

Comprehensive Microbial Functional Gene Microarrays (FGAs) for the Study of Microbial Diversity and Processes in the Environment

Christopher W. Schadt, Jost Liebich, Song C. Chong, Terry J. Gentry, Zhili He and Jizhong Zhou

Based on our previous studies (Wu et al. 2001; Zhou 2003; Schadt et al 2004; Rhee et al. 2004) we have designed a comprehensive functional gene microarray (FGA) for use in the study microorganisms in environmental samples. The designed probes encompass the variation in >14,000 known microbial functional genes involved in nitrogen (e.g. denitrification and nitrogen fixation), carbon (e.g. carbon dioxide fixation and cellulose degradation) and sulfur (e.g. dissimilatory sulfur reduction) cycling processes, as well as methane oxidation and reduction, organic contaminant degradation and metals reduction and resistance (Table 1). These consist of gene specific probes as well as group probes that encompass the variation in closely related sequences for which specific probe design was not possible. In most cases we were able to design multiple probes for each target sequence, bringing the total number of designed probes to 23,864. We have been able to incorporate the design probes in several potential microarray layouts, such that we may create arrays in different formats. For example one design incorporates single probes for each gene for a wide variety of functional process (all design categories) genes can be arrayed together (Figure 1). Such arrays are useful for a broad overview of the functional capabilities of any given microbial community. Additionally more exhaustive and detailed probe sets may be used to create arrays with multiple probes for any given target gene. We have incorporated this strategy into to layout an array that contains up to three probes for each gene involved in carbon, nitrogen and sulfur cycling processes. Thus these arrays may be used to thoroughly and robustly characterize microbial functional diversity between samples for a particular process using DNA or RNA hybridizations.

Table 1. Summary of probe numbers by category for a comprehensive FGA.

Gene Category	Unique Probes	Group Probes	Total
Denitrification	1805	501	2306
Nitrification / N Metabolism	865	902	1767
Nitrogen fixation	1225	0	1225
Sulfur reduction	1286	329	1615
CH ₄ oxidation / production	437	333	770
Carbon polymer degradation	2532	276	2808
Carbon fixation	584	215	799
Metal resistance / reduction	4039	507	4546
Organic contaminant degradation	6920	1087	8007
<i>Total</i>	<i>19714</i>	<i>4150</i>	<i>23864</i>

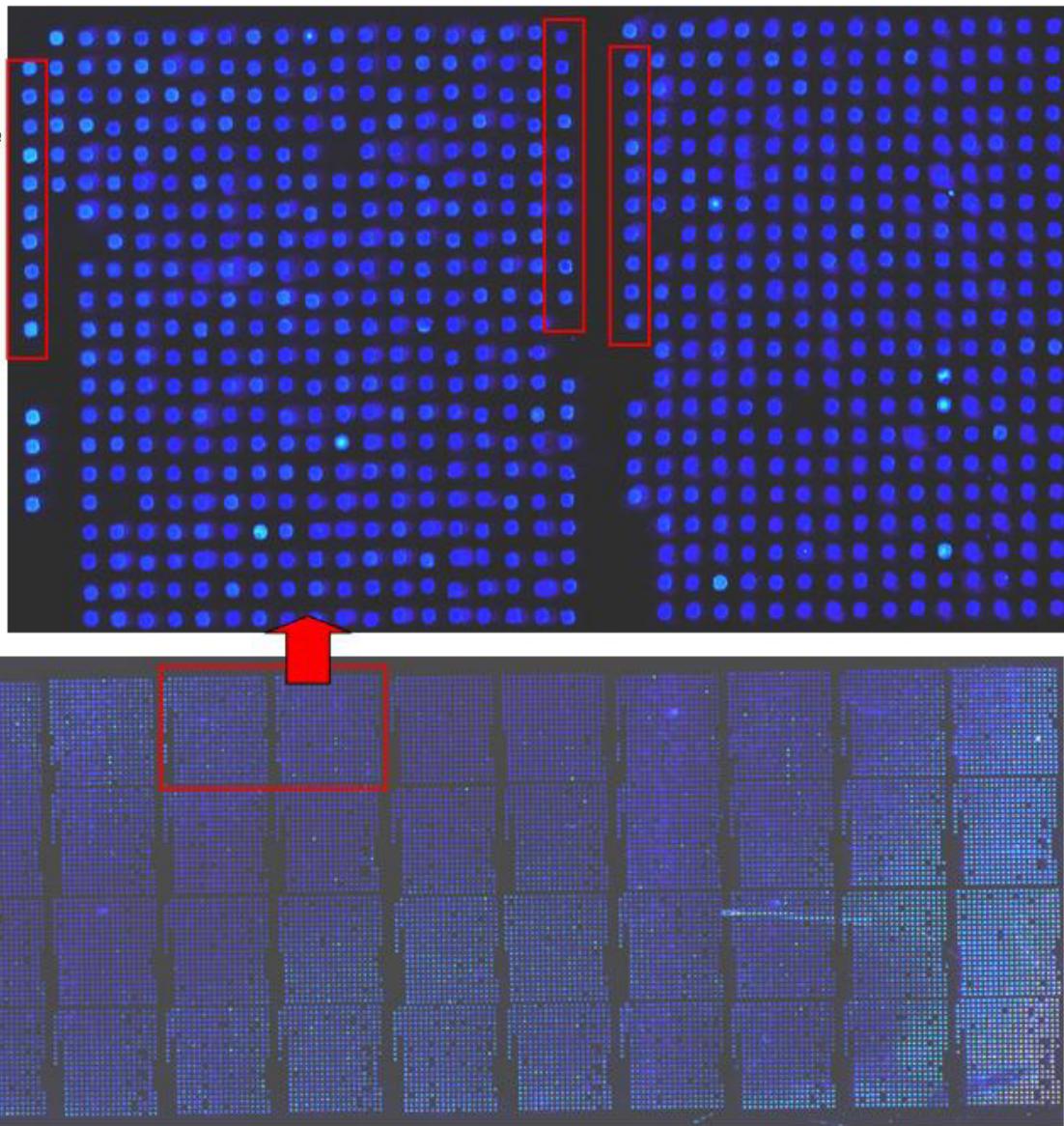
Figure 1. DNA stain showing the layout of an 18,560 feature array.

-8800 experimental probes in duplicate on each slide

-6 human & 4,16S control spots duplicated in each subgrid

-18560 total probe features

-High uniformity



Rhee, S.K., Liu, X., Wu, L., Chong, S.C., Wan, X., and Zhou, J. (2004). Detection of biodegradation and biotransformation genes in microbial communities using 50-mer oligonucleotide microarrays. *Appl. Environ. Microbiol.* 70:4303-4317.

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