Trends in Biotechnology | Industry Highlight

Absolute quantification of cell-free DNA for prenatal genetics and oncology

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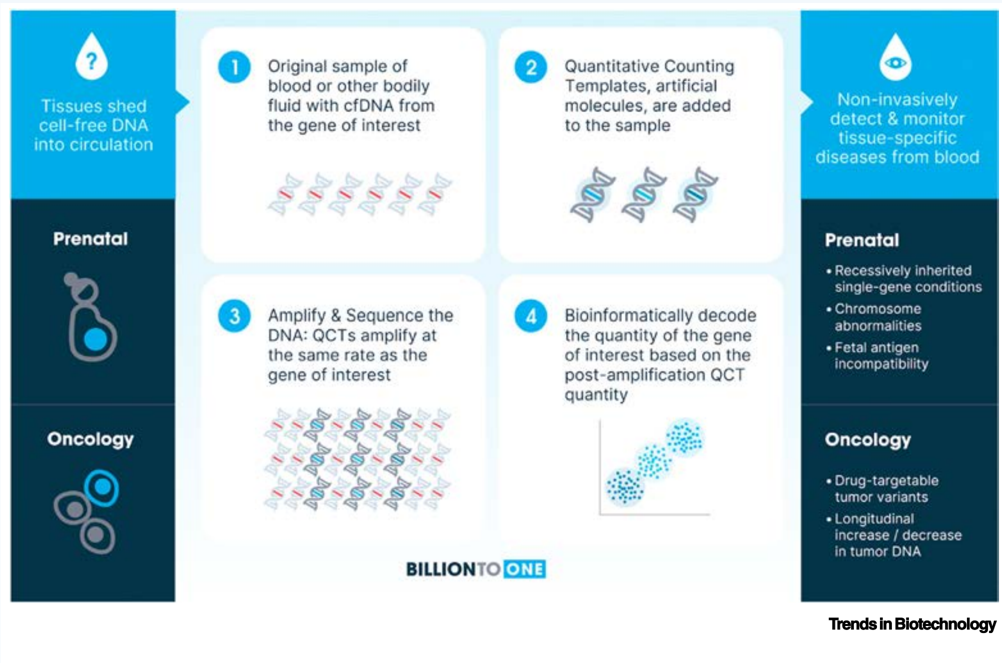

KEY FACTS:

cfDNA are highly fragmented DNA fragments (~150bp) that are shed by apoptotic cells and found in blood.

cfDNA has been used as an analyte to noninvasively detect diseases in diverse tissues: fetal and placenta DNA in pregnancy, tumor DNA in cancer patients, or donor-derived DNA in transplant patients to monitor organ rejection.

Both genetic and epigenetic properties of cfDNA reflect the originating tissue and are used to measure disease.

The use of cfDNA in diagnostics and screening is burdened by low concentration (<10 copies/ml in plasma), requiring ultrasensitive testing methods.

QCTs enable absolute quantification of cfDNA at low concentrations across 1000 genomic loci.

There are 3.6M births per year and 17M patients living with cancer in the USA, all of whom can benefit from noninvasive cfDNA-based testing.

Noninvasive cfDNA-based testing in at-risk pregnancies enables early diagnosis and treatment, including *in utero* and perinatal gene-based treatments.

New cancer treatments are increasingly directed at molecular targets. There are >70 FDA-approved cancer therapies, with 12 new biomarker-directed indications approved in 2023 alone.

Quantification of tumor DNA in blood has shown great promise for predicting relapse, progression, and response to therapy.

