



# ZymoBIOMICS™

Standardizing Microbiomics



# Standardizing Microbiomics



## Optimizing Microbiomics Workflows

Advances in DNA sequencing and other genome-enabled technologies have lowered the cost and time requirements needed to sequence any organism. Next-Generation Sequencing of microbial communities has supercharged our ability to research and explore both human and environmental microbial ecosystems. This advancement in technology has allowed, for the first time, large-scale, multi-lab studies of microbial communities<sup>1</sup>.

Early quality control studies of microbiomic research suggest that this vast new frontier of research is littered with potential sources for error and bias. From collection to sequencing, the potential for variation at each step in the microbiomic workflow is enormous.

During the collection and transportation phase, depending on the quality of the method, microbes can continue to grow and decay. Nucleic acids can also degrade during this phase. These two factors can lead to overall misrepresentation of the original community profile, causing downstream bias. In the extraction phase, inferior forms of lysis can fail to extract DNA from tough-to-lyse microbes (e.g. Gram-positive bacteria and yeast), leading to the underrepresentation of tough-to-lyse microbes and overrepresentation of easy-to-lyse microbes (e.g. Gram-negative bacteria). During sequencing, different library preparation methods can cause biased coverage depending on the GC content of the organisms.

As multi-lab, longitudinal microbiomic studies become more common, there is an urgent need for standards to establish validated methods for reproducible data<sup>2</sup>.

At Zymo Research, we have made it our goal to eliminate bias across the entire microbiomic workflow. Our new product line, ZymoBIOMICS™, is a complete workflow, from collection to analysis, which offers streamlined collection, purification, and the first microbial community standards.

Zymo Research has endeavored to develop standards for the advancement and optimization of microbiomic workflows to allow researchers to have increased confidence in their microbiomics data. ZymoBIOMICS™ Microbial Community Standards are groundbreaking community standards, containing a well-defined and characterized community of Gram-positive bacteria, Gram-negative bacteria and yeast. They are ideal for validating and optimizing microbiomics and metagenomic workflows from extraction to analysis.

At collection, DNA/RNA Shield™ stabilizes nucleic acids and inactivates all organisms — including pathogens — in your sample, allowing for accurate community profiling. There is no degradation of nucleic acids, creating a perfect molecular snapshot of your sample at the time of collection.

Our ZymoBIOMICS™ DNA Mini Kit ensures accurate community profiling by enabling unbiased lysis from any sample, including feces, soil, water, biofilms, swabs, body fluid, etc. DNA extracted from this kit is ultra-pure, inhibitor-free, and ready for all downstream applications, including PCR 16S rRNA gene sequencing and shotgun sequencing.

If you would prefer to leave the work to us, we offer a comprehensive list of ZymoBIOMICS™ Services covering all steps of a microbiomics workflow from collection to analysis. Simply send us your sample and we will handle the rest. All workflows are validated to be non-biased using the ZymoBIOMICS™ Microbial Community Standards and provide publication-ready data.

ZymoBIOMICS™ - Standardizing Microbiomics

1. Sinha, Rashmi, et al. "The microbiome quality control project: baseline study design and future directions." *Genome biology* 16.1 (2015)

2. Stulberg, Elizabeth, et al. "An assessment of US microbiome research." *Nature Microbiology* 1 (2016): 15015.

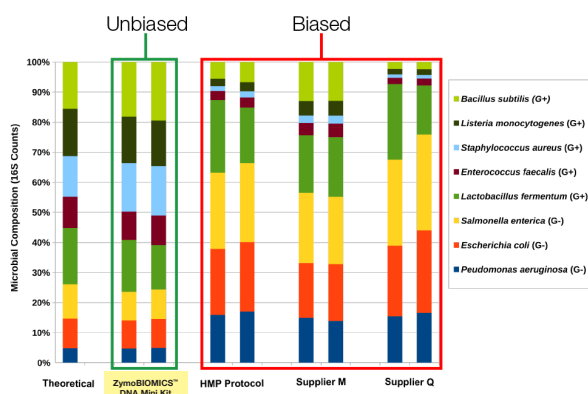
# ZymoBIOMICS™ Microbial Community Standards



