Healthcare industry BW

A complete exome sequence in one week

The exome is the part of the genome that is formed by exons, i.e. the DNA regions that code for proteins and other functional products. Though comprising only about 1% of the total genome, the majority of disease-causing mutations occur in the exome. Selectively sequencing the coding regions of the genome is quicker than whole-genome sequencing. Therefore, exome sequencing plays an important role in the diagnosis of genetic diseases and cancer. Konstanz-based GATC Biotech AG has designed InViewTM 1-Week Exome Diagnostic, an exome sequencing service with the world's fastest turn-around time.

Compared to the complete human genome which comprises more than three billion base pairs, the exome with its around 30 million base pairs is relatively small. Nevertheless, exome sequencing is still relatively expensive and time-consuming. Using next-generation sequencing (NGS) technologies, it takes about four to eight weeks to sequence the entire exome. "Although next-generation sequencing has revolutionised opportunities for research and clinical settings, four to eight weeks is still rather a long time, and makes it hard for patients who are waiting for a diagnosis to cope," says Peter Pohl, CEO of DNA sequencing provider GATC Biotech AG in Konstanz. "Therefore, there is huge demand for faster and cheaper analysis methods."

GATC Biotech AG has long-standing experience in exome sequencing. It is part of the International Cancer Genome Consortium (ICGC), one of the world's largest interdisciplinary large-scale biomedical projects aimed at elucidating the molecular causes of cancer, and has deciphered more than 1000 exomes since 2009. The company went on to design InView™ 1-Week Exome Diagnostic for the early and more accurate diagnosis of diseases, putting its comprehensive experience in exome sequencing to good use in the provision of this service.

Peter Pohl, CEO of GATC Biotech AG in Konstanz, explaining the simple operation of the new MiSeq sequencing robot.

The first step towards making exome sequencing more time- and cost-efficient was the purchase of a new sequencing platform, the MiSeq sequencing system from Illumina. The system's big brother, Illumina's HiSeq sequencing system, is used for next-generation sequencing as well as for exome sequencing.

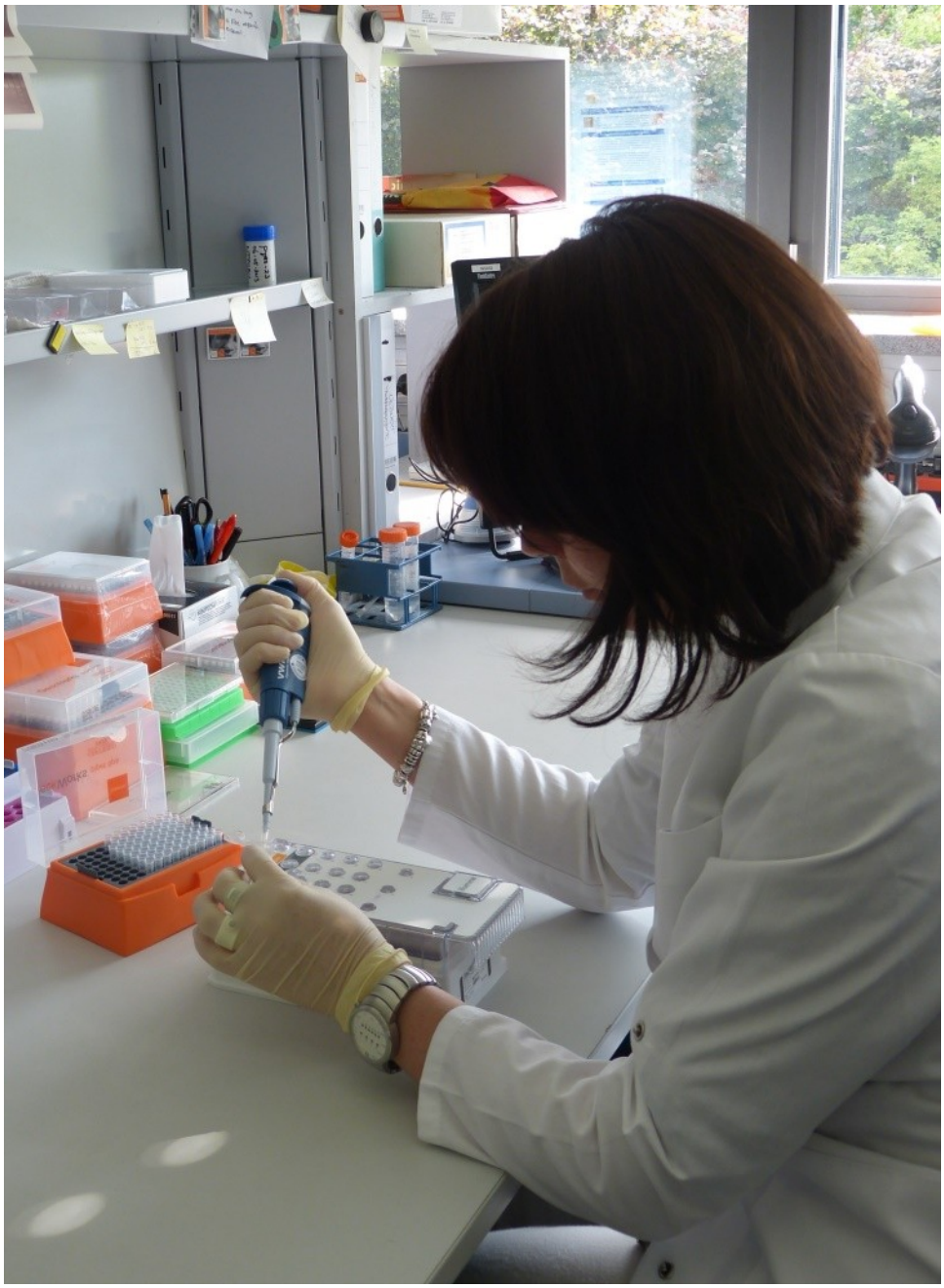
The HiSeq system can sequence up to 600 billion nucleotides in the form of 6 billion reads of 100 nucleotides each, but requires around a week to complete a sequencing run. The MiSeq personal sequencing system does not achieve this output, but is much faster. "The MiSeq sequencer has the ability to sequence across a wide range of applications, and is specifically suited for small genome sequencing," Pohl says. In the dimensions of modern sequencing technologies, the human exome counts as a small genome.

