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High-throughput low-cost DNA sequencing using surface-coating technology

Yanzhe Qin
qinyanzhe@genomics.cn
BGI-shenzhen
Stephan Koehler
Harvard University
Shengming Zhao
BGI-shenzhen
Ruibin Mai
MGI Tech Co., Ltd.
Zhuo Liu
MGI Tech Co., Ltd.
Hao Lu
MGI Tech Co., Ltd.
Chengmei Xing
BGI-shenzhen

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Abstract

The speed, expense and throughput of genomic sequencing impose limitations on its use for time-sensitive acute applications, such as rare or antibiotic resistant infections, and large-scale testing that is necessary for population-wide source-tracing, as in the COVID-19 pandemic. A major bottleneck for increasing throughput and decreasing operating costs of next-generation sequencers (NGS) is the flow cell that supplies reagents for the biochemical processes; this subsystem has not significantly improved since 2005. Here we report a new method for sourcing reagents based on surface coating technology (SCT): the DNA adhered onto a biochip that is directly contacted by a reagent-coated polymeric strip. Compared with flow cells, reagent layers are an order of magnitude thinner while both the reagent exchange rate and biochip area are orders of magnitude greater. For whole genome sequencing (WGS), these improvements reduce turn-around time from days to twelve hours, reduce cost from about $1000 to $15, and increase data throughput by orders of magnitude. This makes NGS more affordable than many blood tests while rapidly providing detailed genomic information about microbial and viral pathogens, cancers and genetic disorders for targeted treatments and personalized medicine. The resulting data can be pooled in population-wide databases for accelerated research and development as well for providing detailed real-time data for tracking and containing pandemic outbreaks.

Introduction

High-throughput DNA sequencing is becoming ever more commonplace for public health[16-18], and is an effective tool for dealing with pandemics[19], such as tracing the origins of SARS-CoV-2 outbreaks[7,20] and following the virus's evolution[6,21,22]. Inside China several COVID-19 outbreaks were successfully contained using source-tracing based on NGS sequencing, because these outbreaks were sourced by viral strains outside of China[8,9]. The fastest NGS systems can generate about 3Tb (30 WGS) per day with a typical cost of about $1000/person and turn-around time of 2 days[23]. But on the scale of the ever-growing current COVID-19 pandemic that is approaching 100 million infections and millions of deaths[24], both data throughput and cost limit contact tracing[19].

The core component of NGS is the flow cell where the bioreactions take place. Significant improvements occurred in 2005, but over the last five years the operating costs and throughput have remained relatively unchanged (Fig. S1). The flow cell is a chamber with a gap of about 100 μm and contains multiple lanes of attached single-stranded DNA molecules[12]. A series of reagents is cycled through this microfluidic device to perform the biochemical reactions necessary for fluorescent imaging and base calling[25]. The majority of operational costs are due to reagent usage[26], and thus approaches to optimize reagent usage have been developed. Recent approaches by Illumina are to recycle 70% of the reagents[23], and tripling the number of DNA molecules in the flow chamber by reducing the spacing between attachment points beyond the optical diffraction limit[27,28]. Although this reduction in spacing improves reagent usage and increases data size, it requires super-resolution imaging for base calling which is slower. Further miniaturization is constrained because the size of the nanowells and their separation are limited by the DNA properties. These approaches have lowered reagent cost from about $80 to about $30 per Gb[29]. Another way to improve throughput and reduce cost is increasing the size of the flow cell[30] and boosting its flow rate. But there are limits to the flow cell's structure: larger flow cells have a propensity for non-uniform reagent flows (Fig. S2). Higher flow rates increase speed and smaller gaps decrease reagent use; however both increase the internal pressure[31] which bends the coverslip and increases the risk of its delamination. To overcome limitations of flow cells, BGI has developed the DNBSEQ-TX sequencer. It employs an alternative technology for supplying biochemical reagents that involves dipping the biochip directly into reagent baths using a

