Product Profile

QIAamp® DNA Microbiome Kit

For isolation of bacterial microbiome DNA from mixed samples

The QIAamp DNA Microbiome Kit is a dedicated solution for the purification and enrichment of bacterial microbiome DNA from swabs and body fluids. Effective depletion of host DNA during the purification process maximizes bacterial DNA coverage in next-generation sequencing analysis and allows highly sensitive 16S rDNA-based microbiome analysis and metagenomic shotgun sequencing studies.

The QIAamp DNA Microbiome Kit provides:

- Isolation of bacterial DNA from swabs and body fluids
- Effective depletion of host DNA
- Optimized mechanical and chemical cell lysis
- Ultra-clean columns to minimize contamination risk

Effective host DNA depletion for deeper microbiome analysis

Swabs and body fluids contain DNA from host human or animal cells as well as microbial cells. In metagenomic studies, total DNA is standardly isolated and analyzed, however, host human or animal DNA greatly outweighs the microbial DNA, which can hamper microbiome analysis. In fact, a key finding of the whole metagenome shotgun sequencing of different sample types conducted by the Human Microbiome Project was that up to 99% of the sequencing reads corresponded to the human genome, so that at most 1% were of microbial nature (1). In contrast to 16S rDNA sequencing, whole metagenome shotgun sequencing can add valuable insight into microbiome studies such as the presence of virulence factors, antibiotic resistance or metabolic networks. Thus, maximized coverage of microbial reads greatly enhances analysis power. ▷

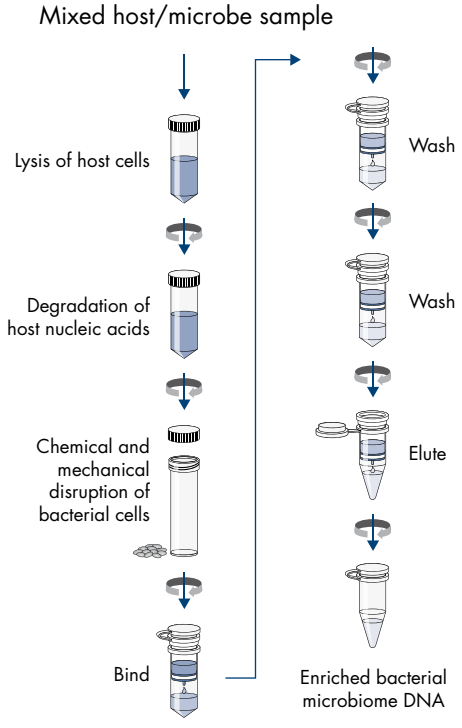

Figure 1. Purification procedure with integrated host DNA removal. Differential lysis and enzymatic treatment remove host DNA during the purification process enabling enrichment of bacterial microbiome DNA.

