

Feedback from a Customer

Product: **FastGene® 0.2 ml PCR tubes with flat caps (FG-021F)**

Manufacturer: **NIPPON Genetics Co., Ltd**

Application: **Comparing PCR-tubes for Multiplex Probe-based Assay on a Rotor-Gene® Q**

The here presented data was provided by the courtesy of Dr. Birgit Klinkert, ARDEYPHARM GmbH, Herdecke, Germany

Background

The non-pathogenic *Escherichia coli* strain Nissle 1917 (EcN) is used as a probiotic drug against intestinal disorders and diseases as pharmaceutical preparation MUTAFLOOR® (Ardeypharm). Certain fitness factors, such as adhesive structures as fimbriae and flagella enables EcN to colonize the human intestine. The duration of this colonization depends on factors predetermined by the host and his microbiome.

The detection of EcN in stool samples is standardly performed in this laboratory using strain specific TaqMan® Probes. Here, the signal of the manufacturer's original plastic was compared to the FastGene® PCR tubes from NIPPON Genetics EUROPE.

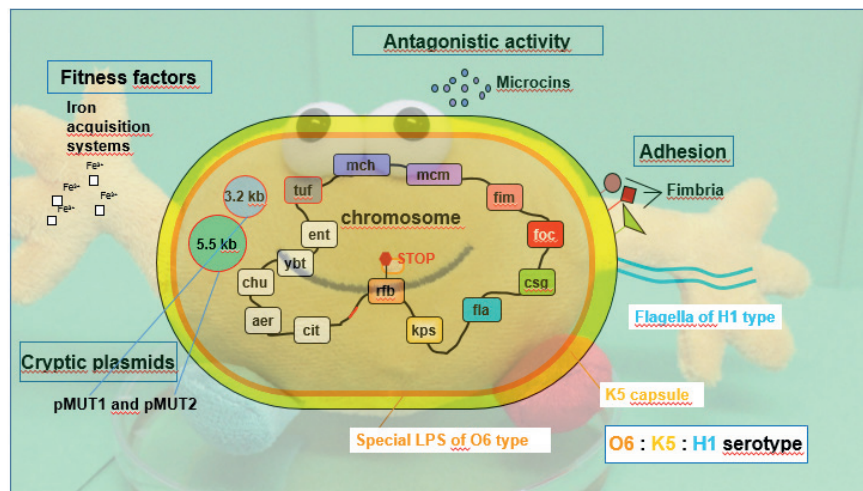


Fig. 1: *Escherichia coli* Nissle 1917 (EcN).

Methods

• PCR-Tubes

- 1) FastGene® 0.2 ml PCR tubes with flat caps (Cat.No. FG-021F)
- 2) Original 0.2 ml Rotor-Gene® tubes (Cat.No. 981005)

• Probes-labels

- 1) FAM™ (green channel) - Reporter primer designed to detect specific EcN plasmids
- 2) HEX (yellow channel) - Reporter primer designed to detect specific EcN plasmids
- 3) ROX™ (orange channel) - Reporter primer designed to detect specific regions in the EcN genome
- 4) Cy5 (red channel) - Reporter primer as a positive PCR control and designed to detect common enterobacteriae sequences

• qPCR Instrument

QIAGEN® Rotor-Gene® Q Mdx 5plex

• qPCR Cycling Conditions (TBC)

- 95 °C 5 min
- 94 °C 15 sec } x 30 cycles
- 60 °C 30 sec }

• qPCR Volumes

25 µl reaction volume



Results

The fluorescence curve of the four different reporter probes were analysed over 30 cycles in the original PCR tube (dark color) or in Fg-021F (light color) and are shown in the figure 2. The C_T -value detected by the qPCR instrument are summarized in Table 1.

