Microsynth

Custom Contract Research Services

Contract research capabilities in the field of molecular genetics delivered how, where and when you need it.

Introduction

Microsynth has been founded more than 30 years ago as a DNA and RNA oligo synthesis service company. Step by step, additional services such as Sanger sequencing, polymerase chain reaction (PCR), real-time PCR (qPCR) and digital PCR services, and Next-Generation Sequencing (NGS) were added. By now, a comprehensive toolbox covering the realm of molecular genetics is at Microsynth's disposition.
Microsynth's skills in combining the different methods to establish a tailored workflow to successfully meet the customer's demand has constantly grown with each project and challenge mastered. Here, we want to provide an overview how Microsynth can suit customers with different needs in contract research capabilities, including clients who need to comply with regulatory demands.
In a general view, the relevant core parameters in molecular genetics are the sequences of DNA and RNA molecules, as well as by the abundance of DNA and RNA molecules with a given sequence identity. Or, in other words, the main analytical tasks are (1) to decipher or to produce the correct DNA and RNA sequences, and (2) to count or quantify DNA and RNA molecules with a given sequence. In addition (3), data interpretation consists of comparing sequences and their abundances to references and/or among different samples.
In the light of this, the main methods of our portfolio are shortly discussed, with advantages and limitations.

Scientific Methods Expertise

Sanger sequencing is useful to decipher the DNA sequence of molecules up to 1'000 nucleotides per reaction. A DNA primer and sufficient template DNA are required for its success. Therefore, for each reaction, some sequence information must be known, and it cannot be directly applied on genomic DNA without a preceding PCR amplification. Sanger sequencing is relatively fast and cheap, and it is mostly limited to homogenous samples.

Next Generation Sequencing (NGS), typically performed on the Illumina sequencing platforms (see ***Figure 1***), in turn provides massive parallel sequencing of millions, even billions of molecules. This allows the determination of up to 2'400 Gb in one sequencing run. In principle, no prior sequence information is required, however, the focus on defined sequences via amplicon sequencing represent a widespread application. NGS has revolutionized molecular genetics and opened a wealth of new applications. However, the read length of Illumina sequencing reaction is limited to 300 nucleotides paired-ended. This means that long DNA molecules, for example genomes, are fragmented into multiple small pieces for sequencing. The sequence information of the long original fragments needs to be reconstructed with computational methods, which are limited, especially due to the presence of repetitive sequence. More recently, long-read sequencing technologies such as Oxford Nanopore or PacBio have been developed to overcome these limitations and are increasingly popular to complement or even substitute Illumina short-read sequencing. The disadvantage of short Illumina reads does not apply to short templates such as cDNA derived from transcribed RNA, or PCR amplicons of up to about 500 base pairs (bp).
In addition to identifying DNA and RNA sequences, NGS can also be used to count molecules. For example,

Microsynth AG, Switzerland
Schützenstrasse 15 · P.O. Box · CH-9436 Balgach · Phone +41 71 722 83 33 · Fax +41 71 722 87 58 · info@microsynth.ch · www.microsynth.com

using RNA sequencing the abundance of a given mRNA is determined by assessing the number of normalized reads. Similarly, the abundance of a given microbial species is determined by the relative number of matching sequences using an amplicon metagenomics approach. A similar logic applies to the fraction of successful edits in a CRISPR/Cas9 experiment.

To precisely determine the number of specific RNA or DNA molecules at relatively lower cost and in higher sample throughput settings compared to NGS, **PCR methods** are recommended, specifically quantitated real-time PCR (qPCR) or digital PCR. With qPCR, the amount of amplified DNA is measured upon each PCR cycle, taking advantage of the activation of fluorescence upon product formation. The number of PCR cycles required to reach a certain signal threshold correlates with the amount of starting template. However, the actual efficiency of each amplification cycle must be considered to correctly infer the amount of starting material. In contrast, digital PCR is almost insensitive to reaction efficiency, as long as the reaction produces a signal discernable from background. Unlike qPCR, dPCR reactions are compartmentalized into 20'000 distinct sub-reactions (droplets), each of which contains a single template molecule on average. The outcome of each sub-reaction is then qualified as either positive or negative. The number of positive reactions directly relates to the number of template present in the reaction. Thus, dPCR allows direct counting of template molecules and is less susceptible to PCR inhibition effects from the matrix. These advantages translate to increased robustness and precision of dPCR compared to qPCR. On the other hand, dPCR is more costly; thus, qPCR will remain useful where precision and robustness is not of prior importance.

For example, Figure 1. *Operator using Illumina MiSeq Sequencer.*

Figure 2. *Operator loading the liquid handler for DNA / RNA isolation.*

These analytical procedures are supported by our **isolation services** that perform DNA and RNA extractions from diverse matrices including environmental samples such as soil or air, animal and plant tissue biopsies, stool, blood and urine samples, to name a few (see ***Figure 2***). Furthermore, primers and oligos needed for NGS and PCR can be synthesized in house, which facilitates shorter production timelines and flexibility.

