Digital PCR at IMR

From Deep Sequencing to Single-Copy Precision

Integrated Next-Generation Sequencing & Digital PCR Services now Under One Roof

IMR.bio
Integrated Microbiome Resource

Reliable microbial quantification using dPCR

From assay selection to validation

Assay selection:

Primer or probe-based

Tailored to your target of interest

Use your validated qPCR primers and probes

Absolute quantification:

Endpoint signal + Poisson statistics

No standard curve requirement

High sensitivity:

Detects rare/low-abundance targets with high precision even for host-associated samples.

Cross-platform validation:

Compare dPCR copy numbers with NGS-based measurements



What we tested and what we recommend

We tested multiple primer and probe sets for 16S, ITS2 and 18S on mock communities, human-DNA spike-ins and real samples, and compared dPCR copy numbers with NGS read counts. Our recommended assays:

16S Bacteria

RECOMMENDED

Pan Bacteria Probe 1 (QIAGEN)

- Matches copy numbers from ~400 bp variable-region primer sets — no need to choose a region
- Detects bacteria down to 0.1% microbial DNA in a human background
- Much lower off-target signal from human DNA than V3–V4 primers

ITS2 Fungi

RECOMMENDED

Pan Fungi Probe (QIAGEN)

- Tested on soils, fish swabs, mock communities and pure fungal cultures
- Copy numbers track ITS amplicon read depth from sequencing

18S Microeukaryotes

RECOMMENDED

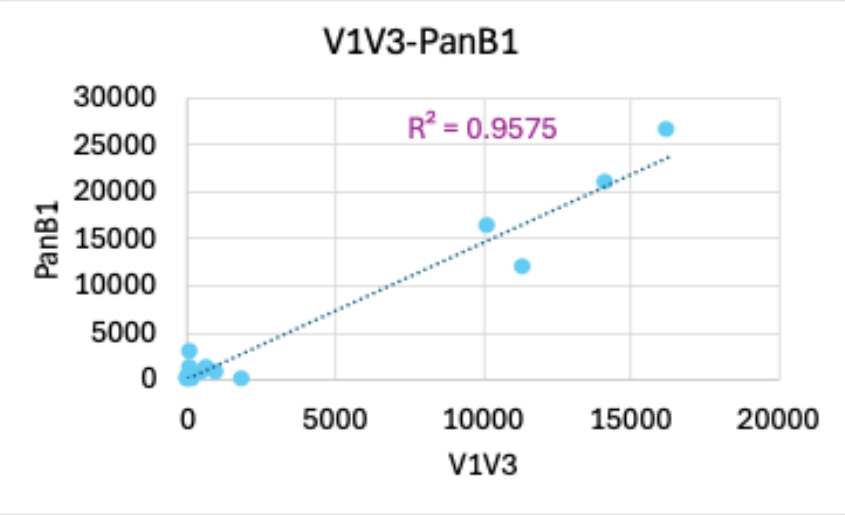
Pan MicroEuk V4 Probe (IMR)

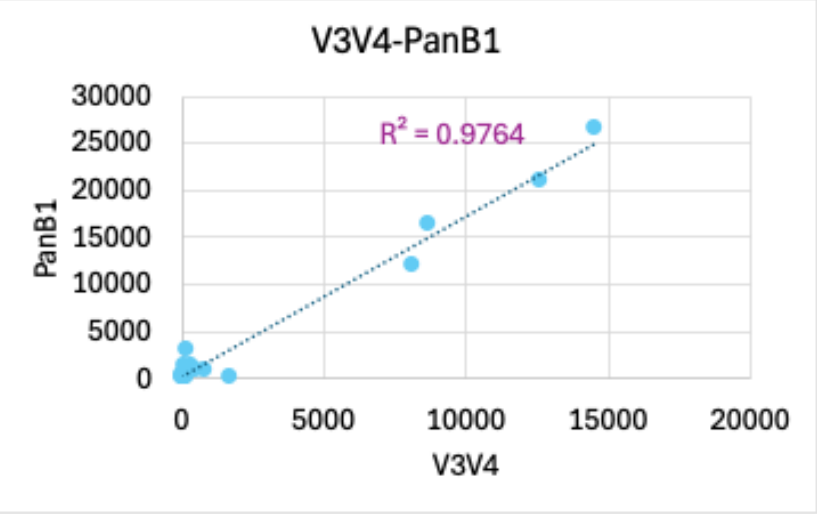
- Detects diverse microeukaryotes in coral, marine, lake and host-associated samples
- Low off-target signal from human DNA

