

chondrial DNA, messenger RNA and transfer RNA. Nucleic acids can also be foreign to the host and contaminate a cell as an infectious agent, e.g. bacteria, viruses, fungi or single celled organisms and infecting multicellular organisms (parasites). Recently, detection and analysis of the presence of nucleic acids has become important for the identification of single nucleotide polymorphisms (SNPs), chromosomal rearrangements and the insertion of foreign genes. These include infectious viruses, e.g. HIV and other retroviruses, jumping genes, e.g. transposons, and the identification of nucleic acids from recombinantly engineered organisms containing foreign genes, e.g. Roundup Ready™ plants.

**[0008]** The analysis of nucleic acids has a wide array of uses. For example, the presence of a foreign agent can be used as a medical diagnostic tool. The identification of the genetic makeup of cancerous tissues can also be used as a medical diagnostic tool, confirming that a tissue is cancerous, and determining the aggressive nature of the cancerous tissue. Chromosomal rearrangements, SNPs and abnormal variations in gene expression can be used as a medical diagnostic for particular disease states. Further, genetic information can be used to ascertain the effectiveness of particular pharmaceutical drugs, known as the field of pharmacogenomics. Genetic variations between humans and between domestic animals can also be ascertained by DNA analysis. This is used in fields including forensics, paternity testing and animal husbandry.

**[0009]** Methods of extracting nucleic acids from cells are well known to those skilled in the art. A cell wall can be weakened by a variety of methods, permitting the nucleic acids to extrude from the cell and permitting its further purification and analysis. The specific method of nucleic acid extraction is dependent on the type of nucleic acid to be isolated, the type of cell, and the specific application used to analyze the nucleic acid. Many methods of isolating DNA are known to those skilled in the art, see for example the general reference Sambrook and Russell, 2001, "Molecular Cloning: A Laboratory Manual". For example, the prior art contains examples of chemically-impregnated and dehydrated solid-substrates for the extraction and isolation of DNA from bodily fluids that employ lytic salts and detergents and which contain additional reagents for long-term storage of DNA samples e.g. U.S. Pat. No. 5,807,527 detailing FTA paper and U.S. Pat. No. 6,168,922 detailing Isocard Paper. The prior art also contains examples of particle separation methods, e.g. U.S. RE 37,891.

**[0010]** Methods of isolating RNA, particularly messenger RNA (mRNA) are well known to those skilled in the art. Typically, cell disruption is performed in the presence of strong protein denaturing solutions, which inactivate RNAses during the RNA isolation procedure. RNA is then isolated using differential ethanol precipitation with centrifugation. As is well known, RNA is extremely labile and is sensitive to alkaline conditions, as well as RNAses, which degrade RNA. RNAses are ubiquitous within the environment and it has been found that they are difficult to remove from solutions and containers used to isolate RNA.

**[0011]** Methods and Apparatuses for Amplification of Nucleic Acid

**[0012]** Polymerase Chain Reaction (PCR) is inhibited by a number of proteins and other contaminants that follow through during the standard methods of purification of genomic DNA from a number of types of tissue samples. It is known that additional steps of organic extraction with phenol,

chloroform and ether or column chromatography or gradient CsCl ultracentrifugation can be performed to remove PCR inhibitors in genomic DNA samples from blood. However, these steps add time, complexity and cost. This complexity limits incorporation into a simple disposable cartridge useful for nucleic acid analysis. Therefore, the development of new simple methods to overcome inhibitors found in nucleic acid samples used for nucleic acid amplification processes is desirable.

**[0013]** Nucleic acid hybridization is used to detect discernible characteristics about target nucleic acid molecules. Techniques like the "Southern analysis" are well known to those skilled in the art. Target nucleic acids are electrophoretically separated then bound to a membrane. Labeled probe molecules are then permitted to hybridize to the nucleic acids bound to the membrane using techniques well known in the art. This method is limited, because the sensitivity of detection is dependent on the amount of target material and the specific activity of the probe. As the probe's specific activity may be fixed, to improve the sensitivity of these assays, methods of amplifying nucleic acids are employed. Two basic strategies are employed for nucleic acid amplification techniques; either the number of target copies is amplified, which in turn increases the sensitivity of detection, or the presence of the nucleic acid is used to increase a signal generated for detection. Examples of the first approach are polymerase chain reaction (PCR), rolling circle (see U.S. Pat. No. 5,854,033), and nucleic acid system based amplification (NASBA). Examples of the second include, cycling probe reaction, termed CPR (see U.S. Pat. No. 4,876,187 and U.S. Pat. No. 5,660,988) and SNPase assays, e.g. the Mismatch Identification DNA Analysis System (see U.S. Pat. No. 5,656,430 and U.S. Pat. No. 5,763,178).

**[0014]** The PCR reaction is well known to those skilled in the art and was originally described in U.S. Pat. No. 4,683,195. The process involves denaturing nucleic acid, a hybridization step and an extension step in repeated cycles and is performed by varying the temperature of the nucleic acid sample and reagents. This process of subjecting the samples to different temperatures can be effected by placing tubes into different temperature water baths, or by using peltier-based devices capable of generating heating or cooling, dependent on the direction of the electrical current as described in U.S. Pat. No. 5,333,675 and U.S. Pat. No. 5,656,493. Many commercial temperature cycling devices are available, sold for example by Perkin Elmer, Applied Biosystems and Eppendorf. As these devices are generally large and heavy they are not generally amenable to use in non-laboratory environments, e.g. at the point-of-care.

**[0015]** A microfabricated device for performing the polymerase chain reaction is described in U.S. Pat. No. 5,639,423 though it is silent on providing an integrated means for extracting nucleic acids. A device for performing the polymerase chain reaction is described in U.S. Pat. No. 5,645,801 which has an amplification chamber that can be mated in a sealable manner to a chamber for detection. U.S. Pat. No. 5,939,312 describes a miniaturized multi-chamber polymerase chain reaction device. U.S. Pat. No. 6,054,277 describes a silicon-based miniaturized genetic testing platform for amplification and detection. A polymer-based heating component for amplification reactions is described in U.S. Pat. No. 6,436,355. U.S. Pat. No. 6,303,288 describes an amplification and detection system with a rupturable pouch containing reagents for amplification. U.S. Pat. No. 6,372,