Bringing it All Together

To address the specific customer needs, different methods are combined in customized analysis workflows. All experimental results are critically evaluated to determine how reliably they describe and represent the actual samples. Thereby can Microynth contribute to the safety of drug products for the consumer and patients, and to fulfill regulatory documentation and experimental guidelines requested by US and European authorities. An important resource is issued by the *The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use* (ICH) (https://www.ich.org/). At Microsynth, we identify the relevant experimental parameters and assays to be addressed based on ICH guidelines. For example, when setting-up a method, Microsynth will evaluate its robustness. This allows identifying how parameters such as temperature, reagent concentrations, DNA or RNA isolation procedures, etc. affect the results. Precision refers to how variable the results are when a given assay is repeated on different days with different operators, reagent lots, or even in different laboratories (intermediate precision). Accuracy describes how close the determined results is to the true result, which refers to the value obtained with an alternative method that could be considered the gold standard. Different questions are addressed for distinct drug products: Identity confirmation, impurity detection or quantification and quantification of an active compound.

As outlined above, in molecular genetics, we work with DNA and RNA sequences and with sequence counts and concentrations of DNA and RNA molecules, which simplifies the task if for example compared with poorly defined drug products arising from complex chemical or biological synthesis workflows.

Accordingly, we design the experimental workflow accompanied by appropriate documentation to meet the regulatory requirements. The foundation for the formal reporting is represented by our quality management system with the ISO 9001:2015 and EN ISO 13485:2016 certifications, the accreditation according to ISO/IEC 17025:2017 (STS 0429) for Sanger Sequencing, Next Generation Sequencing and Fragment Length Analysis and the GMP Compliance issued by Swissmedic for our Sanger Sequencing department. Processes are clearly defined, validated, and controlled, and described in procedures and Standard Operating Procedures (SOPs), which are reviewed periodically. Instruments are qualified with periodic re-qualification periods, and corresponding instrument test documents are archived. Changes that affect the quality are validated. In cases where the quality cannot be covered by verification, the production process is validated. Operators are trained regularly in practical work and documentation procedures. All laboratory processes, including the bioinformatic analyses, are documented in lab data sheets, where time and date, operator, working procedures, reagent lot numbers and machine serial numbers are recorded. Finally, a summary report is produced describing the findings with reference to all primary data and analysis. The documentation and related records are maintained in a controlled manner. This includes the approval and release by our quality assurance unit. Below we will describe a few cases to illustrate our approaches.

Case 1: Sequence Stability of a Bacterial Production Strain

To verify that no unwanted mutations accumulated in a bacterial production strain, the genomes of the reference master strain and several derived production strains were determined. For this, a long-read next generation sequencing approach with PacBio was chosen in parallel with short-read Illumina sequencing. The long reads, up to about 30 kb in length, were used to produce the de novo chromosome assemblies. In parallel, Illumina short reads were generated and used to reduce the remaining sequencing errors in the contigs. The final sequencing contig of the reference master strain was then used to identify and confirm the species identity, using average nucleotide identify and digital to digital sequence hybridization strategies. Publicly available database resources provided the references. Finally, the newly produced genome sequences from the strains in the production process were compared to the genome of the reference strain. Any nucleotide changes were listed according to position. These bioinformatic analyses have confirmed that no unwanted mutations arose during the production process. Conceptually, this task may be regarded as identity test, where identity refers to the genome sequence of the test item.

Case 2: Transgene Copy Number in Production Strains

Production of some vaccines are based on the production of specific proteins, for which transgenic production strains are used. One of the requirements is to confirm that the copy number of the transgene is stable through the production process. In the early development step, Microsynth designed and tested the functionality of a duplex dPCR assay to measure transgene copy number for the respective vaccine program of the sponsor. In a second step, reference material was constructed and qualified before a full qualification was run addressing the parameters: specificity (specific amplification of locus and matrix effects), accuracy, intermediate precision, repeatability, linearity, and range, as well as limit of detection and limit of quantification. Results of the study were reported in a qualification report.

Case 3: Measuring CRISPR/Cas9 Off-Target Editing

Although CRISPR/Cas9 editing is far more precise than previous genome editing technologies, some off-target editing may occur and must be tested in drug products. The editing rates observed in the off-target loci are significantly lower than for the on-target locus. Thus, accurate and precise measurements below 2 % editing rates are required, which is challenging. The method of choice for such low-frequency editing rates is amplicon deep-sequencing. Based on discussions with our sponsor we qualified and validated the amplicon sequencing strategy that included an elaborated robustness testing and the validation of the method against pre-defined criteria set by the sponsor.

Contact

Hopefully, we have been able to provide you with a better understanding how we can help you with your projects and we are looking forward to advising you and designing a work procedure to support your research or drug product development.

To request information or arrange to visit Microsynth, please contact us at:
info@microsynth.ch