DNA IN THE PRESENCE OF GELLAN

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 10/718,488, filed Nov. 20, 2003; which application claims the benefit of U.S. Provisional Patent Application No. 60/427,987 filed Nov. 20, 2002; which applications are incorporated herein by reference in their entireties.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 850136_422D1_SEQUENCE_LISTING.txt. The text file is 1 KB, was created on Oct. 23, 2009 and is being submitted electronically via EFS-Web.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is related to the copying or amplification of nucleic acid molecules in the presence of gellan gum.

2. Description of the Related Art

The PCR method of DNA amplification is widely used in research, forensic investigations, and medical diagnostics. PCR will amplify even a few molecules of a target DNA in a sample to the level of 10^7 to 10^8 molecules within a short period (1-2 hours). PCR is one of a few technologies that will detect or amplify relatively few target nucleic acid molecules. There is great interest in enhancing this ability of PCR reactions to amplify a few target nucleic acid sequences, or its "sensitivity". Many such techniques to optimize PCR reactions are described, for example, in Section 15, Vol. 3, "Current Protocols in Molecular Biology" (Edit. Ausubel, F. M., et al), John Wiley & Sons).

For several applications of nucleic acid amplification, it is desirable to carry out the amplification within, or associated with, a gel matrix. Many types of gel material suitable for use as electrophoresis medium have been the subject of intense research as the gel is often the determining factor for a successful separation. The gels may be composed of natural materials, e.g., agarose or synthetic polymers, e.g., polyacrylamide.

When the PCR ingredients are polymerized within a polyacrylamide gel, the resulting PCR amplifications are inconsistent, perhaps due to damage to enzymes and/or nucleic acids by the free radicals necessary to polymerize the acylamide (*BioTechniques* 33(1), 150-156, 2002; Chetverina, H. V. et al. "Molecular Colony Diagnostics: detection and quantification of viral nucleic acids by in-gel PCR."; and *Nucleic Acid Research,* 27(24), e34, 1999; R. D. Mitra and G. M. Church. "In situ localized amplification and contact replication of many individual DNA molecules.").

Agarose inhibits PCR amplification when the agarose concentration is above 0.15%. (*Biotechiques* 33(2), 282-283, 2002. Yamaguchi, Y, et al "Inhibitory effects of Agarose Gel and LB medium on DNA sequencing."). Agar contains uncharacterized inhibitory components that block PCR even when present in only trace amounts in DNA purified from cells growing on the surface of agar plates. (*Journal of Clinical Microbiology* 36, 275-276, 1998 "Inhibition of PCR by agar from bacteriological transport media").

Therefore, there exists a need in the art for methods of enhanced PCR sensitivity so as to be able to amplify from samples that contain low levels of nucleic acid molecules.

BRIEF SUMMARY OF THE INVENTION

The present invention provides methods and compositions for nucleic acid amplification in the presence of gellan. The gellan may be intact or digested into small molecules.

In one aspect, the present invention provides a method for enzymatically amplifying a target nucleic acid or a fragment thereof that comprises (a) providing a nucleic acid amplification reaction mixture having a water-based liquid phase and a gellan gel matrix phase, wherein the liquid phase comprises a target nucleic acid and is entrapped in the gel matrix phase; and (b) subjecting the reaction mixture to conditions suitable for amplifying the target nucleic acid or a fragment thereof, whereby the target nucleic acid or the fragment thereof is amplified. In certain embodiments, the sample undergoing enzymatic amplification contains at least 200, 220, 240, 260, 280, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, or 2000 molecules of target nucleic acids. The nucleic acid amplification may be performed using PCR, LCR, TAS, NASBA, 3SR, RACE, one-sided PCR or the like. In some embodiments, the present method may further comprise the step of isolating a target nucleic acid from cells (e.g., bacterial cells) grown on gellan-containing medium.

The present invention provides methods for the amplification of nucleic acid in both purified and contaminated gellan. In one preferred embodiment, the gellan is purified using a method comprising the steps of (a) combining gellan and a DNase, where the gellan contaminated with nucleic acid, thereby providing a mixture; and (b) maintaining the mixture of (a) under conditions where the DNase degrades at least some of the nucleic acid, thereby providing purified gellan.

In another aspect, the present invention provides a method for enzymatically amplifying a target nucleic acid or a fragment thereof, comprising (a) providing a nucleic acid amplification reaction mixture that comprises a target nucleic acid, and gellan at a concentration at least or above 0.001 wt % based on the weight of water; and (b) subjecting the reaction mixture to conditions suitable for amplifying the target nucleic acid or a fragment thereof, whereby the target nucleic acid or the fragment thereof is amplified. In certain embodiments, the amplification reaction mixture comprises gellan at a concentration at least, or above, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.011, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.25, 0.3, 0.35, or 0.4 wt % based on the weight of water.

The present application also provides composition suitable for use in nucleic acid amplification comprising gellan at a concentration at least or above 0.001 wt % based on the weight of water, DNA polymerase, dNTPs, a target nucleic acid, and water. In certain embodiments, the present composition comprises gellan at a concentration at least, or above, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.011, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.25, 0.3, 0.35, or 0.4 wt % based on the weight of water.