Optical Genome Mapping

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Abstract
Optical mapping is a method to find the locations of a specific base-pair sequence and its reoccurrences on particular single DNA molecules using fluorescence labeling and light microscopy. Using optical maps combined with the known sequence from the reference genome, or from a raw sequence assembly produced by any genome sequencing technique, one can rapidly identify the target gene and determine the existence of mutations or sequencing errors in the samples. Here we review some recent advances in optical genome mapping, including a brief introduction of optical mapping and its applications in DNA profiling, clinical DNA sensing, and genome sequencing.

Keywords: Commercial optical mapping platforms; DNA sensing; DNA sequencing; Software development.

OVERVIEW

Optical mapping combined with a few other sequencing techniques such as PacBio, Oxford Nanopore, Nabsys, and next-generation sequencing (NGS) techniques provides a solution to *de novo* assembly of reference-quality whole genome sequencing.[1,2] Optical genome mapping is a single molecule method to find the ordered length of sections of DNA between sequence specific labels. Fluorescence microscopy is used to visualize the entirety of single DNA strands using backbone stains. Various labeling methods,[3] traditionally restriction enzymes, are used to find a small portion of the sequence of the DNA strand and cut it at each labeling point. The information acquired during optical mapping can be used to aid in sequencing of DNA, identifying pathogens, testing for diseases, and forensics. Optical mapping has the potential to supplant electrophoresis as the dominant technique in DNA profiling due to the additional information gained in addition to the benefits of using reduced sample sizes. Extensive reviews of optical mapping have been published before and a few recent ones are listed.[1,4–9] This review focuses on two commercial platforms and their applications in recent few years, especially the past two years.

TECHNIQUE DEVELOPMENT

In the past two decades, two main platforms for optical mapping have been commercialized by two companies: OpGen and BioNano Genomics.

Origins of OpGen

The traditional method for optical mapping, developed by Dr. David Schwartz's group in the 1990s (Fig. 1),[10–12] has been commercialized by the company OpGen, which was founded in 2002. The technique is based on stretching the negatively charged DNA by fluid flow and immobilizing it onto a positively charged surface commonly functionalized with polylysine or 3-aminopropyltriethoxysilane in microfluidic channels,[11–13] after which restriction enzymes are added to cut the DNA at site specific sequences and a backbone stain is added to visualize the DNA with clearly defined gaps created by the cuts. OpGen developed Argus, an instrument for automated high-resolution whole genome analysis. This system allows for mapping without the need for polymerase chain reaction, cloning, paired-end libraries, or genomic specific reagents.[14]

The resolution of the original technique was limited to ~1000 base pairs (bp) due to the diffraction limit of light.[15] Replacing restriction enzymes with fluorescent dyes allowed the use of super resolution techniques to be used to increase the resolution of the technique down to 100 bp.[16–18] More information on the resolution improvements made to optical mapping can be found in Levy-Sakin and Ebenstein's review.[15]

Origins of BioNano Genomics

In 2012, a company called BioNano Genomics founded in 2003 published a paper with the Kwok group describing a nanofluidic device to stretch nick-labeled DNA

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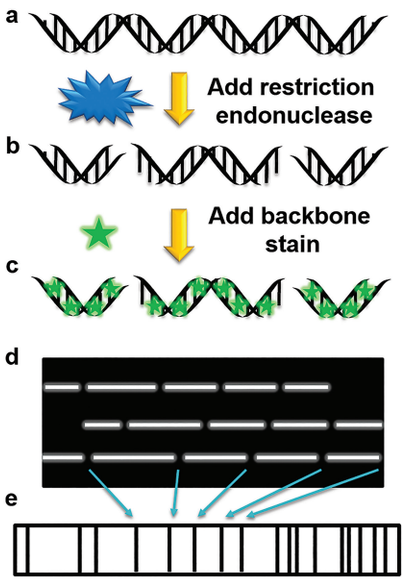