Known application in a new system

As the MiSeq and HiSeq systems are based on the same data format and concept, HiSeq exome sequencing was simply transferred to the MiSeq sequencing system obviating the need for developing a new system from scratch. “We initially had to find out which applications were suitable for the MiSeq system,” says Dr. Kerstin Stangier, Director of Next-Generation Sequencing at GATC Biotech. Sample preparation and bioinformatics were adapted to the new sequencing system and validated in order to obtain ISO certification for the new application.

The exome sequencing workflow always starts with enrichment, i.e. the selective capturing of genomic regions of interest (in this case the exons) from a DNA sample. Only this part of the genome is later sequenced. The entire exome, i.e. small fragments of between 350 and 400 base pairs, is amplified in order to produce enough material for subsequent sequencing. Each DNA fragment is sequenced several times in order to compensate for potential sequencing errors. This leads to a high exome coverage and enables the correct sequence to be derived from the majority of fragments sequenced, even though a mistake might appear somewhere. “InViewTM 1-Week Exome Diagnostic has a coverage of 30 to 120, which means that each fragment is sequenced between 30 and 120 times,” Dr. Stangier says. The speed of the application does not therefore impact on the quality and reliability of the results.

From mutation to diagnosis



Dr. Kerstin Stangier, Director of Next-Generation Sequencing at GATC Biotech, loading a MiSeq sequencing system sample cassette.

Subsequent sequence analysis enables the detection and annotation of single base pair variations (single nucleotide polymorphisms, SNPs) as well as smaller insertions and deletions. This is achieved by comparing the sequenced DNA fragments with corresponding fragments of a human reference genome.

Subsequent analyses provide information on the effects of a mutation. “Amongst other things, we are checking which gene the mutation is located in and how the change in the nucleotide sequence affects the amino acid sequence of the corresponding protein,” Dr. Stangier says. At best, the mutation might be a silent one that does not lead to any change in the amino acid sequence; but the mutation might also generate a stop codon, a nucleotide triplet that signals termination of protein synthesis, resulting in a defective protein. “Moreover, some SNPs are known to be associated with a particular disease,” Dr. Stangier says.

The further interpretation of the data still remains a huge challenge that – at least for the time being – can only be overcome by human geneticists with

specialist knowledge rather than by GPs. “Knowledge is increasing, and I am sure that a new generation of bioinformatics tools that support researchers and doctors in the medical assessment and interpretation of genetic changes will be developed,” says Pohl.

Personalised medicine as future standard

The demand for exome sequencing is already immense and continues to grow. Exome sequencing opens the way to a more personalised patient care in the field of cancer and helps select therapies adapted to particular types of cancer. “In theory, this spares patients ineffective, burdensome treatments, and generates savings for the healthcare system; although exome sequencing has its price, it is nevertheless cheaper than chemo- and radiotherapies that elicit no response in patients with particular cancers,” Dr. Stangier says.

Exome sequencing generates a plethora of information, which has the advantage that where a patient is suspected to have a particular genetic disease, further analyses will then focus on the disease-causing gene as well as on all other genes whose expression might have an effect on the disease-causing one. This could be transcription factors or any type of other modulator. This also assists in the detection of untypical and synergistic changes. “The presence of a single gene mutation usually only means that a person might be at a higher risk of acquiring a particular disease. However, whether the disease develops or not depends on many other factors,” Dr. Stangier explains.

The ever-growing possibilities opened up by the new sequencing technologies will also in future contribute to the increasing importance of genetic diagnostics. “This is the beginning of an era with new concepts, new companies and new tools. I am convinced that exome sequencing will become a medical standard in the diagnosis and personalisation of medicine within the next few years,” says Pohl.

Further information:

GATC Biotech AG
Jakob-Stadler-Platz 7
78467 Konstanz
Tel.: +49 (0) 7531 81 60 0
www.gatc-biotech.com