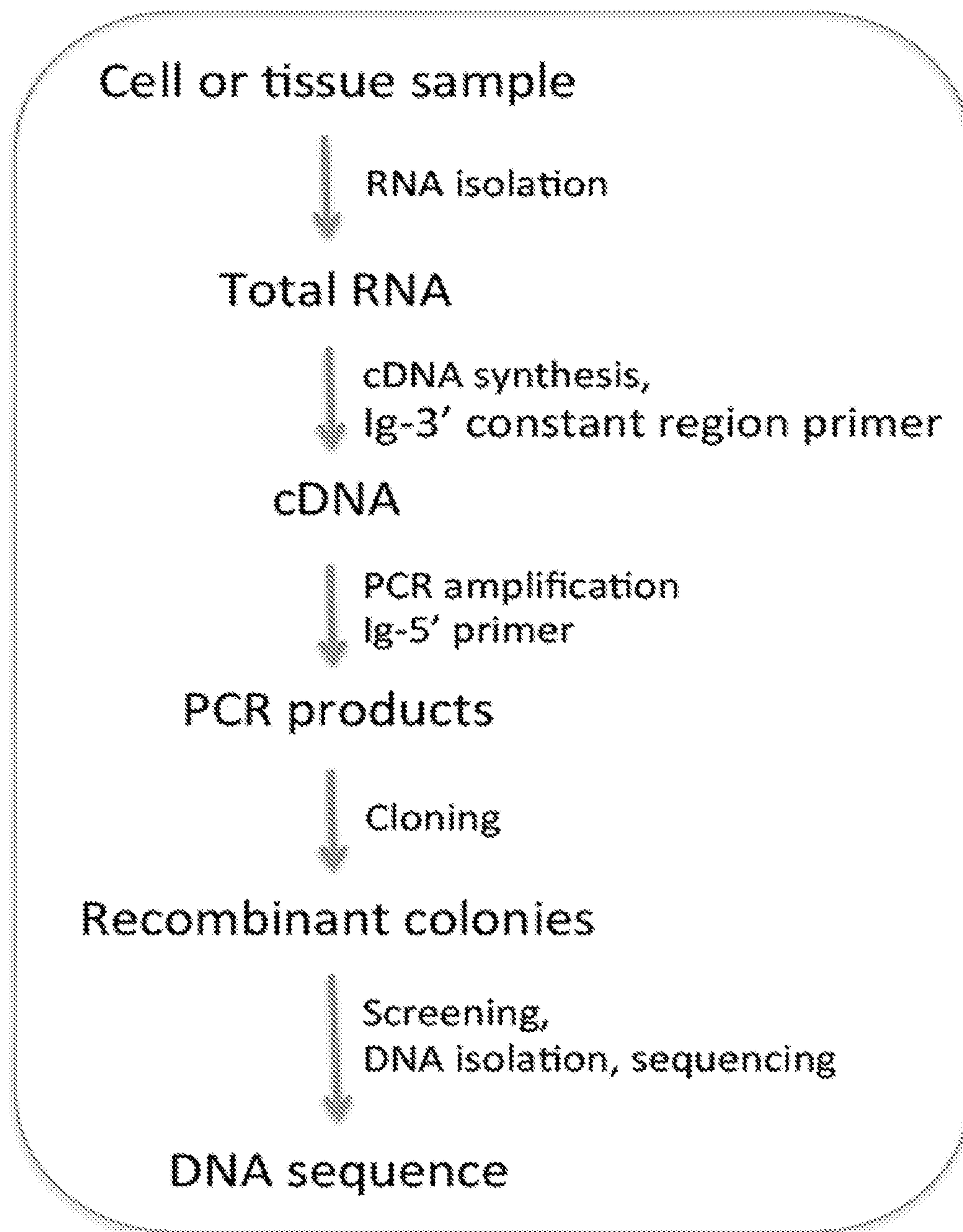
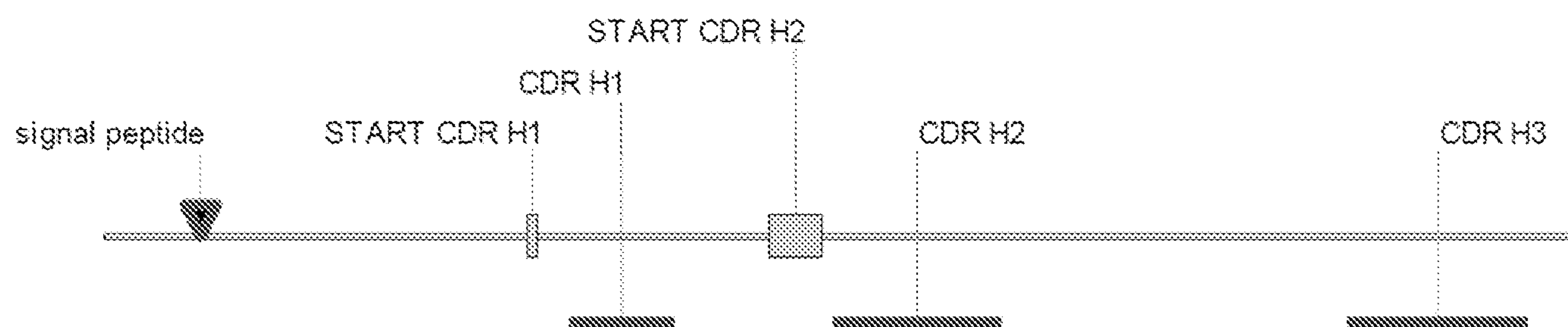


FIG. 12A

B.

1	MDSRNLVFL	VLILKSVQCD	VQLVESGGGL	VQPGGSRKLS	CAASEFTTSS
51	WVRQAP	EKGLEWVAY	ESGRTIAA	DTTTRFTIS	RDNPKNTLFL
101	QMKLPSLCYG	LLGSRNLSNP	LLSGNTPIR	LSIGENKLGI	

C.