robotic arm[32]. After performing a cycle of biochemical reactions via dipping in reagent slots and buffer slots for washing, the robotic arm places the biochip into the imaging subsystem for base calling. This removes size limitations on the flow cell; the biochip area has increased by over two orders of magnitude, which means data production has increased by the same amount. The DNBSEQ-TX sequencer has been deployed for the “10 Million Single-Cell Transcriptome Project (scT10M)” [33] in Latvia as well as an ambitious health initiative by the United Arab Emirates[34] for sequencing genomes of the entire population (1 million). Moreover by washing the biochip between biochemical steps, the reagent baths can be used many times which decreases reagent waste, and the savings in reagent usage drops the price to about $1 per Gb or $100 per WGS[35]. However, the turn-around time for completing PE100 sequencing increases to about four days.

Here we present a surface-coating technique (SCT) that dramatically improves data throughput and reagent usage. We use SCT to rapidly apply thin reagent coatings on a large biochip covered with a microarray of $5x10^{10}$ DNA molecules. This approach increases the data size and exchange rates by orders of magnitude (Fig.1). A PET polymeric strip coated with reagents directly contacts the biochip for fast sourcing of reagents to the DNA. Bands of reagents are arranged sequentially on the strip so that step-wise lateral displacements provide the appropriate reagents for each step of the sequencing-by-synthesis biochemical reactions[25,36](Fig. 2a). This approach sidesteps issues associated with rapidly flowing reagents through a thin flow cell, such as uneven reagent distribution and failure due to high liquid pressures. Our prototype biochip area is 225 cm^2, which is two orders of magnitude larger than current single-lane flow cells and can be further increased several orders of magnitude (Fig. 1a). Moreover, the reagent layer thickness is far smaller than that of flow cells, which reduces the fraction of reagent not used in biochemical processes from 99.9% to about 99% with the possibility of applying thinner layers and further reduction to about 90% (see calculation section in supplementary information); this is significant because reagents currently account for 80-90 percent of operation costs[26]. The speed of coating the biochip with reagents is 80 m/min, which is over an order of magnitude faster than that in a flow cell (Fig. 1b), and in principle can be increased to industry speeds of 300 m/min[37]. A robotic arm transfers the biochip from the prototype SCT subsystem to the commercial imaging subsystem (DNBSEQ-T10 by MGI) for base calling between biochemical reaction cycles (Fig. 2b). The data quality is comparable to that of a flow cell (Fig. 3d). For 20 cycles our unfiltered Q30 exceeds 80% and other key parameters such as lag and run on are acceptable (Figs. 3e and f).

Results And Discussion

The main challenges for replacing the NGS flow cell with SCT were achieving uniform coatings on the PET strip, maintaining the reagent-filled gap between the PET strip and the biochip, and mitigating effects of evaporation resulting from our open-cell approach. The SCT subsystem was designed to be compatible with base calling using our imaging subsystem (DNBSEQ-T10 by MGI) with minimal modifications by using DNA arrays similar to those of commercial NGS platforms, and the main challenge was adapting a robotic arm for transferring the biochip between the two subsystems.

To create uniform reagent coatings on the corona-pretreated PET strip we used slot dies on an industrial roller and matched the reagents’ surface properties to that of the strip (Fig. 2a, S3, and S4a). There are six slot dies for each of the reagents (including buffer) required for sequencing, that apply 20 um reagent layers to the strip with roller speeds of 80 m/min and motor response times of 10 ms. The biochip is placed downstream from the slot dies with a gap that matches the reagent layer thickness (Fig. 2b). To maintain the layer’s uniformity, we placed

adhesive tape of the same thickness at the edges of the biochip (Fig. S5). Drying is not an issue because the tape slows evaporation and the NGS reaction steps take less than a minute.

The length of the moving reagent band necessary for flushing and supplying reagents scales with that of the biochip. We find reagent bands that are twice the length of the biochip replace previous liquid and effectively recoat the biochip (video clip 1 and simulation 1 in supplementary information). Directly coating the PET strip for contact with the biochip is far more efficient than using flow cells: the reagent layer is much thinner (20 μm, which for our reagent formulations can in principle be reduced to 2 μm[38]) and there is no tubing that needs to be flushed (simulations 2 and 3 in SI).