Fig. 1 Scheme of a traditional optical mapping protocol. (a) DNA before modification. (b) DNA with gaps after being cut by the restriction endonucleases. (c) Cut DNA with a backbone stain added to visualize the fragmented DNA strands. (d) Scheme of a fluorescence image of the digested DNA molecules. (e) A barcode generated from aligning fragments of cut DNA where each line represents a gap and the box represents the whole genome

molecules.[19] Briefly, the DNA molecules are both nick-labeled and backbone-stained, then are electrophoretically stretched and imaged in nanofluidic channels with <50 nm cross-section diameters as shown in Fig. 2. The demonstration is explained as "seeing FISH swimming", where FISH stands for the well-established fluorescent *in situ* hybridization technique. This summarized a decade of research in obtaining optical maps in a nanoconfined environment and brought it into commercialization.[20–23] The physics of DNA stretching for optical mapping on these surfaces has been covered by Gupta et al.[6] For general physics of DNA in nanochannels there are several good reviews by Mannion and Craighead,[24] Persson and Tegenfeldt,[25] and Rems et al.[26]

Currently, the Irys system from BioNano Genomics is now widely used in many fields especially *de novo* sequencing of large Eukaryote genomes. The major advances of this platform compared to the original platform is that it has orders of magnitude higher throughput, but with a more complicated experimental process and a higher reagent/device cost.

Instrumentation

Both companies provide commercial instruments (Fig. 3) for roughly $300,000 (USD $, and here after).[29] OpGen discontinued their Argus system in 2016. Both companies' systems utilize a fluidic chip, where the sample is loaded and processed. OpGen runs on a microfluidic platform with flow stretching, while BioNano runs nanofluidic chips. A microscope and a camera set are used for collecting the fluorescence imaging data, which is processed using the integrated software IrysSolve for BioNano, or MapSolver for OpGen. More details on the software development are discussed in "Software" section.

Experimental Cost

OpGen's Argus system gives a cost estimation per microbial sample, between 1 and 6 million basepairs (Mbp), which totals up to $400 consisting of a $20 sample preparation cost and a $100–$300 data generation cost.[30] The total time required is 1–2 days consisting of 6–10 h of sample preparation, ~1.5 h of hands-on work, and 10 min of assembly time.[30] Recently OpGen has upgraded their automation which enabled a ~10 billion basepairs per hour (Gbp/h) assay speed, similar to the BioNano system.[31]

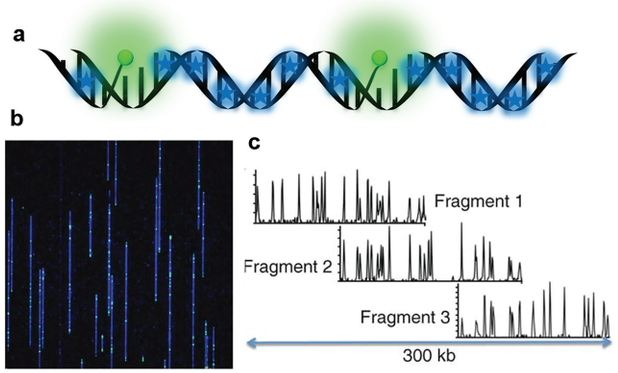

Fig. 2 Scheme of the second generation optical mapping protocol. (a) Scheme shows the DNA strand, which has green fluorophores attached through nick labeling to sequence specific sites and a blue backbone stain to visualize the whole strands. (b) Experimental image of the nick labeled DNA which is confined in parallel 50 nm nanofluidic channels (not visible in the image). (c) The intensity patterns of the fragments are then aligned to produce the DNA's unique barcode. Images (b) and (c) are adapted from the reference with permission[19]

Fig. 3 The left shows the Argus system from OpGen,[27] and the right shows an Irys system from BioNano Genomics[28]

BioNano Genomics offers a system with an estimated cost of ~$2000 per chip plus other reagent and handling costs.[19,22] The total cost to assemble whole genome optical maps can range from $3000 to $100,000 depending on the size of the genome and the depth of mapping.[2,32] For example, the Kansas State University Bioinformatics Center offers BioNano Genomics maps service estimating ~$8000 for a 500 Mbp genome.[33] BioNano's Irys system can process several Gbp/h, and 5–200 Gbp/day.[28,34]

