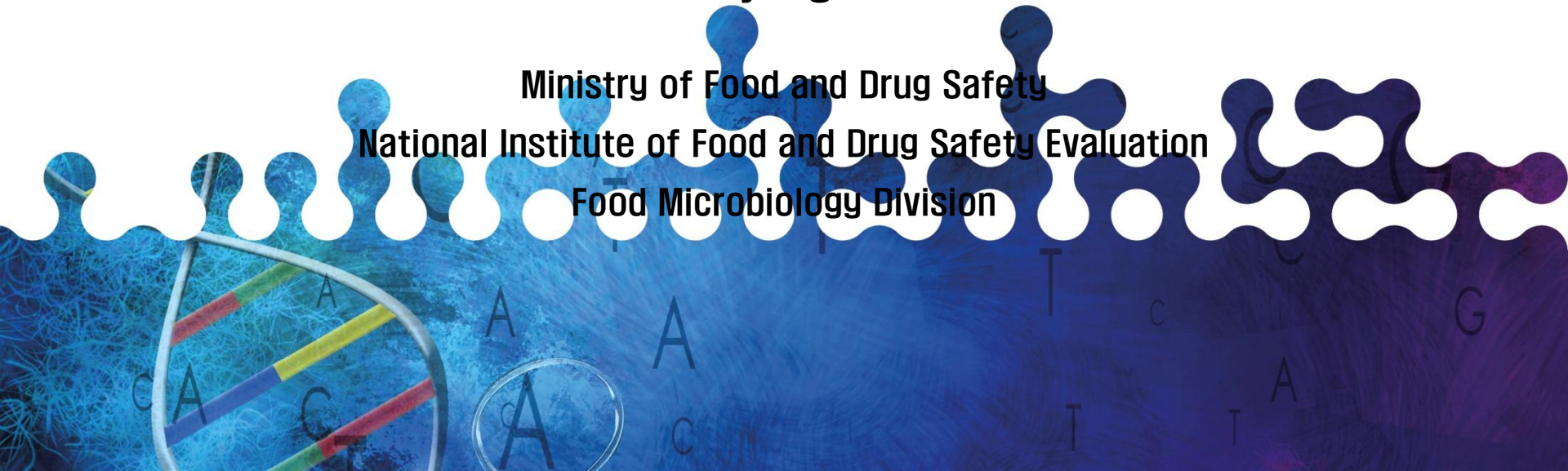


Identification of Probiotic Bacteria in Foods through Metagenomic Approach

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**Ministry of Food and Drug Safety
National Institute of Food and Drug Safety Evaluation
Food Microbiology Division**



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- I Probiotics and Quality control system**
- II MFDS Metagenomic Analysis Pipeline**
- III Applications of MFDS Pipeline in foods**

I . Probiotics and Quality control system



MINISTRY OF FOOD AND DRUG SAFETY

National Institute
of Food and Drug Safety Evaluation

What are Probiotics?



The Food and Agriculture Organization of United Nations (FAO) and World Health Organization (WHO) defined **Probiotics** as

“Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.”

A.

Definition in 2001

American Córdoba Park Hotel,
Córdoba, Argentina
1-4 October 2001

Food and Agriculture Organization
of the United Nations

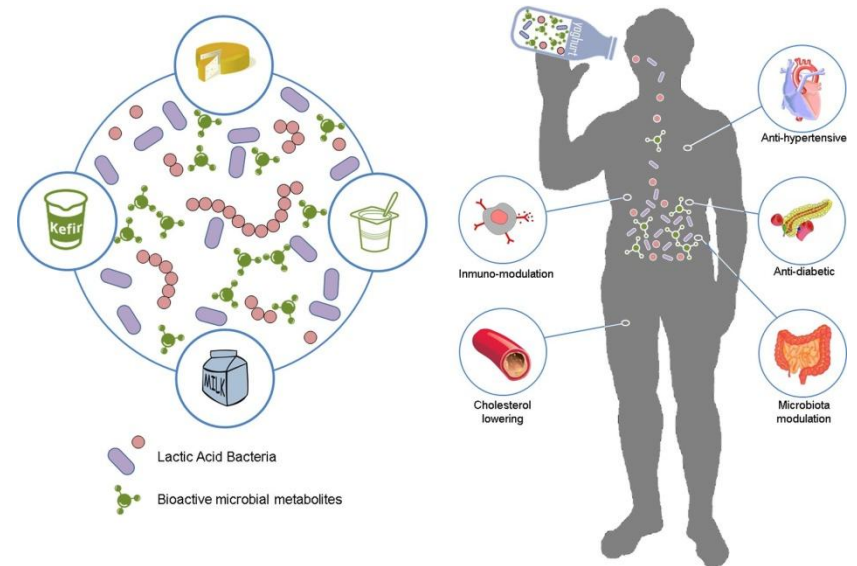
World Health Organization

Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria

Report of a Joint FAO/WHO Expert Consultation on
Evaluation of Health and Nutritional Properties of Probiotics in Food Including
Powder Milk with Live Lactic Acid Bacteria

‘Live micro-organisms which,
when administered in adequate amounts,
confer a health benefit on the host’

B.



Front Microbiol. 2017 May 18;8:846.

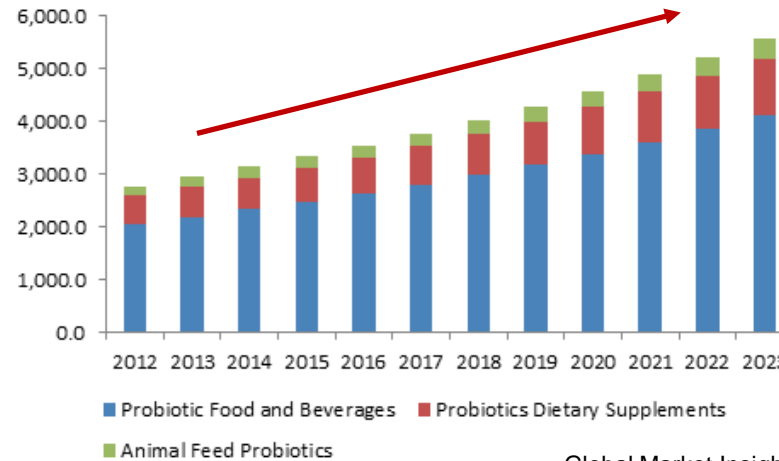
➤ Probiotics are novel functional ingredients that can influence the host microbiota and play an important role in the nutrition, development and health.