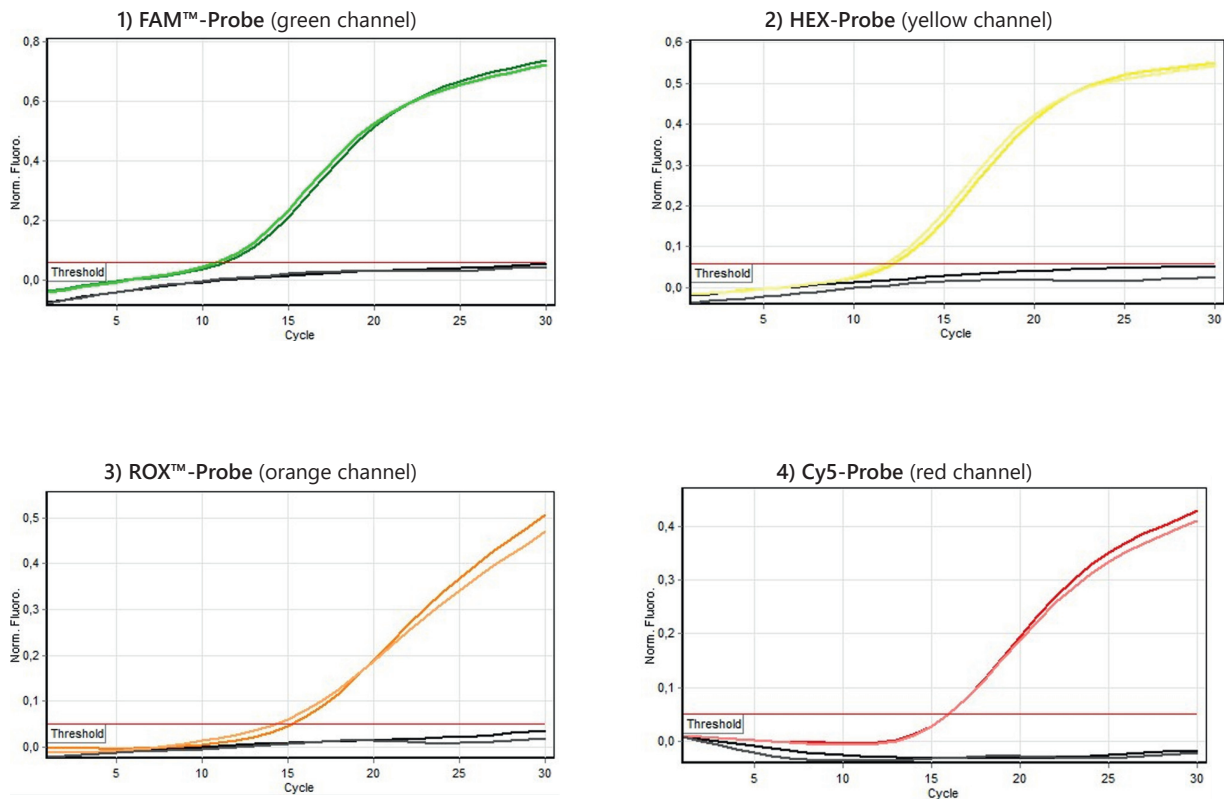


Fig. 2: Fluorescence measurement of four different probes in a multiplex reaction.

Reporter Dye	Filter-Channel	C_T QIAGEN® tube	C_T FastGene® tube	NTC
1) FAM™-Probe	green	11.31	10.83	n.d n.d
2) HEX	yellow	12.16	11.74	n.d n.d
3) ROX™	orange	15.37	14.28	n.d n.d
4) Cy5	red	15.99	15.98	n.d n.d

<Conclusion>

The FastGene® 0.2 ml PCR tubes does not interfere in the detection of the fluorescence of probes. The measured C_T -values were comparable or better. Hence, the plastic tubes can be used for Multiplex-qPCR using a Rotor-Gene® qPCR instrument.

<Customer's comments>

The lid of the FastGene® 0.2 ml PCR tubes are different from the original. Nonetheless, the lock mechanism of the Rotor-Gene® Q Mdx worked perfectly with them. The fluorescence of the probes in the reaction are measured at the tip of the tubes. We can recommend to replace the original tubes for the here tested fluorescent probes without any restriction.