FERROCENE LABELLED PRIMERS FOR ELECTROCHEMICAL DETECTION

FIELD OF THE INVENTION

The present invention relates generally to the fields of electrochemical detection technology and molecular diagnostics.

BACKGROUND OF THE INVENTION

Access to diagnostic tests is critical to help limit the spread of disease. Yet, during the COVID-19 pandemic, access to effective diagnostic tests was a significant problem especially during the initial outbreak in 2020. Accelerated development and scaled up manufacturing and delivery of COVID-19 tests was critical in the fight against COVID-19. There remains a need to quickly develop accurate, fast assays and manufacture those assays in order to get them quickly to market when additional COVID-19 variants and new infectious diseases arise.

SUMMARY

Provided herein are methods, devices, and systems for amplifying and detecting nucleic acids, such as DNA and/or RNA, using a signal primer for use in electrochemical detection systems. Amplifying and detecting nucleic acids (such as DNA and/or RNA) using a signal primer accelerates the development of detection assays, improves their accuracy, speeds up the time to result and simplifies manufacturing in order to ensure that diagnostic kits can be quickly developed and delivered to patients in need. Rapid development and distribution of diagnostic kits will help reduce the spread of disease.

In one example, a method of detecting the presence or absence of a target nucleic acid in a sample is provided, the method comprising (a) combining a solution comprising target nucleic acid or suspected to comprise target nucleic acid with amplification reagents to amplify the target nucleic acid if present, the amplification reagents comprising a labeled primer capable of hybridizing to the target nucleic acid; (b) amplify the target nucleic acid to produce a double stranded labeled amplicon if the target nucleic acid is present; (c) incubating the double stranded labeled amplicon with exonuclease to form a single stranded labeled amplicon; (d) hybridizing the single stranded labeled amplicon with a first capture probe; and (e) detecting the presence or absence of the target nucleic acid in the sample using electrochemical detection. In some examples, the labeled primer, double stranded labeled amplicon, and/or single stranded labeled amplicon comprise at least one label selected from the group consisting of ferrocene, methylene blue or osmium. In certain examples, the at least one label is ferrocene. In some examples, the labeled primer comprises a ferrocene label on the 5'-end, on any one nucleotide of nucleotides 1 to 3 at the 5'-

end, on the 3'-end, on any one nucleotide of nucleotides 1 to 3 at the 3'-end or on both, the 5'- and the 3'-end. In some examples, the labeled primer comprises a ferrocene label internal to the labeled primer. In some examples, the labeled primer, double stranded labeled amplicon, and/or single stranded labeled amplicon comprise a plurality of ferrocene labels at at least two locations. In some examples, the single stranded labeled amplicon comprises a first section capable of hybridizing to the first capture probe, a second section that is not capable of binding to the capture probe and a third section comprising the at least one label. In certain examples, the label is selected from the group consisting of ferrocene, methylene blue or osmium. In some examples, the labeled primer comprises a first section capable of hybridizing to the first capture probe, a second section comprising a linker and a third section comprising the at least one label, wherein the linker connects the first section and the third section. In some examples, the method further comprises separating the labeled primer and the single stranded labeled amplicon prior to electrochemical detection. In another example, the method further comprises separating the labeled primer and the double stranded labeled amplicon prior to electrochemical detection. In some examples, the labeled primer consists of a first section capable of hybridizing to the capture probe, and a second section comprising the at least first label. In some examples, the amplification reagents comprise nucleic acid polymerase, deoxynucleotide triphosphates (dNTPs), reaction buffer necessary for the function of the nucleic acid polymerase and a divalent cation (such as, e.g., Mg^{2+} and/or Mn^{2+}). In some examples, the target nucleic acid is a DNA. In some examples, the nucleic acid is an RNA and the method further comprises the step of reverse transcribing RNA to generate cDNA using a reverse transcriptase. In some examples, the nucleic acid polymerase is a DNA polymerase. In some examples, the nucleic acid polymerase further exhibits reverse transcriptase activity.

In one aspect, the present disclosure refers to a method of detecting the presence or absence of a target nucleic acid in a sample, the method comprising, a. combining a solution comprising target nucleic acid or suspected to comprise target nucleic acid with amplification reagents to amplify the target nucleic acid if present, the amplification reagents comprising a signal primer comprising an electrochemically detectable label and a nucleic acid sequence complementary to at least a portion of said target nucleic acid thereby being capable of hybridizing to the target nucleic acid; b. amplify the target nucleic acid to produce a double stranded labeled amplicon if the target nucleic acid is present; c. incubating the double stranded labeled amplicon with exonuclease to form a single stranded labeled amplicon; d. hybridizing the single stranded labeled amplicon with a first capture probe; and e. detecting the presence or absence of the target nucleic acid in the sample using electrochemical detection.