Probiotics market share



- ✓ As public awareness of well-being is gradually increasing, the market size of probiotics is growing steadily
- ✓ Identifying microorganisms in probiotic products is an important issue for products quality control

U.S. probiotics market size, by application, 2012 – 2023 (USD Million)



Global Market Insights Inc.



Probiotic labeling & Quality control system



- ✓ MFDS manages the probiotic product with the Food Code and the Health Functional Food Code

Health Functional Food Code & Labeling Standards

- (Regulations and standards) Probiotics number: 1×10^8 CFU/g or more, Coliform: Negative
- (Label requirements) The name of raw material which represents the functionality of the relevant product shall be declared first

- Genus, species and strain
- Minimum viable numbers of each probiotic strain (CFUs) at the end of the shelf-life
- Suggested serving size
- Health benefits
- Proper storage conditions
- Corporate contact details for consumer information

Lactomin advance+ probiotics

Made in Korea

Recommended Dosage:
*For Adults and Kids (2yr & Above)
*Take after meals

Build & Support Immunity 1 Sachet Daily	Relieves stomach upset Adults: 3-4 sachets daily Kids (> 2yrs): 2-3 sachets daily
---	--

Directions:
- Recommended to be taken directly
- Can be taken dissolving in room temperature water
Lactomin advance+ not only provide many benefits to our intestinal gut, it has added vitamins B1, B2, B5, B6, B9, D3, prebiotics and a pleasant taste which makes it easy to consume.

Storage:
- Store in a cool, dry place
- Avoid direct sunlight
- No refrigeration necessary
- Safe and gentle, appropriate for regular use
- Health Supplement
- Not Recommended to dissolve in hot water
- This product is not intended to treat, diagnose, cure or prevent any disease.

30 sachets

Great Taste 100% Natural Probiotics

Ministry of Food and Drug Safety

Health Functional Food

Quality Standard KFDA KOSHA KKFDA

Product of Korea

Imported by: **NOVARTIS**

Distributed by: **www.vetkem.com.sg**

For further information and questions, please visit us at www.lactomin.com.sg

Lactomin advance+ probiotics

Made in Korea

Supplement Facts
Serving size: 1 sachet / 3g Servings per box: 30

	Amount Per Serving
Double microencapsulated active:	3 Billion (3 X 10 ⁸ CFU) Sachet
Probiotic cultures:	
- Lactobacillus acidophilus	
- Bifidobacterium longum	
- S. faecalis	
- Lactobacillus rhamnosus	
- Lactobacillus plantarum	
Vitamins: B1 (0.2 mg), B2 (0.18 mg), B5 (0.6 mg), B6 (0.24 mg), B9 (0.05 mg), D3 (27 IU)	
Vegetable cream powder (125 mg)	
Fruits oligosaccharide (Prebiotics) (390 mg)	
Yoghurt flour powder (60 mg)	
Yoghurt concentrate powder (450 mg)	
Isomalt (300 mg)	
Manganese, Zinc (3 mg), Ferrous fumarate (9 mg), Casein phosphopeptide (15 mg)	

Supports Immune System

100% Natural Probiotics + Prebiotics

Great Tasting Probiotic Powder

Product of Korea

Imported by: **NOVARTIS**

Distributed by: **www.vetkem.com.sg**

For further information and questions, please visit us at www.lactomin.com.sg

Lactomin advance+ probiotics

Made in Korea

Promotes Better Digestion and Immunity

Lactomin advance+ probiotics
With added Vitamins + Prebiotics

Strengthens Immunity

Improves Digestion

Relieves Stomach upset

- 3 Billion Double Microencapsulated
- Active Probiotic Cultures: Probiotics Supplement with: Lactobacillus acidophilus, Bifidobacterium longum, S. faecalis, Lactobacillus plantarum, Lactobacillus rhamnosus
- Noltec® Double Microencapsulation for optimal release into the gut
- Vitamins B1, B2, B5, B6, B9 and D3

Each Lactomin Advance+ probiotic has:

- Noltec® Double Microencapsulation for Optimal release
- 1st Protective Coating for high survival rate to the gut
- 1st Primary Coating (Noltec®)
- 2nd Secondary Enteric Coating
- Probiotic Strains

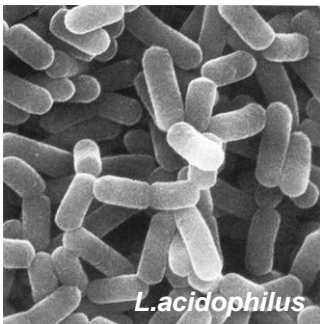
30 Sachets

Probiotics approved in MFDS (1)

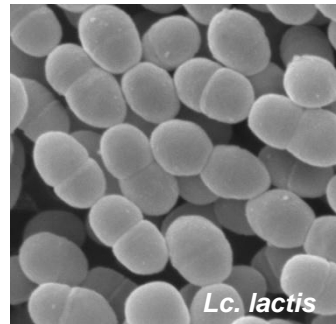


- ✓ First type is notified in the codes of Health Functional Foods
- ✓ A manufacturer can use notified functional ingredients for its health functional food products in accordance with the standards and specifications prescribed in this Code

