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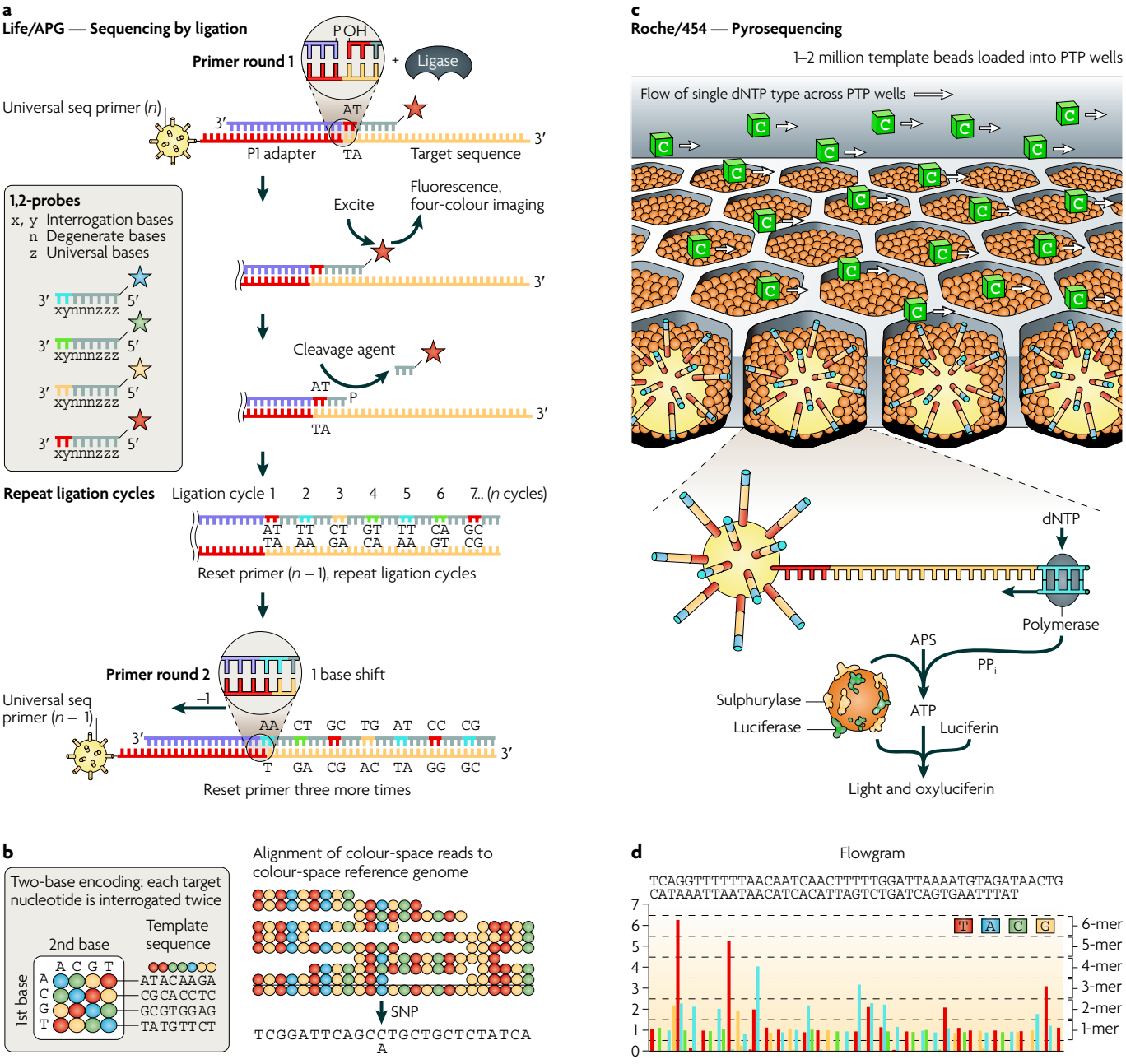


Figure 3 | **Next-generation sequencing technologies that use emulsion PCR. a** | A four-colour sequencing by ligation method using Life/APG's support oligonucleotide ligation detection (SOLiD) platform is shown. Upon the annealing of a universal primer, a library of 1,2-probes is added. Unlike polymerization, the ligation of a probe to the primer can be performed bi-directionally from either its 5′-PO_4 or 3′-OH end. Appropriate conditions enable the selective hybridization and ligation of probes to complementary positions. Following four-colour imaging, the ligated 1,2-probes are chemically cleaved with silver ions to generate a 5′-PO_4 group. The SOLiD cycle is repeated nine more times. The extended primer is then stripped and four more ligation rounds are performed, each with ten ligation cycles. The 1,2-probes are designed to interrogate the first (x) and second (y) positions adjacent to the hybridized primer, such that the 16 dinucleotides are encoded by four dyes (coloured stars). The probes also contain inosine bases (z) to reduce the complexity of the 1,2-probe library and a phosphorothiolate linkage between the fifth and six nucleotides of the probe sequence, which is cleaved with silver ions[106]. Other cleavable probe designs include RNA nucleotides[107,108] and internucleosidic phosphoramidates[107], which are cleaved by ribonucleases and acid, respectively. **b** | A two-base encoding scheme in which four dinucleotide sequences are associated with one colour (for example, AA, CC, GG and TT are coded with a blue dye). Each template base is interrogated twice and compiled into a string of colour-space data bits. The colour-space reads are aligned to a colour-space reference sequence to decode the DNA sequence. **c** | Pyrosequencing using Roche/454's Titanium platform. Following loading of the DNA-amplified beads into individual PicoTiterPlate (PTP) wells, additional beads, coupled with sulphurylase and luciferase, are added. In this example, a single type of 2′-deoxyribonucleoside triphosphate (dNTP) — cytosine — is shown flowing across the PTP wells. The fibre-optic slide is mounted in a flow chamber, enabling the delivery of sequencing reagents to the bead-packed wells. The underneath of the fibre-optic slide is directly attached to a high-resolution charge-coupled device (CCD) camera, which allows detection of the light generated from each PTP well undergoing the pyrosequencing reaction. **d** | The light generated by the enzymatic cascade is recorded as a series of peaks called a flowgram. PP_i, inorganic pyrophosphate.