FIGs. 12B-12C

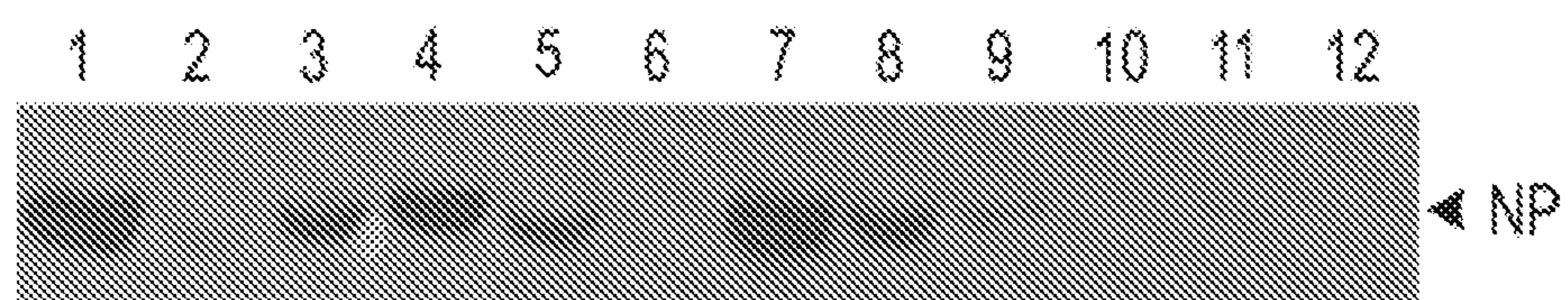
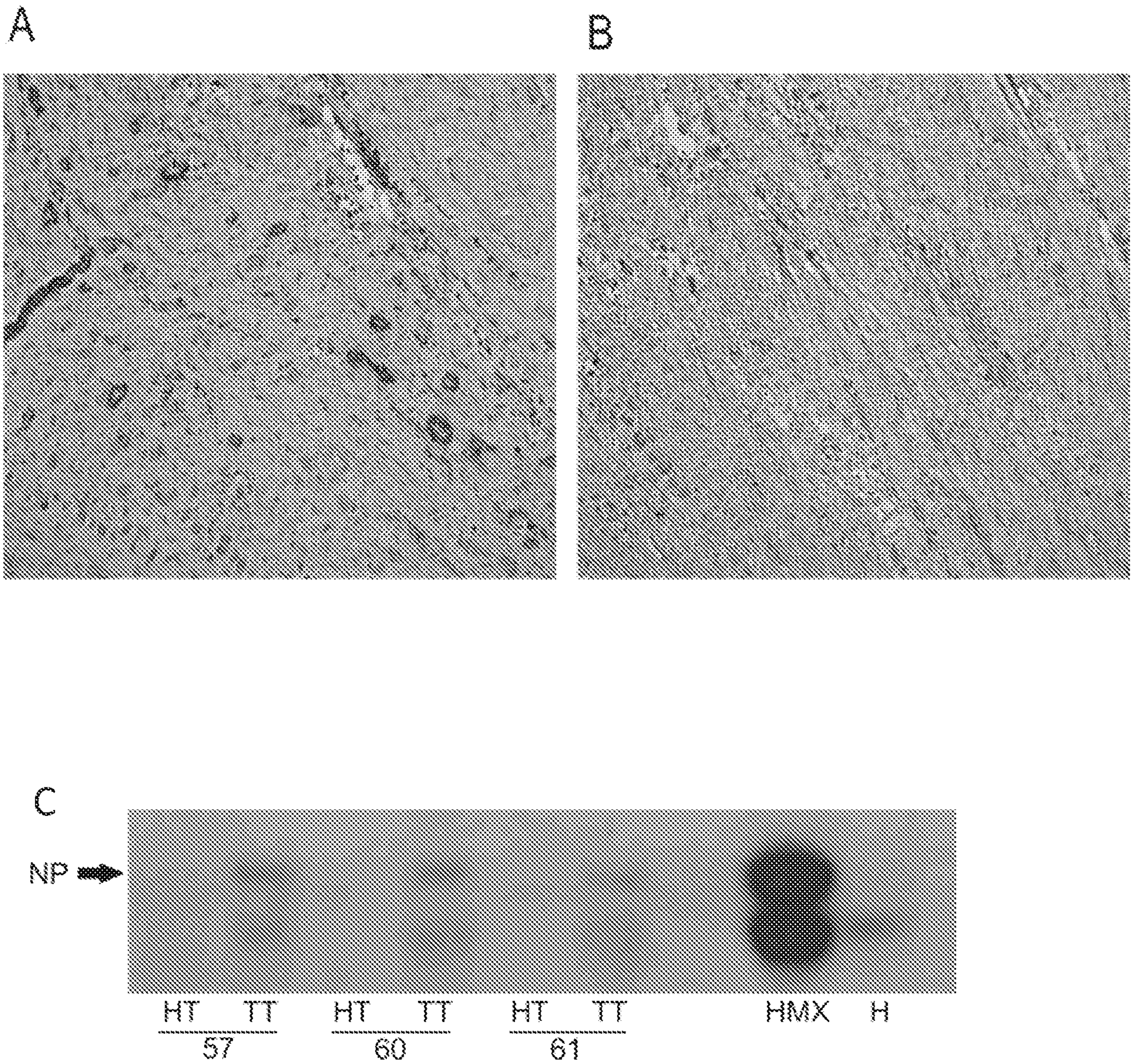
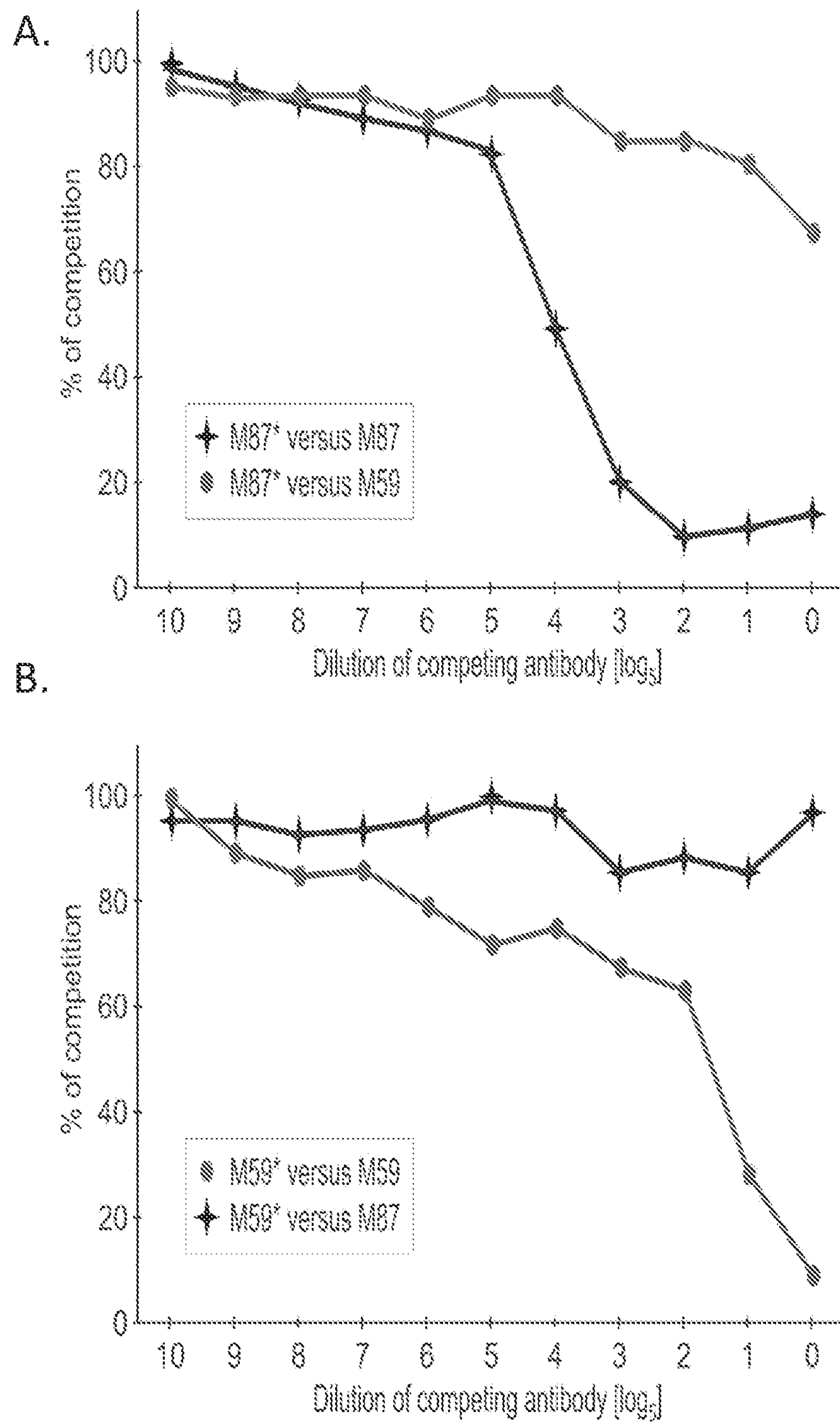