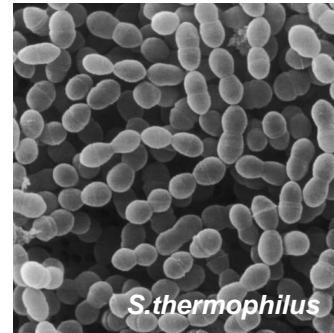
Genus	Species
<i>Lactobacillus</i> (11)	<i>L.acidophilus</i> , <i>L.casei</i> , <i>L.gasseri</i> , <i>L.delbrueckii</i> ssp. <i>bulgaricus</i> , <i>L.helveticu</i> , <i>L.fermentum</i> , <i>L.paracasei</i> , <i>L.plantarum</i> , <i>L.reuteri</i> , <i>L.rhamnosus</i> , <i>L.salivarius</i>
<i>Lactococcus</i> (1)	<i>Lc. lactis</i>
<i>Enterococcus</i> (2)	<i>E.faecium</i> , <i>E.faecalis</i>
<i>Streptococcus</i> (1)	<i>S.thermophilus</i>
<i>Bifidobacterium</i> (4)	<i>B.bifidum</i> , <i>B.breve</i> , <i>B.longum</i> , <i>B.animalis</i> ssp. <i>lactis</i>



L.acidophilus



Lc. lactis



S.thermophilus



B.animalis

Probiotic labeling & Quality control Issue



Quality control of probiotics is lacking, study suggests

By Louisa Richards 20-Nov-2015
Last updated on 24-Nov-2015 at 20:07 GMT



Related tags: Bifidobacterial, Study,

A new study by scientists at the University of California and University of Massachusetts in the US and University of Modena and Reggio Emilia in Italy.

The researchers say there is cause for concern for consumers and those involved in clinical trials.

"These results suggest that quality control of probiotics is lacking," they wrote.

The study published in *Pediatric Research* was conducted by scientists at the University of California and University of Massachusetts in the US and University of Modena and Reggio Emilia in Italy.

Species confusion

Bifidobacterium longum (*B. longum*) has two subspecies found in humans - *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* - that are challenging to distinguish using common methods.

- Identifying microorganisms in probiotic products is an important issue for products quality control and public health. Furthermore, misidentification of the probiotics might cause several health issues to consumers.
- There is common needs of quality control on the probiotic products around the world and growing demand for the research related to.

(A) **VSL#3[®]**
Dispensing Pack
Probiotic Food Supplement

Store in a refrigerator at 2 - 8°C
Can be stored at room temperature (up to 25 °C)
for up to 7 days without adversely affecting potency
Best before: See base of pack

Ingredients: Bacteria blend *, maltose, anti-caking agent: silicon dioxide.
*** Product information.**
Each 4.4g sachet provides a blend of 450 billion bacteria, containing: *Streptococcus thermophilus* DSM 24731, bifidobacteria (*B. longum* DSM 24736, *B. breve* DSM 24732, *B. infantis* DSM 24737), lactobacilli (*L. acidophilus* DSM 24735, *L. plantarum* DSM 24730, *L. paracasei* DSM 24733, *L. delbrueckii* subsp. *bulgaricus* DSM 24734).

Directions: For adults and teenagers, take 1 to 4 sachets daily.
Open the sachet and stir the contents into cold water or any cold non-fizzy drink or food and consume immediately.
Do not exceed the recommended daily intake.
Food supplements should not be used as substitute for a balanced and varied diet and a healthy lifestyle.
Do not use if the sachet is broken or damaged.
Store out of reach of young children.

This product does NOT contain soy, gluten, lactose or milk products.

Distributed in the UK by:
FERRING Pharmaceuticals Ltd.
Drayton Hall, Church Road, West Drayton UB7 7PS.

Distributed in the Republic of Ireland by:

Manufacturer:
S.I.T. srl
Via Anzole - 50060
00090 Treczano sul Naviglio, (MI) Italy

015919 450094

- 8°C
temperature (up to 25 °C)
by alteration.
ie of pack

Streptococcus thermophilus BT01,
Lactobacillus (*L. acidophilus* BA05,
D08).

Directions: For adults and teenagers, take 1 to 2 sachets daily.
Open the sachet, pour the content into cold water or any cold non-fizzy liquid or food, stir and consume immediately. Do not exceed the recommended daily dose.
Warnings: Food supplements should not be used as substitute for a balanced and varied diet and a healthy lifestyle.
Do not use if the sachet is broken or damaged. Store out of reach of young children.

This product does NOT contain soy, gluten, lactose or milk products

Distributed in the UK by:
FERRING Pharmaceuticals Ltd.
Drayton Hall, Church Road, West Drayton UB7 7PS

Distributed in the Republic of Ireland by:
FERRING Ireland Limited, United Drug House,
Magna Business Park, Citywest, Dublin 24

VSL#3[®] is a registered trademark of Actial Farmaceutica Lda

5 015919 450094

FIGURE 20.1 Packaging of VSL#3. (A) Original DS formulation; (B) lately marketed product.

URL : <http://www.nutraingredients.com>

The Microbiota in Gastrointestinal Pathophysiology, 2017, 171-178

Identification of Probiotics using NGS (FDA)

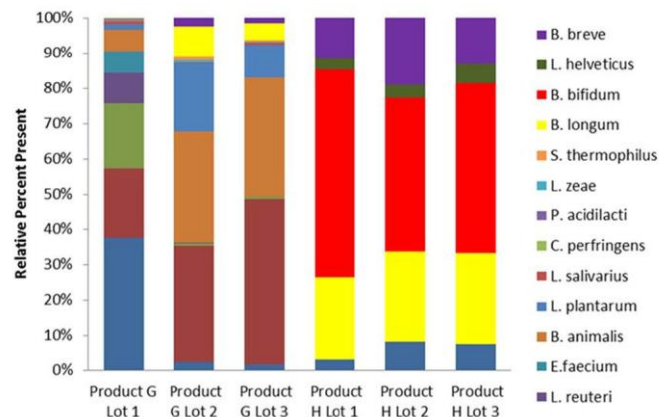


✓ Researches on the test of LAB by metagenomic sequencing and analysis

Dietary Supplement Brand Name		Strains																			
		Bifidobacterium animalis subsp lactis	Bifidobacterium bifidum	Clostridium perfringens	Bifidobacterium longum subsp infantis	Bifidobacterium longum subsp longum	Enterococcus faecium	Lactobacillus acidophilus	Lactobacillus bulgaricus	Lactobacillus casei group	Lactobacillus delbrueckii subsp bulgaricus	Lactobacillus plantarum	Lactobacillus reuteri	Lactobacillus rhamnosus	Lactobacillus salivarius	Bacteroides fragilis subsp commensale	Lactobacillus zeae	Paenibacillus coelicolor	Lactococcus lactis subsp lactis	Bifidobacterium breve	
Product A	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product B	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product C	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product D	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product E	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product F	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product G	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product H	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product I	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				
Product J	Lot 1																				
	Lot 2																				
	Lot 3																				
	PCR*																				
	Viability**																				