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hybridized to templates amplified by emPCR (FIG. 1a). The SOLiD cycle of 1,2-probe hybridization and ligation, imaging, and probe cleavage is repeated ten times to yield ten colour calls spaced in five-base intervals (FIG. 3a). The extended primer is then stripped from the solid-phase-bound templates. A second ligation round is performed with an '*n* – 1' primer, which resets the interrogation bases and the corresponding ten colour calls one position to the left. Ten ligation cycles ensue, followed by three more rounds of ligation cycles. Colour calls from the five ligation rounds are then ordered into a linear sequence (that is, the colour space) and aligned to a reference genome to decode the DNA sequence (FIG. 3b). SOLiD uses two slides per run; each can be partitioned into four or eight regions called spots. TABLE 1 depicts the current sequencing statistics of the Life/APG platform operating at the BCM-HGSC (D. Muzny, personal communication). Substitutions are the most common error type. Similar to the genome analysis of Illumina/Solexa reads, SOLiD data have also revealed an underrepresentation of AT-rich and GC-rich regions[26]. Shen and colleagues[38] recently showed that MAQ alignment of SOLiD data may be undercalling true variants.

Single-nucleotide addition: pyrosequencing. Pyrosequencing is a non-electrophoretic, bioluminescence method that measures the release of inorganic pyrophosphate by proportionally converting it into visible light using a series of enzymatic reactions[39,40] (FIG. 3c). Unlike other sequencing approaches that use modified nucleotides to terminate DNA synthesis, the pyrosequencing method manipulates DNA polymerase by the single addition of a dNTP in limiting amounts. Upon incorporation of the complementary dNTP, DNA polymerase extends the primer and pauses. DNA synthesis is reinitiated following the addition of the next complementary dNTP in the dispensing cycle. The order and intensity of the light peaks are recorded as flowgrams, which reveal the underlying DNA sequence (FIG. 3d).

Margulies and colleagues[41] described the first NGS platform to integrate pyrosequencing using their PTP device. Commercialized by Roche/454, the instrument uses DNA templates prepared by emPCR (FIG. 1a), with 1–2 million beads deposited into PTP wells. Roche/454 recently released a titanium-coated PTP design, which substantially increases read length and improves data quality by reducing crosstalk between adjacent wells containing single clonally amplified beads (T. Harkins, personal communication). Smaller beads, which have sulphurylase and luciferase attached to them to facilitate light production, are loaded into wells surrounding the template beads. Individual dNTPs are then streamed across the wells and dispensed in a predetermined sequential order. The bioluminescence is imaged with a charge-coupled device (CCD) camera (FIG. 3c). TABLE 1 shows the current sequencing statistics of the Roche/454 platform operating at the BCM-HGSC (D. Muzny, personal communication). Unlike platforms that produce shorter read lengths, the Roche/454 platform does not require the run time to be doubled for the sequencing of mate-pair templates. For homopolymeric repeats of up to six nucleotides, the number of dNTPs added is directly proportional to the light signal. Insertions are the most common error type, followed by deletions.

Real-time sequencing. The next technology method to hit the commercial sector is likely to be real-time sequencing, and Pacific Biosciences is currently leading this effort[15]. Unlike reversible terminators, real-time nucleotides do not halt the process of DNA synthesis. Simply put, the method of real-time sequencing involves imaging the continuous incorporation of dye-labelled nucleotides during DNA synthesis[42]. With the Pacific Biosciences platform, single DNA polymerase molecules are attached to the bottom surface of individual zero-mode waveguide detectors (ZMW detectors)[43] (FIG. 4a) that can obtain sequence information while phospholinked nucleotides (BOX 1) are being incorporated into the growing primer strand[15] (FIG. 4b).