Circulating cell-free DNA (cfDNA) are short, extracellular DNA fragments (<200bp) that are found in body fluids such as blood, urine, and cerebral spinal fluid. cfDNA in blood primarily originates from apoptosis and has a short half-life of ~1–2 h. Thus, the concentration of cfDNA provides a real-time snapshot of cellular turnover due to diverse physiological and pathological processes. The accessibility of blood as a specimen type is also attractive for clinical translation as a convenient ‘liquid biopsy’. Diagnostics utilizing cfDNA as an analyte are gaining adoption in clinical practice to noninvasively assess fetal genetics, genetic profiling of tumors, and rejection of transplanted organs.

cfDNA-based diagnostic and screening tools require robust sequencing, precise quantitation, and scalability to be clinically impactful. However, DNA samples undergo numerous enzymatic, amplification, and other biochemical steps prior to sequencing. Each of these steps introduces technical noise that makes it harder to quantify the absolute and relative abundance of cfDNA sequences in the biological specimen, resulting in lower sensitivity and specificity.

BillionToOne has developed Quantitative Counting Templates (QCT) to obtain precise DNA quantification data across thousands of genomic loci. QCTs are synthetic DNA fragments that mimic the biochemical properties of the gene of interest and also incorporate a sequence tag to uniquely identify each synthetic DNA molecule. QCTs are added to the specimen at an early point in the testing process to act as internal controls for downstream molecular biology techniques [such as DNA extraction, PCR amplification, and next-generation sequencing (NGS) library construction and sequencing]. Each sample’s sequencing data then undergoes bioinformatic analysis to determine systematic biases specific to sequence, sample, and batch effects. Once these biases are determined, correction factors are then applied to obtain accurate quantification measurements, with the result of increasing assay sensitivity.

QCTs enable absolute quantification when integrated with NGS workflows. PCR is highly efficient at exponentially amplifying DNA via thermal cycling. However, target quantification based on the endpoint amplified PCR product is challenging because slight perturbations in initial conditions are also compounded exponentially. Since QCTs co-amplify with the target DNA in each PCR cycle, both the amplification rate and starting amount of target DNA is determined by bioinformatically analyzing the amplified and sequenced QCT DNA. The precise number of QCT molecules added to each specimen is calculated bioinformatically through sequence tags. This method is therefore ‘calibration-free’ and does not require careful measurement and titration of spike-in DNA concentrations, a feature that is particularly advantageous for quantitative assessments across multiple timepoints in longitudinal monitoring contexts.

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The precision and scalability of QCT-powered assays make them ideal for addressing clinically significant challenges where rare-variant detection/screening coupled with high sensitivity are essential. Here are two key areas where QCTs are driving innovation.

Noninvasive prenatal testing (NIPT): during pregnancy, approximately 10% of cfDNA in maternal blood originates from the fetus. QCT-enabled NIPTs provide actionable genetic information, including detection of fetal aneuploidies, single-gene disorders, and blood antigen incompatibilities, all from a simple blood draw from the pregnant individual. These tests reduce the need for invasive procedures like amniocentesis and have been validated in over 50 000 patients, demonstrating exceptional sensitivity and specificity.

Oncology: due to the low circulating tumor DNA (ctDNA) concentration in this population (0.01–10.0%), highly sensitive and precise liquid biopsies are valuable when time is critical or when tissue biopsies are not feasible. Increased sensitivity allows for more alterations to be found, which may be clinically actionable and enable therapy selection and initiation sooner. Similarly, highly sensitive and precise tools are required for therapy response monitoring as the abundance of ctDNA correlates with tumor burden and reflects dynamic changes in response to therapy. Longitudinal monitoring with QCTs often detects changes in tumor burden at the molecular level, well before conventional imaging techniques like magnetic resonance imaging (MRI) or computed tomography (CT) scans can provide visual confirmation, thereby providing clinicians with a powerful and timely tool for personalized treatment decisions.

The discovery of cfDNA, coupled with advancements in sequencing technologies, has revolutionized diagnostics over the past decade. However, most clinical applications have been limited to presence/absence detection, leaving the potential of precise cfDNA quantification largely untapped. Absolute quantification by QCT technology is bridging this gap, enabling highly sensitive and accurate measurements that expand the scope of cfDNA diagnostics.

Declaration of interests

D.S.T. is a co-founder of BillionToOne, has a >5% ownership stake, and is the inventor of patents assigned to the company.

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