* 'v' indicates a positive PCR identification using strain specific primers
 ** 'v' indicates single colony growth on selective media

Included on product label; Found during sequencing
 Included on product label; Not found during sequencing
 Not on product label; Found during sequencing
 Sequencing values are too low to definitively confirm strain presence



B. The relative abundance of each bacterium in a product also shows the inconsistency in different lot of the same product

- This study gave us a good reason why we need to test and control the quality of the commercial probiotic products.
- The NGS clearly demonstrated its utility for quickly analyzing commercially available products containing multiple microbes to ensure consumer safety.

A. It is notable that some of the indicated LAB and bifidobacteria ingredients from 5 probiotic products were not detected

II. MFDS Metagenomic Analysis Pipeline



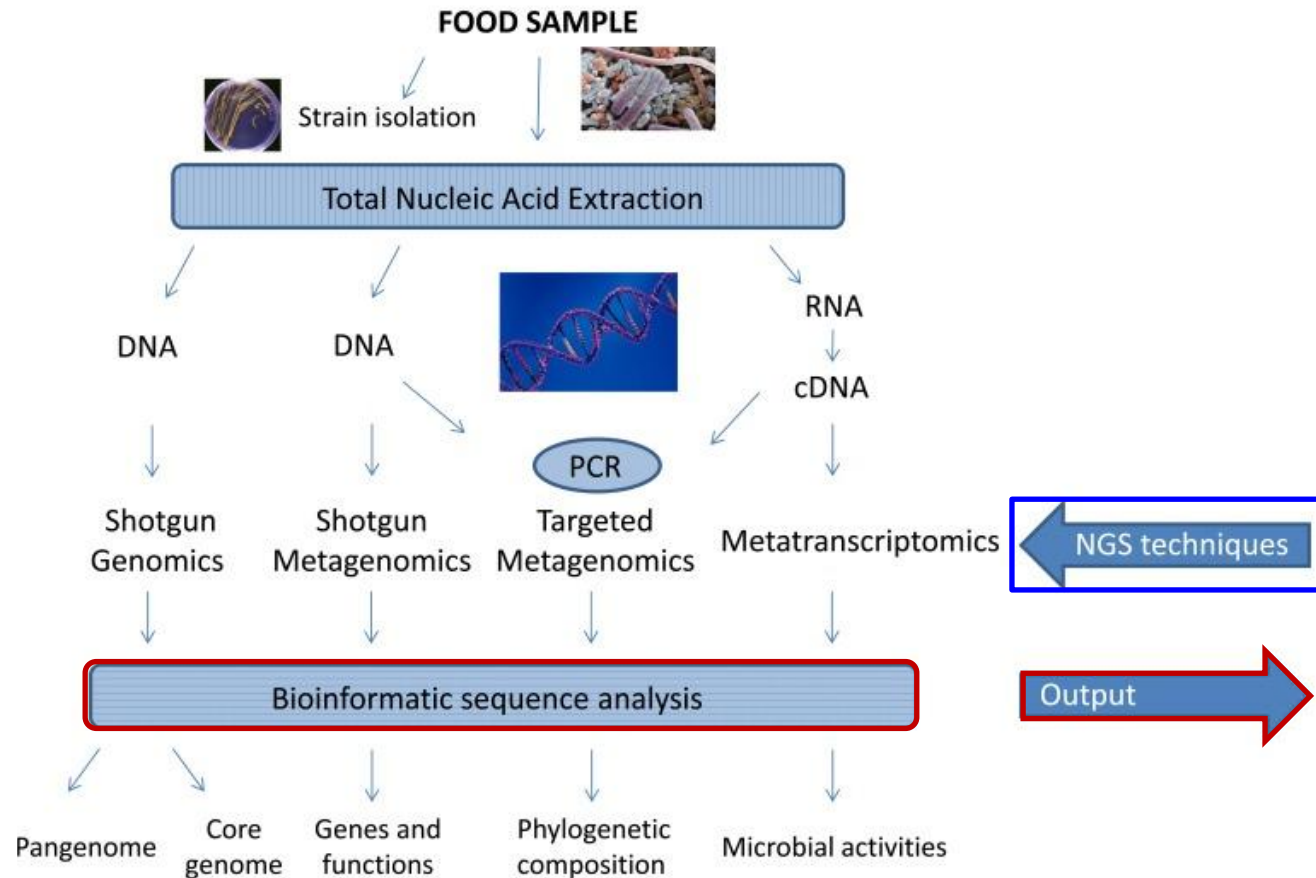
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Bioinformatic Analysis of Sequence Data