After executing a bio-chemical reaction cycle the biochip is removed from the PET strip with a robotic arm and placed in our commercial DNBSEQ-T10 imaging subsystem for base calling, see video clip 2 in SI. As the biochip is exposed to air in this step the robotic arm must move quickly to prevent drying of the DNA. During imaging the biochip is placed under an objective and coated with an imaging solution. When the biochip is neither subject to biochemical reactions nor being imaged, we place it in a buffer bath to prevent evaporation.

We confirmed the accuracy of the genomic sequences from our SCT subsystem. Aside from the biochip's edges where the presence of the tape disrupts reagent flow and heat transfer, the fluorescence signal was sufficient for the remaining 92% of the biochip for base-calling (Figs. 3a and 3b). We verified that the fluorescent channels had good separation (Fig. 3c). Similar to our commercial NGS platform[39], our Q30 remains above 80% after 20 cycles, and both lag and run on are comparable (Figs. 3d, e and f).

Finally, we compare our method with state-of-the-art NGS genomic sequencing in terms of turn-around time, bandwidth and cost (Fig. 1b). Since the reagent exchange is an order of magnitude faster than flow cells, PE100 or 212 cycles and reads can be completed within 12 hours (10 hours minimum with optimistic parameters for all subsystems) rather than two days. A single lane chip system such as DNBSEQ-G50 produces 300Mb per day while our single chip system produces 20 Tb, which is due to much faster reagent exchange and larger biochip area. Behind the slot dies the SCT strip has a 2m x 0.4m region available for coating biochips, which is sufficient for 266 of our 225 CM^2 biochips that would generate 8500 Tb per day. Since the data production of a single state-of-the-art imaging subsystem is 20Tb per day, 425 such subsystems running in parallel would be required, see video clip 2. Implementation of faster TDI sensors may largely reduce the number of imaging systems. SCT has a much thinner reagent layer and has no tubing which compared to flow chips decreases reagent usage by orders of magnitude. Since reagent cost accounts for 80-90% of current NGS platforms, this decrease in reagent usage of SCT drops the operating costs for WGS from about $1000 to about $15.

Conclusions

We developed a new NGS subsystem to replace the flow cell with SCT without compromising data integrity that increases throughput by two orders of magnitude and decreases turn-around time from several days to 12 hours. Moreover, SCT uses less reagents and thereby decreases operating costs by almost two orders of magnitude. We anticipate that further improvements to our SCT prototype can be made in terms of scaling up the biochip size to increase output, and scaling down the reagent layer thickness to decrease cost. This makes SCT an attractive approach for all aspects of gene research including work involving large-scale single cell sequencing[40]. SCT rivals standard diagnostics, such as blood tests and biopsies, in terms of cost and speed while providing vastly more detailed and comprehensive information in terms of the whole genome that can be collected in massive

population-wide databases[41]. This in turn will democratize personalized medicine, dramatically accelerate the pace of research and development of therapies, as well as provide epidemiologists and policy makers with detailed data for combating outbreaks, such as those of the current COVID-19 pandemic.

Materials and Methods

Preparation of DNA nanoballs (DNBs)

We used the library of single-stranded circle (ssCir) Escherichia coli (Ecoli) and Standard Library Kit (MGI Tech Co. Ltd) to prepare DNA fragments about 300bp. A 20μL of a 40fmol ssCir DNA solution was mixed with 20μL Make DNB Buffer solution from the BGISEQ-500RS DNB Make Load Reagent Kit (MGI Tech Co. Ltd.). It was annealed using a Bio-Rad S1000TM thermocycler at 95℃ for 1 min and 65℃ for 1 min and 40℃ for 1 min. To this we added 40μL of Make DNB Enzyme mix I and 2μL of Make DNB Enzyme mix II from the BGISEQ-500RS DNB Make Load Reagent Kit (MGI Tech Co. Ltd.) and used the solution for rolling circle amplification (RCA) at 30℃ for 20 min. To stop the amplification, we added 20μL of Make DNB Stop Buffer. The DNBs were verified using the Qubit dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific)[36].