FIG. 13



FIGs. 14A-14C



FIGS. 15A and 15B

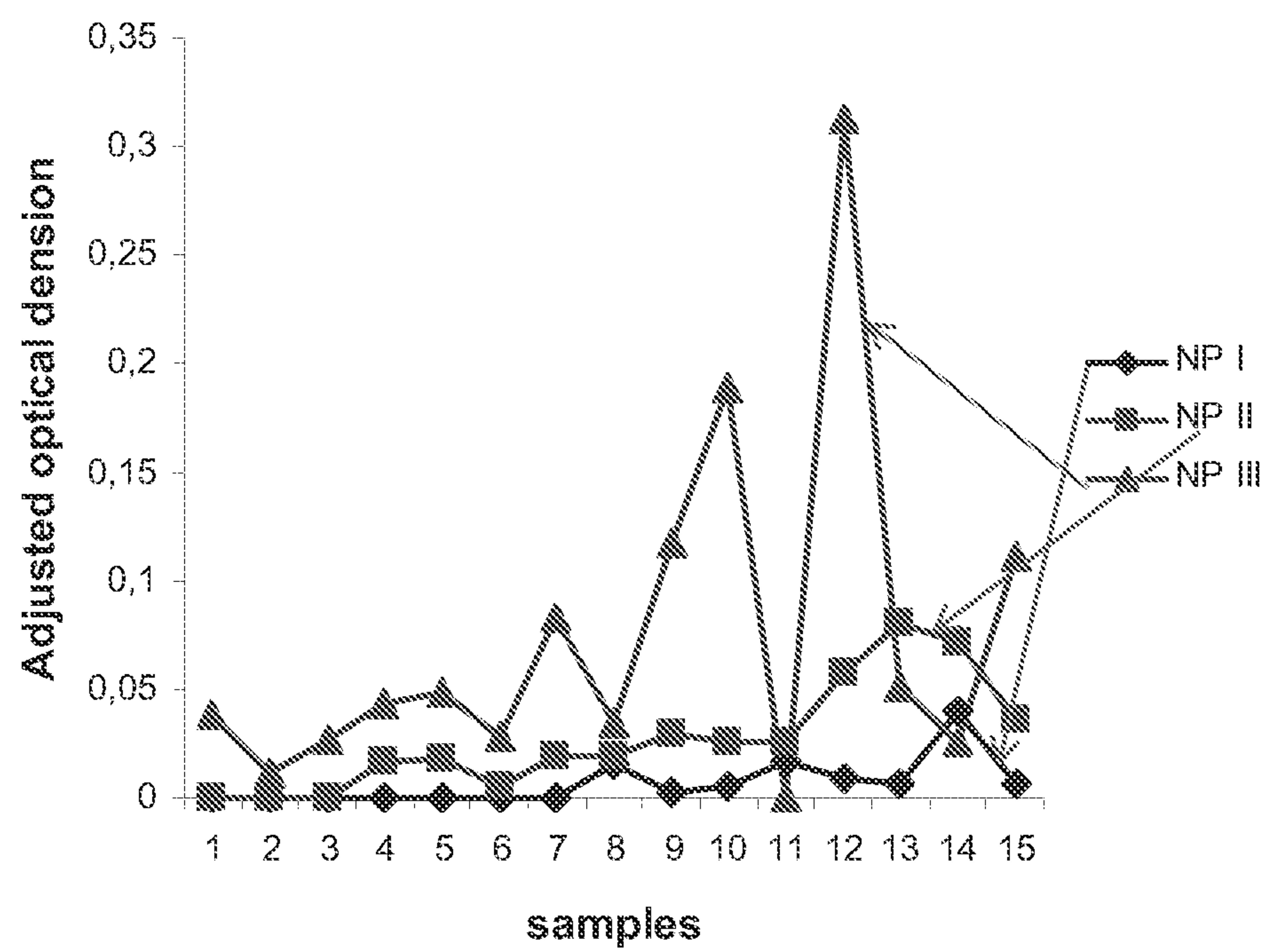


FIG. 16