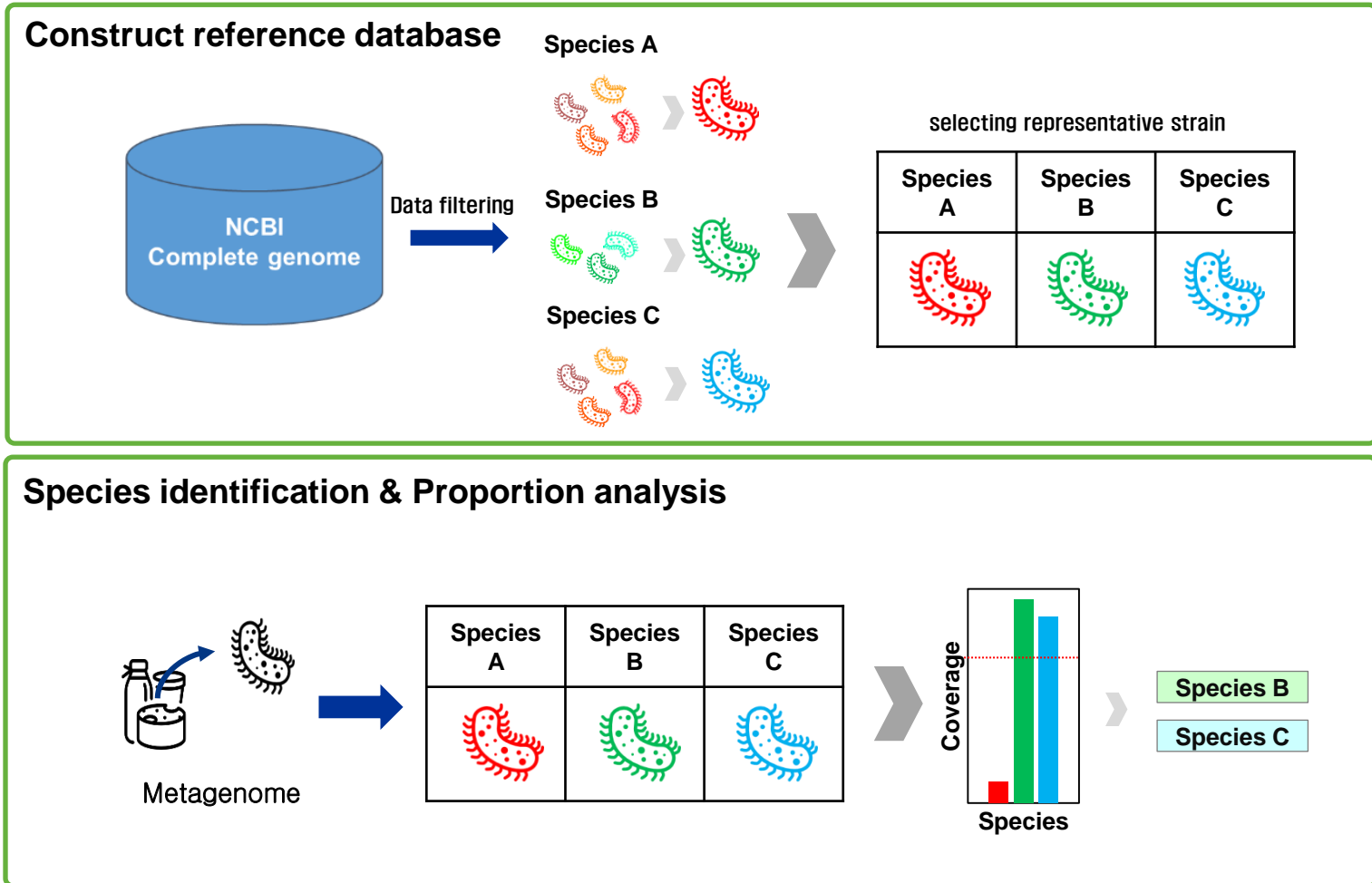
- ✓ NGS is massively parallel and sequences millions of fragments per run simultaneously
- ✓ Bioinformatic sequence analysis is an essential part of the genome sequence analysis



Overview of MFDS pipeline



- ✓ The development of the pipeline was done in two steps which are building a representative genome database and a software for LAB detection and species proportion analysis

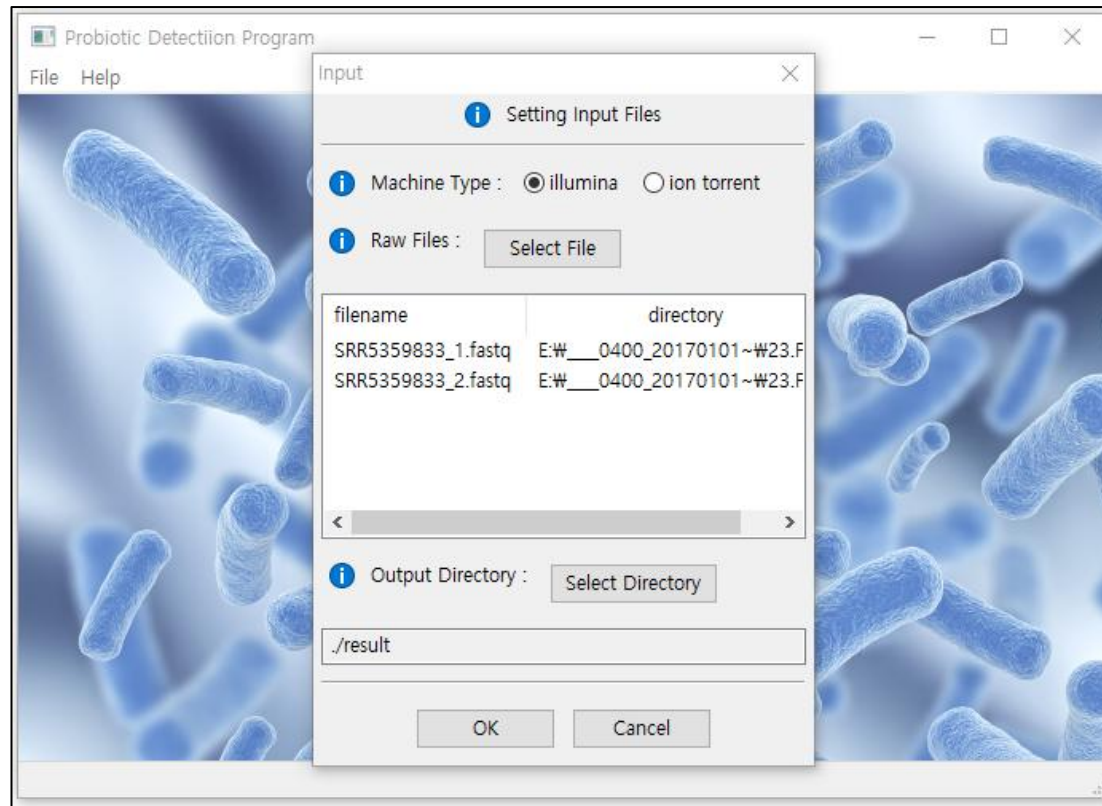


Automated analysis



- ✓ Establish a windows based automated application that detects the probiotics in probiotic products
- ✓ With this software, we can get the result of species identification and probiotics proportion of a probiotic product with fully automatic way