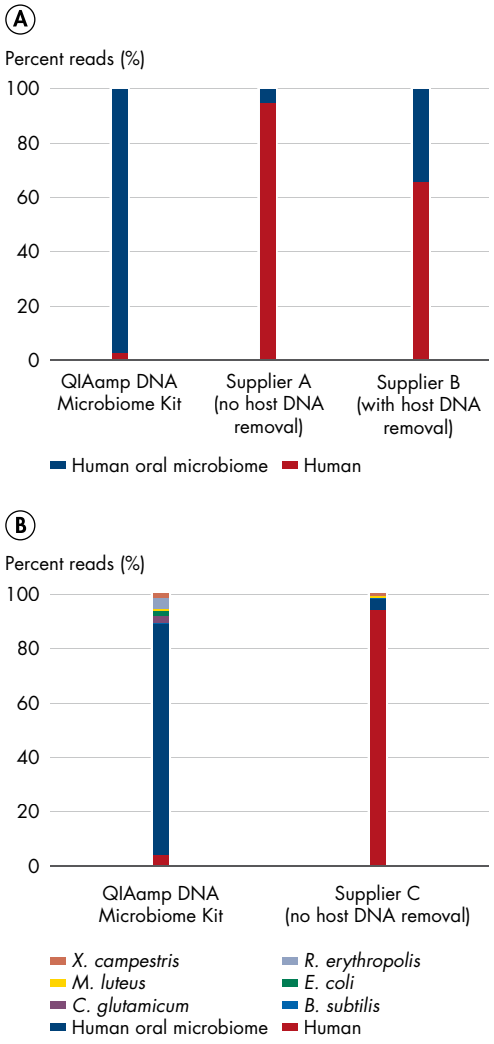

Figure 2. Effective host DNA removal enhances whole metagenome shotgun sequencing results. DNA isolated from buccal swabs using the QIAamp DNA Microbiome Kit or solutions from alternative suppliers was analyzed by whole metagenome shotgun sequencing on the Illumina® MiSeq® platform. **A** The percentage of human-derived reads was quantified and compared between the QIAamp DNA Microbiome Kit and alternative solutions with (Supplier B) and without (Supplier A) host DNA depletion protocols. Without host DNA removal, over 90% of reads mapped to the human genome. Compared to the kit from Supplier B, the effective removal of host DNA afforded by the QIAamp DNA Microbiome Kit resulted in over 90% reads for microbiome analysis. **B** To monitor recovery of bacterial species, buccal swab samples were spiked with an artificial sample of 6 bacterial species. Again, the effective host DNA removal achieved with the QIAamp DNA Microbiome Kit increases the coverage of bacterial species compared to a solution that affords no host DNA depletion (Supplier C).

Removal of host DNA increases the coverage of microbial reads in sequencing experiments. The QIAamp DNA Microbiome Kit efficiently removes host DNA by differential lysis of host cells and subsequent enzymatic digestion of host DNA. Then, using a combination of mechanical and chemical lysis, intact cells are efficiently lysed and the released bacterial DNA is purified using proven QIAamp chemistry and decontaminated QIAamp UCP spin columns (Figure 1).

Enhanced resolution of bacterial DNA in whole metagenome shotgun sequencing experiments

The high percentage of reads attributed to the host genome in whole metagenome shotgun sequencing experiments makes proper assembly of microbial datasets time consuming and resource intensive, even with sophisticated bioinformatics tools. By removing host DNA in a sample, the QIAamp DNA Microbiome Kit provides samples with enriched bacterial composition for whole metagenome shotgun sequencing. Figure 2A compares reads from whole metagenome shotgun sequencing experiments performed on DNA isolated from human buccal swabs, where samples were prepared with either the QIAamp DNA Microbiome Kit or solutions from 2 other suppliers. Sequencing of samples prepared with the QIAamp DNA Microbiome Kit resulted in less than 5% human reads, greatly reduced compared to over 90% reads with a kit that does not include host DNA removal and 35% with a kit that does. In a second experiment, samples were spiked with known cultured bacteria. Bacterial DNA was recovered with higher representation compared to a solution without host DNA removal (Figure 2B).

Improved amplification for 16S rDNA sequencing

16S rDNA sequencing is commonly used to determine relative microbial community composition. The QIAamp DNA Microbiome Kit enables the most efficient amplification of the V4 region of DNA purified from buccal swabs when compared to 3 other purification kits (Figure 3A). Sequencing results of the amplified V4 region from 2 samples purified with the QIAamp DNA Microbiome Kit exhibited the expected bacterial composition representative of a human oral microbiome (Figure 3B).