Preparation of biochip

Biochips were fabricated from 8-inch silicon wafers and handles were glued on for robotic handling. Deep ultraviolet (DUV) lithography was used to fabricate a 15 x 15 cm array of circular wells with diameter of ~250 nm with centre-to-centre separation of 700 nm. Aminosilane was vapor deposited in the holes to provide attachment sites for DNBs. DNBs were loaded onto the biochip and after 30 min were treated by the post-loading solution from the DIPSEQ DNB Make Load Reagent Kit (MGI Tech Co. Ltd.). To avoid drying the DNB-loaded biochips were stored in buffer. For more detailed information regarding biochip preparation, please refer to reference 36 and 42.

Reagents for biochemistry

We used the DNBSEQ-T10x4 RS High-throughput Sequencing Reagent (FCL PE100) Kit (MGI Tech Co. Ltd.) for Combinatorial Probe-Anchor Synthesis (cPAS)[42]. There are three biochemical reactions to replicate a complementary base of the DNB template and remove its fluorescent cap for the next cycle. For each of these three reagents the kit provides a specific buffer washing solution. To prevent beading up on the biochip and PET strip the surface tension of all six solutions was lowered to less than 42 mN/m using Tween-20 surfactant.

SCT set-up

We purchased rolls of corona - pretreated poly (ethylene terephthalate) (PET) transparent membrane that were 100 um thick and 20 cm wide. The length of the rolled membrane is more than 1000 meters and supports up to 300 cycles. We load these rolls onto our industrial roll coater with a tension of 20 kg to keep the membrane flat. The coater has speeds that are adjustable from 0.1 to 80 m/min with response times of 10 ms. It is equipped with 6 slot dies made by Foshan Edge Development Mold Mechanical Technology Inc, where each of the three biochemistry reagents has a dedicated slot die together with another dedicated slot die containing the specific washing buffer solution.

SCT operation

To stabilize the PET strip in the roller, we first applied 20 kg of tension. Prior to operation the slot dies are primed, and a 20 μm thick film is inserted for creating 20 μm thick layers. Dedicated software operates the roller and slot dies via user-adjustable diagrams. The biochemical processes, which are base adding and fluorescent cap cutting, take 30 sec each at a temperature of 60 ℃ during which the strip is stationary. Upon completion the biochip is washed by displacing the PET strip coated with the appropriate washing buffer solution for a distance twice the length of the biochip at a speed of 80 m/min. Washing takes about 3 seconds, whereas flow cells take about one minute.

NGS operation

A robotic arm, C8XL by Epson, moved the biochip between the SCT subsystem, the imaging subsystem taken from the commercial DNBSEQ-T10 by BGI, and the storage buffer bath (Fig. 2b). One complete cycle for base calling takes about 2 minutes: 3 seconds for washing, 60 seconds for heating, 10 seconds for the robot arm to transfer the biochip to the imaging subsystem, and 60 seconds for base calling imaging - see the video clip 2 in supplementary information.

Reagent layer uniformity and thickness

In order to achieve uniform coatings, we matched the reagents' chemistry to that of the polymeric strips' surface (i.e., surface wettability, surface energy and surface charge). Corona pretreatment increases the membrane's surface tension, which ensures that the reagents spread and do not bead up. We used Acrotest Pens and found that for surface tension values below 42 mN/m beading up was absent. The layer thickness was set to 20 μm by adjusting the roller speed, flow rate of the slot dies and spacers. These were strips of 3M tape, 20 μm thick one cm wide, that were stuck to the biochip (Fig. S5). Layer thickness was measured using the Keyence sensor LKG 150, which has linearity of ±0.05% of F.S. and repeatability of 0.5 μm.

