Molecular methods – Practical seminar

- Our goal is to identify bacterial species which contaminated our food samples, using molecular methods. The workflow consists of following steps. Due to time and other constraints, we will perform only those in **bold.**

1) DNA isolation – you will be provided with ready isolates

2) Polymerase chain reaction – amplification of a portion of bacterial 16S rRNA gene; frequently used for bacterial species identification

3) Gel electrophoresis – verification that the PCR was successful

4) Purification of PCR product – getting rid of unwanted compounds, which could inhibit or interfere with sequencing

5) Sequencing

6) evaluation and editing of acquired DNA sequences

7) comparison of resulting sequences with GenBank database

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- Polymerase chain reaction – preparation of the PCR mixture

6 µl of PCR water - pure water, used for filling out the reaction volume, important for correct concentration of ohter components

10 µl of PCR master mix – contains DNA polymerase, nucleotides, salts, dye etc.

1 µl primer FF

1 µl primer FR

2 µl DNA isolate

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- **Evaluation and editing of acquired DNA sequences**

  We need to check the raw data:

  - evaluate quality/usability
  - edit if necessary
  - delimit the representative portion of sequence

- **Comparison of resulting sequences with database**

  - using an interface in MEGA software, we can easily compare our sequences with hundreds of millions of sequences stored in GenBank

  - we look for 100% identity with database sequence -> taxonomical identity with known bacterial species