Other Methods

In the 1990s, a technique called fiber-FISH was developed.[35] Briefly, aggregations of DNA molecules, which are like a big balls of yarn, are FISH labeled. Some individual DNA molecules are pulled out of the yarn when dried on a glass slide. These molecules are then optically imaged yielding optical maps. Fiber-FISH has been further developed/modified and applied in many genomic projects.[36–40]

Another method uses methyltransferase enzyme labeling which has been well studied and reviewed by Neely et al.[41] and Levy-Sakin et al.[42]

An additional technique using YOYO-1 as a site specific label has been developed with either denaturation[23,43] or competitive netropsin binding[44,45] to limit the labeling to GC segments of the DNA as shown in Fig. 3a–c for denaturation and Fig. 4d for competitive netropsin binding.

APPLICATIONS

Genome Sequencing

Genome sequencing is a technique used to obtain the genome sequence of an organism focusing on the variations of the genetic codes among different species and among different individuals of the same species. Many reference databases, high-quality sequences obtained from one or a group of individuals for particular species, have been established in the past two decades. In the past decade, there is a fundamental shift away from the traditional first generation automated Sanger sequencing method to the second generation methods called the next-generation sequencing (NGS) which has already been significantly reviewed.[46–50] One of the major challenges in NGS is to assemble the small fragments (called the library) into contigs (segments of the genome that are nearly completely resolved), then into scaffolds (groups of contigs that are properly placed and oriented with respect to each other), and fill gaps with high accuracy and high confidence (Fig. 4). A statistical value called scaffold N50 is often used to measure the quality of a particular unfinished assembly. When all scaffolds are ordered from long to short regardless of their real position on the genome, the length of the scaffold at 50% of the whole genome length is the N50 value. Assuming a Poisson distribution of the lengths, an increasing N50 value correlates to a smaller number of gaps.

With the booming of NGS, optical mapping has been heavily valued by the genome sequencing community since 2008, when it was used to successfully confirm eight

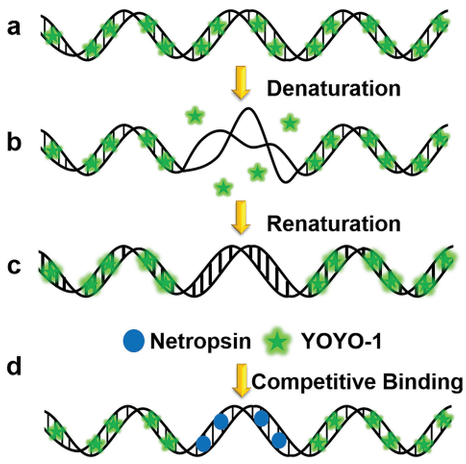

Fig. 4 (a–c) Depicts the process for denaturation mapping, (a) where the DNA is fully stained with YOYO-1, an intercalating dye. (b) YOYO-1 is released from the AT segments after denaturation from a combination of increased temperature and formamide treatment. (c) The resulting stained DNA after renaturation where only the GC segments are labeled. (d) Depicts a competitive binding map where only GC segments are labeled with YOYO-1 because Netropsin binds more strongly to AT segments

large insertions identified by fosmid one-end-anchoring to the human reference assembly NCBI35.[51,52] Compared to the other physical mapping methods,[53–55] optical mapping provides an independent experimental route to generate a highly accurate *de novo* scaffold on which to align short-read sequencing data (Fig. 5).[52] This technique is now routinely used by the Genome Reference Consortium's (GRC) mission in maintaining reference human, mouse, zebrafish and chicken genomes.[52]

The application of optical mapping in genome sequencing has been reviewed.[6,52] This section summarizes some new reports in the past two years in human, animal, plant, and microbial genome projects.