Data quality

Lag and run-on are due to imperfections of the biochemical synthesis procedure. Lag indicates that one or more bases failed to be synthesized in current sequencing cycle, while run-on indicates that one or more bases are synthesized prematurely. To correct lag and run-on[43], we used with the BGI's commercial software which also automatically provides interpretable figures and key indicators such as Q30[44].

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Declarations

Conflicts of interest

The authors are employee of BGI-Shenzhen or MGI Tech Co., Ltd. and bound by confidentiality agreements.

Contributions

Y.Q. conceived the idea, conducted experiments to prove the concept, built the automated prototype and direct the project. S.K. discussed and helped with writing of the paper. S.Z. helped with imaging on DNBSEQ-T10. R.M oversaw electrical engineering and coding of the project. Z.L. oversaw mechanical engineering of the project. H.L. helped with fluid simulation and C.X. helped with DNB loading of the biochips.

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Figures

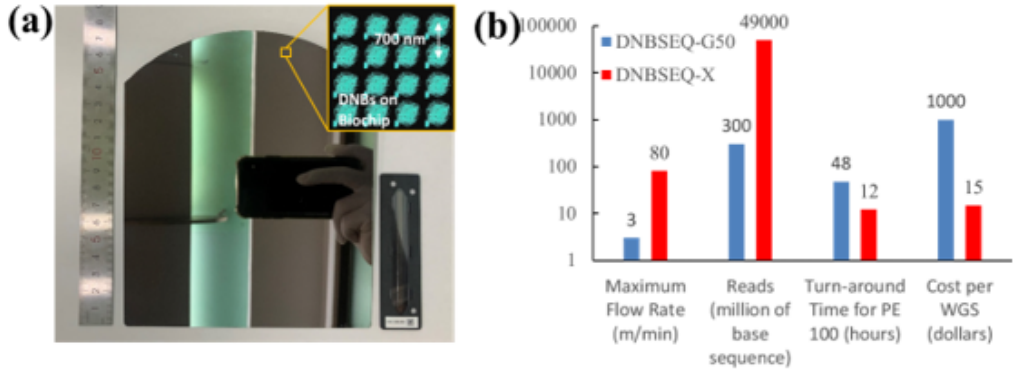


Figure 1

Advantages of SCT. (a) Size comparison of biochips for SCT and an NGS flow cell. Inset show pattern array loading with DNBs. (b) Comparison of the flow rate, reads, turn-around time and cost for a WGS for a conventional DNBSEQ-G50 single-lane flow cell and our SCT prototype.