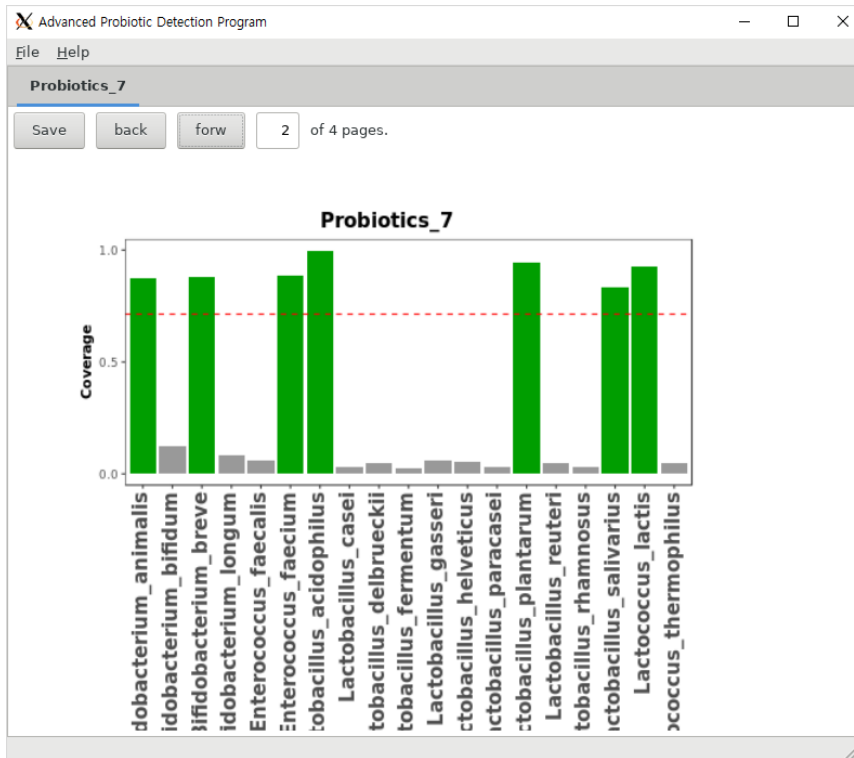
Automated analysis program (Windows 10)



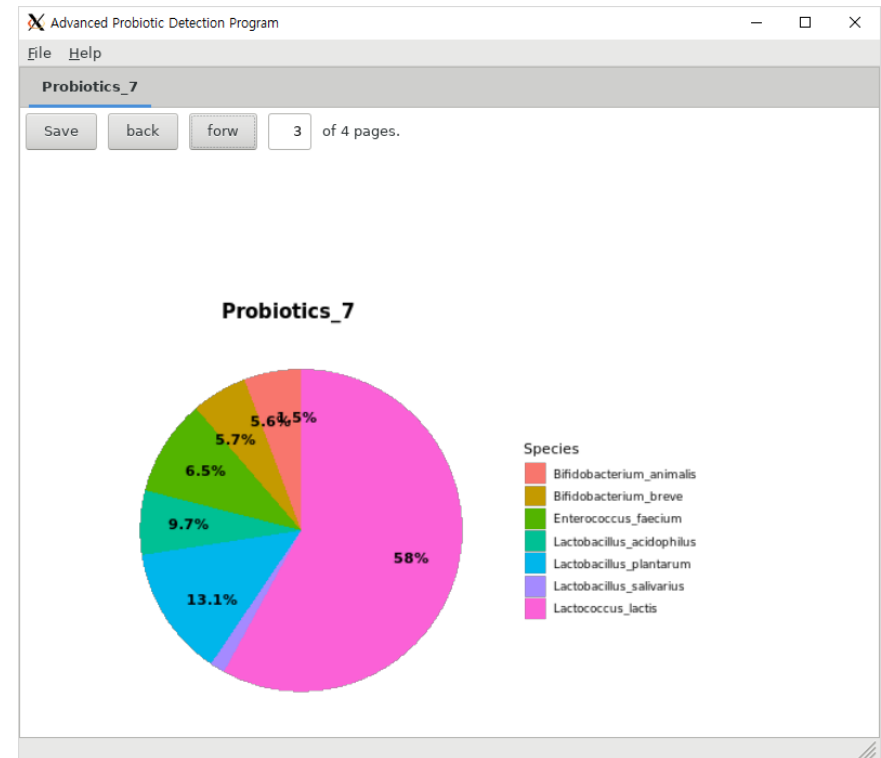
Automated analysis



Species Identification



Species Proportion



III. Applications of MFDS Pipeline in Foods



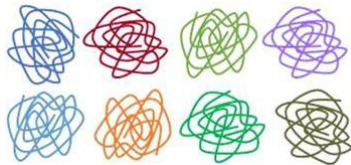
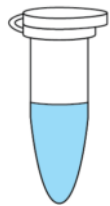
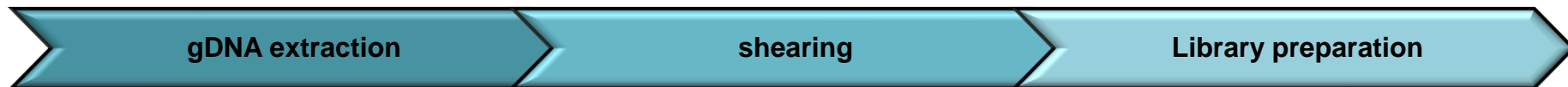
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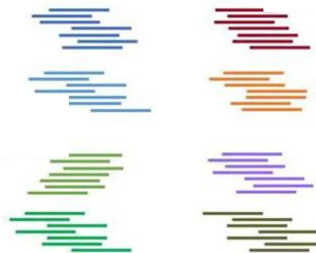
Sample Preparation



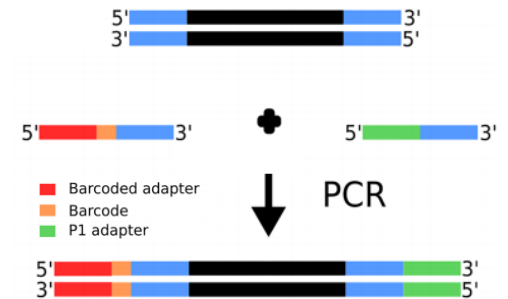
✓ To make sequencing sample, we performed gDNA extraction, shearing and library preparation