Other approaches have been proposed to enhance signal-to-noise measurements in real-time sequencing using more conventional detection schemes. For example, Life/VisiGen has engineered DNA polymerases with an attached fluorescent dye that, upon incorporation of their β-labelled nucleotides, produce an enhanced signal by fluorescence resonance energy transfer[16]. LI-COR Biosciences, whose technology was acquired by Pacific Biosciences, has been developing dye-quencher nucleotides (BOX 1), which in their native state produce low signals owing to the presence of a quencher group attached to the base. The release and diffusion of the dye-labelled pyrophosphate analogue away from the immobilized DNA polymerase produces a fluorescent signal[17].

Pacific Biosciences used the highly processive, strand-displacing φ29 DNA polymerase because it efficiently incorporates phospholinked nucleotides and enables the resequencing of closed circular templates[15]. To assess the accuracy of this method, a four-colour sequencing experiment was conducted using a known 150 bp linear template. Base calls from the real-time reads were determined from their corresponding fluorescence pulses (FIG. 4b). When the reads were compared to a known sequence, 27 errors consisting of deletions, insertions and mismatches were identified, corresponding to a read accuracy of approximately 83% (131/158). Factors that led to sequencing errors included extremely short interphase intervals between two incorporation events, and the binding and release of nucleotides in the active site before incorporation into the primer strand. Given that most errors appear as stochastic events, the authors showed that repeated sequencing of the same template molecule 15 times or more could improve the consensus read accuracy to >99%[15]. At the 2009 AGBT meeting, Pacific Biosciences reported improvements to their platform; when it was used to sequence the *E. coli* genome at 38-fold base coverage, 99.3% genome coverage was obtained. The consensus accuracy reached was >99.999% for the entire genome, with read lengths averaging 964 bases (S. Turner, personal communication).

Adjacent valid colour
A nucleotide substitution will have two colour calls, one from the 5′ position and one from the 3′ position of the dinucleotide sequence. When compared with a reference genome, base substitution in the target sequence is encoded by two specific, adjacent colours. In Figure 3b, the sequence 'CCT' is encoded as blue-yellow ('CC' = blue; 'CT' = yellow), but substituting the middle 'C' for 'A' would result in two colour changes to green-red. Any other colour sequence can be discarded as an error.

Colour space
With two-base-encoded probes, the fluorescent signal or colour obtained during imaging is associated with four dinucleotide sequences having a 5′- and 3′-base. Colour space is the sequence of overlapping dinucleotides that codes four simultaneous nucleotide sequences. Alignment with a reference genome is the most accurate method for translating colour space into a single nucleotide sequence.

Zero-mode waveguide detectors
This nanostructure device is 100 nm in diameter, which is smaller than the 532 nm and 643 nm laser wavelengths used in the Pacific Biosciences platform. Light cannot propagate through these small waveguides, hence the term zero-mode. These aluminium-clad waveguides are designed to produce an evanescent wave (see the 'total internal reflection fluorescence' glossary term) that substantially reduces the observation volume at the surface of the polymerase reaction down to the zeptolitre range (10^{-21} l). This provides an advantage for the polymerization reaction, which can be performed at higher dye-labelled nucleotide concentrations.

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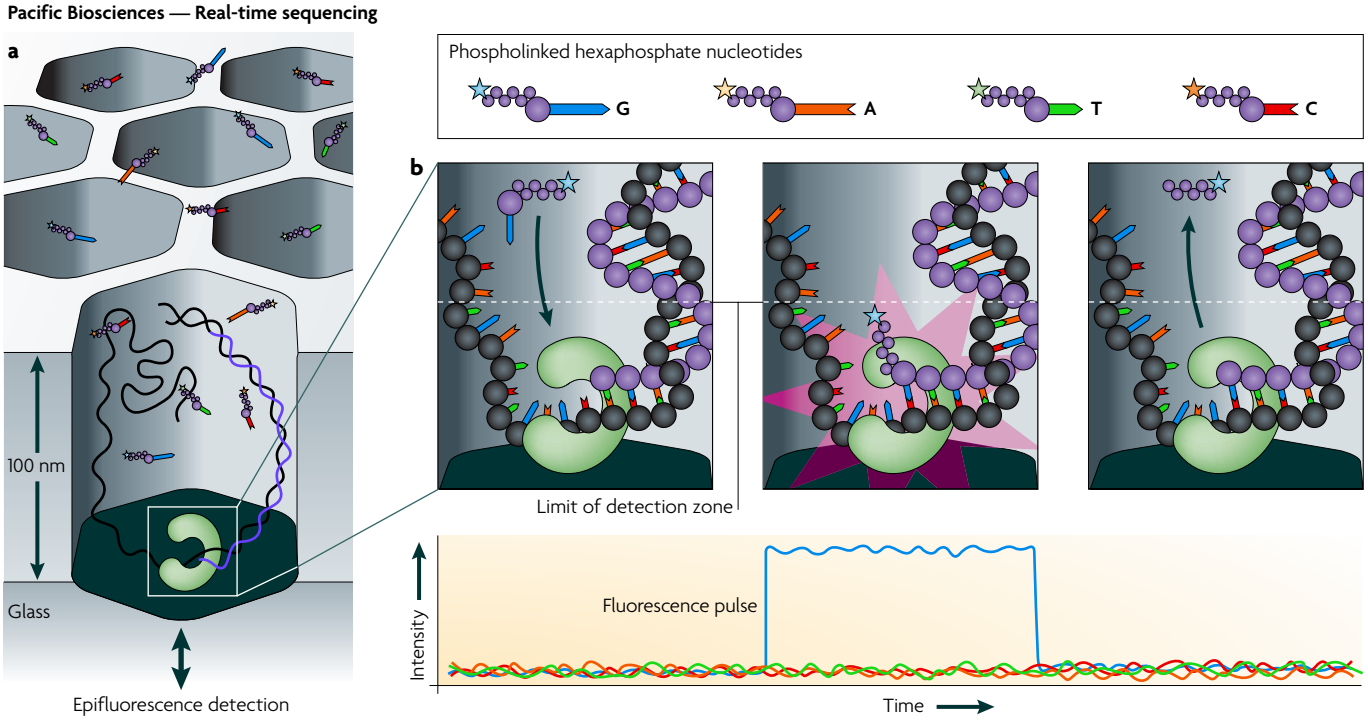


