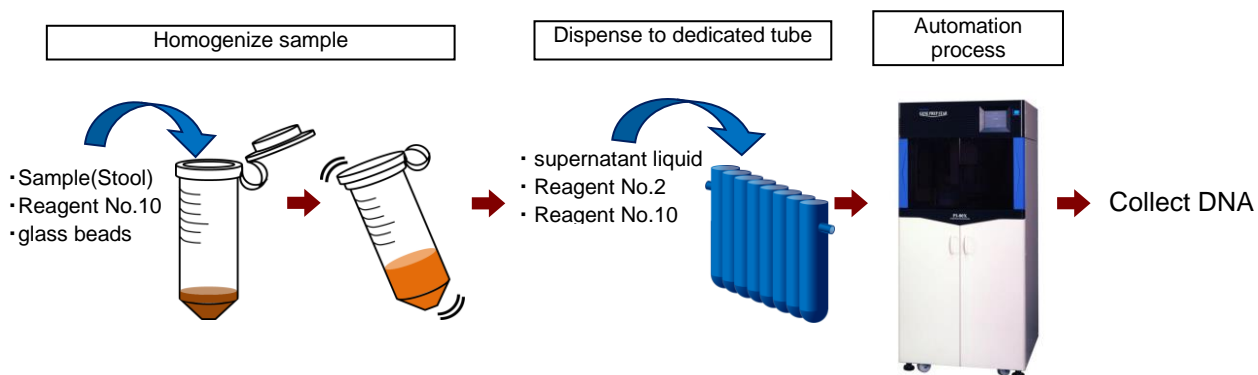


PI-480 Tissue DNA Protocol

**Bacterial genomic DNA extraction from Stool
/ 16S rDNA metagenomic analysis**

The result of DNA isolation from stool



Experiment

Sample	Stool (using sampling kit)
Sample volume	40 mg / 200µl
Number of sample	Maximum 48 sample / 1 run
Isolation system	DNA isolation system GENE PREP STAR PI-480 Tissue DNA protocol
Reagent kit	Tissue DNA kit (NR-201)
Consumable kit	Sample tube (PT-8000), DNA Collection tube (NT-8000)
Chemical principle	Lysate : Beads beating and Proteinase K digest Extraction : Kurabo original, using phenol Precipitate : Alcohol precipitation method

Manual process

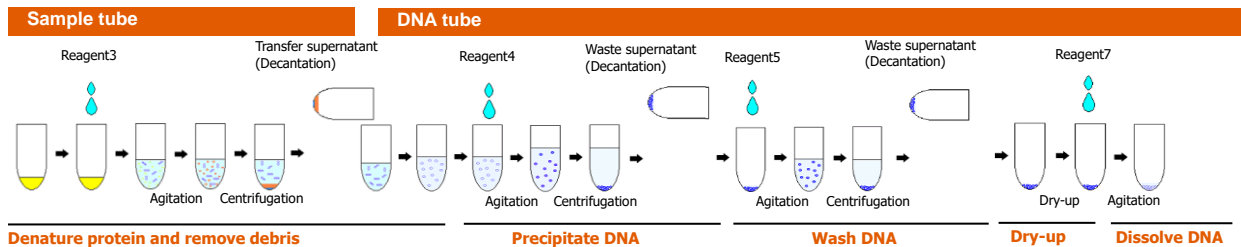
- (1) Add 0.1mm glass beads (0.5g) and Reagent No. 10 (300μl) to suspended stool sample (200ml)
- (2) Homogenize (4800 rpm, 50sec).
- (3) Centrifugation (12000 rpm, 5min)
- (4) Transfer supernatant liquid (200μl) to dedicated tubes (PT-8000)
- (5) Transfer Reagent No. 2 (NR-2025+Proteinase K 0.4 mg/ml) 150μl and Reagent No. 10 (NR-10025) 150μl to (4)
- (6) Set the sample tube (5) and collection tube into PI-480
 - ※After Step (6), process with standard Tissue protocol
- (7) Transfer DNA to storage tube and centrifuge (12000 rpm, 5min, 4°C) after the automated process finished.
- (8) Freezer storage with -30°C or Analysis (supernatant)

Automated process

- Step 1. Denature protein and remove debris
- Step 2. Precipitate DNA
- Step 3. Wash DNA
- Step 4. Dry up
- Step 5. Dissolve DNA

Elution volume : 100μl

Workflow of Tissue DNA protocol



Analysis method

- Yield and purity** : Measured absorbance of 260nm with spectrometer
 • DNA yield is calculated as follows:
 「A260 x 50 x dilution factor x elution volume」
 • DNA purity is evaluated by the ratio of A260 / A280
- Electrophoresis** : Load DNA solution (5ul) into 0.7% agarose gel.
- PCR** : Use a primer amplifying V3-V4 of 16S gene
 Forward; 5' - TCGTCGGCAGCGTCAGATGTGTATAAGCGACAGCCTACGGGNGGCWGCAG - 3'
 Reverse; 5' - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC - 3'
- 16S rDNA metagenomic analysis**
 : Use a Illumina sequencer Miseq, and Analyze the data with Illumina Cloud Service
 Basespace

