Set up the optimal TaqMan qPCR method for the screening of human gut microbiota

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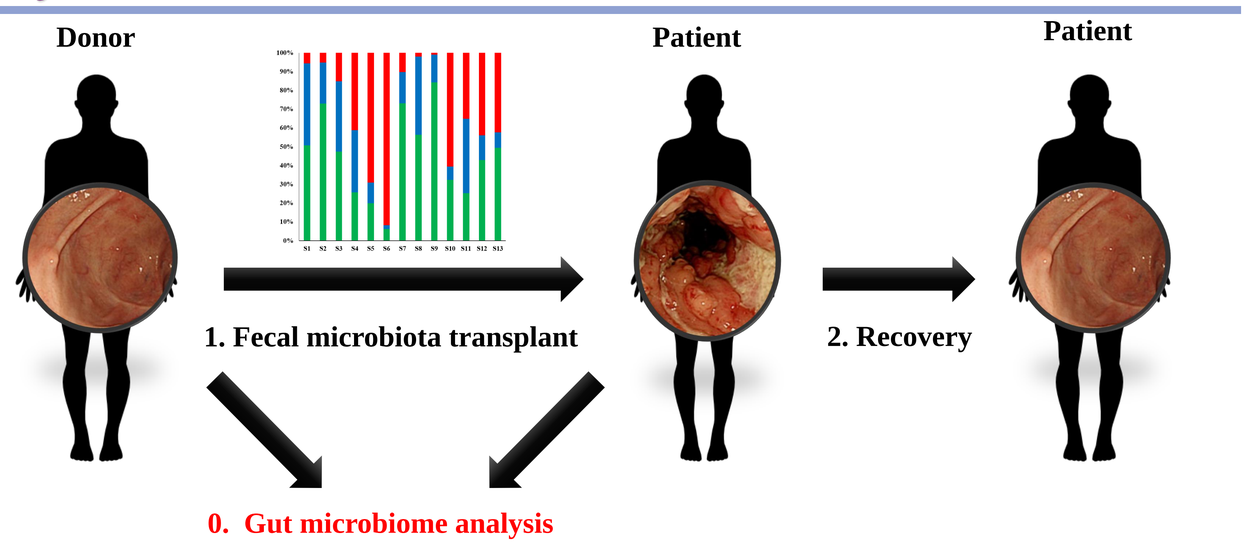
Abstract

Healthy gut microbiota are composed mostly of the Bacteroidetes and Firmicutes. When dysbiosis occurs, Proteobacteria are increased and the balance between Bacteroidetes and Firmicutes is broken. To solve this problem, Fecal Microbiota Transplantation (FMT) is used as an innovative treatment. For successful FMT processing, it is necessary to analyze the donors' and patients' gut microbiota. Next generation sequencing (NGS) has been used to analyze gut microbiome. Nevertheless, NGS method is expensive and takes time to get the results that it is not beneficial in emergency cases. Therefore, a cheaper and faster detection method is necessary and we set up TaqMan qPCR method to replace NGS. For this purpose, we designed the best specific probes and primers by in silico PCR. And adjusted TaqMan qPCR condition temperature, time, cycle of amplification step and an optimum condition was established. when the same samples were processed using of NGS and Taqman qPCR, samples contaminated with Proteobacteria that were detected by NGS were also found in TaqMan qPCR method. Furthermore, by confirming the relative abundance between phyla in the gut microbiota is consistent with the NGS results, we believe this TaqMan qPCR method could be used instead of NGS providing cheaper and faster result.

Objective



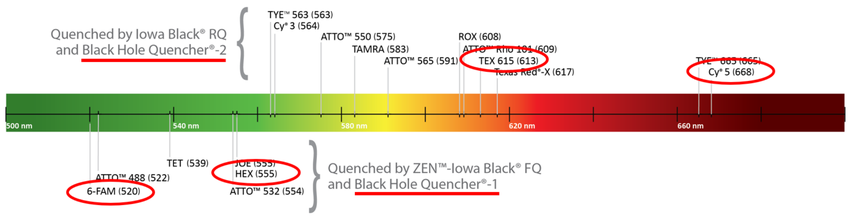
NGS has been used to analyze gut microbiome for decade However NGS is expensive and takes time to get result therefore It is necessary to find other cheaper and faster method

Materials & Methods

❖ Primer, Probe designing

- ✓ **Multiple sequence alignment (MSA)**
- ✓ **Search each common regions in 16s rRNA gene region (Bacteroidetes, Firmicutes, Proteobacteria)**

❖ Used primer and probe



❖ Comparison NGS and TaqMan qPCR

- **Accuracy**
- **High price**
- **Take long times**
- **Not suit for quantity analysis**