A breakthrough in human genomics is reported by a group of scientists from several institutes using NGS, PacBio Single Molecule, Real-Time (SMRT) sequencing, and BioNano Genomics optical mapping to analyze a diploid human genome NA12878.[2] It generates a *de novo* sequence approaching reference quality, with scaffold N50 values as long as 30 Mbp. The PacBio SMRT depth of sequencing is ~20× coverage/depth (random local coverage, but total effective data length of 20× the 3 Gbp long human genome), the BioNano optical mapping coverage is ~80×, and two Illumina (one of the NGS techniques) data sets are 50× and >30× sequencing depth respectively. The reagent and device cost is estimated at ~$30,000 for PacBio, ~$3000 for BioNano in this report, and Illumina sequencing may cost an additional few thousand dollars as claimed by Illumina in their $1000 human genome project.[56] The greatest cost is the amount of time needed to assemble PacBio reads (over 30 years of CPU time) and BioNano reads (~2 years of CPU time). Illumina has estimated a CPU time of ~2 years for a human genome assembly.[57] Therefore, it takes more than two months for a 200-CPU cluster to analyze all the data separately.

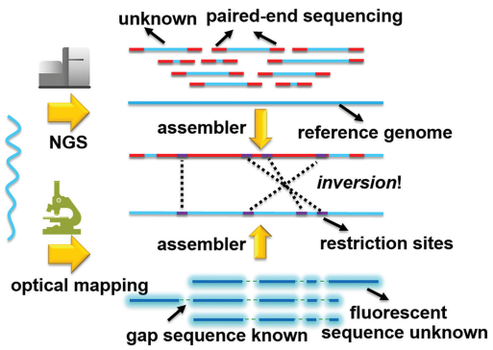

Fig. 5 A scheme illustrating the concept of optical mapping as a tool for genome sequencing. Copies of the genome are sent separately through the NGS pipeline and through the optical mapping pipeline. The results from both methods are analyzed independently with or without a reference genome. The assembled sequence from NGS, and genetic maps from optical mapping are then combined to generate a more accurate sequence of the genome. If no reference is involved, the process is called *de novo*

Optical mapping also helps the first generation Sanger sequencing and NGS finish animal genomes. Using an OpGen system, a high quality optical map of an ostrich (*Struthio camelus*) genome (1.23 Gbp) is obtained by ultra-deep optical mapping (500× coverage), with the scaffold N50 value of 17 Mbp (five times longer than reported before).[58] This optical map is expected to serve as a scaffold reference for future Ostrich genomic projects and, more generally, bird genome projects. Recently, NGS, optical mapping (OpGen's system), and fiber-FISH techniques were combined in a pig genome project to improve the X-chromosome sequence and also to generate the first Y-chromosome sequence, which had not been done in previous projects.[59] In another report, an optical map (obtained from 500× coverage using the traditional optical mapping method) of a clone-free cow genome is able to estimate relative errors in two cow genome references.[60] This optical map can be used to improve cow genome assembly in the future.

The applications of optical mapping in plant genome sequencing have been recently reviewed.[4,8,61,62] Many plant genomes contain complex sequences and repeats which are extremely challenging for genomic sequencing, especially for NGS. The unique ability of optical mapping in assembling the alignment and orientation of long range scaffolds makes it an ideal tool to assist plant genome shotgun sequence assembly.

Optical mapping was first demonstrated on microbial genomes and continues to be applied in various microbial genome projects in finishing genomes and identifying new species. Recently, PacBio (as little as 20× coverage of long reads) and optical mapping (OpGen) together showed the ability to generate the complete *de novo* sequence of a fungal genome,[63] and surprisingly NGS was not required. Very recently, a new bacterium was found and isolated from the West Desert of China. Whole genome optical mapping and NGS were used to assemble a finished *de novo* genome sequence (4.6 Mbp).[64] The OpGen Argus system was used to generate the optical map with ~30× coverage. In another report, NGS (shot reads), PacBio (long reads), and optical mapping (extremely long reads from OpGen's system) were used to assemble a *de novo* yeast genome (~17 Mbp).[65] A pipeline to treat and combine the data obtained from the three platforms is proposed and demonstrated in this report to generate a nearly completed yeast sequence.