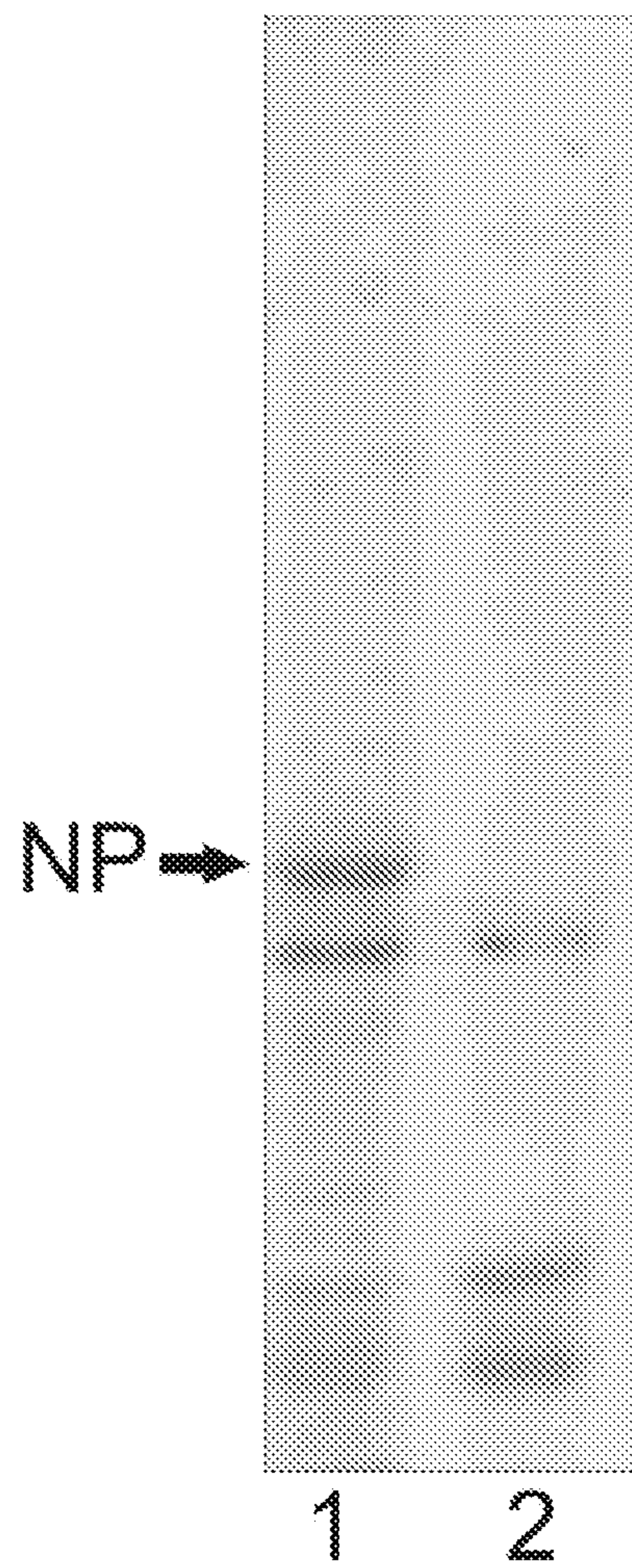
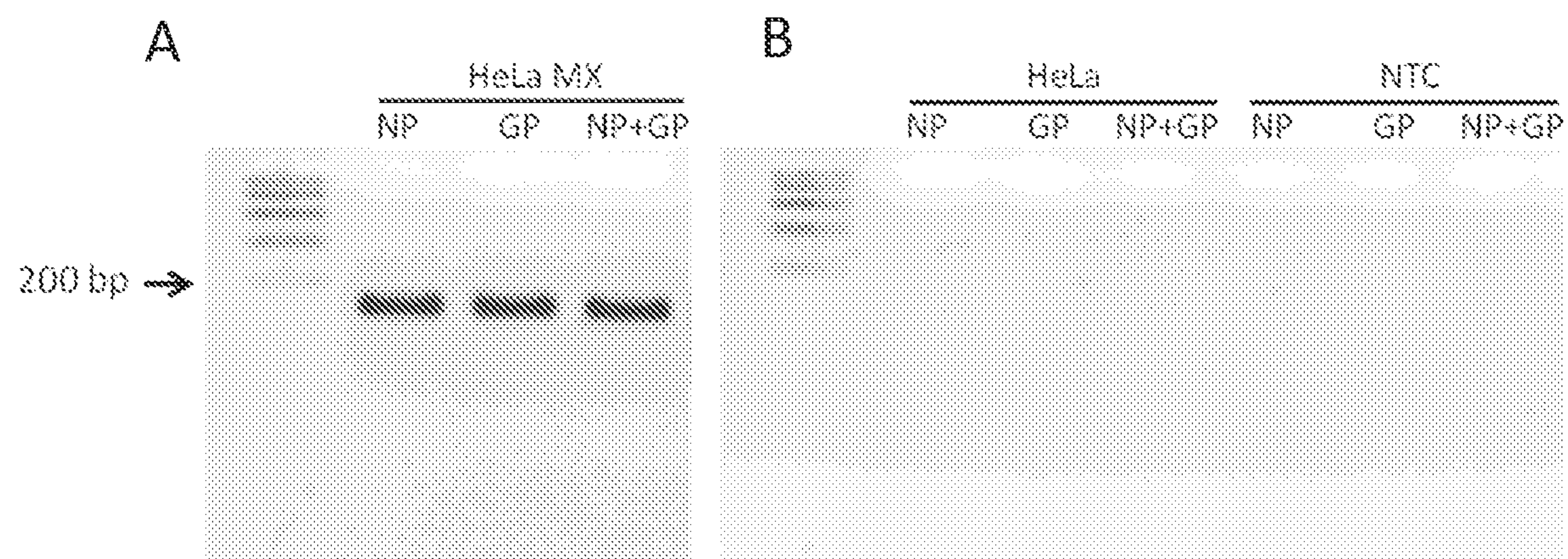
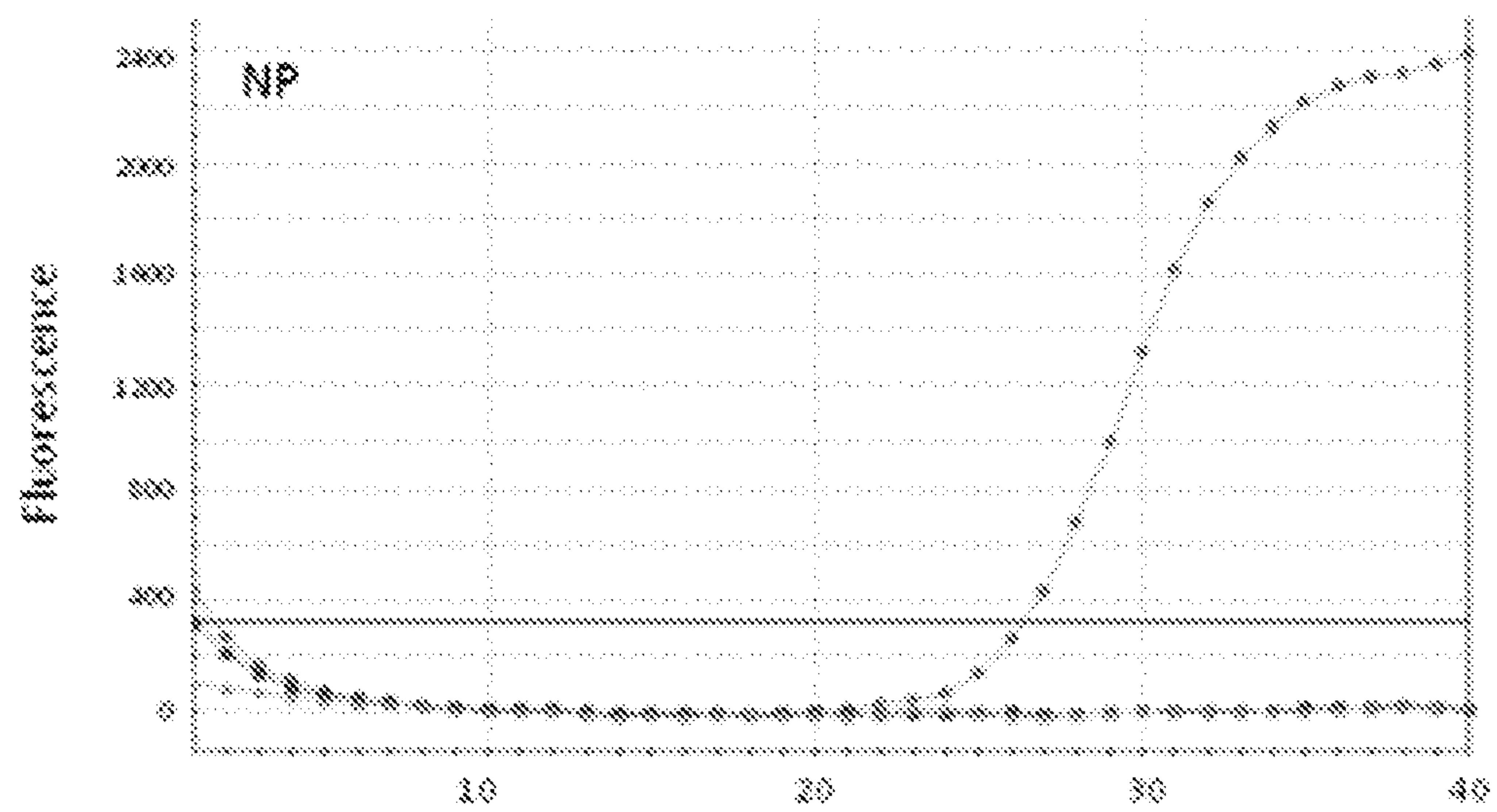