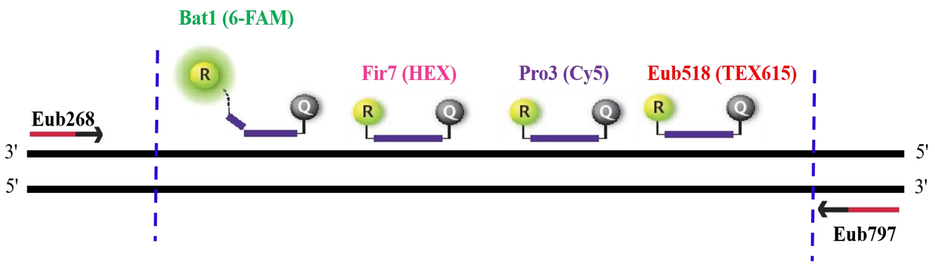
VS

- **Accuracy**
- **Low price**
- **Able to rapid quantity analysis**

❖ In silico PCR

Calculate theoretical polymerase chain reaction (PCR) results using a given set of primers (probes) to amplify DNA sequences from a sequenced genome

❖ Multiplex TaqMan qPCR layout



Results

❖Result of MSA

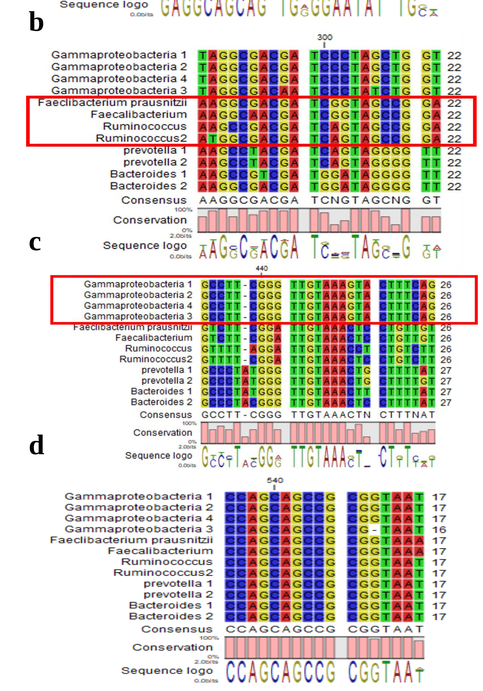


Figure 1. Multiple sequence alignment (MSA) for designing primers and probes
All sequences from NCBI and all sequences are aligned on CLC Main Workbench ver.8.1. **(a)** Bacteroidetes common region, **(b)** Firmicutes common region **(c)** Proteobacteria common region, **(d)** Eubacteria common region

❖ Specificity test

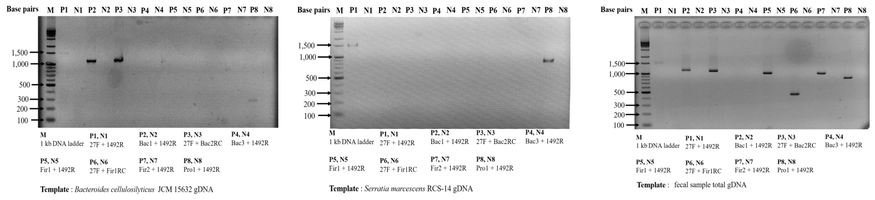


Figure 2. The designed candidate sequences specificity is confirmed by PCR with each phylum's template
(a) Two of three Bacteroidetes candidate sequences amplified DNA and Firmicutes, Proteobacteria candidates didn't amplify Bacteroidetes DNA **(b)** No Bacteroidetes and Firmicutes candidate sequences amplified Proteobacteria DNA on the other hand Proteobacteria did **(c)** All candidate sequences amplified fecal DNA