Disease Diagnostics and Pathogen Detection

Whole genome sequencing with the help of optical mapping has been widely applied in genetic disease diagnostics, especially in cancer diagnostics.[66–69] Optical mapping and sequencing can detect mutations such as copy number variations and single nucleotide polymorphisms

respectively on their own. When combining the approaches more structural variations can be confidently obtained such as deletions, insertions, and inversions. Recently, Gupta et al. used traditional optical mapping to study the genomic structural variations in multiple myeloma (MM) complimented with DNA sequencing on an Illumina platform.[68] By integrating the findings of both techniques and studying the genome of MM cells at two stages during the progression of cancer they were able to identify a large number of mutations. In another report, NGS and traditional optical mapping have been used to study the genomic mutations in the 2q13 region of the human chromosome, which cause Nephronophthisis 1, a genetic disorder of the kidneys that affects children.[70] Optical mapping is a necessity for this study due to the high number of low copy repeats in the 2q13 region and without it the accuracy of assembly from NGS would be greatly reduced. After combining the mapping and sequencing data, three haplotypes were found that may protect deletion of genes that cause Nephronophthisis 1.

Another major application of optical mapping in clinics is detecting pathogens. Optical mapping plus genome sequencing is a rapid way to identify bacterial or virus strains. After the 2006 *Escherichia coli* (*E. Coli*) bacterial outbreak in the United States, optical mapping (OpGen) plus DNA sequencing is able to find the connection among the bacterial strains in animal, human, and fresh spinach.[71] During another *E. Coli* outbreak in Europe 2011, optical mapping and NGS is able to resolve the whole genome within just 62 h.[72] *Staphylococcus aureus* (*S. aureus*) is a bacterium that can cause diseases ranging from mild skin infections to fatal infections. Approximately 25% of *S. aureus*'s genome is variable between strains. Shukla et al. showed that this margin is enough to identify different strains as they identified an inversion between two different strains of *S. aureus* using optical mapping, and further confirmed through DNA sequencing.[73] In another study, Xiao and coworkers used nicking enzymes to label DNA for optical mapping to distinguish human adenovirus and rhinovirus genomes.[74] They were also able to use this method to successfully identify four different strains of the human rhinovirus. Nyberg and coworkers used competitive binding optical mapping, described earlier, to create a method for plasmid identification.[75] A theoretical *in silico* fingerprint is created using available sequencing data. The plasmid can be determined by a statistical comparison of the experimental fingerprint to the theoretical fingerprint database. Plasmids with a length of 70–300 kilobase pairs (Kbp) can be identified using this method. It is important to note that plasmids around 180–190 Kbp are identified with less confidence due to high sequence similarities in plasmids of this length. This method helped contain a recent *Enterobacteriaceae* outbreak at a hospital in Sweden.[76] No sequence information is needed and the mapping steps, after plasmid extraction, can be accomplished in less than 1 h. It was determined that all fingerprints from the sampled patients had a shared plasmid that differed only slightly, due to different strains of bacteria. Four of the plasmids were 10–15 Kbp longer suggesting an insertion mutation. Since each bacterium had a shared plasmid, it is likely it was the cause of the disease. In another report, Grunwald et al. used methyltransferase, M.TaqI, for quick detection of λ and T7 bacteriophages out of a pool of 20 phages of similar lengths.[77] M.TaqI catalyzes the transfer of the TAMRA dye to adenine base of the TCGA sites recognized by M.TaqI. This labeling method allows for up to 60% more information gained from phage genomes.