In one example, a method of detecting the presence or absence of a target nucleic acid in a sample, the method comprising (a) receiving a sample; (b) extracting nucleic acids from the sample, wherein the nucleic acids are suspected of comprising target nucleic acid; (c) combining the nucleic acid with amplification reagents to amplify the target nucleic acid if present, the amplification reagents comprising a signal primer capable of hybridizing to the target nucleic acid; (d) amplify the target nucleic acid to produce a double stranded signal amplicon if the target nucleic acid is present; (e) incubating the double stranded signal amplicon with exonuclease to form a single stranded signal amplicon; (f) hybridizing the single stranded signal amplicon with a first capture probe; and (g) detecting the presence or absence of the target nucleic acid in the sample using electrochemical detection. In some examples, the signal primer comprises an electrochemically detectable label. In some examples, the signal primer comprises at least one

label selected from the group consisting of ferrocene, methylene blue or osmium. In certain examples, the at least one label is ferrocene. Herein, the detectable label may act as a signaling moiety. In some examples, the signal primer does not bind/hybridize to the first capture probe. In some examples, a first portion of the single stranded signal amplicon binds/hybridizes to the first capture probe and a second portion of the single stranded signal amplicon binds/hybridizes to a second capture probe. In some examples, the labeled primer, double stranded labeled amplicon, and/or single stranded labeled amplicon comprise at least one label selected from the group consisting of ferrocene, methylene blue or osmium. In certain examples, the at least one label is ferrocene. In some examples, the labeled primer comprises a ferrocene label on the 5'-end, on any one nucleotide of nucleotides 1 to 3 at the 5'-end, on the 3'-end, on any one nucleotide of nucleotides 1 to 3 at the 3'-end or on both, the 5'- and the 3'-end. In some examples, the labeled primer comprises a ferrocene label internal to the labeled primer. In some examples, the labeled primer, double stranded labeled amplicon, and/or single stranded labeled amplicon comprise a plurality of ferrocene labels at at least two locations. In some examples, the single stranded labeled amplicon comprises a first section capable of hybridizing to the first capture probe, a second section that is not capable of binding to the capture probe and a third section comprising the at least one label. In certain examples, the label is selected from the group consisting of ferrocene, methylene blue or osmium. In some examples, the labeled primer comprises a first section capable of hybridizing to the first capture probe, a second section comprising a linker and a third section comprising the at least one label, wherein the linker connects the first section and the third section. In some examples, the method further comprises separating the labeled primer and the single stranded labeled amplicon prior to electrochemical detection. In another example, the method further comprises separating the labeled primer and the double stranded labeled amplicon prior to electrochemical detection. In some examples, the labeled primer consists of a first section capable of hybridizing to the capture probe, and a second section comprising the at least first label. In some examples, the amplification reagents comprise nucleic acid polymerase, deoxynucleotide triphosphates (dNTPs), reaction buffer necessary for the function of the nucleic acid polymerase and a divalent cation (such as, e.g., Mg^{2+} and/or Mn^{2+}). In some examples, the target nucleic acid is a DNA. In some examples, the nucleic acid is an RNA and the method further comprises the step of reverse transcribing RNA to generate cDNA using a reverse transcriptase. In some examples, the nucleic acid polymerase is a DNA polymerase. In some examples, the nucleic acid polymerase further exhibits reverse transcriptase activity.

In one aspect, the present disclosure refers to a method of detecting the presence or absence of a target nucleic acid in a sample, the method comprising, a. receiving a sample; b. extracting nucleic acids from the sample, wherein the nucleic acids are suspected of comprising target nucleic acid; c. combining the nucleic acid with amplification reagents to amplify the target nucleic acid if present, the amplification reagents comprising a signal primer comprising an electrochemically detectable label and a nucleic acid sequence complementary to at least a portion of said target nucleic acid thereby being capable of hybridizing to the target nucleic acid; d. amplify the target nucleic acid to produce a double stranded signal amplicon if the target nucleic acid is present; e. incubating the double stranded signal amplicon with exonuclease to form a single stranded signal amplicon; hybridizing the single stranded signal amplicon with a first capture probe; and g. detecting the presence or absence of the target nucleic acid in the sample using electrochemical detection.

In one example, a process for detecting the presence of a single-stranded or double-stranded

