UNIVERSITY OF COPENHAGEN
DEPARTMENT OF FOOD SCIENCE

Food spoilage bacteria identification and quantification using Nanopore DNA sequencing technology in 1 day

Kavitha Anguluri[1], Susann R. Gröbner[1], Emma Wiedenbein[1], Farhad Panah[1], Susanne Knøchel[1], Dennis Sandris Nielsen[1], Lukasz Krych[1],*
[1]University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark
**Presenting author, E-mail: dn@food.ku.dk*

INTRODUCTION

Microbial spoilage causes loss of food in all stages of the food supply chain. Rapid testing allows rapid corrective actions, but unfortunately available methods are either slow or provide results with insufficient details. There is a strong need for rapid and cost-effective microbiological test procedures providing absolute quantification and taxonomic classification. Nanopore long-read sequencing technology offers high resolution data with rapid bacterial identification, but until now without absolute quantification.

AIM

- To develop a rapid, cost-effective tool based on Oxford Nanopore DNA sequencing technology allowing bacterial identification and absolute quantification simultaneously.
- The protocol should offer the possibility to distinguish dead bacteria (i.e. inactived starter cultures) from live bacteria.
- The protocol should differentiate between reaction inhibition and sample with no or low bacterial count.

EXPERIMENTAL SETUP

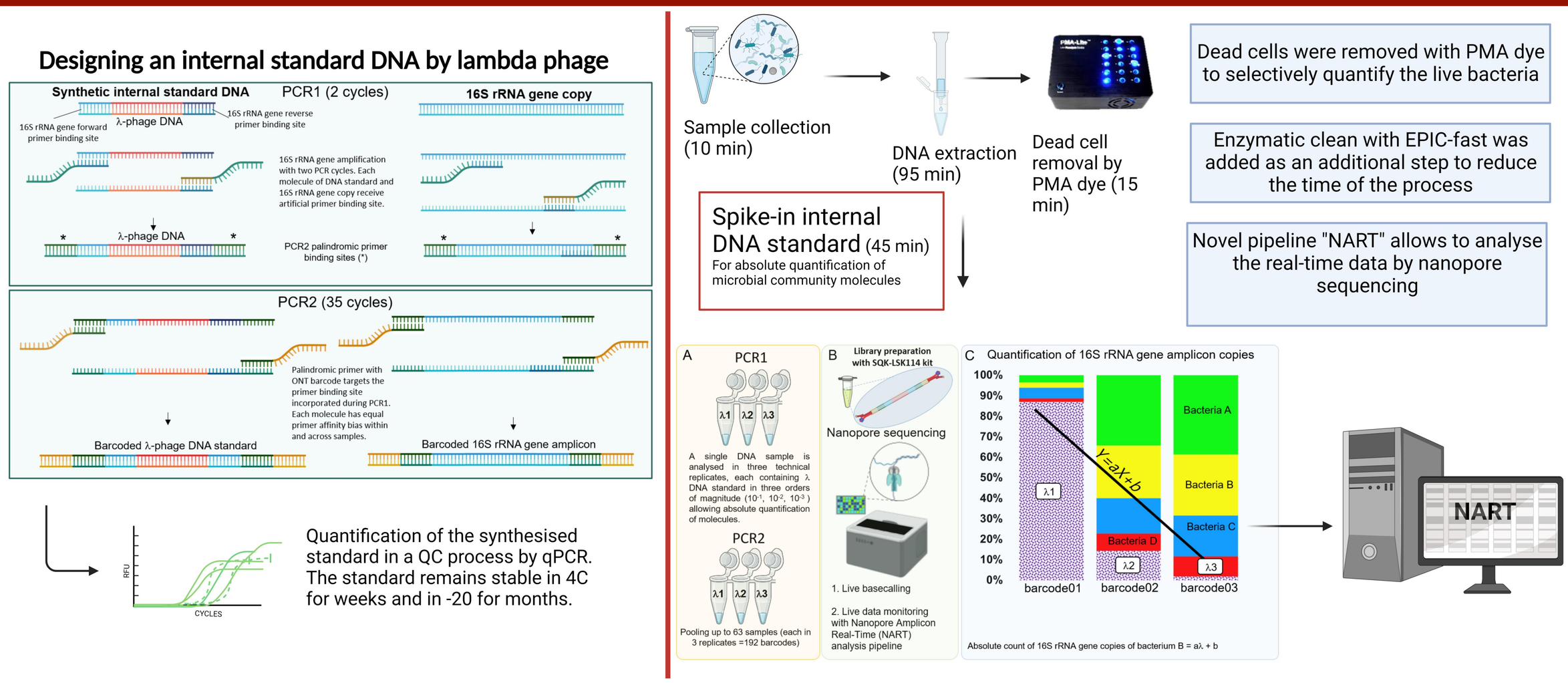

Figure 1: Workflow of the 16S rRNA gene amplicon sequencing from sample collection to data analysis. The process involves the detailed analysis of the samples collected from food and environment considering the most important factors absolute quantification using internal standard, removing dead cell DNA (optional) and the use of enzymes for quick and effective library preparation (<6 hours). The generated data can be analysed by real-time data analysis pipeline “NART” that provides rapid taxonomic classification.

CONCLUSION

- We have developed a novel tool for rapid identification and quantification of bacteria based on 16S rRNA gene amplicon sequencing.
- Synthetic DNA standards from lambda phage containing primer binding sites from the 16S rRNA gene are added in three different concentrations to technical replicates of a tested sample, enabling the extrapolation of 16S rRNA gene copy numbers for absolute quantification. In total, up to 64 samples can be analyzed simultaneously.
- Finally, we have developed a Nanopore Amplicon Real-Time (NART) analysis pipeline offering live sequencing process monitoring.

This work was supported by a grant “DNAPROKON” from the Green Development and Demonstration Programme (GUDP), Danish Agricultural Agency in the Ministry of Food, Agriculture and Fisheries