Fingerprinting and Forensics

In the field of forensics, optical mapping offers benefits over other methods especially in DNA fingerprinting/profiling because it is a single-DNA method, and therefore only requires a small amount of sample. DNA fingerprinting has been widely applied in forensic DNA analysis to trace the origin of a sample with either living organisms or dead cells.[78] Both a single cell and a single genome can be used in optical mapping.[79] Thus, optical mapping is not susceptible to sample contamination and errors introduced by polymerase chain reaction (PCR) amplification (~$1/10^6$ error rate), which is commonly used in other mapping techniques.[80] Optical mapping has a great potential in both human and non-human sample analysis with the continuing decrease in experimental cost and improved automation. The completed 1000 genome project,[81] the 1-million-volunteer precision medicine initiative[82] and other human genomic projects may eventually create a high-coverage human genome database for forensic *in silico* optical mapping if the associated ethical issues are resolved.[83] Optical mapping measures the fingerprint of a genome directly.[84] Soil microbial DNA fingerprints have been demonstrated to trace soil origin with high confidence in criminal investigations,[85–87] but optical mapping has yet to be employed in these studies.

SOFTWARE

Computational algorithms and tools to process optical mapping data have been reviewed before.[7,88–90] The major functions of the software are to analyze the optical images, align and generate optical maps, assemble the whole genome optical map, calculate map-to-sequence alignment, and identify structural variants in the sequence. Some software programs are developed for both OpGen and BioNano Genomics systems. The image analysis parts of the software for these two systems are different but the alignment parts share the same concept. When the data format is unified for the two systems, the software can take data from both systems for the remaining assembly, alignment, and analysis steps.

For OpGen System

OpGen developed commercial software such as MapSolver and Genome-Builder to analyze optical mapping data. Genome-Builder contains Gentig v.2 for overlap alignment. To mention a few recent examples, Genome-Builder has been used in a ~2.66-Gbp genome sequence of a goat and when OpGen improved the automated processing speed to ~10 Gbp/h.[31] Genome-Builder was also used for the analysis of the 1.23-Gbp ostrich genome;[58] and a ~274 Mbp *Rhazya stricta* genome.[91]

Valouev and Waterman et al. developed a software (referred to as Valouev hereafter) that proposed a popular representation of sizing error.[92] This software was developed for both finding the overlaps between optical maps and aligning them to a reference map. They introduced a statistical model that enables them to design a likelihood-ratio-based alignment score to distinguish high-quality alignments from lower ones. This is achieved by assuming that the false cuts and missing cuts by the restriction enzyme on a genome are distributed randomly by Poisson and Bernoulli functions.

Many research groups have developed different algorithms for the OpGen system. [32,93–96] SOMA is an open source free assembler developed by Nagarajan et al.,[95,96] which is used to align sequence contigs from genome sequencing data to a unified optical map. TWIN is an index-based free software developed for analyzing OpGen sequencing data. For a Budgerigar genome (1.2 Gbp), TWIN is estimated to be ~10 times faster than Valouev and more than three orders of magnitude faster than SOMA.[93,94] OPTIMA is a free software developed by Verzotto and Nagarajan et al.,[32] which combines the index-based technology with a statistical method. Recently OPTIMA has been applied to simulated Drosophila and Human genome sequencing, and on a real human GM12878 HapMap cell line. It is estimated to be 2–5 times faster than Gentig, 40 times faster than Valouev, and four orders of magnitude faster than SOMA. An open source software MISSEQUEL was recently developed, which uses optical maps to correct misassembled contigs in NGS assemblies.

For BioNano Genomics System

BioNano Genomics developed an alignment tool called RefAligner around 2014.[97] Many research groups have used it for assembly. For example, in recent years, Bashir's group and BioNano Genomics reported *de novo* NGS assembly of whole human genomes using their optical mapping data generated by RefAligner.[2,98] RefAligner has also been used in several other groups for the yellow croaker,[99] maize,[100,101] banana,[102] and allopolyploid *Brassica juncea* genomes.[103]

Many research groups have also developed their own algorithms to analyze data obtained from the BioNano Genomics system. For example, Brown's group working with BioNano Genomics have recently developed a custom-designed hybrid scaffolding pipeline named Stitch to validate and improve a 7× Sanger draft of the *T. castaneum* genome.[104] Stitch filters low quality alignments using a confidence score to increase the assembly quality. OMWare was developed by Sharp and Udall to offer an alternative user interface to RefAligner and was tested with the optical mapping data collected from a cotton genome.[105] Yiu's group developed a new error estimation algorithm for optical maps obtained from the BioNano Genomics system.[106] This model is potentially useful in estimating the assembly quality and the minimum mapping depth (number of coverage) of each region in the genome.

