System for High-Throughput Analysis of Individual DNA Molecules

Published date: March 14, 2017

Technology description

Now that complete sequence information is available for many organisms, researchers are focusing on the analysis of single genomic DNA molecules. A key issue in this analysis is how to unravel and mark the DNA molecules for high-throughput applications.

One option is to elongate a single DNA molecule and fix it to a substrate by electrostatic attraction. Sequence features of the fixed molecule can be identified by cleaving it with one or more restriction enzymes to produce gaps that can be marked and then visualized. However, although fixing DNA to a substrate simplifies the process of preserving the elongated state, stabilizing the molecule’ s position and preventing fragment shuffling after cleavage, it is difficult to control chemical interactions because the DNA is close to the substrate. Additionally, cleaved DNA may not be suitable for subsequent use.

An alternative is to suspend the DNA molecule in a “nanochannel” without attaching it to the wall. However, nanochannels, which typically have diameters of about 30 nanometers, are costly, difficult to fabricate and not reusable. Their small size makes adding chemical reagents, particularly enzymes, difficult, and it also is difficult to encourage DNA molecules to enter the small cross-sectional area of the nanochannels. Additionally, because DNA is not under significant tension within nanochannels, using restriction enzymes to cut the DNA may not produce visible gaps. UW–Madison researchers have developed improved methods of using channels for tagging, characterizing and sorting individual double-stranded DNA molecules while maintaining the integrity of the biomolecules. In these methods, a single strand of the molecule is broken, or “nicked.” Because the molecule is not cleaved, the nicking process can occur before the DNA is immobilized in a channel. Fluorescently labeled, sequence-specific nucleotides also can be added at specific sites before the DNA is introduced into the channel.

Additionally, nicked DNA molecules can be kept in a low ionic strength buffer to increase their stiffness. This increased stiffness makes it possible for the molecules to remain properly aligned in channels whose height is nanometer scale but whose width is micrometer scale. Using the larger channels simplifies DNA loading, the introduction of reagents and channel construction, reducing costs and making disposable channels practical.

After a nucleic acid molecule is optically analyzed and characterized in a channel, it can be captured, for example by electrostatic attraction, and collected at a defined point in the array. Then the molecule can be released and conveyed for subsequent analysis, such as sequencing.
The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a system for high-throughput analysis of single DNA molecules that couples powerful labeling and identification features with the ability to selectively capture or discard identified molecules.

Jo K., Dhingra D.M., Odijk T., de Pablo J.J., Graham M.D., Runnheim R., Forrest D. and Schwartz D.C. 2007. A Single-Molecule Barcoding System Using Nanoslits for DNA Analysis. Proc. Natl. Acad. Sci. U.S.A. 104, 2673-2678.
Jo K., Dhingra D.M., Odijk T., de Pablo J.J., Graham M.D., Runnheim R., Forrest D. and Schwartz D.C. 2007. A Single-Molecule Barcoding System Using Nanoslits for DNA Analysis. Proc. Natl. Acad. Sci. U.S.A. 104, 2673-2678.

Application area

High-throughput analysis of individual nucleic acid molecules

Advantages

This technology combines powerful labeling and identification features with the ability to selectively capture or discard identified molecules to provide a system for the high-throughput analysis of individual molecules of DNA.
DNA can be nicked and labeled before it is immobilized in a channel, simplifying the marking process. In nanochannels, nick translation labeling is a more effective method than restriction mapping.

Institution

Wisconsin Alumni Research Foundation

Inventors

David Schwartz
Dalia Dhingra
Kyubong Jo

联系我们

叶先生

电 话：021-65679356

手 机：13414935137

邮 箱：yeyingsheng@zf-ym.com