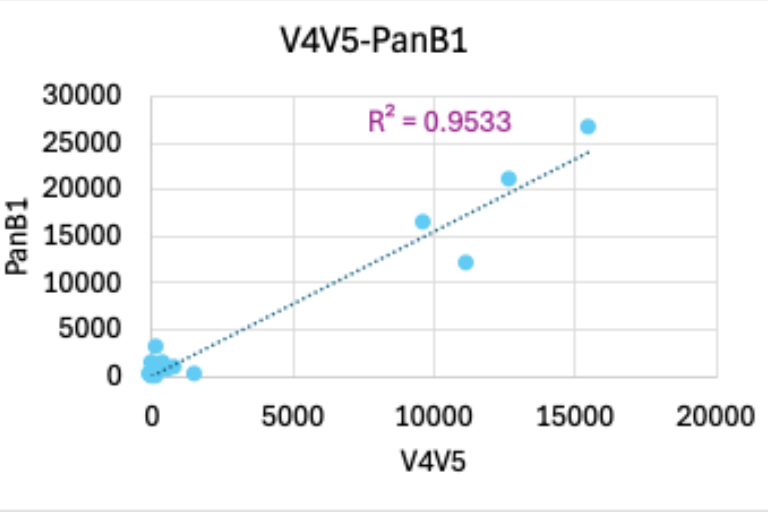
DO I NEED REPLICATES? **Usually not above ~0.5% microbial DNA (more than ~1,000 copies/ng).**

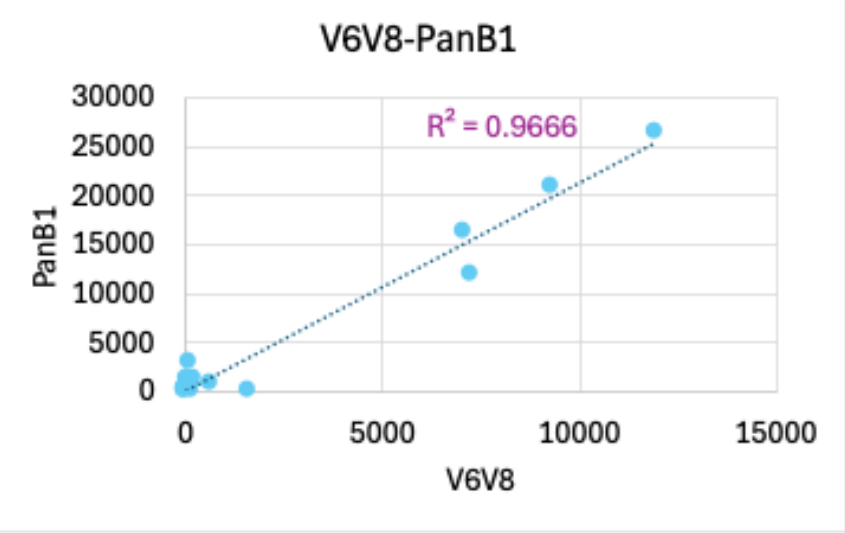
Pan Bacteria Probe 1 for 16S copy number quantification

Spending too much time deciding which variable region primers to use?