Figure 4 | **Real-time sequencing.** Pacific Biosciences' four-colour real-time sequencing method is shown. **a** | The zero-mode waveguide (ZMW) design reduces the observation volume, therefore reducing the number of stray fluorescently labelled molecules that enter the detection layer for a given period. These ZMW detectors address the dilemma that DNA polymerases perform optimally when fluorescently labelled nucleotides are present in the micromolar concentration range, whereas most single-molecule detection methods perform optimally when fluorescent species are in the pico- to nanomolar concentration range[42]. **b** | The residence time of phospholinked nucleotides in the active site is governed by the rate of catalysis and is usually on the millisecond scale. This corresponds to a recorded fluorescence pulse, because only the bound, dye-labelled nucleotide occupies the ZMW detection zone on this timescale. The released, dye-labelled pentaphosphate by-product quickly diffuses away, dropping the fluorescence signal to background levels. Translocation of the template marks the interphase period before binding and incorporation of the next incoming phospholinked nucleotide.

Genome alignment and assembly

After NGS reads have been generated, they are aligned to a known reference sequence or assembled *de novo*[8,44,45]. The decision to use either strategy is based on the intended biological application as well as cost, effort and time considerations. For example, identifying and cataloguing genetic variation in multiple strains of highly related genomes, such as those found in specific species of bacteria[46–51], *C. elegans*[25,30,38] and *Arabidopsis thaliana*[52], can be accomplished by aligning NGS reads to their reference genomes. This approach is substantially cheaper and faster than Sanger sequencing. SNVs can be readily identified, although in many cases, validation of these findings is required.

There are limitations to the alignment approach, such as placing reads within repetitive regions in the reference genome or in corresponding regions that may not exist in the reference genome[28]; the latter situation may result from gaps in the reference genome or the presence of structural variants (SVs) in the genome being analysed. Mate-pair reads can resolve the correct genome assignment for some repetitive regions as long as one read in the pair is unique to the genome. A study by Egholm, Snyder and colleagues[53] showed that Roche/454 read data (averaging 258 bases), derived from 3-kb sized mate-pair genomic libraries, could capture a larger fraction of SVs in the human genome, although this approach still identified fewer SVs than traditional fosmid-end sequencing approaches[54].

De novo assemblies have been reported for bacterial genomes and mammalian bacterial artificial chromosomes[55–59], but substantial challenges exist for their application to human genomes. A reasonable strategy for improving the quality of the alignment or assembly has been to increase the read coverage. An article by Frazer and colleagues[26] challenges this approach by reporting systematic variability in local sequence coverage that was specific to different human genomic regions for the Roche/454, Illumina/Solexa and Life/APG platforms (other commercially available platforms were not evaluated). Because each NGS platform examined produced a uniquely reproducible pattern of variable sequence coverage, the mixing of different NGS read types in the alignment or assembly may remedy this shortcoming. Recently it was reported that mixing Roche/454 and Illumina/Solexa read data resulted in improved *de novo* assemblies of microbial genomes compared with assemblies based on data from either platform alone[60,61].

Fluorescence resonance energy transfer
This is generally a system that consists of two fluorescent dyes, one being a donor dye (a bluer fluorophore) and the other an acceptor dye (a redder fluorophore). When the two dye molecules are brought into close proximity (usually ≤30 nm), the energy from the excited donor dye is transferred to the acceptor dye, increasing its emission intensity signal.

Structural variants
All sequence variants other than single-nucleotide variants, including block substitutions, insertions or deletions, inversions, segmental duplications and copy-number differences.