Genomic DNA



Shearing DNA fragment

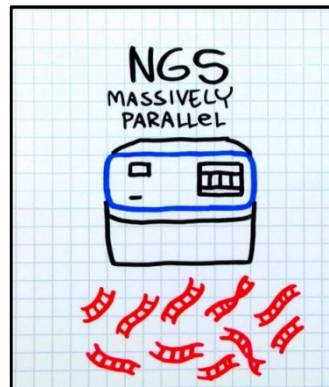
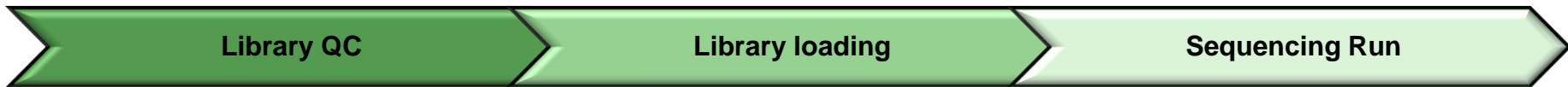


Ligation adaptor and Amplify library

Sequencing Run



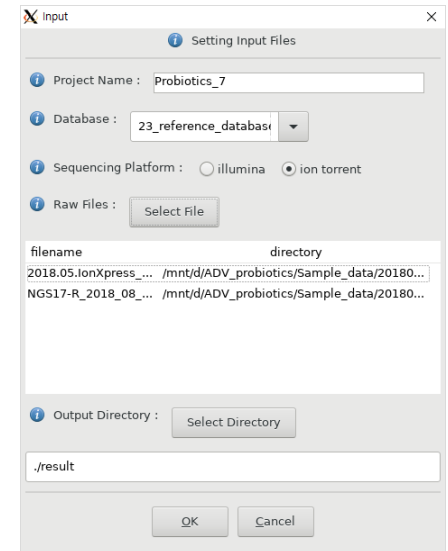
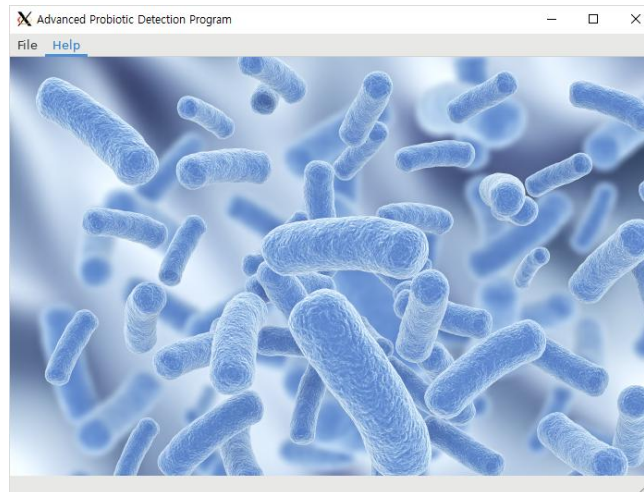
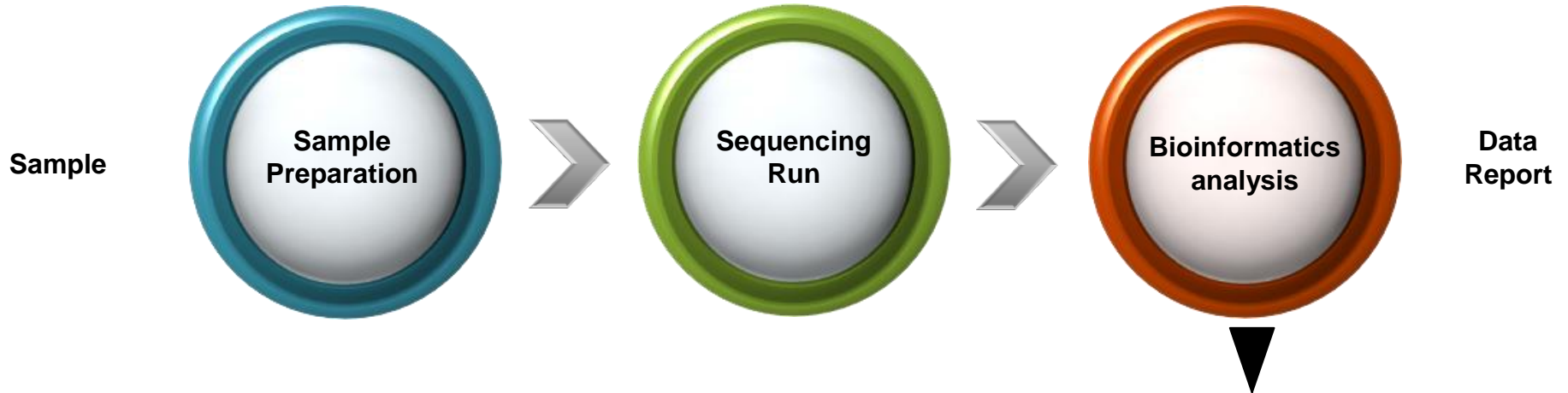
✓ For a sequencing step, we check the library QC, load the library and run the sequencing