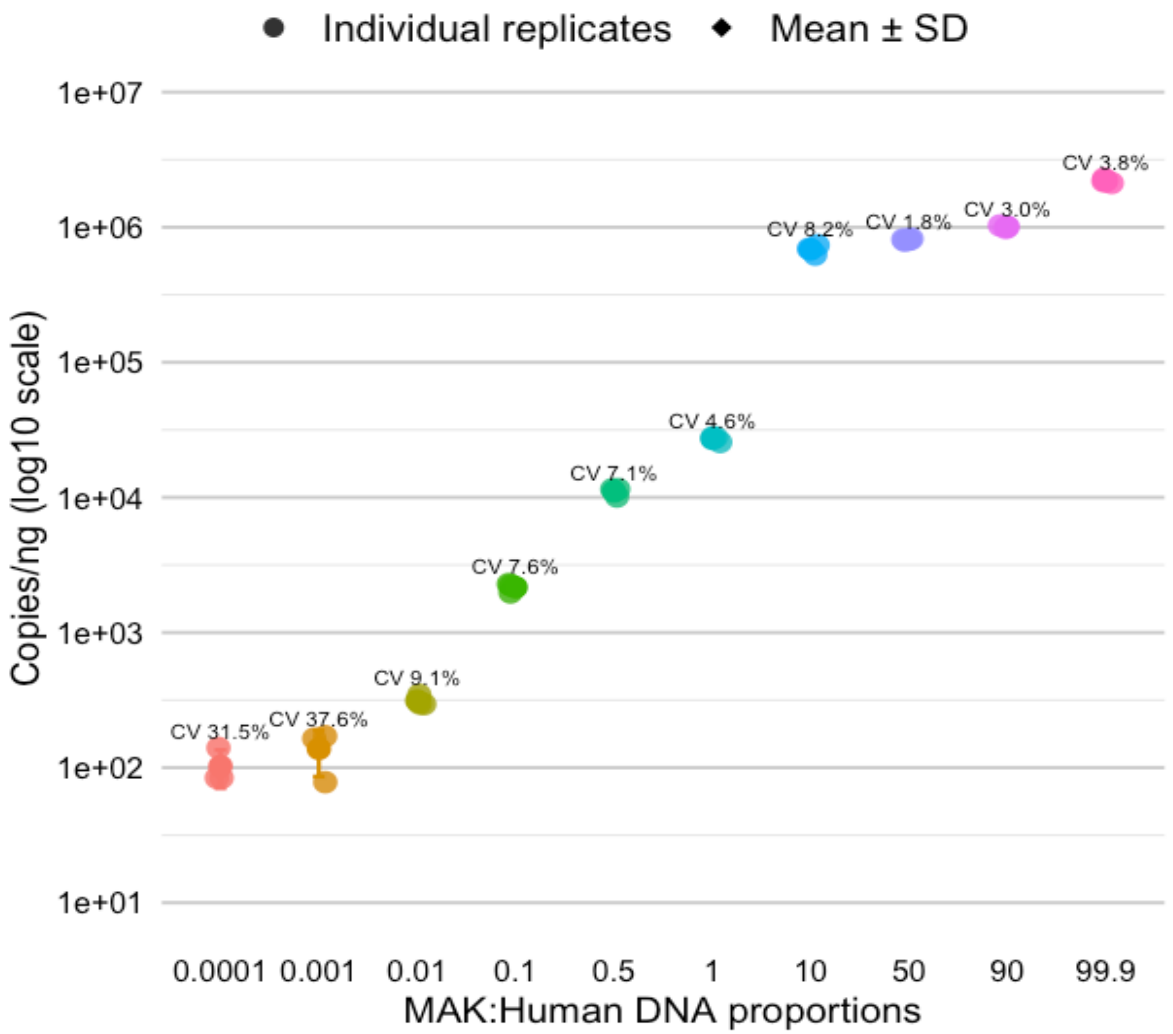




One assay can stand in for many 16S primer choices. In our internal testing, copy numbers from variable-region primer sets (~400 bp amplicons) correlated highly with Pan Bacteria Probe 1.