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Genome enrichment

Despite the substantial cost reductions associated with NGS technologies in comparison with the automated Sanger method[62], whole-genome sequencing is still an expensive endeavour (see below). An interim solution to this problem may be to use NGS platforms to target specific regions of interest. This strategy can be used to examine all of the exons in the genome, specific gene families that constitute known drug targets or megabase-size regions that are implicated in disease[63] or pharmacogenetics effects[64] through genome-wide association studies. The concept of targeting specific regions of the genome is well established, with PCR being the most widely used method, albeit on a small scale. PCR coupled with Sanger sequencing is suitably matched for analysing a handful of candidate genes[65], but coupling PCR with high-throughput NGS platforms for targeting strategies is not practical, as sample preparation would require handling tens of thousands of primers individually or in large multiplex groups to meet the needs of a single instrument run. A recent article by Frazer and colleagues in collaboration with RainDance Technologies reported the simultaneous amplification of 3,976 products using microdroplet PCR technology[66]. Here, a microfluidic device creates aqueous picolitre-volume droplets of forward- and reverse-targeting primers in an oil solution. The primer droplets that are targeted to different regions of the genome merge with separate picolitre droplets that contain fragmented genomic DNA and associated PCR reagents, and these mixed droplets are thermal cycled in a single tube (FIG. 5a). The authors reported an 84% capture efficiency with 90% of the targeted bases showing uniform coverage using the microdroplet PCR method sequenced with either the Roche/454 or Illumina/Solexa platform[66].

Custom-designed oligonucleotide microarrays and solution-based hybridization strategies have also been used for targeting regions of interest. For example, Roche/NimbleGen[67] oligonucleotide microarrays have been designed to provide complementary sequences for solid-phase hybridization to enrich for exons[68,69] or contiguous stretches of genomic regions[68,70] (FIG. 5b). The BCM-HGSC[68] and Cold Spring Harbor Laboratory[69] groups reported capture efficiencies of 65–77% (Roche/454 platform) and 53% (Illumina/Solexa platform), respectively, with targeted exons being covered by at least one NGS read. Recently, the BCM-HGSC group reported >90% capture efficiency with at least 10× base coverage (Roche/454 and Life/APG platforms) by chip optimization that decreases the number of probes in higher coverage regions while increasing those in low coverage regions (R. A. Gibbs, personal communication).

Other groups have captured specific genomic regions by using solution-based hybridization methods, such as molecular inversion probes (MIPs)[71–73] and biotinylated RNA capture sequences[74] (FIG. 5c). Shendure, Church and colleagues[71] were the first group to use MIPs in exon targeting by designing specific oligonucleotide ends that flank the exons of interest. However, in duplicate experiments only 20% of the targets were captured and even fewer exon regions (11%) were found in both data sets (Illumina/Solexa platform)[71]. Mindrinos, Davis and colleagues[72] recently described several technical improvements that increased the efficiency of MIP capture to >90%, with approximately 70% of targets falling within a 10-fold range in 485 exons. Shendure and colleagues[73] have also described improvements that enabled them to capture 91% of their initial 55,000 targets (Illumina/Solexa platform). An alternative method has been reported by Nusbaum and colleagues[74], who created biotinylated RNA capture sequences that are hybridized to genomic targets and subsequently enriched with streptavidin-coated magnetic beads (Illumina/Solexa platform) (FIG. 5c). This group estimated a capture efficiency of 60% for exons and 80% for genomic regions. The 1000 Genomes Project and The Exome Project have adopted microarray-based and solution-based strategies that use Roche/454, Illumina/Solexa and Life/APG platforms to target regions of interest.

Although any of the NGS platforms can perform the sequencing phase of targeted capture, some may be better suited than others (TABLE 1). Initial reports raised concerns as to the readiness of targeted capture for routine use[75,76] owing to the high degree of variability in coverage and representation, the capturing of off-target fragments, the requirement for microgram quantities of starting material and the high cost for microarrays, but improvements are being made to address these limitations[76].

Next-generation sequencing applications

The production of large numbers of low-cost reads makes the NGS platforms described above useful for many applications. These include variant discovery by resequencing targeted regions of interest or whole genomes, *de novo* assemblies of bacterial and lower eukaryotic genomes, cataloguing the transcriptomes of cells, tissues and organisms (RNA–seq)[5], genome-wide profiling of epigenetic marks and chromatin structure using other seq-based methods (ChIP–seq, methyl–seq and DNase–seq)[4], and species classification and/or gene discovery by metagenomics studies[77]. With this many applications, which platform is best suited for a given biological experiment? For example, the Illumina/Solexa and Life/APG platforms are well suited for variant discovery by resequencing human genomes because gigantic volumes of high-quality bases are produced per run (TABLE 1). Furthermore, the Helicos BioSciences platform is well suited for applications that demand quantitative information in RNA–seq[78] or direct RNA sequencing, as it sequences RNA templates directly without the need to convert them into cDNAs[79]. TABLE 1 provides an overview of NGS technologies, instrument performance and cost, pros and cons, and recommendations for biological applications; however, the rapid pace of technological advances in the field could change this information in the near future. Readers are directed to several excellent reviews on RNA–seq[5], ChIP–seq[80] and metagenomics[77].

In the following section, I highlight recent advances in sequencing personal genomes using NGS, as demand in this area is driving rapid technological developments and competitive pricing.

1000 Genomes Project
A project aimed at discovering rare sequence variants with minor allele frequencies of 1% in normal genomes derived from HapMap samples.

The Exome Project
A project aimed at developing and validating cost-effective, high-throughput technologies for resequencing all of the protein-coding regions of the human genome.

