Advantages and Disadvantages of 16S-rRNA gene and 5,8S-/ITS-rRNA gene region sequencing for Identification of unknown cultures

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A fast, reliable and cost effective method to identify unknown microorganisms is very important in many areas of research. It is also important for commercial organizations. For more than 20 years, a PCR based method has been established to differentiate bacterial cultures: 16S/ITS-rRNA gene sequencing.

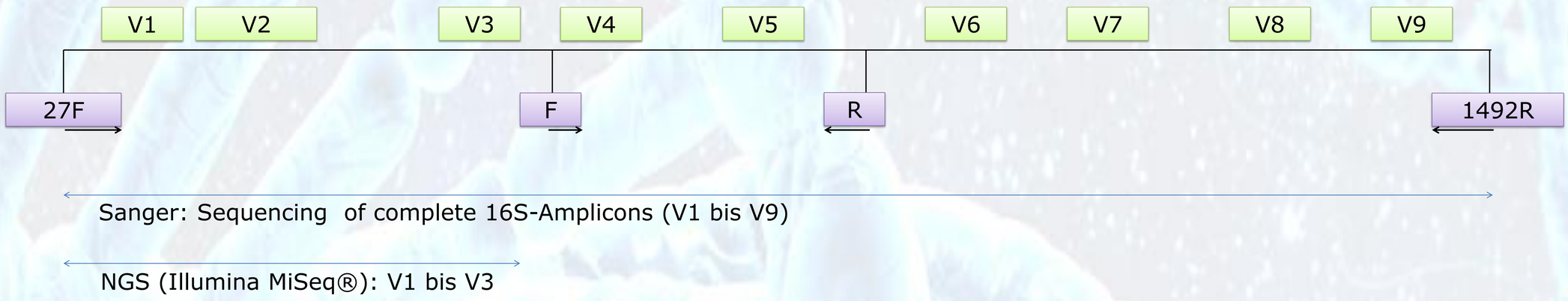
Is 16S/ITS-rRNA gene analysis as a stand alone method sufficient to identify unknown cultures?

Methodolgy of 16S-rRNA-Genesequencing

The 16S/ITS-rRNA gene is located on the ribosomal DNA which allows, due to small genome size, the reproducible amplification of genes starting from very low amounts of template DNA. Since the 16S/ITS-rRNA genes are flanked by highly conserved regions, universal PCR primer sets can be used for broad range amplification. A special primer mix developed by selekt-ID can amplify approximately 98% of all gram-positive and gram-negative bacteria in one reaction batch.

The first step is **the extraction of the genomic DNA**. Afterwards the target region is amplified by **PCR**: 16S-rRNA gene for bacterial cultures or ITS/5,8S-rRNA gene region (Internal Transcripet Spacer) for fungi and yeasts, followed by a **gel electrophoresis** as quality check and **purification** to clean out the PCR primers and nucleotides prior **sequencing** reaction. In case of pure cultures Sanger sequencing of amplicons is performed and the sequences were compared against internal and external databases to identify the sample by homology. Most databases are based on NCBI (National Center for Biotechnology Information) entries. If a mixed-culture is object of inquiry the partial sequencing of 16S/ITS rRNA gene by Next Generation Sequencing is recommend, e.g. MiSeq.

Schematic representation of bacterial 16S-rRNA-gene

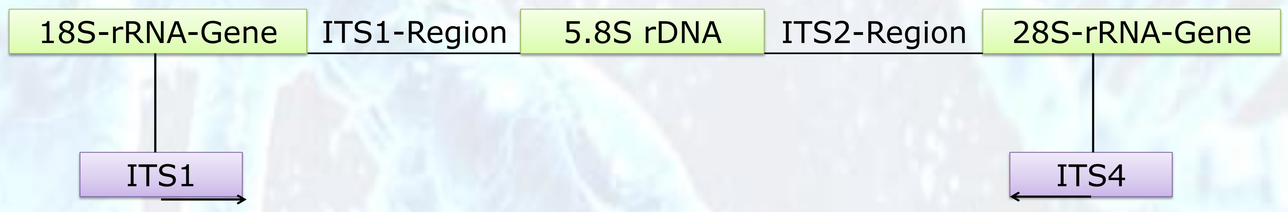


Pure Cultures: Sequencing of whole 16S rDNA (about 1450 bp).

