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MERIEUX NutriSciences

Long Read Sequencing for Food Safety Applications

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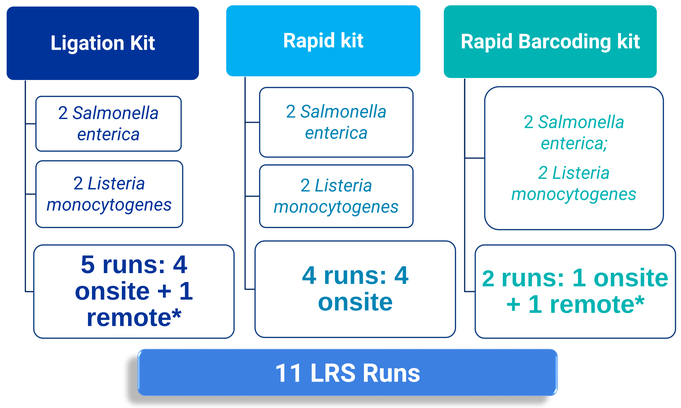
INTRODUCTION

With over a decade of development, high throughput sequencing (HTS) has become indispensable and approachable. Second- and third-generation technologies have taken sequencing affordability and flexibility to the next level. The third generation sequencers are particularly captivating with their long read sequencing (LRS) capabilities, rapid and remote sequencing, and real-time analysis features.

OBJECTIVES

This study evaluated the application of LRS for the food safety industry, using Oxford Nanopore MinION sequencer.

MATERIALS AND METHODS



•Remote sequencing was used to simulate LRS in the field (e.g., location with limited lab supplies) by using a laptop to sequence and classify reads.

Software and Data Analysis

•MinKONW was used for LSR sequencing.
•What's in my port (WIMP) was used for real-time LRS reads classification.
•GraphMap (Sovic et al., 2015) was used to assemble MinION data.

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Species and serotype identification using rapid long read sequencing technology is promising.

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RESULTS

Long Read Sequencing Classification

➢ It took as little as 30 min to obtain the correct species and serotype (for *Salmonella)* information using MinION and WIMP.
➢ Onsite and remote sequencing mode had the same sequencing performance, in terms of sequencing /base-calling speed, reads quality, and classification result.
➢ WIMP requires a stable internet connection for real-time data analysis and classification.

DNA Input	Library Kit	WIMP Result	Accuracy
Salmonella Typhimurium	**Ligation** and **Rapid**	*Salmonella* Typhimurium	**Correct**
Salmonella Montevideo	**Ligation** and **Rapid**	*Salmonella* Montevideo	**Correct**
Listeria monocytogenes	**Ligation** and **Rapid**	*Listeria* monocytogenes	**Correct**
Listeria monocytogenes	**Ligation** and **Rapid**	*Listeria* monocytogenes	**Correct**
Multiplexing of 4 strains	**Rapid Barcoding**	*Salmonella* Typhimurium, *Salmonella* Montevideo, *Listeria* monocytogenes and *Listeria* monocytogenes	**Correct**

Oxford Library Kit Comparison

➢It is a trade off between preparation time and data quality/yield. Depending on needs, one may spend more time preparing the library and generate sequencing reads of higher quality and yield; or use the rapid kit to obtain result within an hour.
➢Although multiplexing 4 strains accurately classified the species and serotypes (for *Salmonella*), barcoding/debarcoding inefficiency was observed, losing 24% reads.

Library Kit	Ligation	Rapid	Rapid Barcoding
Cost per Sample	$135*	$100	$109
Hands-on/Total Time	1 h / 1.5 h	10 min / 20 min	0.5 h / 1 h
DNA Input	1000 ng	400 ng	1.5 ng
Ave. Read Size (Bases)	6.0 k	1.8 k	3.5 k
Ave. Yield (Bases)	4.7 G	1.5 G	3.6 G
Ave. QS	10.5	9.2	9.5
Pros	•Higher data quality.	•Less library preparing steps . •Shorter preparing time.	•Sequencing up to 6 samples at a time. •Less library preparing steps. •Shorter preparing time.
Cons	•Longer library preparing time. •Requires 3rd party consumables.	•Lower data quality. •Prone to damage sequencing pores.	•Losing reads due to barcoding/debarcoding. •Lower data quality.

REFERENCE

Sovic I, Sikic M, Wilm A, Fenlon SN, Chen S, Nagarajan N. Fast and sensitive mapping of error-prone nanopore sequencing reads with GraphMap. bioRxiv. 2015 Jan 1:020719.

FOOD SAFETY & QUALITY

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