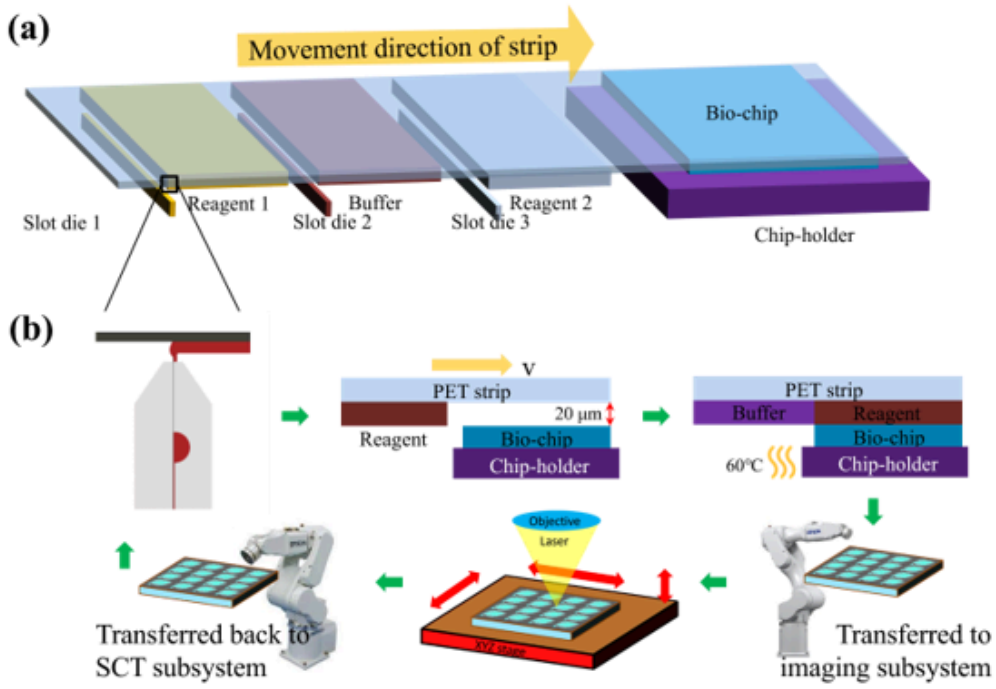


Figure 2

Principle and set-up of the strip-coating technique (SCT) subsystem. (a) Schematic of the coating and replacement process for sequencing reagents, using a moving hydrophilic PET strip to drive flow. (b) Schematic of the workflow for SCT NGS: coating the strip, advance the strip to source reagents, apply heat, transfer the biochip to the imaging subsystem, and return the biochip for the next biochemical cycle.

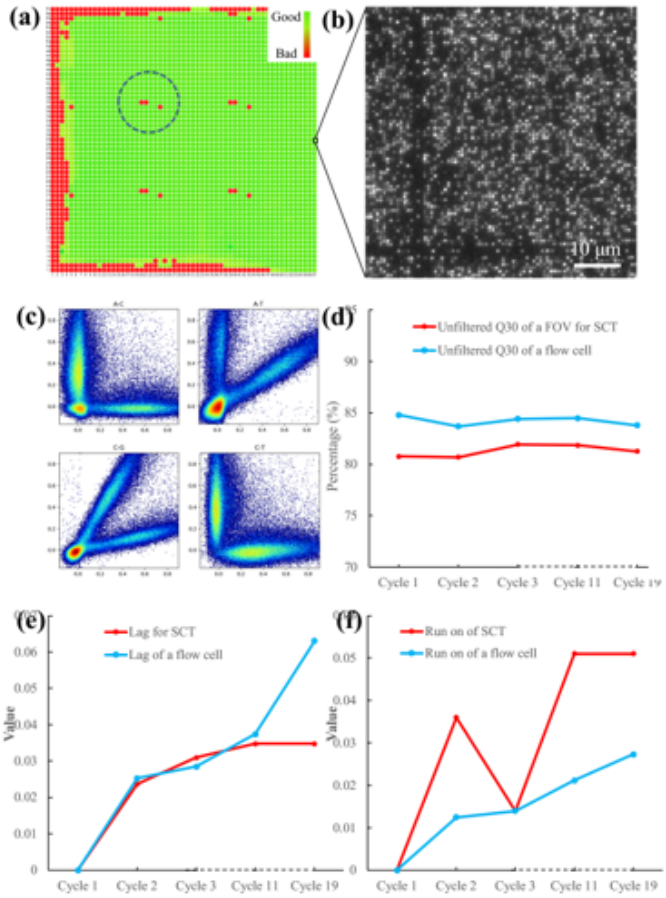


Figure 3

Data quality achieved with our SCT prototype. (a) Base calling information content (BIC) heatmap for a sequencing chip. Green indicates acceptable quality for base calling. The dashed white circle indicates one of the four regions used for image registration. (b) A fluorescent image of the square region of micropatterned DNBs. (c) Crosstalk plots for the A-C, A-T, C-G, and C-T fluorescent channels, respectively, which show good channel separation. (d) Comparison of unfiltered Q30 of a FOV and that of a flow cell for the first 19 cycles. (e) The lag and (f) run on of the first 19 cycles.

Supplementary Files

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- Simulation3.0.508mminnerdiameterbufferexchangeratiois5Xonesecondissetforsingletotalvolumepassage.mp4
- Videoclip1.SCTmachine.mp4
- Videoclip2.automationwithDNBSEQT10.mp4