FIG. 17

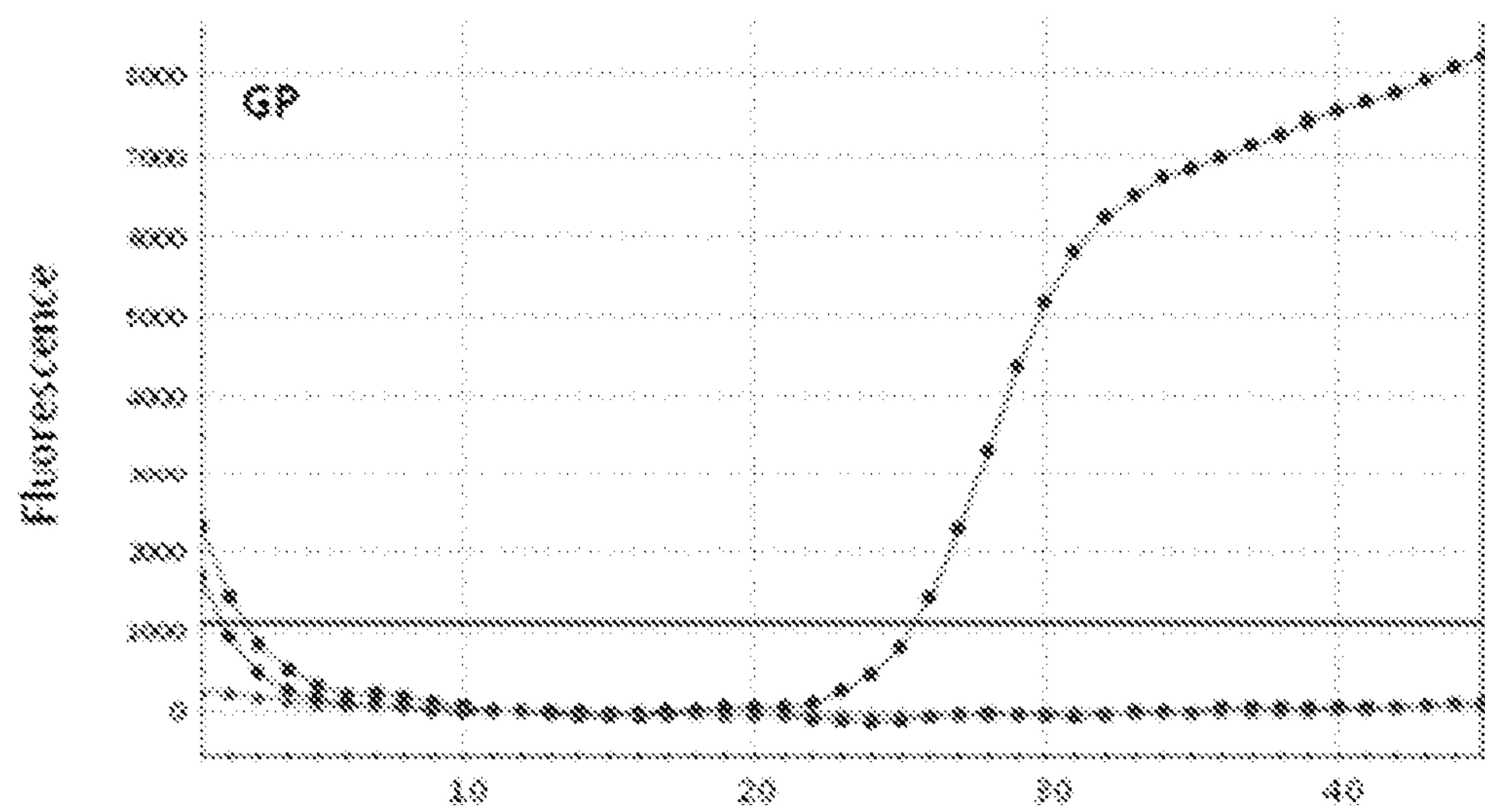


FIGs. 18A-18B

A



B



Thermal cycles

FIGs. 19A-19B

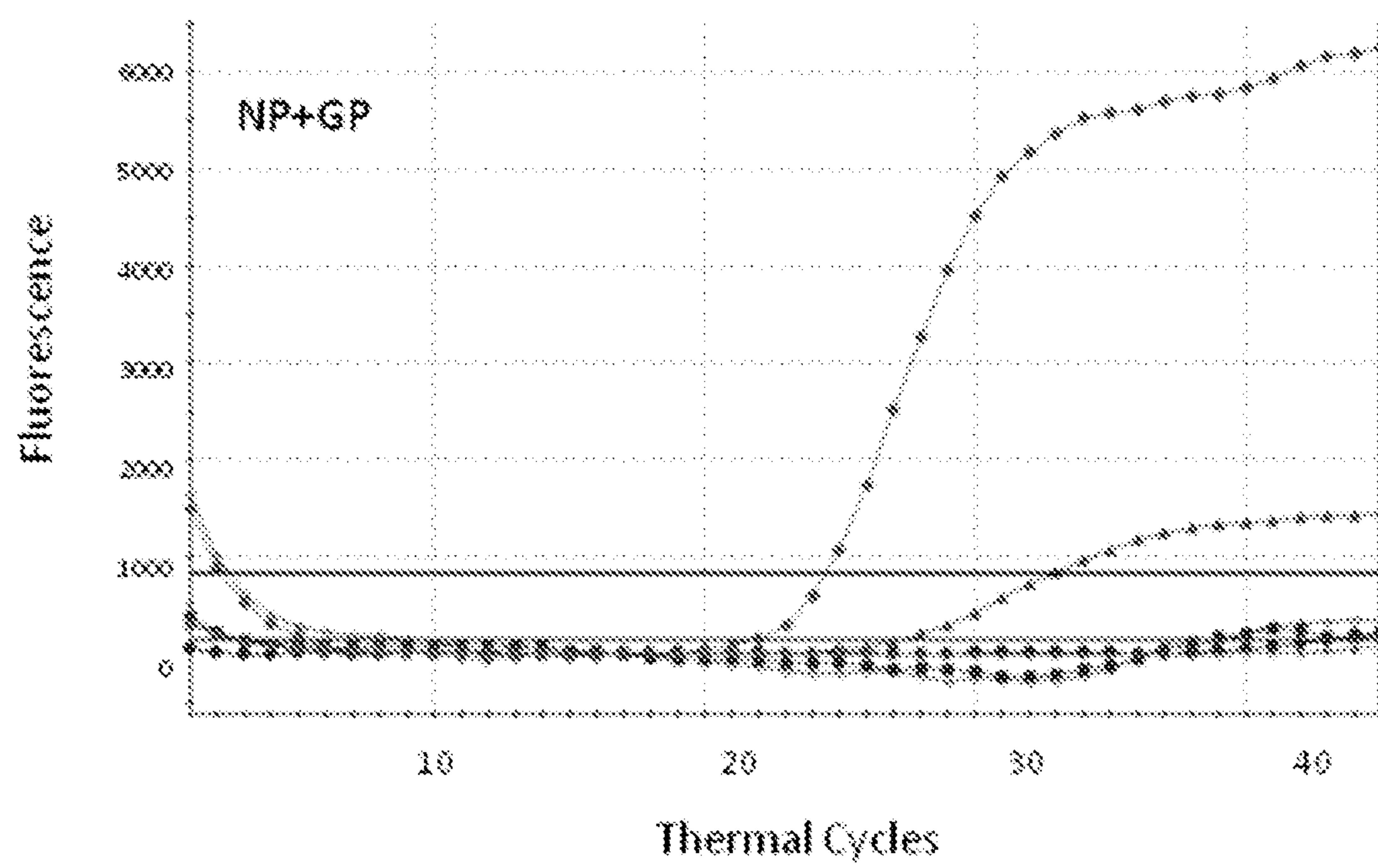
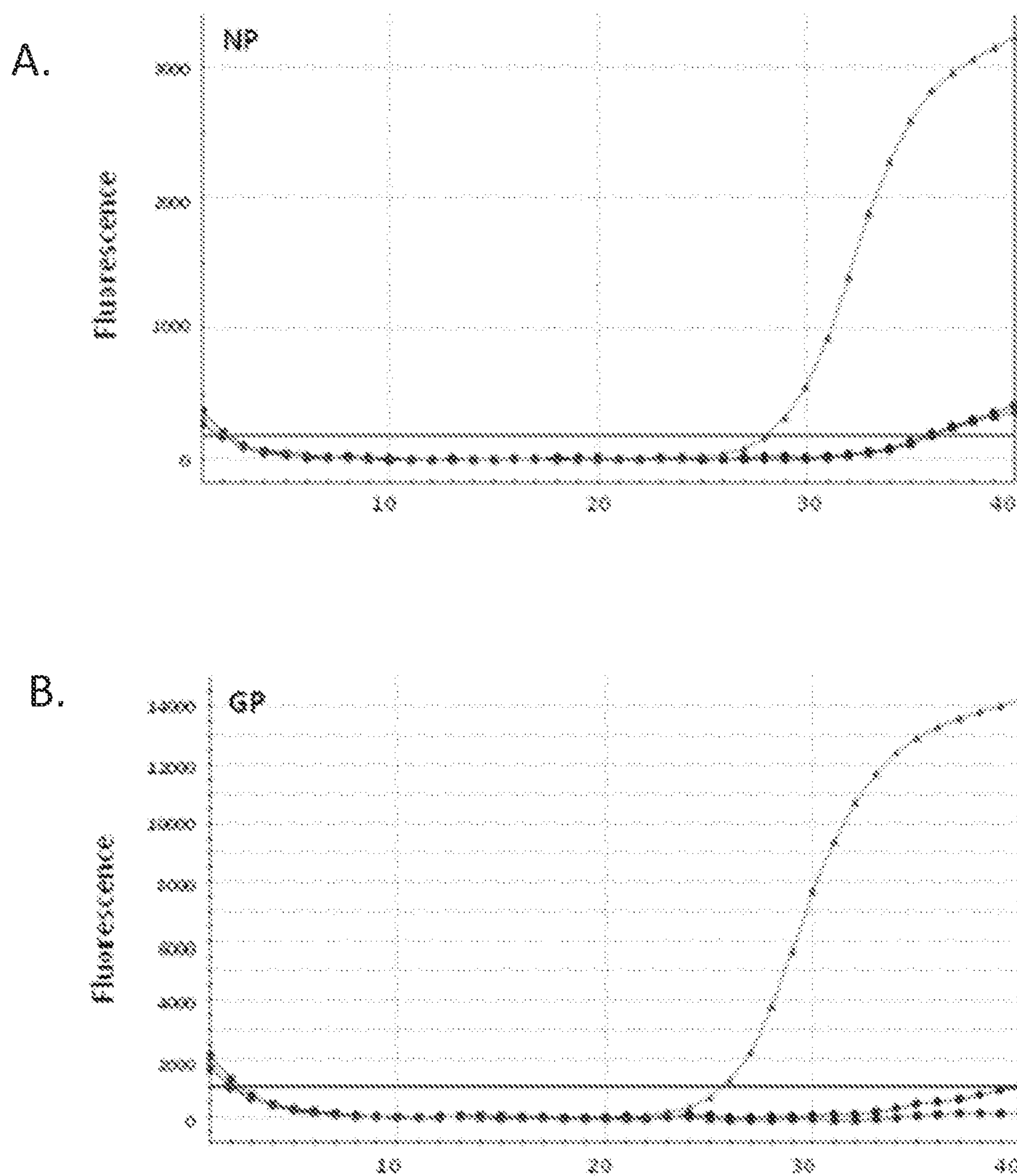