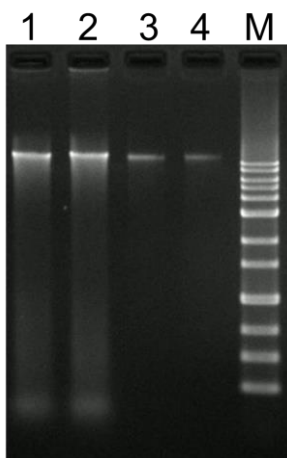
Results

Yield and Purity

Protocol	No.	A260/A280	yield (ng/μl)
KURABO PI-480	1	1.84	50.6
	2	1.82	52.0
A DNA Isolation kit from stool	3	1.58	10.0
	4	1.93	9.9

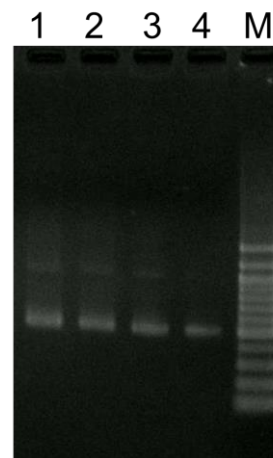
Manual process on same condition

Electrophoresis



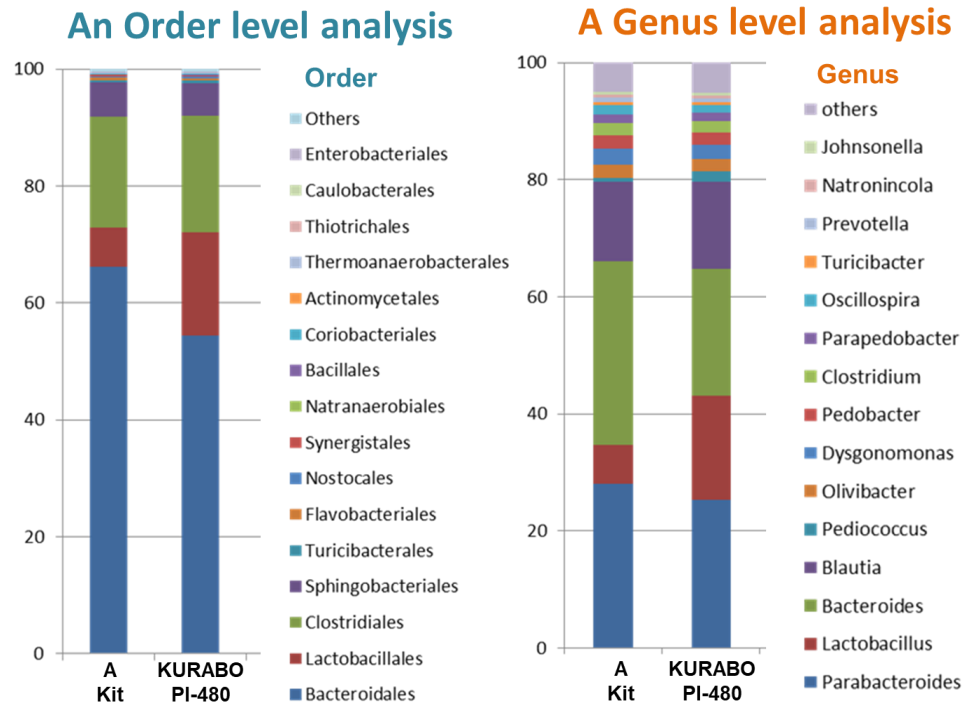
M: 1 kb DNA Ladder One (nacalai tesque)

PCR amplification



M: 100 bp DNA Ladder One (nacalai tesque)

16S rDNA metagenomics analysis



Species diversity comparison

	Shannon species diversity	The number of detected species of Bacteria
KURABO PI-480	2.130	266
A kit	1.973	214

Conclusion

From the results of spectrophotometry and electrophoresis, we found our application is able to isolate high molecular DNA from stool samples using PI-480. The isolated DNA was of a quality that could be used for PCR and 16S rDNA metagenomic analysis. Compared to other products dedicated to DNA extraction from stool, DNA isolated with PI-480 has high yield, high Shannon species diversity, which is an indicator of bacterial flora diversity, and many bacterial species can be identified.

Cooperation

National Institutes of Biomedical Innovation, Health and Nutrition, : Vaccine material project

Project Leader Mr. Jun Kunisawa

Researcher Mr. Koji Hosomi

Ordering Information

DNA isolation system : GENE PREP STAR PI-480、Tissue DNA protocol

Reagent kit : Tissue DNA kit , NR-201

Kit constitution	Reagent No.	Code	Content
Proteinase dissolving reagent	2	NR-2025	1
Deproteinizing reagent	3	NR-3025	1
Precipitation reagent	4	NR-4050	1
Washing reagent	5	NR-5050	3
DNA dissolving reagent	7	NR-7025	1
Tissue suspending reagent	10	NR-10025	1

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