Metagenomics
The study of communities of mixed microbial genomes that reside in animals, plants and environmental niches. Samples are collected and analysed without the need to culture isolated microbes in the laboratory. The Human Microbiome Project aims to characterize a reference set of microbial genomes from different habitats within the human body, including nasal, oral, skin, gastrointestinal and urogenital regions, and to determine how changes in the human microbiome affect health and disease.

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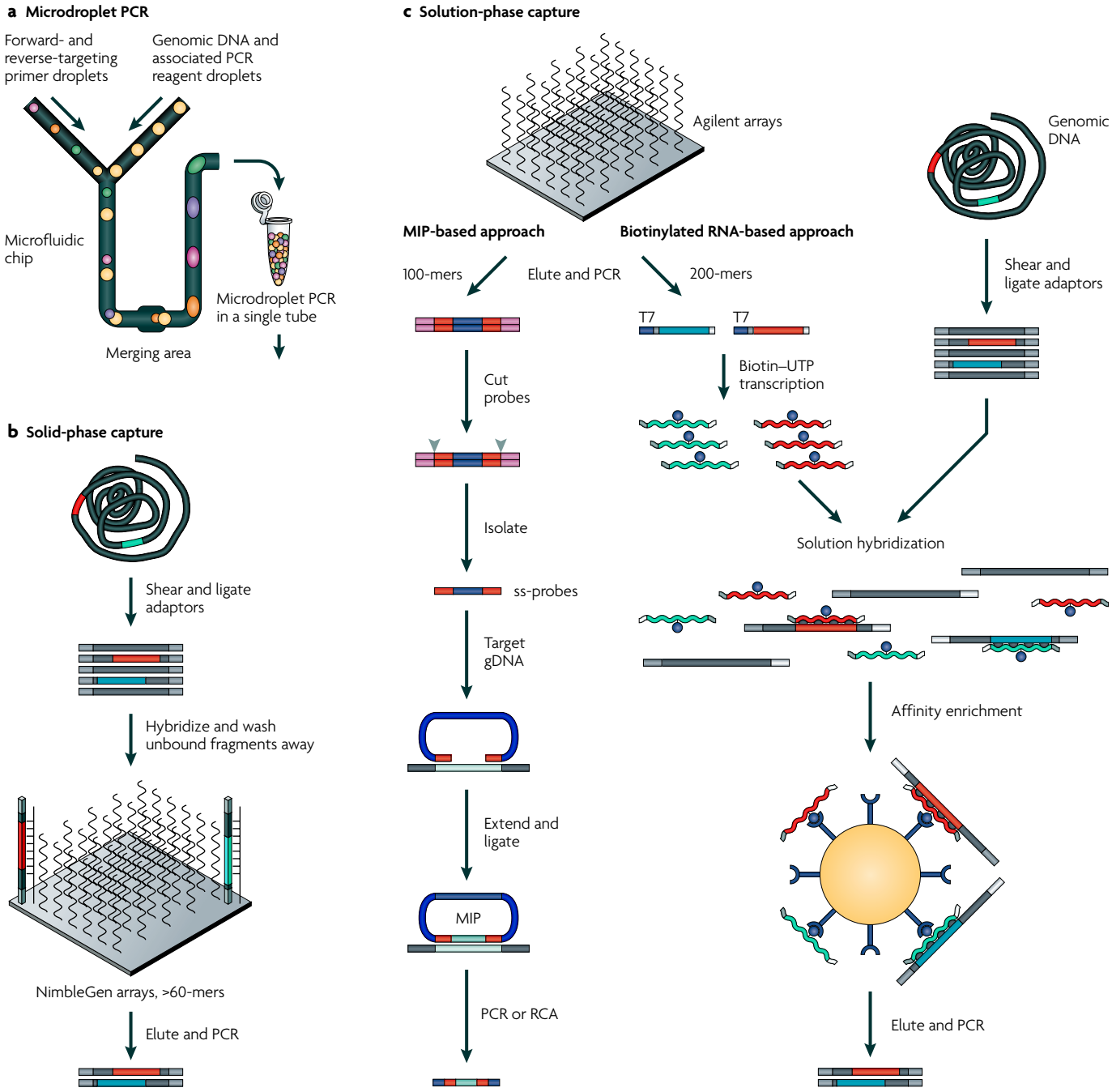


Figure 5 | **Targeted capture scheme. a** | A microfluidic nozzle creates aqueous droplets (8 pl) of forward- and reverse-targeting primers in oil (not shown), which are dispensed into a channel in a microfluidic chip. Fragmented genomic DNA and associated PCR reagents are separately dispensed as aqueous microdroplets (14 pl) and paired together with the primer droplets at a 1/1 ratio. Paired droplets pass through a merging area for coalescence into PCR-competent microdroplets, which are collected into a single tube for thermal cycling. **b** | Solid-phase capture methods ligate adaptor sequences to fragmented genomic DNA before microarray hybridization. Following stringent washing to remove non-selected genomic fragments, the enriched targets are eluted, PCR amplified and sequenced. Adaptor sequences can also be designed specifically for a given next-generation sequencing platform, which eliminates the PCR step. **c** | Solution-phase methods use custom-designed microarrays from Agilent Technologies to synthesize long, target-specific oligonucleotides, which are cleaved as a pool of probes from the support and eluted into a single tube. In the molecular inversion probe (MIP)-based approach, single-stranded probes (ss-probes) are hybridized to their corresponding templates. DNA polymerase extends the 3′-end of the probes across the exon region, and a ligation step creates closed circular DNAs. The biotinylated RNA-based approach introduces a T7 promoter sequence, which allows the incorporation of biotin-labelled uridine-5′-triphosphate (UTP) into the probe sequence by *in vitro* transcription. Following solution hybridization with genomic targets, the RNA–DNA hybrids are enriched by their binding to streptavidin-coated magnetic beads. RCA, rolling circle amplification.