Technical replicates consistency

Replicate consistency: MAK–human DNA spike-ins



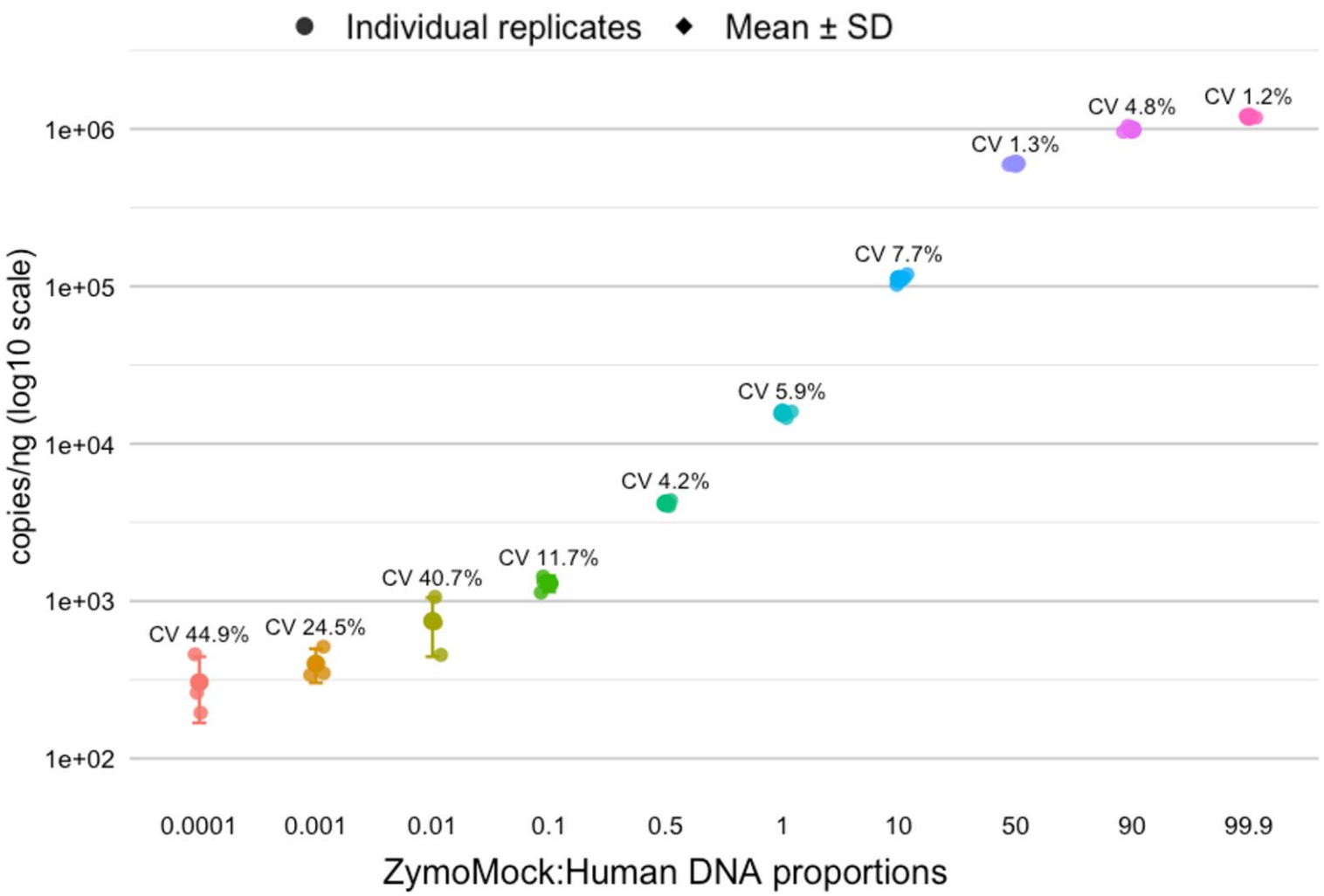
Matrix spike-in experiment performed at IMR:

MAK (*an in-house* stool-derived, complex community) was mixed with human DNA across a gradient of microbial-to-host DNA proportions to test triplicate consistency at low proportions.

Replicates were highly consistent down to 0.01% microbial DNA (CV = 9.1%; ~170 copies/ng). Below ~0.1%, signal approaches the background seen in human-only DNA, so interpret very low values with caution.

