# NEXTGENPCR


## APPLICATION NOTE

**RESEARCH USE ONLY (RUO)**

## NEXTGENPCR™ 16S FULL GENE AMPLIFICATION KIT

### HIGHLIGHTS

- Single-day laboratory workflow suited for standard microbiological laboratories
- Ultrafast amplification of full length 16S rDNA sequences in ≤ 21 minutes
- Complete PCR kit including all necessary chemistries for target sequence amplification
- High fidelity sequence data generation for V1–V9 regions allowing species-level determination
- Suitable for complex and difficult to amplify samples, i.e. polymicrobial specimens

THE WORLD'S FASTEST PATENTED PCR THERMAL CYCLER

## BACKGROUND

Sequencing of the 16S ribosomal DNA (16S rDNA) gene is a well-established method to identify bacterial species in biological specimens. Taxonomic identification using 16S sequence is based on exploiting the unique combination of hyper conserved and variable regions (V1 – V9).
The NextGenPCR™ 16S Full Gene Amplification Kit is a complete PCR assay solution intended for ultrafast analysis of bacterial composition in a biological specimen. The kit includes all necessary materials (primer oligonucleotides, ready-to-use polymerase master mix and genomic bacterial DNA control) to amplify the full length 16S rDNA gene in ≤ 21 minutes total cycling time.

Performance of the NextGenPCR™ 16S Full Gene Amplification Kit was evaluated using DNA template material isolated from the Microbial Community Standard (ZymoBIOMICS) using a MagNA Pure 96 System (Roche). Using 10 ng gDNA template as input and the cycling program listed in Table 1, amplicons were generated on a NextGenPCR™ Thermal Cycler using either NextGenPCR™ Arctic Fox HF Chemistry-2× (Molecular Biology Systems) or a competitor's polymerase mix, of which two were tested. Amplicons were barcoded using a Rapid Barcoding Kit (SQK-RBK114.96) and sequenced using a Flow Cell R9.4.1 on a MinION MK1B sequencer (Oxford Nanopore Technologies). Sequences were analyzed using a custom bioinformatics pipeline (MABA16S, available through GitHub).

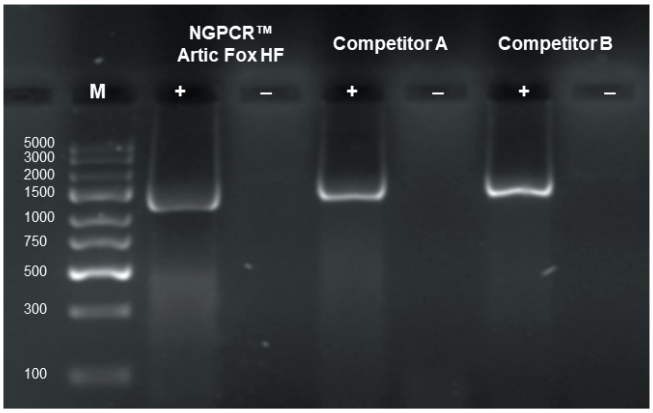
### CYCLING CONDITIONS FOR THE SEQUENCED SAMPLES

| | Cycle № | Time | Temp | Total cycling |
|---|---|---|---|---|
| **NextGenPCR™ Arctic Fox HF** | 1 | 30 sec | 98 °C | 21 min |
| | 30 | 10 sec | 98 °C | |
| | | 15 sec | 54 °C | |
| | | 15 sec | 68 °C | |
| **Competitor A** | 1 | 30 sec | 98 °C | 43 min |
| | 30 | 10 sec | 98 °C | |
| | | 15 sec | 54 °C | |
| | | 60 sec | 68 °C | |
| **Competitor B** | 1 | 30 sec | 98 °C | 36 min |
| | 30 | 10 sec | 98 °C | |
| | | 15 sec | 54 °C | |
| | | 30 sec | 68 °C | |