## Highlights

- Mock microbial community of well-defined composition for workflow validation optimization
- DNA standard of the same mock community for sequencing validation and optimization
- Perfect for assessing bias of DNA extraction methods along with the quality control of microbiome profiling and metagenomic analyses

## Standards for Optimizing Microbiomics Workflows



### Find Your Bias & Eliminate It

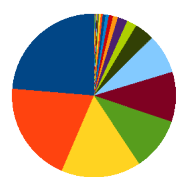
ZymoBIOMICS™ Microbial Community Standard was used to compare different DNA extraction protocols. DNA samples were profiled by 16S rRNA gene targeted sequencing. HMP Protocol stands for the fecal DNA extraction protocol used by the Human Microbiome Project.

### Accurate Characterization

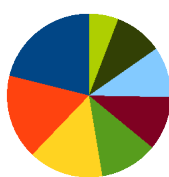
Containing three easy-to-lyse Gram-negative bacteria, five tough-to-lyse Gram-positive bacteria, and two tough-to-lyse yeasts, the ZymoBIOMICS™ Microbial Community Standards are perfect for assessing bias in various DNA extraction methods. The microbial standards are accurately characterized, with a wide GC range (15%-85%) and contain negligible impurities (<0.01%), enabling easy exposure of artifacts, errors, and bias in microbiomics or metagenomic workflows.

Species	Ave. GC %	Gram Stain	gDNA (%)
<i>Bacillus subtilis</i>	43.8	+	12
<i>Listeria monocytogenes</i>	38	+	12
<i>Staphylococcus aureus</i>	32.7	+	12
<i>Enterococcus faecalis</i>	37.5	+	12
<i>Lactobacillus fermentum</i>	52.8	+	12
<i>Salmonella enterica</i>	52.2	-	12
<i>Escherichia coli</i>	56.8	-	12
<i>Pseudomonas aeruginosa</i>	66.2	-	12
<i>Saccharomyces cerevisiae</i>	38.4	Yeast	2
<i>Cryptococcus neoformans</i>	48.2	Yeast	2

## Reduce Noise in Your 16S rRNA Gene Seq. with the ZymoBIOMICS™ Microbial Community DNA Standard



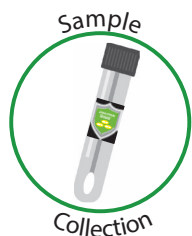
**Before**  
Noise and bias



**After**  
True composition of the standard

Controlling noise/rare biosphere in 16S rRNA gene targeted sequencing with ZymoBIOMICS™ Microbial Community DNA Standard. The pie chart on the left is the microbial composition profile of the standard determined by a regular workflow of 16S sequencing using primers targeting v3-4 region. The pie chart on the right is the profile of the same standard determined using the same primer sets but with an optimized in-house workflow of 16S sequencing. Noise observed on the left panel were mainly caused by PCR chimera, process contamination and reagent contamination, which were controlled in the optimized workflow.

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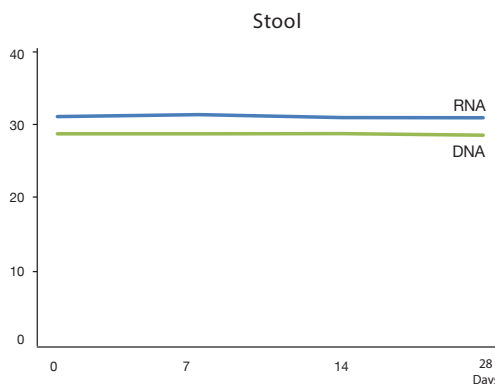
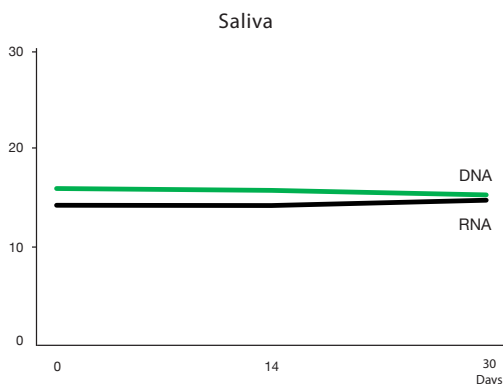