Technical replicates consistency

Replicate consistency: ZymoMock–human DNA spike-ins



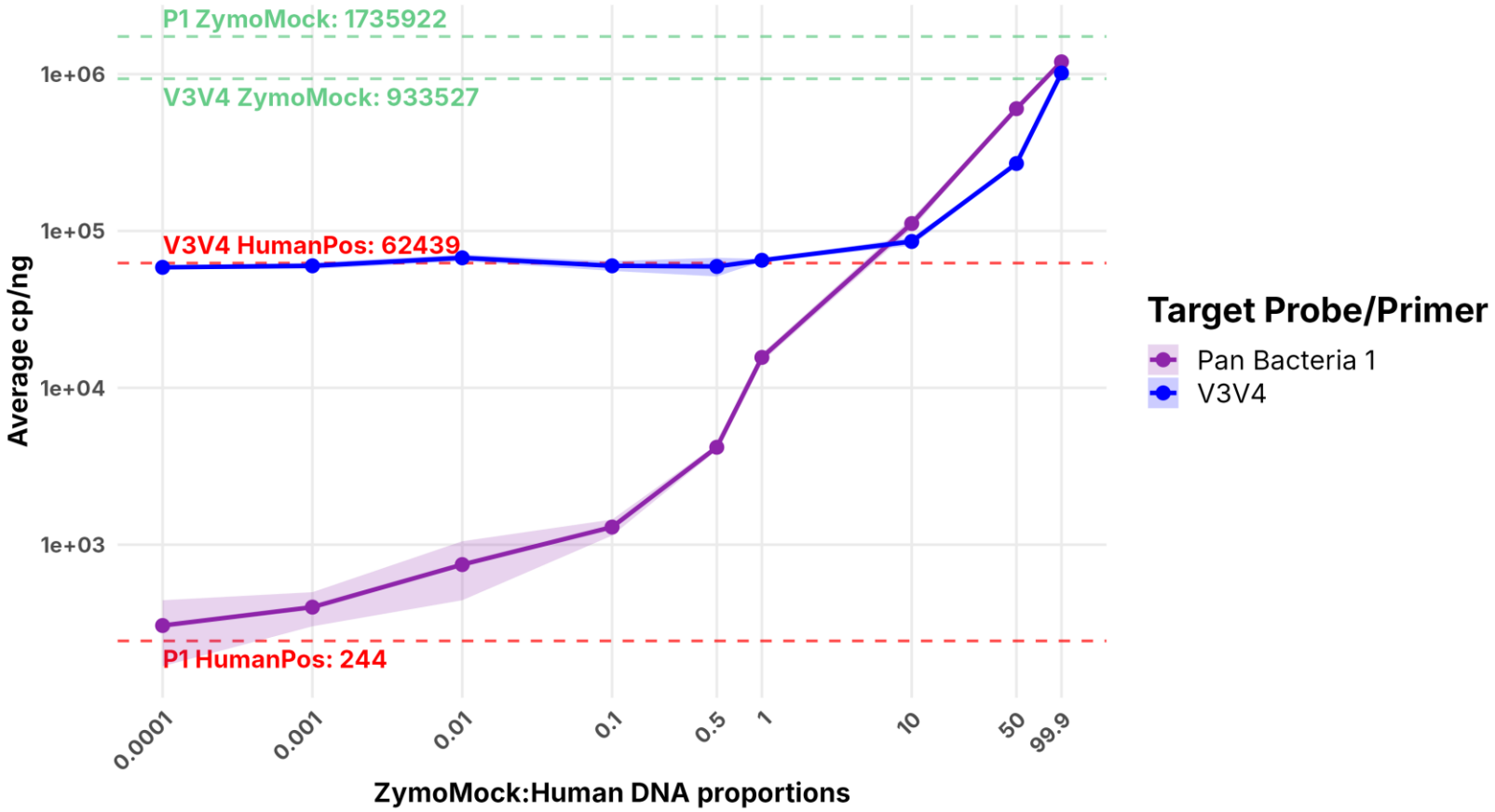
Matrix spike-in experiment performed at IMR:

ZymoMock (a defined and simple community; Zymo Research) was mixed with human DNA across a gradient of microbial-to-host DNA proportions to assess triplicate consistency at lower proportions.

Replicates were most consistent at higher ZymoMock proportions and began to diverge at 0.1% microbial DNA (~1,000 copies/ng).

Conclusion: Samples with ≥0.5% microbial DNA (more than ~1,000 copies/ng) do not necessarily need technical replicates.