FIG. 20



FIGs. 21A-21B

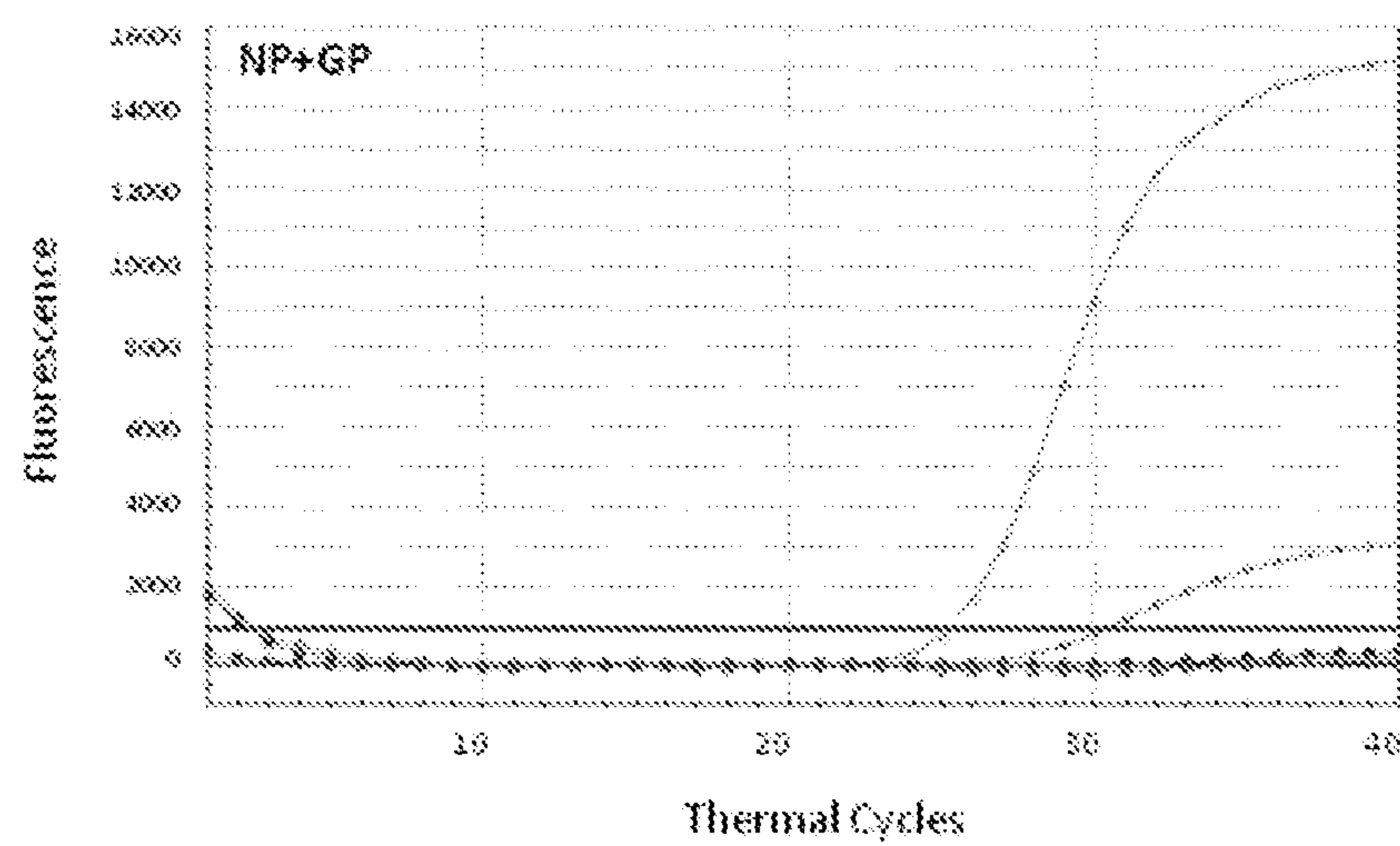
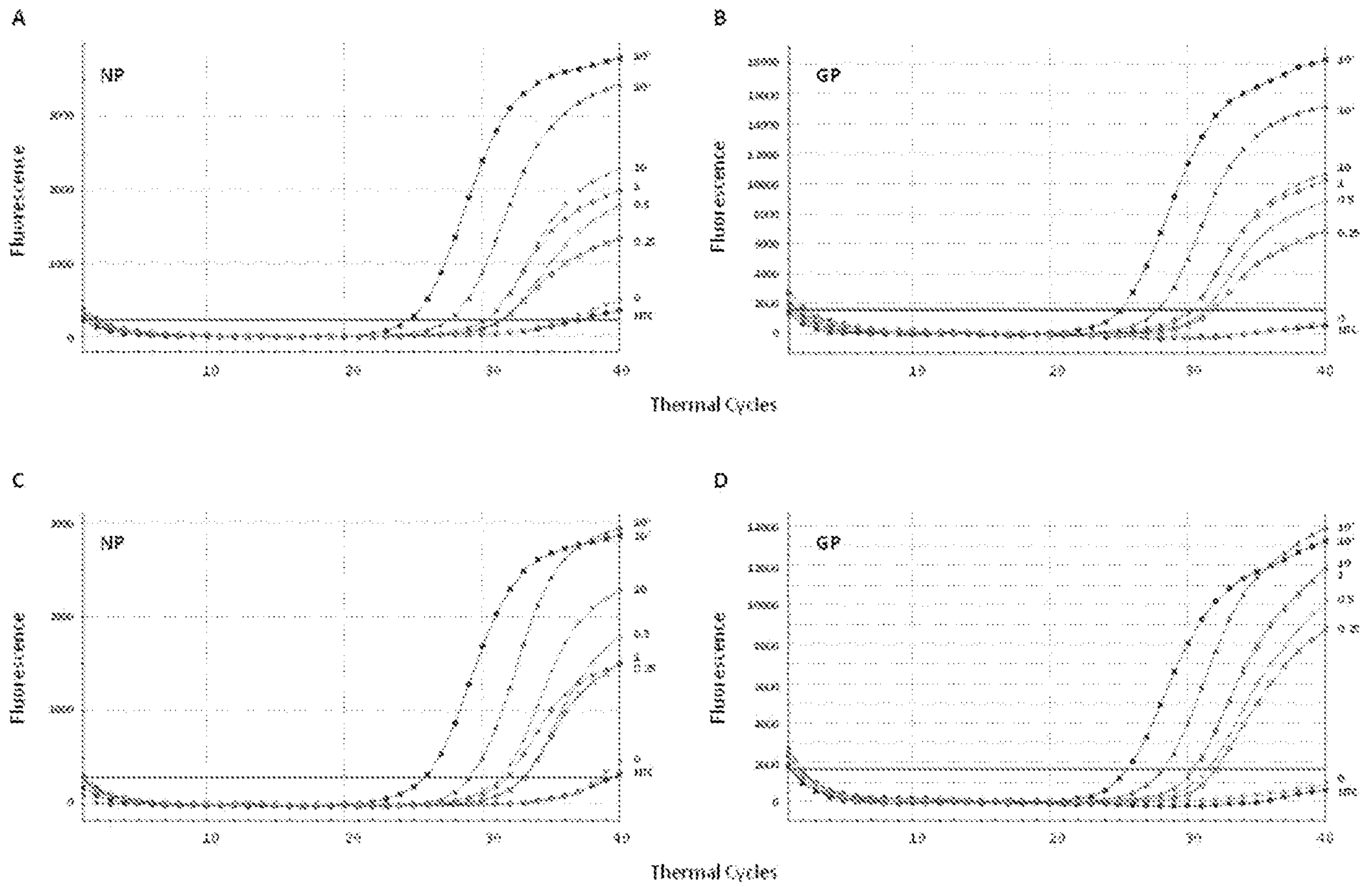