# DNA/RNA Shield™

## Highlights

- Avoid bias or erroneous results due to compositional changes from nucleic acid degradation or microbial growth
- DNA/RNA Shield™ provides an unbiased molecular snapshot of the sample at the time of collection by preserving nucleic acids at ambient temperature and inactivating microbes

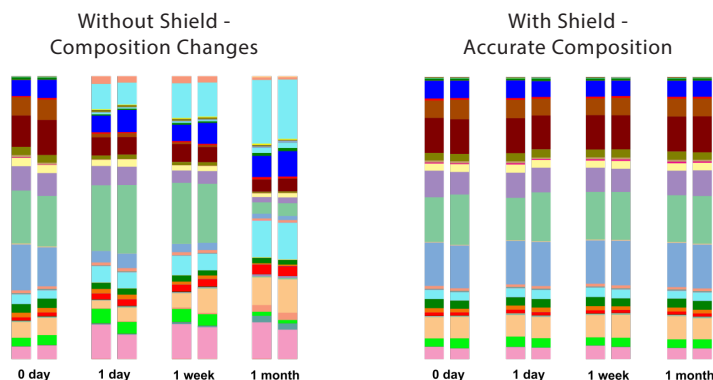
## Nucleic Acid Stabilization at Ambient Temperature



DNA and RNA in saliva and stool is effectively stabilized in DNA/RNA Shield™ at ambient temperature. Cellular RNA from human whole blood and spike-in DNA and RNA controls from saliva, stool and tissue purified at the indicated time points and analyzed by (RT)qPCR. Controls: HSV-1 and HIV (AcroMatrix, Life Technologies).

## DNA/RNA Shield™ Preserves Microbial Composition at Ambient Temperature

Microbial composition of stool is unchanged after one month at ambient temperature with DNA/RNA Shield™. Stool samples suspended in DNA/RNA Shield™ and stored at room temperature were compared to stool without preservative for one month. They were sampled at the indicated time points and processed with ZymoBIOMICS™ DNA Mini Kit. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Graphs show both phylum composition (left) and genus composition (right). Samples stored with DNA/RNA Shield™ had a constant microbial composition while the samples stored without shifted dramatically.



## Break the Cold Chain

Not the bank!

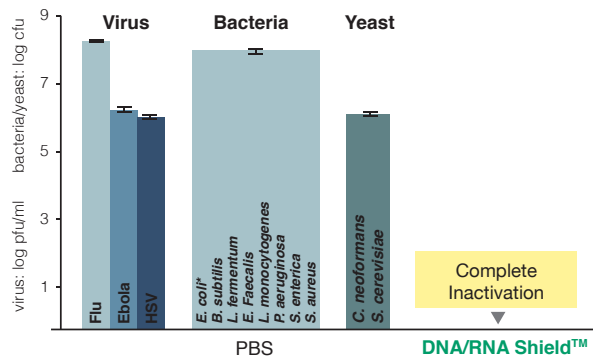


Transport at ambient temperatures

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## Microbial Inactivation



Viruses, bacteria and yeast are effectively inactivated by DNA/RNA Shield™. Samples containing the infectious agent (virus, bacteria, yeast) were treated with DNA/RNA Shield™ or mock (PBS) treated for 5 minutes. Titer (PFU) was subsequently determined by plaque assay. Validated by: Influenza A - D. Poole and Prof. A. Mehle, Department of Medical Microbiology and Immunology, University of Wisconsin, Madison; Ebola (Kikwit) - L. Avena and Dr. A. Griffiths, Department of Virology and Immunology, Texas Biomedical Research Institute; HSV-1/2 - H. Oh, F. Diaz and Prof. D. Knipe, Virology Program, Harvard Medical School; E. coli, L. fermentum, B. subtilis, S. cerevisiae - Zymo Research Corporation).