For Both Systems

OMBlast is an alignment tool developed by Yip's group and Chan's group for optical mapping using a seed-and-extend approach.[107] This software aligns optical maps to a high quality reference and is intended to find structural variants such as insertions, deletions, inversions, and duplications. In its first report, OMBlast aligned an individual human optical mapping dataset (BioNano Genomics) to the human genome reference. It was compared to SOMA, TWIN, Valouev, and RefAligner showing good performance for a few examples tested in both alignment speed and accuracy.[107]

ALLMAPS was developed to combine a variety of mapping information including optical mapping for *de novo* genome assembly.[108] The program is able to combine different references, genetic maps, physical maps, cytological maps, optical maps, or references from evolutionary related species all together to order the scaffolds. Each map is either assigned a weight or allowed variable weight during the assembly. ALLMAPS is potentially useful to both increase and estimate assembly quality.

For Other Systems

New software for the denaturation mapping method, described earlier, has been reported.[23] Reisner used a kymograph alignment algorithm to align the fluorescent images of the DNA strands. Noble and Ambjörnsson et al. developed an algorithm named WPAlign using a pattern recognition method that greatly increases the speed of the kymograph alignment step of these partial denaturation maps.[109] For a simulated human DNA, WPAlign was shown to be five orders of magnitude faster than the method developed by Reisner et al. In another report, a low-cost all-polymer injection molded device has been developed recently for partial denaturation of the DNA in human genome optical mapping.[110] The fabrication cost of the device is estimated to be as small as $3 and is potentially compatible with the OpGen system. This microfluidic device has multiple inlets and junctions that takes human DNA strands (still wound around histones) followed with

washing, staining, partial denaturing, stretching, and imaging. The development of the software and hardware for this system may yield a new commercial platform for optical mapping in the future.

FUTURE PERSPECTIVES

The development of commercial platforms, namely OpGen and BioNano Genomics, has greatly facilitated the applications of optical mapping in genome-related research and clinics. However, fundamental research and engineering in both hardware and software are still highly desired.

In technical development, Lab-on-a-chip devices with high-throughput features continues to be a trend to increase the testing speed and reduce the cost. Major tasks include: 1) further reducing the price of the chips, 2) developing a new chemical labeling method, and 3) further increasing the resolution and offering higher quality optical maps.

In software development, more automatic pattern recognition and optical map assembly algorithms will probably emerge in the near future. Advances in indexing technology have greatly increased the data processing speed, while massive parallel computing and machine learning might also further increase the assembly speed. Smart probe and enzyme selection tools may help the researchers to design optical mapping experiments to address specific applications. For example, to close a gap or to confirm a structural variant on a particular genome sequencing assembly, a particular set of probes or enzymes can be predicted based on the knowledge obtained from the assembly itself and/or a higher-level reference. A key feature desired for software is an accurate estimation of the uncertainty levels, namely false positive probability, false negative probability, and localization errors. An algorithm to distinguish structural variation, heterozygosity and paralogous variation, and miss-assembly errors is highly desired. For the field, a standard benchmark process to test and evaluate the large amount of software programs in terms of performance and speed is also highly desired.

In applied research, fast pathogenesis diagnostics is one of the major goals, such as whole genome sequencing, and bacteria and virus detection. A clear trend for reference quality *de novo* sequencing is combining different techniques such as NGS, PacBio, nanopore sequencing, Nabsys, and optical mapping, which requires a software platform, e.g., Stitch and ALLMAPS to unify all the data, and a database to store and share all resources. It is also possible to see applications of single genome optical mapping for ultrasensitive genealogical forensic tests in the near future.

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