Mixed Cultures: Read length of Next Generation Sequencing (NGS) technologies is limited. Due to this fragments with maximum 500 bp are generated by PCR: Either V1 to V3 or as an alternative V3 to V5.

Yeast and fungi: Analysis of 5,8S-rRNA/ITS-gene region is performed.

Schematic representation of 5,8S-rRNA/ITS gene region



Limiting criteria

- As every method based on database comparison: **The quality of database.** We use BLAST (Basic Local Alignment Search Tool) by NCBI (National Center of Biotechnology Information). This open access database is updated daily. This is an advantage (actual) and also a disadvantage since these data sets are often not validated. Due to this we compare these results with additional internal and external databases to validate the results from each BLAST search.

- **Significant differences in genotype** are necessary to determine the exact species. Some species/subspecies share identical or almost identical 16S-rDNA sequences: Sequences derived from these taxa can not be identified correctly on the basis of 16S-rDNA sequence. These microbes are summed in a taxonomic group which is defined as a group of taxa that cannot be differentiated solely by 16S-rDNA sequences.

- If the 16S-rRNA-Gene shows **more than one copy** no clear sequence information can be generated by Sanger sequencing. Cost intensive additional lab work is necessary.

Advantages for diagnostic usage

In contrast to classical microbiology methods (morphologic, biochemical) PCR based diagnostic methods can identify a strain **short-term** within one working day since no over-night incubation is necessary. Only a very **low amount** of culture is needed to extract the DNA as template for PCR. Also (human) pathogen microbes can be processed **without any risk for lab staff**. In addition non cultural microbes were analysed by this molecular biological analysis. Based on a robust PCR the results are reproducible and by high throughput processing (Multitier plates) a **cost-efficient** routine is realisable.

Future prospects

By using NGS technology, a fast analysis of biodiversity of mixed cultures is possible. E.g. **microbioms** can be characterized. Also qualitative and semi-quantitative analysis of e.g. **environmental samples** or **forage** regarding microbiological hazard presentable. The interaction of single species among each other can be obtained: This is a very important aim regarding **pathogenicity** (which strain influences other pathogen strain) and also regarding inhibiting factors during antibioses in human and veterinary medicine.

Conclusion

16S/ITS-rRNA gene sequencing can characterize a strain **rapid** (within 24 hours). It can be done in 96 well plates which reduces cost significant: This method represents a **cost-effective high throughput analysis**. In contrast to classical taxonomic methods like morphological analysis the molecular biological based 16S/ITS-rRNA gene sequencing method can also **differentiate non-cultivable isolates and pathogen strains without risks for lab staff**. The database is often a limiting factor since NCBI is a huge and actual database, but not in all cases are all entries validated.
For an exact identification of strains a significant difference in genotype is necessary. If the genotype of 16S/ITS-rRNA gene shows no significant differences the strains were summarized in taxonomic groups.

Also a qualitative and semi-quantitative analysis of mixed cultures can be done which allows details analysis of biodiversity studies of **microbioms**.

Since the 16S-rRNA gene does not show significant differences in genotype for all species this method cannot be used as stand-alone method for differentiation of subspecies. Additional methods are required (such as MALDI-TOF, API, RAPD-PCR and MLST) to differentiate the isolates exactly. The best method differs by genus: The optimal combination of these methods is currently the main focus in the development activities of selekt-ID BIOLABS GmbH.

However, for routine diagnostics the 16S/ITS-rRNA gene sequencing is a very attractive method since it can identify/analyse an unknown culture up to species level rapid and cost-effective.

Profile of selekt-ID BIOLABS GmbH

selekt-ID BIOLABS GmbH was founded in December 2014 ,and offers molecular biology services as business to business solution for companies, universities and other research organisations. Located in Berlin we offer high quality services with short turnaround times. Our Portfolio comprises several PCR based analytical services:

•Identification of microorganisms (bacteria, yeast, fungi) by 16S-rRNA-gene or 5,8S-rRNA/ITS-gene region analysis
• Identification of pork, horse, and many other species in meat products
• Identification of GMO

Our main focus is on high customer satisfaction: Due to a direct contact between customer and lab/administration we are able to establish new targets short-term. We always find a solution which meets your needs! In cooperation with our partner lab we also offer a broad range of microbiology services in accomplishment of ISO17025 standards.

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