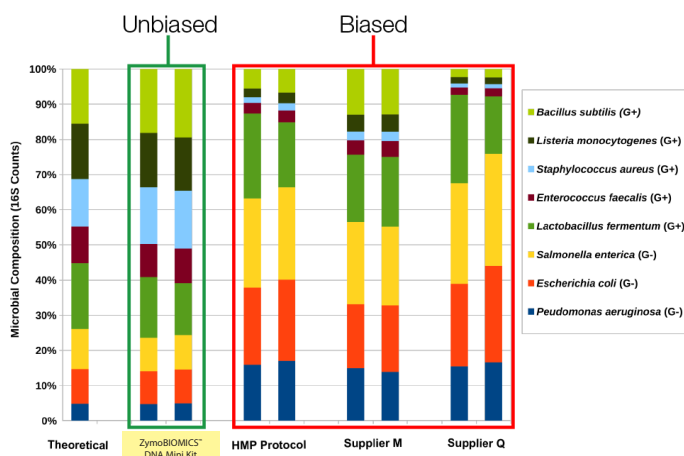
# ZymoBIOMICS™ DNA Isolation Kits



## Highlights

- Ultra-pure, inhibitor-free DNA from any sample (feces, soil, water, biofilms, swabs, body fluid, etc.) that is ideal for all downstream applications including PCR, arrays, 16S rRNA gene sequencing, and shotgun sequencing
- Innovative lysis system enables efficient and unbiased lysis of microbes including Gram-positive/negative bacteria, fungus, protozoans, and algae for accurate microbial community profiling

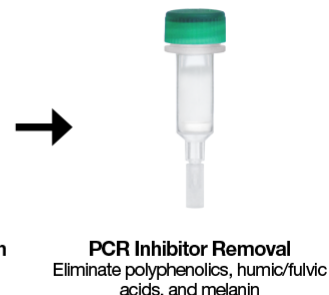
## Accurate Community Profiling



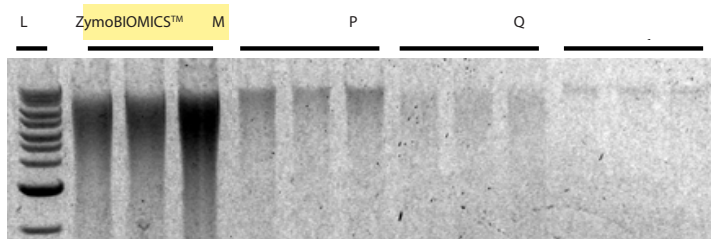
## Validated, Unbiased DNA Isolation

The ZymoBIOMICS™ DNA Mini Kit provides unbiased representation of the organisms extracted from the ZymoBIOMICS™ Microbial Community Standard.

## Innovation. Pure & Simple.™

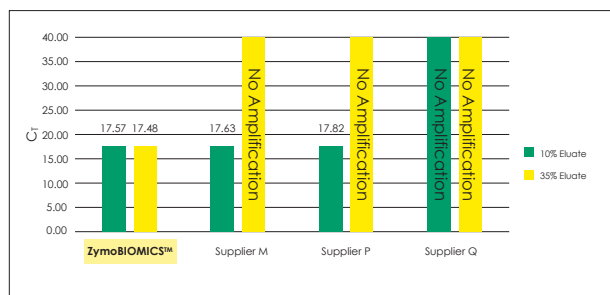


## Superior Yields



The ZymoBIOMICS™ DNA Mini Kit provides superior yields when compared to Suppliers M, P, and Q.

## Ultra-pure DNA from Inhibitor Rich Samples



The ZymoBIOMICS™ DNA Mini Kit provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples.

To learn more, visit  
[www.zymoresearch.com/zymbiomics](http://www.zymoresearch.com/zymbiomics)



# ZymoBIOMICS™ PCR Pre-Mix and Femto™ DNA Quantification Kits

## Highlights

- Use the low-bioburden, ZymoBIOMICS™ PCR Pre-Mix to ensure non-biased quantification and microbiomic analysis
- The Femto™ DNA Quantification Kits can reliably detect and quantify bacterial, fungal, and human DNA with high sensitivity and specificity to as little as 20 fg of DNA in 1 µl of eluate

## Low-Bioburden DNA Polymerase

A single preparation, using pure water as a process control, is guaranteed to contain less than one bacterial genomic copy per 1 µl of eluate as determined by quantitative amplification of the 16S rRNA gene when eluted using 100 µl water.