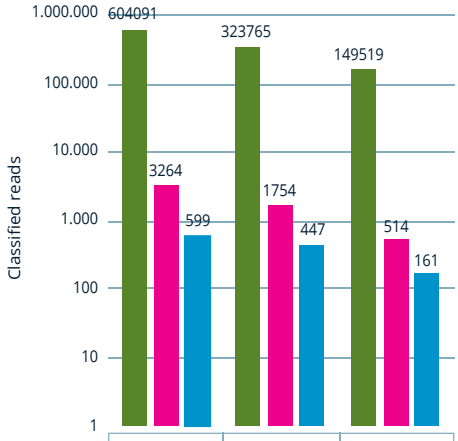
Analysis of the microbial community standard sample mix revealed excellent amplification results for all three tested chemistries, with clear bands at the ~1,500 bp range corresponding to the full length 16S ribosomal DNA gene sequence (Figure 1A). Sequencing amplicons for 26 hours on a MinION flow cell yielded the highest number of classified reads for the NextGenPCR™ Arctic Fox HF Chemistry library (604,091 reads) as compared to competitor A (323,765 reads) and competitor B (149,519 reads) libraries while also having the shortest total amplification time of 21 minutes. In all three cases, the number of reads mapping to other bacterial species was ≤ 0.5% and the number of unmappable reads was ≤ 0.1% (Figure 1B), demonstrating that the NextGenPCR™ Thermal Cycler is compatible with multiple polymerase mixes and generates excellent quality sequence data.

# AMPLIFICATION OF ~1500BP FRAGMENT OF THE 16S FULL GENE SEGMENT

**A**




**B**



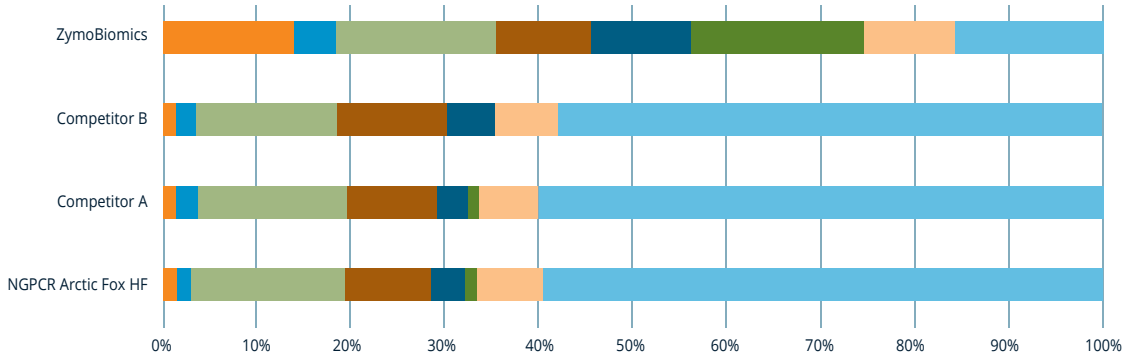

| | NGPCR Arctic Foc HF | Competitor A | Competitor B |
|---|---|---|---|
| N reads TARGET SP. | 604091 | 323765 | 149519 |
| N reads OTHER BAC. | 3264 | 1754 | 514 |
| N reads NO HIT | 599 | 447 | 161 |

***Figure 1.***

***(A)*** *Amplification of ~1500bp fragment of the 16S full gene segment using different polymerases and cycling programs on the NextGenPCR™ Thermal Cycler. The starting template was ~10 ng input Microbial Community Standard (+), with a non-template control (-) for each condition.*

***(B)*** *Number of classified reads was highest using the NextGenPCR Arctic Fox HF Chemistry as compared to competitors.*