Improved specificity in low-biomass samples



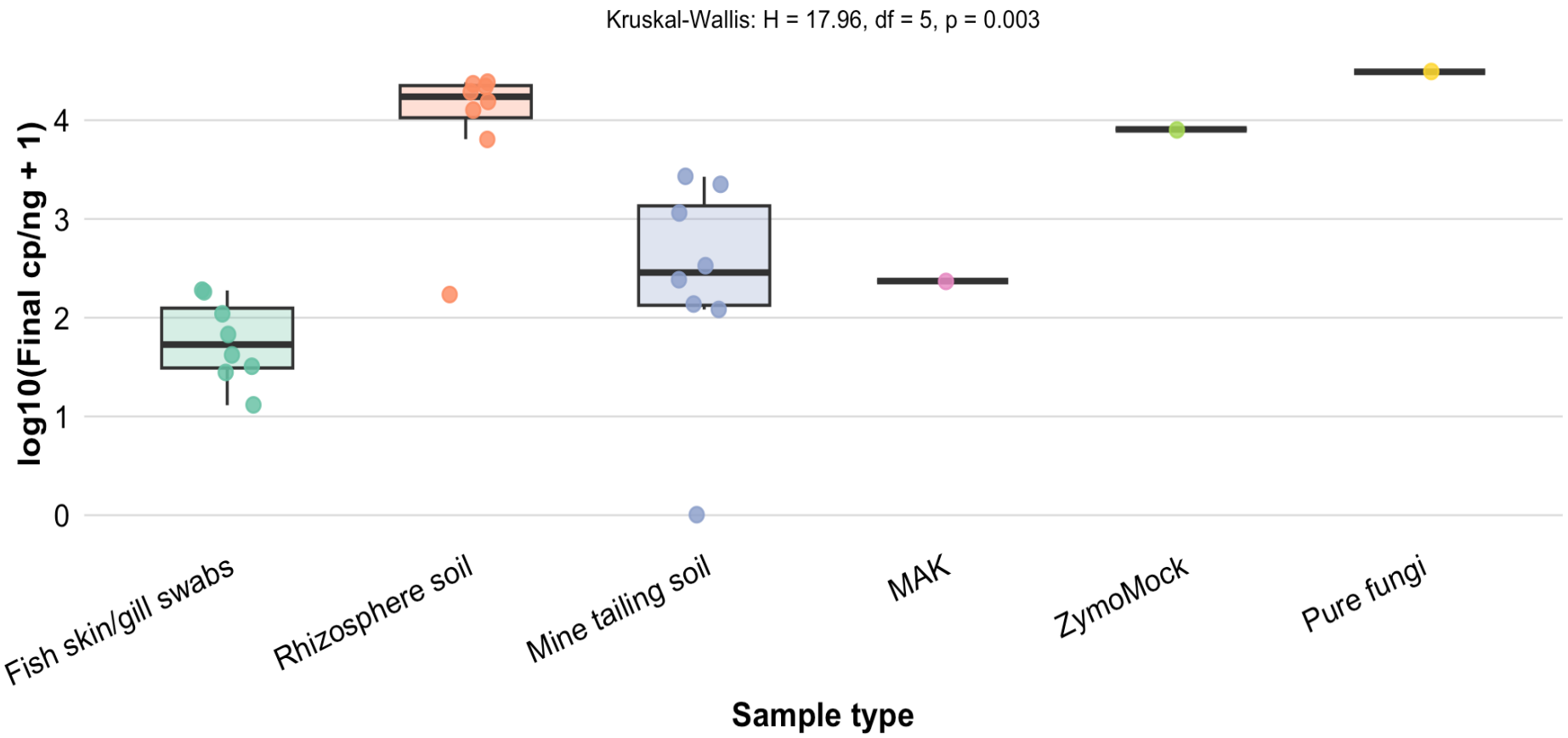
Matrix spike-in experiment performed at IMR:

ZymoMock mixed with human DNA across a gradient of microbial-to-host DNA proportions to assess dPCR specificity and detection limits.

Pan Bacteria Probe 1 accurately detected ZymoMock down to 0.1% microbial DNA, whereas the V3–V4 assay showed high off-target amplification of human DNA (likely mitochondrial).