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Table 2 | **Sequencing statistics on personal genome projects**

Personal Genome	Platform	Genomic template libraries	No. of reads (millions)	Read length (bases)	Base coverage (fold)	Assembly	Genome coverage (%)*	SNVs in millions (alignment tool)	No. of runs	Estimated cost (US$)
J. Craig Venter	Automated Sanger	MP from BACs, fosmids & plasmids	31.9	800	7.5	*De novo*	N/A	3.21	>340,000	70,000,000
James D. Watson	Roche/454	Frag: 500 bp	93.2‡	250§	7.4	Aligned*	95‖	3.32 (BLAT)	234	1,000,000¶
Yoruban male (NA18507)	Illumina/ Solexa	93% MP: 200 bp	3,410‡	35	40.6	Aligned*	99.9	3.83 (MAQ)	40	250,000¶
		7% MP: 1.8 kb	271	35				4.14 (ELAND)		
Han Chinese male	Illumina/ Solexa	66% Frag: 150–250 bp	1,921‡	35	36	Aligned*	99.9	3.07 (SOAP)	35	500,000¶
		34% MP: 135 bp & 440 bp	1,029	35						
Korean male (AK1)	Illumina/ Solexa	21% Frag: 130 bp & 440 bp	393‡	36	27.8	Aligned*	99.8	3.45 (GSNAP)	30	200,000¶
		79% MP: 130 bp, 390 bp & 2.7 kb	1,156	36, 88, 106						
Korean male (SJK)	Illumina/ Solexa	MP: 100 bp, 200 bp & 300 bp	1,647‡	35, 74	29.0	Aligned*	99.9	3.44 (MAQ)	15	250,000¶,#
Yoruban male (NA18507)	Life/APG	9% Frag: 100–500 bp	211‡	50	17.9	Aligned*	98.6	3.87 (Corona-lite)	9.5	60,000¶,**
		91% MP: 600–3,500 bp	2,075‡	25, 50						
Stephen R. Quake	Helicos BioSciences	Frag: 100–500 bp	2,725‡	32§	28	Aligned*	90	2.81 (IndexDP)	4	48,000¶
AML female	Illumina/ Solexa	Frag: 150–200 bp‡‡	2,730‡,‡‡	32	32.7	Aligned*	91	3.81‡‡ (MAQ)	98	1,600,000‖‖
		Frag: 150–200 bp§§	1,081‡,§§	35	13.9		83	2.92§§ (MAQ)	34	
AML male	Illumina/ Solexa	MP: 200–250 bp‡‡	1,620‡,‡‡	35	23.3	Aligned*	98.5	3.46‡‡ (MAQ)	16.5	500,000‖‖
		MP: 200–250 bp§§	1,351‡,§§	50	21.3		97.4	3.45§§ (MAQ)	13.1	
James R. Lupski CMT male	Life/APG	16% Frag: 100–500 bp	238‡	35	29.6	Aligned*	99.8	3.42 (Corona-lite)	3	75,000¶,¶¶
		84% MP: 600–3,500 bp	1,211‡	25, 50						

*A minimum of one read aligning to the National Center for Biotechnology Information build 36 reference genome. ‡Mappable reads for aligned assemblies. §Average read-length. ‖D. Wheeler, personal communication. ¶Reagent cost only. #S.-M. Ahn, personal communication. **K. McKernan, personal communication. ‡‡Tumour sample. §§Normal sample. ‖‖Tumour & normal samples: reagent, instrument, labour, bioinformatics and data storage cost, E. Mardis, personal communication. ¶¶R. Gibbs, personal communication. AML, acute myeloid leukaemia; BAC, bacterial artificial chromosome; CMT, Charcot–Marie–Tooth disease; Frag, fragment; MP, mate-pair; N/A, not available; SNV, single-nucleotide variant.

Personal genomes. Human genome studies aim to catalogue SNVs and SVs and their association to phenotypic differences, with the eventual goal of personalized genomics for medical purposes. In 2004, the International Human Genome Sequencing Consortium published the first, and still only, finished-grade human reference genome (currently National Center for Biotechnology Information (NCBI) build 36)[1]. Its cost was estimated at US$300 million. In October 2007, Venter and colleagues[81] described the genome sequence of J. Craig Venter using a whole-genome shotgun approach coupled with automated Sanger sequencing (TABLE 2). When the Venter genome was compared with the reference genome, 3.2 million SNVs were identified. In addition, there were over 900,000 SVs, which altogether accounted for more variant bases than the SNVs.