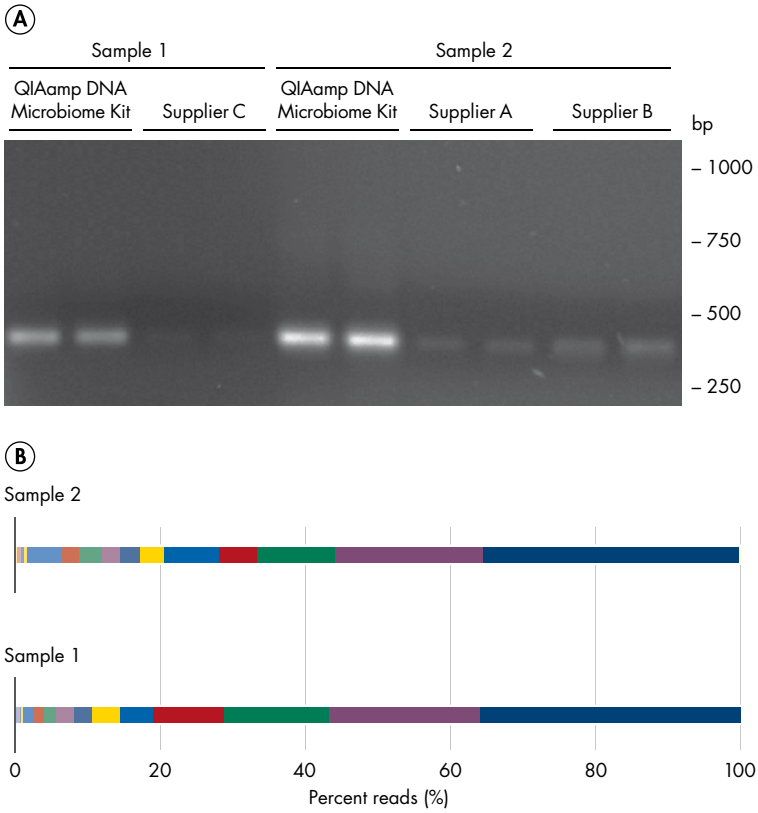

Figure 3. Efficient amplification and sequencing of 16S rDNA. **A** DNA (10 ng) was purified from buccal mucosa swabs with the QIAamp DNA Microbiome Kit or kits from 3 alternative suppliers and the V4 region was amplified. After 25 cycles, amplification of samples isolated with the QIAamp DNA Microbiome protocol was most efficient, demonstrating the benefit of host DNA removal. **B** After sequencing on the Illumina® MiSeq® platform, the V4 amplicons of 2 samples purified with the QIAamp DNA Microbiome Kit were mapped to reference genomes derived from the Ribosomal Database Project. Bacterial composition was representative of the human oral microbiome. Primers for the V4 region were modified from Caporaso, J.G., et al. (2).

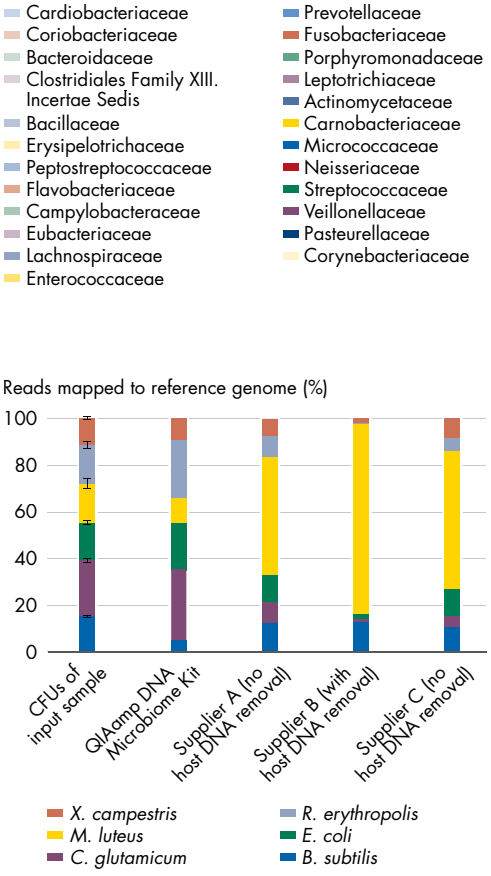

Figure 4. Minimal sample preparation bias. The optimized protocol of the QIAamp DNA Microbiome Kit reduces bias due to differential susceptibility of microbes to lysis. A model microbiome was created by adding 6 cultured bacterial species to buccal swab samples. Community composition was quantified by plate counts. Whole metagenome shotgun sequencing was performed on the Illumina MiSeq platform and output reads were compared to input amounts. Relative composition was maintained most closely using the QIAamp DNA Microbiome Kit for sample preparation.

Optimized cell lysis to minimize sample preparation bias

Differences in cell wall morphology render microbes differentially susceptible to different lysis methods. For example, bacteria with thick cell walls, rich in lipids and polysaccharides, tend to be underrepresented in samples prepared via enzymatic lysis. The result is that any one lysis method can introduce a bias in the representation and relative composition of a microbiome. The QIAamp DNA Microbiome Kit uses a combination of chemical and mechanical lysis, optimized to minimize bias introduced by sample preparation. Furthermore, the Ultra Clean Production (UCP) spin columns provided in the QIAamp DNA Microbiome Kit undergo a proprietary cleaning process to minimize the risk of contamination. To examine sample preparation bias, a model community with known numbers of colony forming units (CFUs) of 6 different bacteria was created from cultures. Samples of the model microbiome were prepared for sequencing using the QIAamp DNA Microbiome Kit or alternative kits. The samples processed with the QIAamp DNA Microbiome Kit exhibited the best overall representation of the model microbiome composition compared to all other methods used (Figure 4).

References

1. The Human Microbiome Project Consortium. (2012) A framework for human microbiome research. Nature **486**, 215.
2. Carporaso, J.G., et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. **6**, 1621.

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Microbiome Kit	For 50 preps: 50 QIAamp UCP columns, 50 Pathogen Lysis Tubes L, buffers and reagents	51704
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes with small beads, 1 vial Reagent DX	19091
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes with large beads, 1 vial Reagent DX	19092

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