nucleic acid of interest in a sample, said process comprising the steps of (a) providing (i) a sample suspected of containing said nucleic acid of interest, (ii) a nucleic acid primer that comprises a nucleic acid sequence complementary to at least a portion of said nucleic acid of interest, and a first electrochemically detectable label and (iii) reagents for carrying out nucleic acid strand extension; (b) forming a reaction mixture comprising (i), (ii), and (iii) above; (c) contacting under hybridization conditions the nucleic acid primer with the nucleic acid of interest if present; (d) extending the nucleic acid primer, thereby incorporating the electrochemically detectable label into an amplicon to form an electrochemically labeled amplicon if said nucleic acid of interest is present; (e) denaturing the electrochemically labeled amplicon; (f) hybridizing the electrochemically labeled amplicon with a capture probe bound to an electrode surface; (g) detecting the presence of the nucleic acid of interest by detecting energy transfer between the electrochemically labeled amplicon and the electrode surface. In some examples, the electrochemically detectable label is selected from the group consisting of ferrocene, methylene blue or osmium. In certain examples, the at least one label is ferrocene. Herein, the detectable label may act as a signaling moiety. In some examples, the nucleic acid primer comprises a first portion that binds/hybridizes to the capture probe and a second portion that does not bind/hybridize to the capture probe. In some examples, the nucleic acid primer further comprises a second electrochemically detectable label and the first electrochemically detectable label and the second electrochemically detectable label are different. In some examples, the nucleic acid primer is a signal primer that does not bind/hybridize to the first capture probe. In some examples, a first portion of the single stranded signal amplicon binds/hybridizes to the first capture probe and a second portion of the single stranded signal amplicon binds/hybridizes to a second capture probe. In some examples, the nucleic acid primer, double stranded labeled amplicon, and/or single stranded labeled amplicon comprise at least one label selected from the group consisting of ferrocene, methylene blue or osmium. In certain examples, the at least one label is ferrocene. In some examples, the nucleic acid primer comprises a ferrocene label on the 5’-end, on any one nucleotide of nucleotides 1 to 3 at the 5’-end, on the 3’-end, on any one nucleotide of nucleotides 1 to 3 at the 3’-end or on both, the 5’- and the 3’-end. In some examples, the nucleic acid primer comprises a ferrocene label internal to the labeled primer. In some examples, the nucleic acid primer, double stranded labeled amplicon, and/or single stranded labeled amplicon comprise a plurality of ferrocene labels at at least two locations. In some examples, the single stranded labeled amplicon comprises a first section capable of hybridizing to the first capture probe, a second section that is not capable of binding to the capture probe and a third section comprising the at least one label. In certain examples, the label is selected from

the group consisting of ferrocene, methylene blue or osmium. In some examples, the nucleic acid primer comprises a first section capable of hybridizing to the first capture probe, a second section comprising a linker and a third section comprising the at least one label, wherein the linker connects the first section and the third section. In some examples, the process further comprises separating the nucleic acid primer and the single stranded labeled amplicon prior to electrochemical detection. In another example, the process further comprises separating the nucleic acid primer and the double stranded labeled amplicon prior to electrochemical detection. In some examples, the nucleic acid primer consists of a first section capable of hybridizing to the capture probe, and a second section comprising the at least first label. In some examples, the reagents for carrying out nucleic acid strand extension comprise nucleic acid polymerase, deoxynucleotide triphosphates (dNTPs), reaction buffer necessary for the function of the nucleic acid polymerase and a divalent cation (such as, e.g., Mg^{2+} and/or Mn^{2+}). In some examples, the target nucleic acid is a DNA. In some examples, the nucleic acid is an RNA and the method further comprises the step of reverse transcribing RNA to generate cDNA using a reverse transcriptase. In some examples, the nucleic acid polymerase is a DNA polymerase. In some examples, the nucleic acid polymerase further exhibits reverse transcriptase activity.

In one aspect, the present disclosure refers to a method for detecting the presence of a single-stranded or double-stranded target nucleic acid of interest in a sample, said method comprising the steps of: a. providing i. a sample suspected of containing said target nucleic acid of interest; ii. a signal primer that comprises: 1. a nucleic acid sequence complementary to at least a portion of said target nucleic acid of interest, and 2. a first electrochemically detectable label; iii. reagents for carrying out nucleic acid strand extension; b. forming a reaction mixture comprising (i), (ii), and (iii) above; c. contacting under hybridization conditions the signal primer with the target nucleic acid of interest if present; d. extending the signal primer, thereby incorporating the electrochemically detectable label into an amplicon to form an electrochemically labeled amplicon if said target nucleic acid of interest is present; e. denaturing the electrochemically labeled amplicon; f. hybridizing the electrochemically labeled amplicon with a capture probe bound to an electrode surface; g. detecting the presence of the target nucleic acid of interest by detecting energy transfer between the electrochemically labeled amplicon and the electrode surface.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1a shows a typical sandwich assay used in electrochemical detection. In FIG. 1a, the sandwich comprises three main elements, the capture probe (**2**), the signal probe (**3**) and the target (**4**). The target can be synonymous with a PCR amplicon sequence in nucleic acid embodiments. The target can have a portion (**4b**) which binds or hybridizes specifically to a desired portion of the signal probe (**3b**), a portion (**4a**) which binds or hybridizes to a corresponding capture probe portion (**2b**) and optionally one or more flanking portions, *e.g.*, (**4c**). The capture probe can include a linker (**2a**) that links, joins or binds the capture probe (**2**) to the electrode surface (**1**). As pictured, the signal probe has a detectable-labeled portion or labels (**3a**) that are in close proximity to the electrode surface. The labeled portion **3a** is conjugated or internal to the signal probe-binding portion (**3b**). Not pictured is a self-assembled monolayer (SAM), which is also attached to the electrode surface in some variants via one or more linkers in similar format to the capture probe linkers (**2a**), and which serves to prevent or lessen undesired electron transfer events (“noise”) to the electrode surface.

FIG. 1b shows a typical signal complex as disclosed herein. In FIG. 1b, the assay comprises just two main elements, the capture probe (**2**), and the target (**4**). The target can be synonymous with a PCR amplicon sequence in nucleic acid embodiments. The target can have a portion (**4a**), which