BIPM Capacity Building & Knowledge Transfer Programme

2022 BIPM - TÜBİTAK UME Project Placement

REPORT

Project Name	BIPM - TUBITAK Project Placements
Description	Program of Training of Bioanalysis Laboratory
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Motivation & Introduction

One of the tasks force National Measurement Standards of Indonesia is to support Halal law, currently Indonesia laboratories gives DNA detection services using individually developed methods. Our methods are different for target genes, primers and PCR protocols. Therefore, different measurement assays may resulting in different results for the same products, which causes consumers to be confused and doubtful in the measurements. As a results, stakeholders are confused in making decisions, and the industry suffers economic losses. Attendance to BIPM – TÜBİTAK UME Project Placement Programme would be very important for my Biology Laboratory NMI Indonesia to improve our knowledge, capability and capacity according to requirements DNA analysis. How to produce meat CRMs with high purity is very critical. Calculating measurement uncertainty is required for the reporting of measurement results. TÜBİTAK UME has all equipment and facilities for DNA measurements and CRM production in addition to the experts in this area.

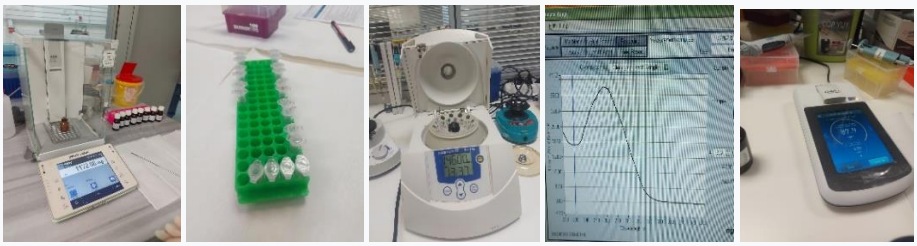
Research

We must understand the theoretical and practical knowledge of DNA analysis. Nucleic acid analysis laboratory must meet several requirements as to divide the laboratory to several rooms such as DNA extraction room, PCR preparation room, weighing room and instrument room to avoid DNA cross contamination. The results of DNA measurement relies on the DNA extraction protocol. The DNA extraction protocol should be efficient, inexpensive and give high purity and high DNA yield. I learned the details of the methods for my research by theory and practical applications of DNA extraction from meat sample, measurement principles of DNA, primer and probe design for PCR, principles of real time PCR quantification, principles of Digital PCR quantification, method validation and uncertainty calculations.

A. DNA extraction

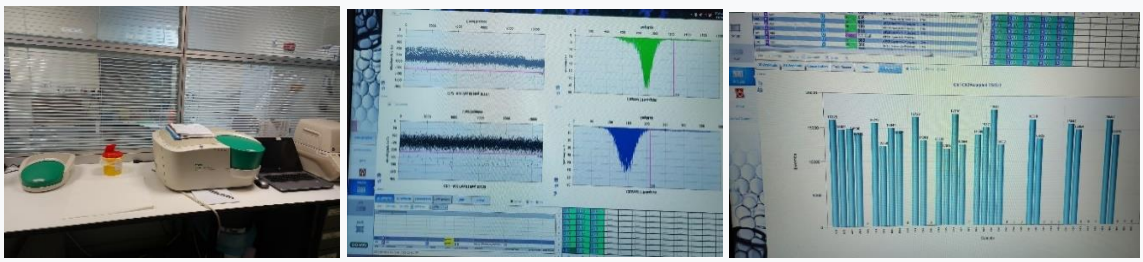
I performed salt DNA extraction method from different meat matrices and evaluated the concentration and purity of the extracted DNA. Extraction method steps are given below in brief.

- First I took a 50 mg meat sample, added 150 ul of water, 400 ul of melting buffer and 40 ul of 20% (m/v) SDS.
- The tubes were vortexed. Then, I added 20 ul proteinase K (20 mg/ml) into tube, incubated for an hour at 65°C.
- After that, I added 300 ul 5,8 M NaCl, vortexed for 30 sec, centrifuged for 10 min at 10000 g.
- The supernatant was poured off into a clean tube (750 ul), an equal volume of cold isopropanol was added incubated -20°C at 10 min.
- The tubes were mixed upside-down few times.
- The tubes were centrifuged for 15 mins at max 14,000 g at 4°C
- The supernatant were removed, added 500 ul 70% ethanol.
- Centrifuged for 10 mins at max 14,000 g.
- The supernatant were removed. The pellet was air dried and dissolved the pellet with 400 ul TE.
- Measured the concentration and purity with NanoDrop Spectrophotometer.



B. Digital PCR analysis

PCR is the process of amplifying DNA fragments to determine the number of DNA copies. The initial stage is the primer and probe design of the target gene. The primers and probes are optimized to differentiate between positive and negative signals. Then, the samples are diluted to fit in to the linear range of the digital PCR. The samples were measured with the digital PCR.



C. Calculating Uncertainty

In order to comply with the requirements of ISO/IEC 17025 clause 7.6 Evaluation of measurement uncertainty, the laboratory shall identify the contribution to measurement uncertainty. In this project I learned how to calculate the uncertainty of the measurement data from the analysis results.

D. <u>Production of Certified Reference Material (CRM) from meat</u>

Meat reference material is important to meet ISO/IEC 17025 requirements to ensure the quality of laboratory test results. To produce certified reference material, first we should describe and plan what kind of reference material to produce. The preparation of meat CRMs has several steps for material processing such is milling, sieving, drying, filtering, homogenization, short term and long term stability test, and finally characterization. In addition to the Material Data Sheet, the value and uncertainty of the CRM should be determined. After sales, post monitoring measurements continue until all CRM finishes.

Conclusions and Future Work

After the training, I will share my knowledge to our personnel at NMI Biology laboratory about the theoretical and practical applications of DNA extraction, the practical applications of digital PCR analysis, uncertainty calculations and how to produce reference materials from meat. With this knowledge transfer, we are going to produce meat reference material in my laboratory.

Acknowledgements

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