Pan Fungal probe

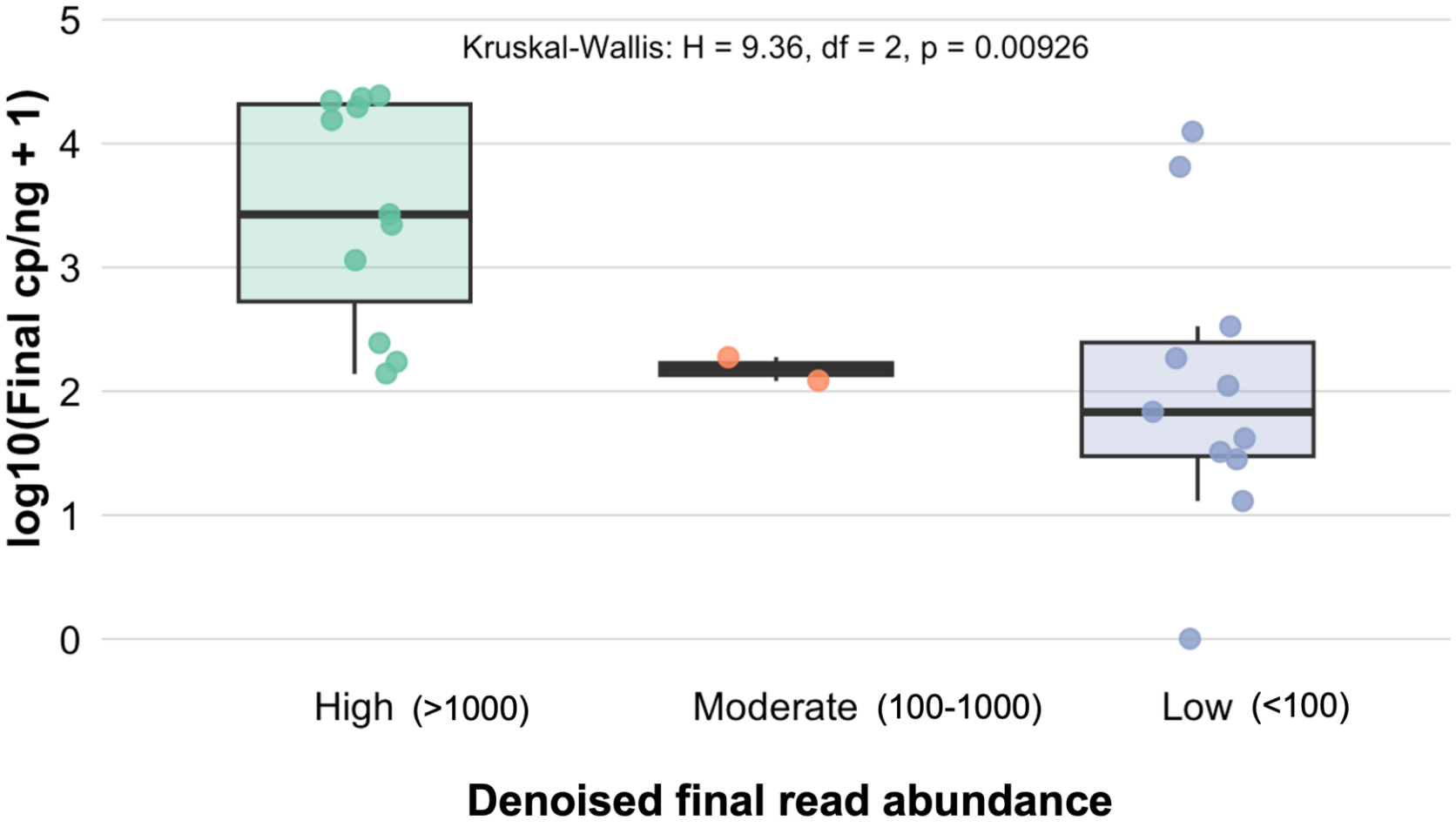
Pan Fungal probe dPCR, grouped by sample type



The Pan Fungal probe (QIAGEN) was tested on a range of sample types and on in-house and commercial microbiome controls.