Bioinformatics Analysis



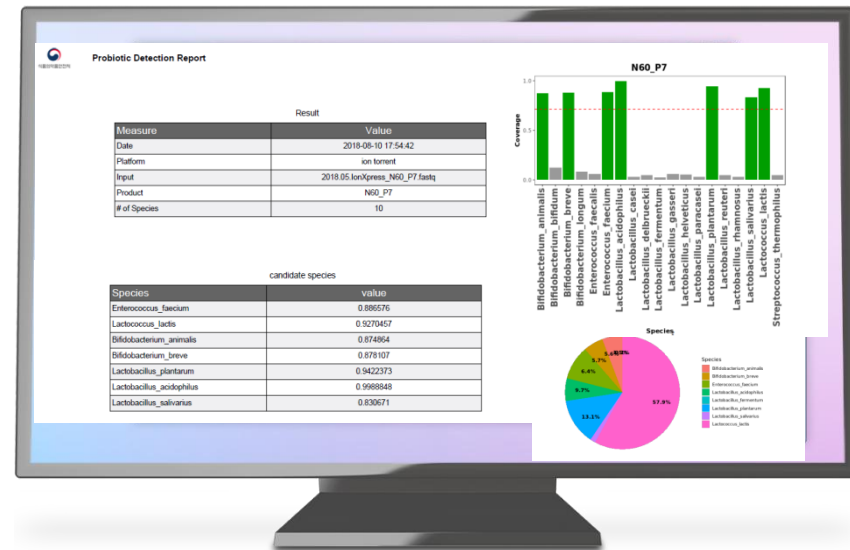
✓ To analyze sequencing data, we used the MFDS metagenomic analysis pipeline



DATA REPORT



✓ The analyzed data was summarized and displayed





• Probiotics and Quality control system

- ✓ In order to manage the probiotic market, it is necessary to check whether the probiotic products actually contains the lactic acid bacteria (LAB) and bifidobacterial as it labeled.
- ✓ The **genome-based method** has been added to **the official microbiological testing methods** of Food Code.

• Development of MFDS Metagenomic Analysis Pipeline

- ✓ A bioinformatics analysis pipeline is required for efficient analysis of NGS data.
- ✓ **MFDS metagenomic analysis pipeline** provided new criteria for the presence or absence of LAB in the sample to **adequately control false detection and manifested the high accuracy in the proportion analysis.**

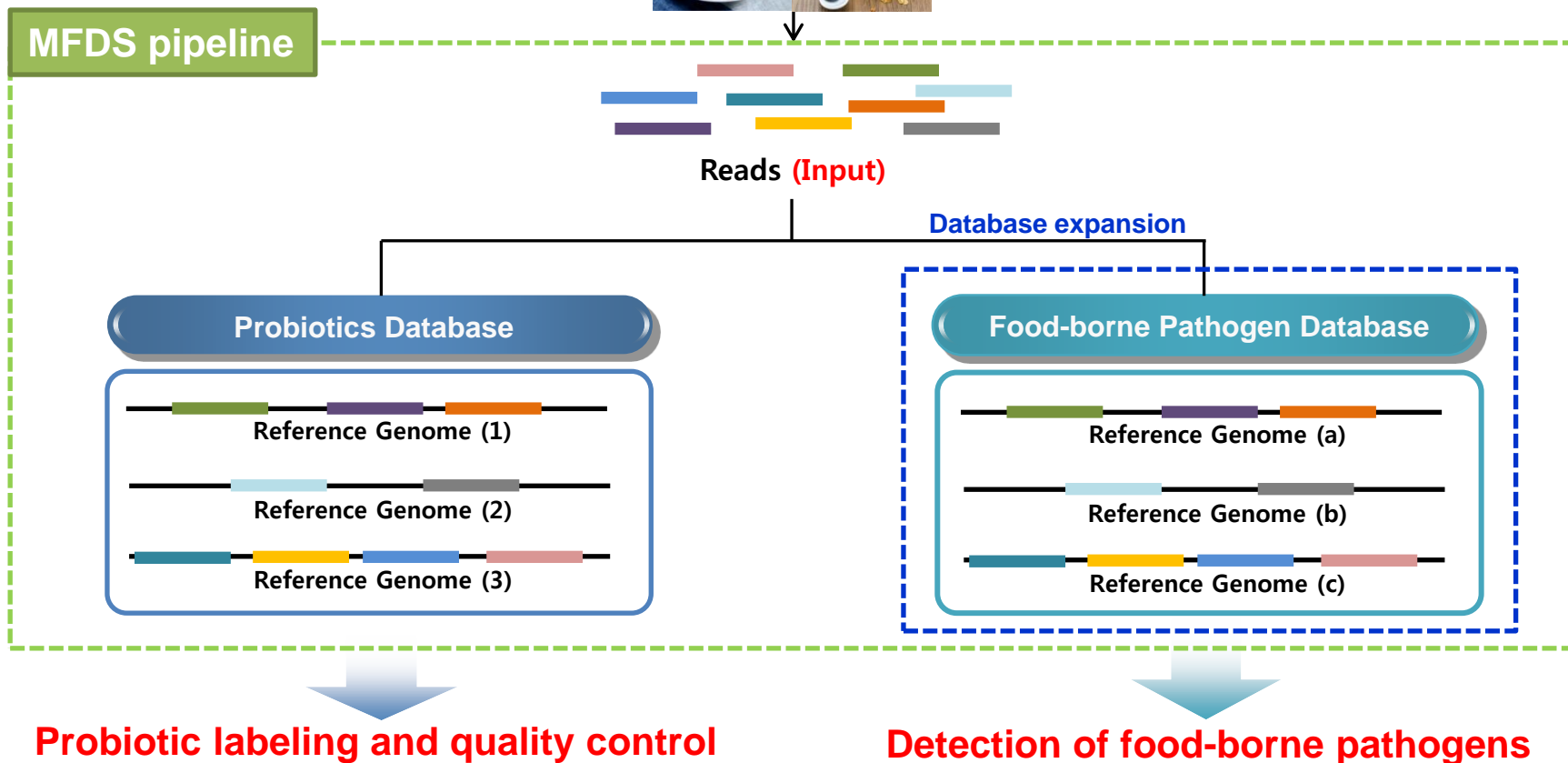
• Applications of MFDS Pipeline in Foods

- ✓ MFDS pipelines can provide appropriate assistance for **probiotic quality control, including labeling issues.**
- ✓ The pipeline will also **be open to the probiotic product manufacturers** so that those companies keep the quality of their products consistently.

Further study



- ✓ We will improve pipeline continuously and use it for the detection of food poisoning bacteria in food



Acknowledgement



MINISTRY OF FOOD AND DRUG SAFETY
National Institute
of Food and Drug Safety Evaluation

Food Microbiology Division



Bioinformatics analysis : Woori Kwak, Donghyeok Seol



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Thank you for Listening