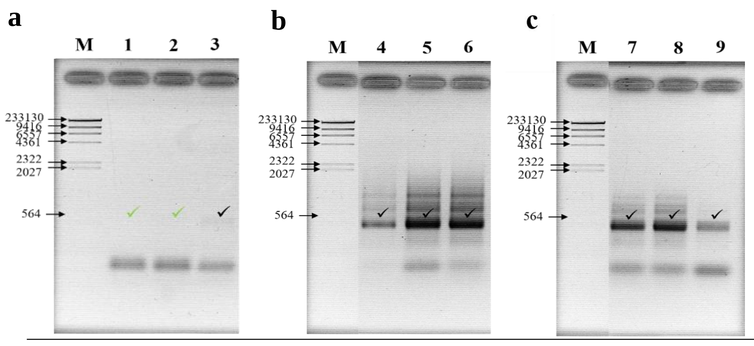
Table 1. The result of In silico PCR

Target phylum (Family)	Sequence	Bacteroidetes detect	Firmicutes detect	Proteobacteria detect
Bacteroidetes	5'-GATGAACTGCAGTGAGGAATATTGGT-3'	87%	9.3%	0.21%
Firmicutes	5'-AAGGCGAAATCGCGGGGCCGRM-3'	0.5%	81%	2.7%
Proteobacteria (Enterobacteriaceae)	5'-GCCTTCATACCACGGAGTACTTTCAGC-3'	0.01%	0.02%	34% (96%)
Eubacteria probe	5'-ATTACCGCGGCTGCTGG-3'		98%	
Eubacteria primer	Forward primer : 5'-ATTACCATATCGCTGG-3' Reverse primer : 3'-GGACTANAGTATCTAATCCTGTT-5'		88.9%	

All selected probe sequences are run by In silico PCR based on Silva database (SILVA 132). The probe for detect Bacteroidetes show 87% predicted detection ratio of all Bacteroidetes also this probe will detect only 9.3% of Firmicutes and 0.21% Proteobacteria. In probe for detect firmicutes case, this probe show 81% predicted detection ratio of all firmicutes also this probe will detect only 0.5% of Bacteroidetes and 2.7% Proteobacteria. In probe for detect Proteobacteria case, this probe show 34% predicted detection ratio of all Proteobacteria but detect 96% Enterobacteriaceae furthermore this probe is predicted detecting 0.01% Bacteroidetes and 0.02% Firmicutes.

Figure 4. Comparison NGS and TaqMan qPCR
Both NGS and TaqMan qPCR data show same Firmicutes/Bacteroidetes ratio and When Proteobacteria is detected by NGS, we can confirm TaqMan qPCR method also can detect Proteobacteria with similar ratio

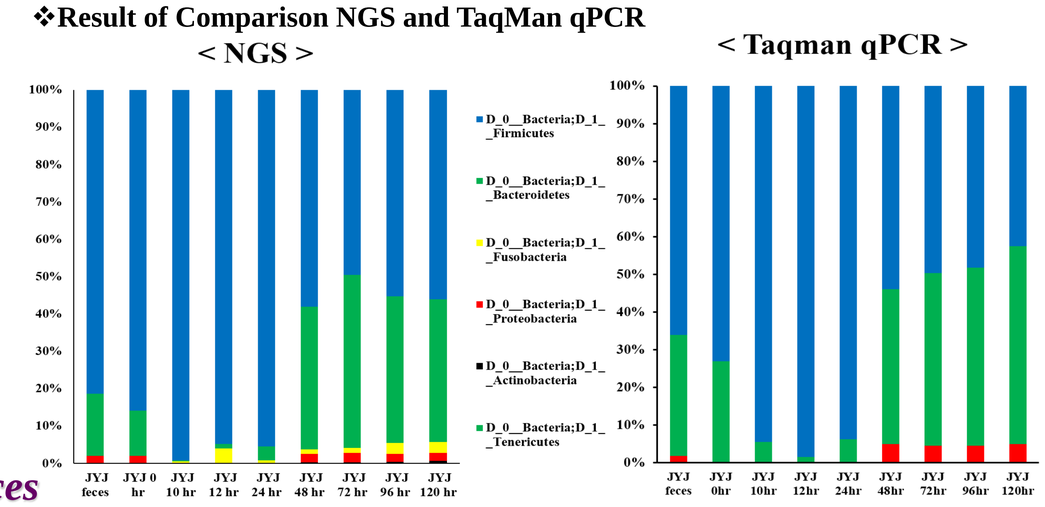
❖ Set up optimal TaqMan qPCR condition



Condition	Pre denaturation at 95˚C	Denaturation at 95˚C	Annealing at 60˚C	Extension at 72˚C	Amplification cycle no.
a	5 min	30 sec	1 min	-	40
b	5 min	30 sec	45 sec	15 sec	40
c	5 min	30 sec	30 sec	30 sec	40

Figure 3. Set up optimal TaqMan qPCR condition according to amplification step's temperature and time
(a) There's no band when condition was set as recommended protocol (b) There are band with targeted size however non specific bands are also appeared (c) when the TaqMan qPCR condition set as Condition c, non-specific band were decreased and The qPCR c(t) was constant

❖Result of Comparison NGS and TaqMan qPCR



Conclusion & Discussion

- ✓ This study suggests that this TaqMan qPCR method can replace NGS
- ✓ Furthermore, this method can be used for test safety of FMT medicine

References

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2. Cao, Heping, and Jay M. Shockey. "Comparison of TaqMan and SYBR Green qPCR methods for quantitative gene expression in tung tree tissues." *Journal of agricultural and food chemistry* 60.50 (2012): 12296-12303.