# DISTRIBUTION OF CLASSIFIED SPECIES IN EXPERIMENTAL CONDITIONS




| | NGPCR Arctic Foc HF | Competitor A | Competitor B | ZymoBiomics |
|---|---|---|---|---|
| Listeria monocytogenes | 1,57% | 1,54% | 1,56% | 14,10% |
| Pseudomonas aeruginosa | 1,51% | 2,33% | 2,02% | 4,20% |
| Bacillus subtilus | 16,39% | 15,76% | 15,08% | 17,40% |
| Escherichia coli | 9,22% | 9,48% | 11,58% | 10,10% |
| Salmonella enterica | 3,38% | 3,26% | 3,82% | 10,40% |
| Lactobacillis fermentum | 1,27% | 1,21% | 1,48% | 18,40% |
| Entercoccus faecalis | 7,15% | 6,41% | 6,63% | 9,90% |
| Staphylococcus aureus | 59,52% | 60,00% | 57,84% | 15,50% |

***Figure 2.*** *Distribution of classified species in experimental conditions as compared to the theoretical composition of the ZymoBIOMICS Microbial Community Standard.*

## CONCLUSION

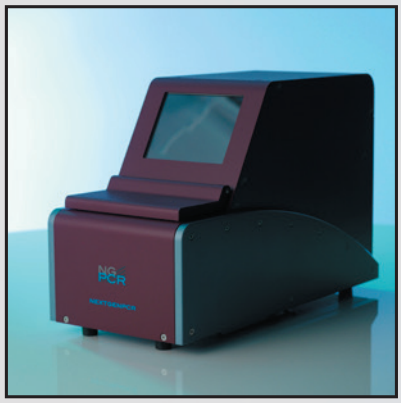
Integration of ultrafast amplification of the 16S gene using NextGenPCR™ products and rapid sequencing provides a promising new avenue for bacterial identification in a biological sample within a single working day. The NextGenPCR™ 16S Full Gene Amplification Kit offers several pronounced advantages over existing bacterial identification methods such as Sanger sequencing and MALDI-TOF, including the capability to simultaneously detect multiple organisms in a sample and the ability to confidently determine negative results if no reads are obtained.

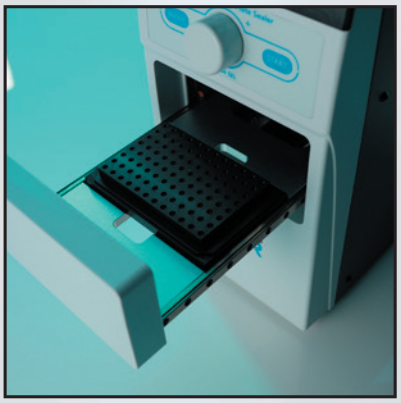
**Find out more about NextGenPCR™ ultrafast PCR products and applications on our website over at:**

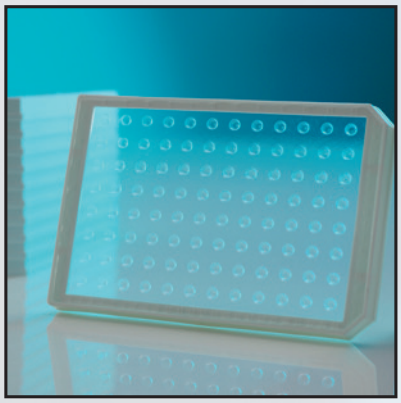
**www.nextgenpcr.com**

## RELATED PRODUCTS

| P/N | Product name | Packaging size |
|---|---|---|
| #10001 | NextGenPCR™ Thermal Cycler | 1 unit |
| #10101/10102 | NextGenPCR™ Semiautomatic Heat Sealer | 1 unit |
| #20102 | NextGenPCR™ Sealing Anvil 96×20 µL | 1 unit |
| #20301/20305 | NextGenPCR™ Pipetting Anvil 96×20 µL | 1 unit in white/black color |
| #33602/33603 | EZtrieve™ Microplates 96×20 µL | 50/500 microplates per pack |
| #30110/30111 | EZtrieve™ Aluminum Heat Seals | 50/500 sheets per pack |
| #50034 | NextGenPCR™ 16S rDNA Full Gene Amplification Kit | 100×20 µL reactions per pack |







## DISCLAIMER

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