FIG. 22



FIGs. 23A-24D

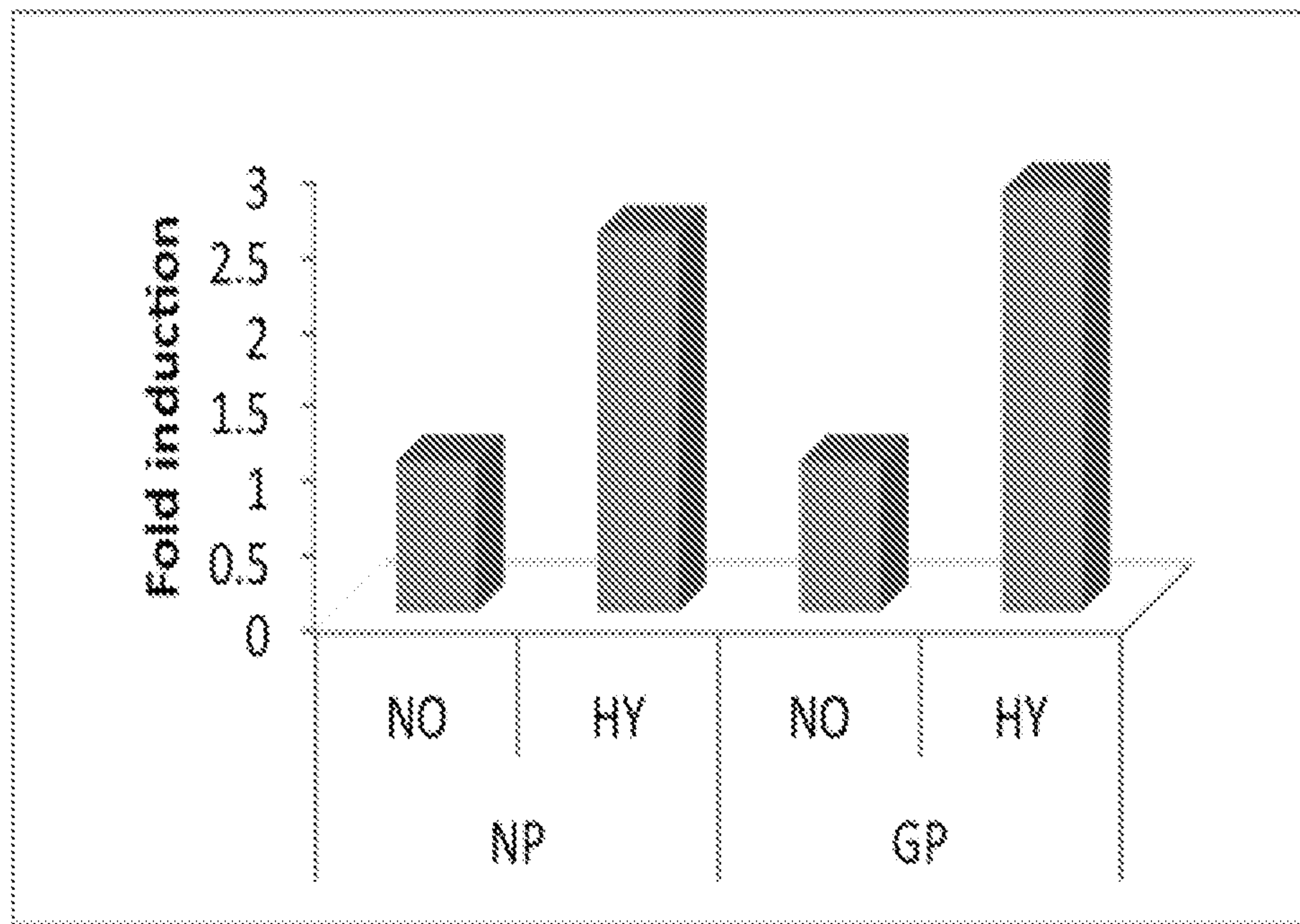
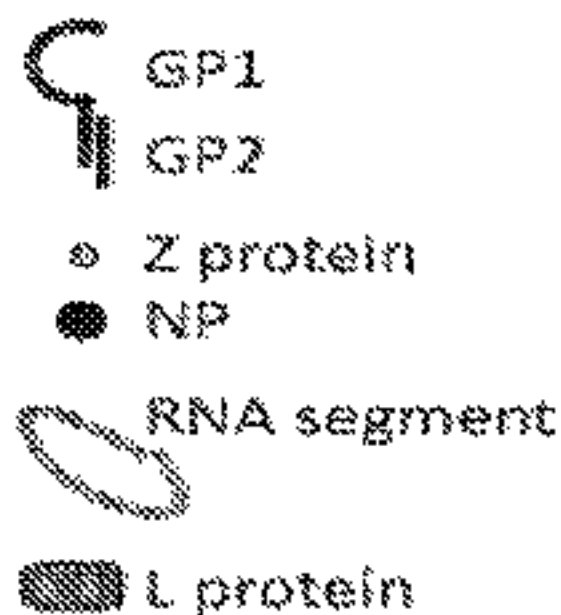
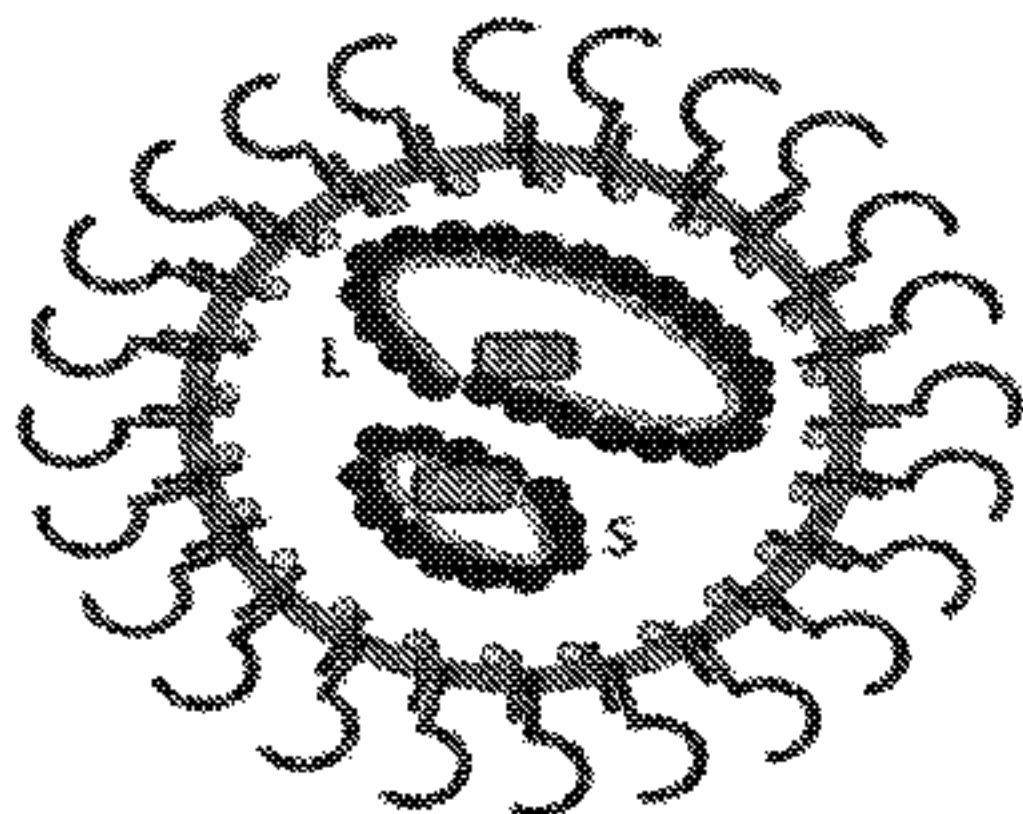
