Droplet Digital™ PCR: Linking Microbial Physiology and Phylogeny Using Bio-Rad's QX100™ Droplet Digital PCR System

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Droplet Digital PCR

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Abstract

For environmental microbiology, Droplet Digital PCR (ddPCR™) provides a new opportunity to investigate linkages between physiology and phylogeny. PCR, quantitative PCR (qPCR), and sequencing-based metagenomics have revolutionized microbiology over the past few decades by providing new insights into microbial diversity and metabolic interactions. We can now expand on these by rapidly and simply determining linkage utilizing the unique properties of ddPCR. Using this simple ddPCR approach, we demonstrate the association of proteorhodopsin with SAR11 16S rRNA signatures without the need for extensive metagenomic analysis.

Introduction

Bio-Rad's ddPCR system opens new avenues for environmental microbiology because it can explicitly determine if two genes are co-localized on the same DNA molecule, and thus originate from a single cell (Figure 1). These co-localization experiments, unlike most single cell analyses, do not require individual bacteria to be isolated prior to analysis. Instead, they rely upon the physical DNA linkage combined with droplet partitioning to characterize a sample. The approach is rapid, simple, and scalable because it is based on PCR assays, but provides linkage information not otherwise available.

We demonstrate how this approach can quickly characterize photoheterotrophs in seawater samples. The discovery of proteorhodopsin within the genome of SAR11 bacteria was one of the first major revelations from metagenomic analysis, leading to the discovery of new metabolic activities that play a major role in global nutrient cycling (Béjà et al. 2000). We demonstrate simultaneous detection of both the SAR11 16S rRNA gene and the functional gene proteorhodopsin. Additionally, we show that these genes were physically linked for a majority of templates, indicating that both originate from the same cells. This simple approach provides direct evidence that SAR11 bacteria are the photoheterotrophs in this natural assemblage.

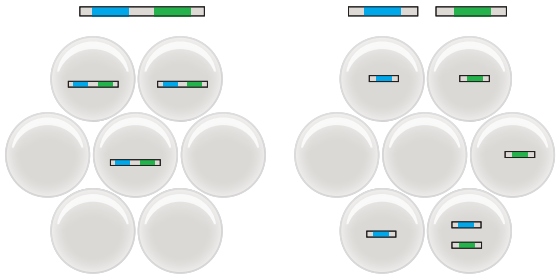

Fig. 1. Comparison of expected co-localization for linked and unlinked targets. In a ddPCR experiment, the DNA strands partition into droplets randomly and then are detected using two separate PCR assays. When both assay targets occur on the same DNA strand, the two assays co-localize in the same droplet more often than expected by random chance. Unlinked copies segregate randomly and co-localize based on the assay target's abundance.

Methods

We used the QX100 ddPCR system and primer sets targeting 16S rRNA (Suzuki et al. 2001) and proteorhodopsin (Campbell et al. 2008) from the SAR11 clade. We added probes to both assays to allow duplex detection. Bacteria were harvested from seawater samples by 0.22 µm filtration. High molecular weight genomic DNA was extracted with phenol:chloroform followed by ethanol precipitation. The resulting DNA was used directly in the ddPCR reactions to investigate linkage, or restriction digested with MseI to separate the two targets and thus unlink the signals. Droplets were generated using the QX100 system. PCR was performed with Bio-Rad's T100™ thermal cycler, then droplets were read in a QX100 droplet reader.

Results and Discussion

Detection of SAR11 Proteorhodopsin and 16S rRNA

To investigate linkage by ddPCR, DNA from coastal seawater was quantified by assays targeting two genes in a duplex reaction. The concentration for each target was similar between the digested and undigested samples, demonstrating that the digestion did not alter the frequency of positive droplets for either assay. Although the concentration was similar for each assay, the number of droplets positive for both targets simultaneously differed between the two treatments.

Only a small number of droplets are positive for both the proteorhodopsin and 16S rRNA targets in the digested samples (Figure 2A). In these samples, the frequency of dual positives is consistent with the probability of both targets localizing to the same droplet by chance. Figure 2B shows the undigested samples with an overabundance of dual-positive droplets. This indicates that SAR11 proteorhodopsin and 16S rRNA show a high degree of linkage due to a shared genomic origin.

The distance between loci and degradation state of the DNA can alter the probability of a break between loci and thus decrease the observed linkage; however, linkage does not need to be complete to be detected. QuantaSoft™ software provides a statistical framework for evaluating observed co-localization. It is also possible to run assays of known distance on the same samples to account for the degree of degradation or shearing and thus get a better estimate of the original linkage.

Conclusions

Droplet Digital PCR is a new and powerful tool with great potential for improving existing experiments and creating novel approaches for microbial science. ddPCR provides a rapid and simple way to investigate linkage that is fully amenable to environmental sampling. As shown here, ddPCR alone can provide unique information that can link physiology and phylogeny, or link two independent physiologies. It can also serve as a cost-effective way to extend metagenomic sequencing analysis by providing a mechanism to determine which genes and pathways exist on a shared genome, even when lack of good genomic scaffolds or variation within samples makes full genome assemblies difficult.

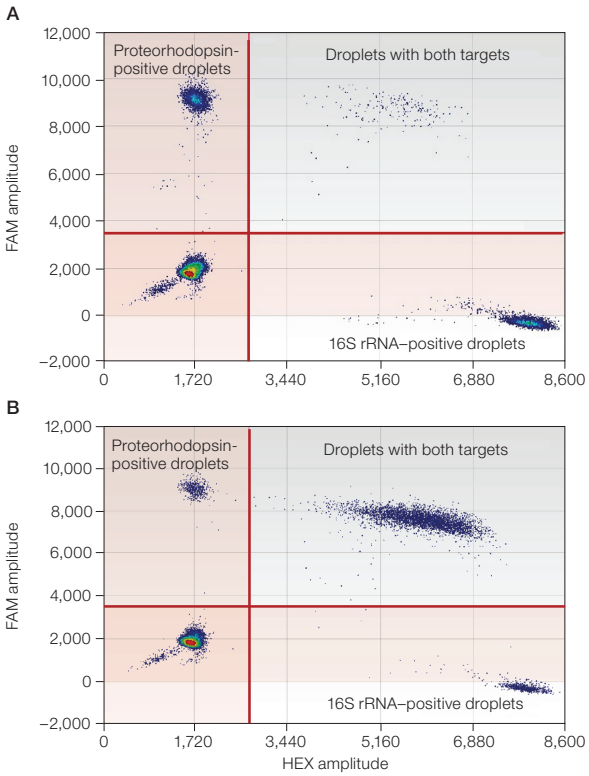

Fig. 2. Two-dimensional fluorescence plots of ddPCR droplets with probes targeting proteorhodopsin (FAM) and 16S rRNA (HEX). Composites of four replicate wells for each treatment are shown. **A**, digested DNA; **B**, undigested DNA.

References

Béjà O et al. (2000). Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. Science 289, 1902–1906.

Campbell BJ et al. (2008). Abundant proteorhodopsin genes in the North Atlantic Ocean. Environ Microbiol 10, 99–109.

Suzuki MT et al. (2001). Quantitative mapping of bacterioplankton populations in seawater: Field tests across an upwelling plume in the Monterey Bay. Aquat Microb Ecol 24, 117–127.

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www.bio-rad.com/web/ddPCRmicrobialtargets.

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