Pan Fungal probe

Pan Fungal probe dPCR, grouped by denoised read abundance



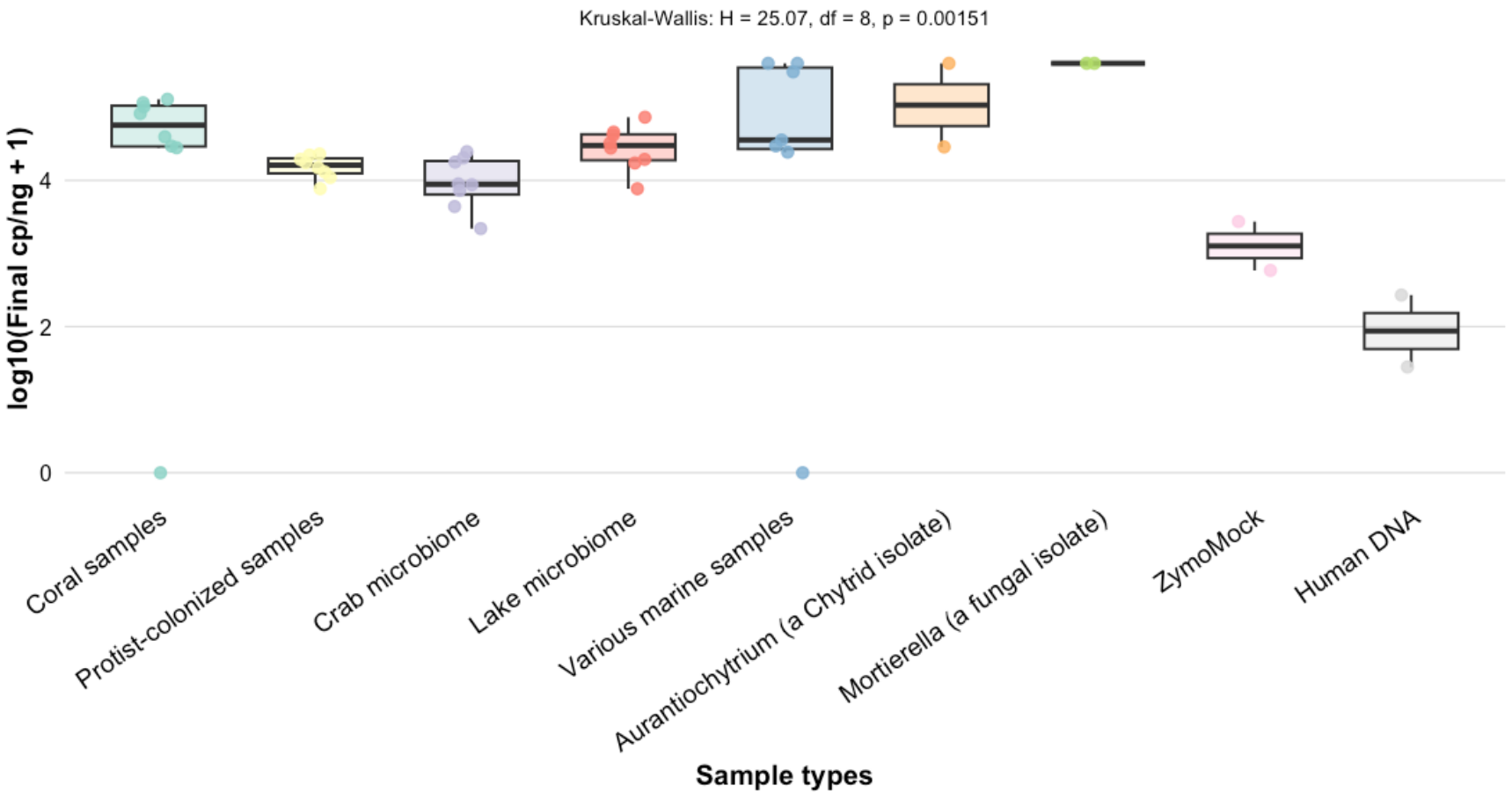
Fungal copies/ng differed significantly across read-abundance categories (Kruskal–Wallis p = 0.009): samples with more denoised reads had higher copy numbers.

Two samples in the low category (<100 reads) had high cp/ng, suggesting PCR inhibition or reduced amplification efficiency with Phusion Plus polymerase during amplicon sequencing.

Note: all samples were sequenced on the Illumina platform.

Pan MicroEuk V4 probe

Pan MicroEuk V4 probe dPCR, grouped by sample type



The Pan MicroEuk V4 Probe (IMR) shows low off-target signal from human DNA while efficiently detecting microeukaryotes.

Note: ZymoMock contains two fungal species, *Saccharomyces cerevisiae* and *Cryptococcus neoformans*.

Read counts were not compared with copies/ng here because these samples were sequenced on two different platforms.

dPCR solutions at IMR

01 Total microbial copy number quantification quantification

For cross-platform validation or metabarcoding

- Bacteria (16S) | Pan Bacteria Probe 1 (QIAGEN)
- Fungi (ITS2) | Pan Fungi Probe (QIAGEN)
- Microeukaryotes (18S) | Pan MicroEuk V4 Probe (IMR)

02 Functional gene copy number quantification quantification

Custom assays

- Bring your own primer-probe mixture (UCP Probe mix, QIAGEN)
- Bring your own primers (EvaGreen mix, QIAGEN)

03 Specific bacterial load quantification

Single-copy gene-based custom assays

- Bring your own primer-probe mixture (UCP Probe mix, QIAGEN)
- Bring your own primers (EvaGreen mix, QIAGEN)

PRICING PER SAMPLE

Academic	Commercial
16 CAD	24 CAD

WHY CHOOSE US?

One lab, complete genomics

Faster time to results

Contact us

Have questions about assay design, experimental setup, sample requirements or pricing?

Contact our team to discuss your project or arrange a consultation.

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SAMPLE SUBMISSION

Ready to get started?

Find sample submission information and guidelines:

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