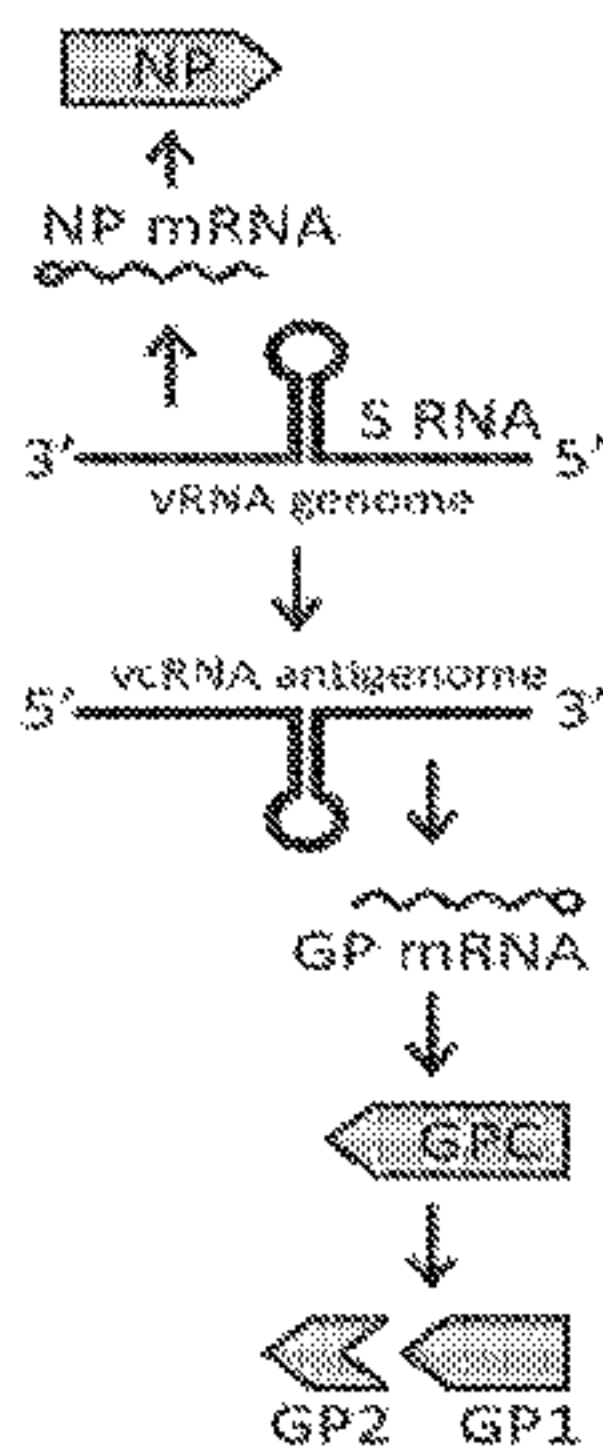
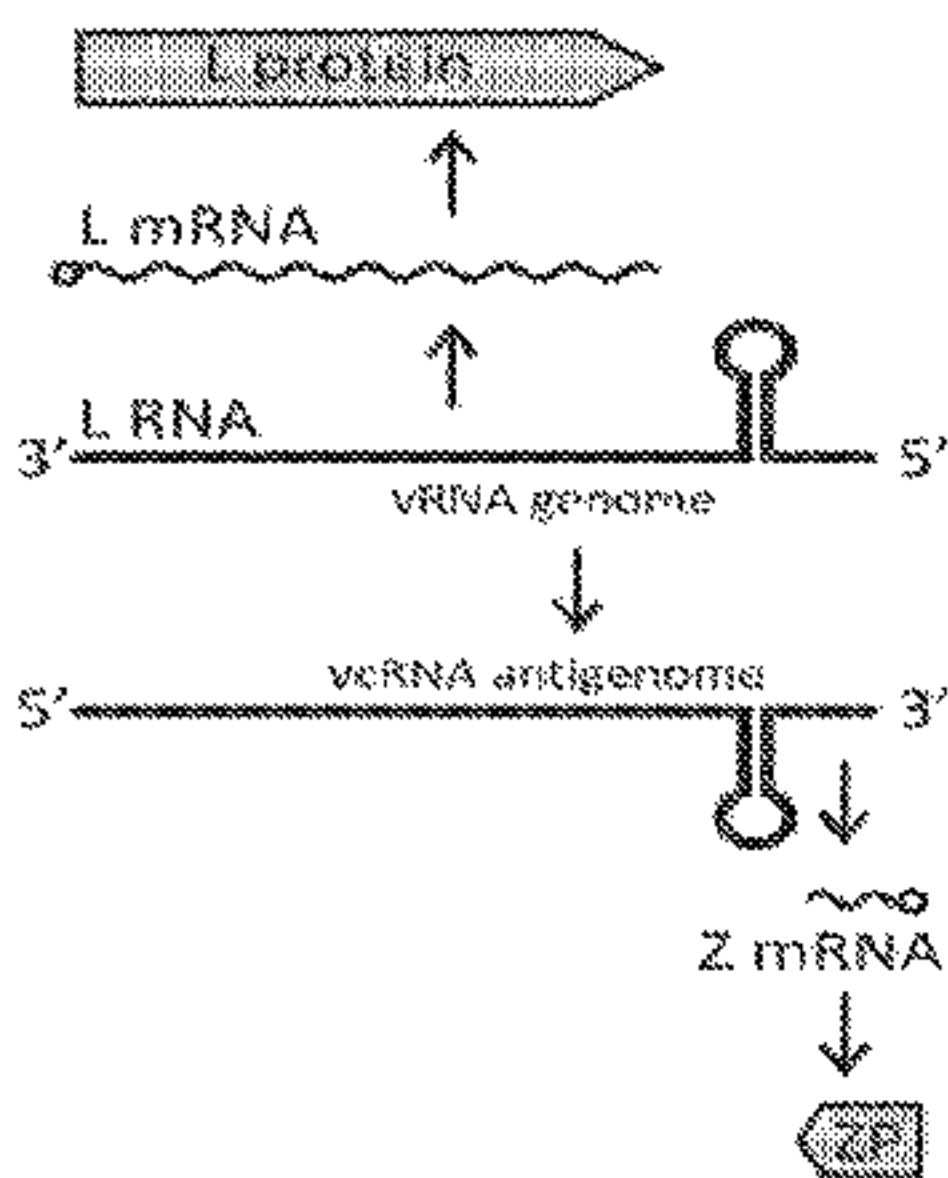


FIG. 24

A



B



FIGs. 1A-1B