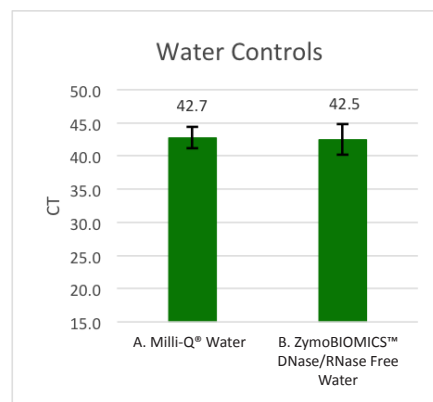
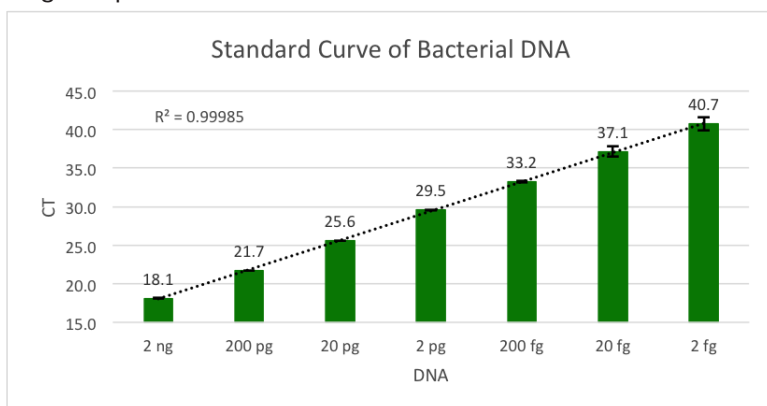
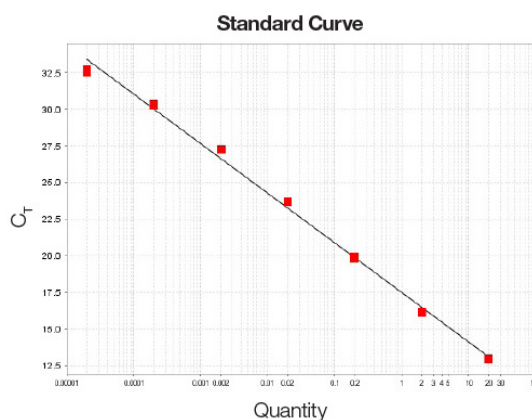
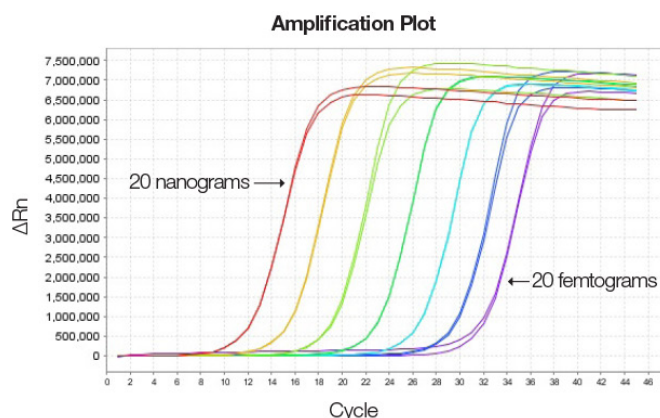


Figure 1 (left). A tenfold serial dilution of *Lactobacillus fermentum* genomic DNA was quantified via real-time PCR, after the addition of 2.5 µM SYTO® 9 to a 20 µl reaction volume. Amplification of the 16S rRNA gene can be quantified down to 2 femtograms of bacterial genomic DNA in a 45 cycle qPCR. Figure 2 (right). Quantification of no template controls (NTCs) via real-time PCR was determined by amplification of the 16S rRNA gene, after the addition of 2.5 µM SYTO® 9 to a 20 µl reaction volume. Real-time PCR was performed for 45 cycles to determine the amount of bacterial contamination. NTCs include (A) Millipore filtered water and (B) DEPC treated Millipore filtered water.

## Accurately Quantify Bacterial, Fungal, or Human DNA



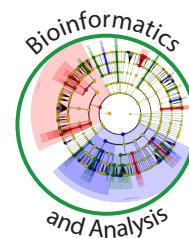
Reliable standards for the quantification of bacterial DNA: Bacterial DNA Standards (measured in duplicates) comprise a 10-fold dilution series ranging from 20 ng to 20 fg.