The first group to apply NGS to whole human genomes was Roche/454 in collaboration with Gibbs and colleagues at the BCM-HGSC, who reported the diploid genome of James D. Watson[82] (TABLE 2). When the Watson genome was compared with the reference genome, roughly 3.3 million SNVs were identified, but far fewer SVs were found than in the study by Venter and colleagues. This is important because undiscovered SVs could account for a substantial fraction of the total number of sequence variants, many of which could be potentially causative in disease[83–86]. Within the past year, five additional human genomes have been described[23,87–91], one of which was sequenced on two different NGS platforms (TABLE 2). As with the Watson genome, far fewer SVs were reported than in the study by Venter and colleagues.

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The Cancer Genome Atlas
A project aimed at discovering single-nucleotide variants and structural variants that are associated with major cancers, such as brain cancer (glioblastoma multiforme), lung cancer (squamous carcinoma) and ovarian cancer (serous cystadenocarcinoma).

Personal Genome Project
A project aimed at providing open access to human genome sequences from volunteers and to develop tools for interpreting this information and correlating it with related personal medical information.

Personal genomics is also being applied to the study of disease. For example, Mardis and colleagues[29,92] have reported the sequencing of two acute myeloid leukaemia cancer genomes using the Illumina/Solexa platform, and both studies identified somatic mutations that may be associated with the disease. Gibbs and colleagues have recently described the elucidation of both allelic variants in a family with a recessive form of Charcot–Marie–Tooth disease using the Life/APG platform[93] (TABLE 2).

Several projects aimed at sequencing more individuals, including The Cancer Genome Atlas[94] and the 1000 Genomes Project, are also using the Illumina/Solexa and Life/454 platforms to sequence whole genomes. Complete Genomics (see below) recently described the first genome sequence from a Caucasian male (PGP1) enrolled in the Personal Genome Project. These projects should lead to a substantial increase in the number of personal genomes sequenced in the near future.

In comparison with automated Sanger sequencing, NGS platforms have dramatically increased throughput and substantially lowered expenditure, with several groups reporting reagent costs of below US$100,000 (TABLE 2). However, there is variability among and within NGS platforms in terms of template size and construct, read-length, throughput, and base and genome coverage (TABLE 2), and such variability makes it difficult to assess the quality (that is, the base accuracy, genome coverage and genome continuity) of genomes based on cost considerations. In June 2009, Illumina announced a personal genome sequencing service that provides 30-fold base coverage for the price of US$48,000. Complete Genomics offers a similar service with 40-fold coverage priced at US$5,000, based on a business model that is reliant on huge customer volume. Recently, Drmanac, Ballinger and colleagues sequenced the whole genomes of three individuals, including PGP1, using a modified non-cleavable probe SBL method known as combinatorial probe anchor ligation (cPAL). The number of SNVs identified was consistent with those reported in TABLE 1 for other genomes, and the group reported an amortized cost of US$4,400 in reagents[95]. Although impressive, it is unclear whether this price will be sustainable as a retail service[96] or whether the Illumina or Complete Genomics business models will see long-term profits. Achieving these cost advantages may also come with a substantial trade-off, namely the production of lower quality genomes that inadequately capture the extent of SVs[83–86]. Closing the gap between $10,000 and $1,000 will be the greatest challenge for current technology developers, and the $1,000 genome might result from as-yet-undeveloped innovations. A timetable for the $1,000 draft genome is difficult to predict, and even more uncertain is the delivery of a high-quality, finished-grade personal genome.

Closing remarks

Since 2004, the National Human Genome Research Institute has awarded more than $100 million for the development of NGS technologies[62], and these awards have facilitated much of the progress in NGS to date, as well as several commercial developments. Many companies, including IBM, Oxford Nanopore Technologies, Intelligent Bio-Systems, LaserGen, Inc. and NABsys, have NGS technologies at various stages of development and commercialization.

The production of billions of NGS reads has also challenged the infrastructure of existing information technology systems in terms of data transfer, storage and quality control, computational analysis to align or assembly read data, and laboratory information management systems for sample tracking and process management. Advances in bioinformatics are ongoing, and improvements are needed if these systems are to keep pace with the continuing developments in NGS technologies. It is possible that the costs associated with downstream data handling and analysis could match or surpass the data-production costs described in this Review.

NGS technologies have an impressive range of applications, and more are being developed. In addition to the applications described above, NGS technologies are being used to characterize the evolutionary relationships of ancient genomes[97,98] and to elucidate the role of non-coding RNAs in health and disease[99,100]. In the not too distant future, it is foreseeable that NGS technologies could be used to obtain high-quality sequence data from a genome isolated from a single cell, which would be a substantial breakthrough, particularly for cancer genomics. For this to occur, advances will be needed in techniques for efficiently isolating intact long DNA molecules and in NGS methods for accurately reading the sequence content of these molecules. The field of NGS development and applications is a fast-moving area of research, which makes this an exciting time for genomic studies.

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Competing interests statement

The author declares competing financial interests: see Web version for details.

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