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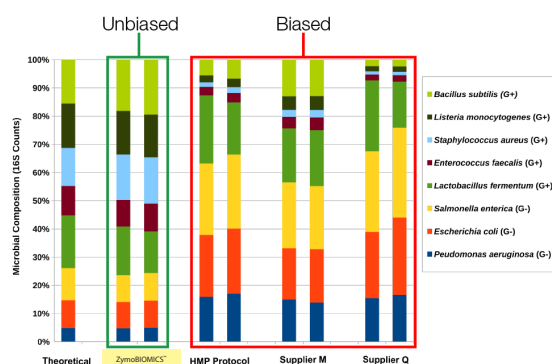
# ZymoBIOMICS™ Services

## Highlights

- Zymo Research offers the most comprehensive services for 16S rRNA and shotgun sequencing from any sample type
- ZymoBIOMICS™ Services are validated using the ZymoBIOMICS™ Microbial Community Standards for unbiased, publication-quality data
- Services include low-bioburden processing and accurate DNA/RNA isolation using the ZymoBIOMICS™ product line for the most accurate taxonomic profiling

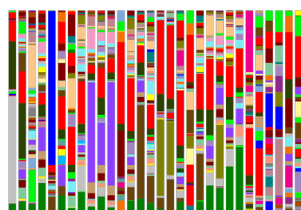


## Validated, Non-Biased Workflows from Collection to Analysis

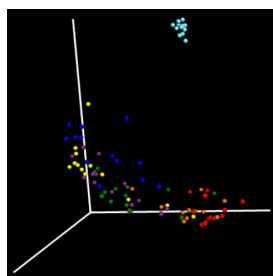


ZymoBIOMICS™ Services are validated using the ZymoBIOMICS™ Microbial Community Standards for unbiased, accurate community profiling

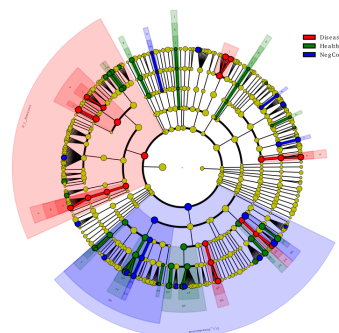
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Composition Barplots



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Services are powered by the latest Next-Gen sequencing technologies.



# Innovation. Pure & Simple.

Since its inception in 1994, Zymo Research has been proudly serving the scientific community by providing innovative, reliable, and high quality research tools at affordable prices. Our vision... “The Beauty of Science is to Make Things Simple” is now truer than ever. Whether it’s epigenetics, DNA, RNA, *E. coli*, or yeast based research, our philosophy remains the same: To provide the highest quality products in the industry while ensuring they are both simple to use and reliable in their performance.

Zymo Research stands on three pillars which form the foundation of our company: Innovation, Quality, and Customer Service. These pillars are fundamental to our culture and ensure our products meet your needs.

## Innovation

Zymo Research is historically recognized for its innovation of high quality nucleic acid purification technologies. Under the branding DNA Purification Made Simple™ and RNA Purification Made Simple™, our technologies are pushing the limits of what is possible with nucleic acid isolation. As The Epigenetics Company™, Zymo Research has also received much attention for its rapidly expanding portfolio of epigenetics products and services. It is our objective to develop and provide the most comprehensive set of tools for DNA, RNA, and epigenetic research and analysis available today. Thousands of peer-reviewed scientific publications from researchers around the world feature our technologies. Through innovation, our scientists have made streamlined DNA methylation detection possible, pioneered the micro-elution column for DNA and RNA purification, developed the simplest and the most sophisticated methods for high-quality plasmid DNA purification, and patented the first RNA purification directly from Trizol® without phase separation among many other leading technologies in the industry.

## Quality

We are committed to quality and guarantee that all of our products and service will meet and exceed your expectations. Our products are constantly evaluated by scientists like you to help ensure their reliability and the highest standard of quality.

## Customer Service

We strive for excellence in how we support your scientific endeavors. We pledge to be honest and responsible for everything we do with you. We will treat you as we would like to be treated. Together, we will build a brighter future.