Comparison of the Use of Plasmid and PCR DNA on Microarray Chips

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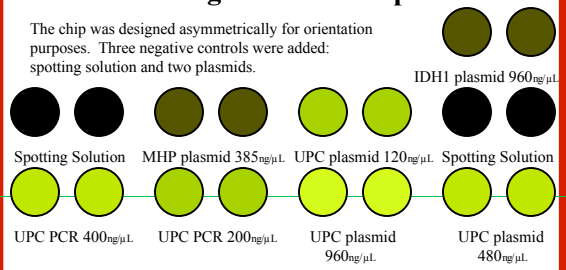
Abstract

Using microarrays for high-throughput DNA detection is one of the fastest growing fields in biology today. However, as of yet there is no universal set of rules that researchers follow in designing experiments and determining results. Because of this there may be avenues of research that have not been utilized.

One of the methods that have not been reported is the use of probing for genes previously inserted within a plasmid. In previous experiment that utilized DNA detection with microarrays, the appropriate DNA has been extracted and amplified before being spotted on a microarray chip. This experiment is designed to test the effectiveness of spotting and probing plasmid DNA and comparing the result to spots of PCR DNA.
A favorable result would indicate a new path that researchers could take when preparing microarray chips. In certain experiments, the step of PCR could be removed, creating much faster, cheaper, and easier means to determine the result.

Design of DNA Chip

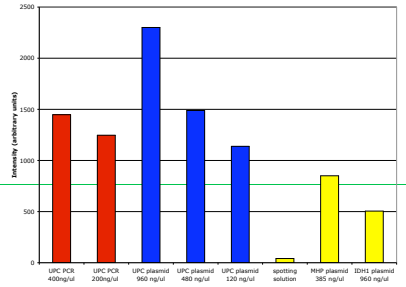
The chip was designed asymmetrically for orientation purposes. Three negative controls were added: spotting solution and two plasmids.



This design was then spotted on a DNA chip using a Microarrayer. The colors above indicate a possible result after the chips have been scanned.

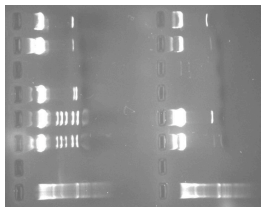
Graph – PCR vs. Plasmid

After scanning, the intensities of the spots were examined using Scanalyze. While the intensities of a single type of spot varied greatly between slides, a graph of the average intensities of light for type still proves useful. Standard deviations are not shown due to their extremely large value.

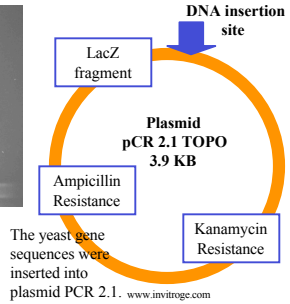


Created using Scanalyze software from the Eisen Lab and Microsoft Excel

Preparation of Plasmid DNA

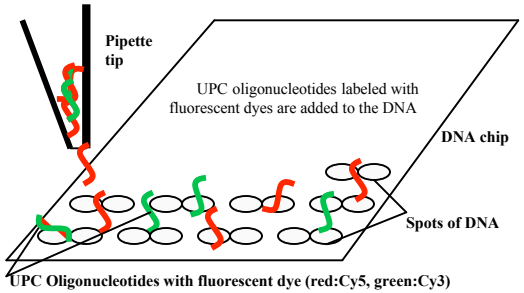


As is evident in this gel electrophoresis, the insert is very clean; when it is present, there is no smearing or question about whether the gene is or is not in the plasmid.



The yeast gene sequences were inserted into plasmid PCR 2.1. www.invitroge.com

Methods: Oligonucleotide Addition



UPC Oligonucleotides with fluorescent dye (red:Cy5, green:Cy3)

Conclusions

Overall, as the graph would indicate, it appears that the intensity of the signal received from spotted plasmid DNA is very comparable to spotted PCR DNA. Also, the average intensities of the plasmid DNA decreased with concentration.

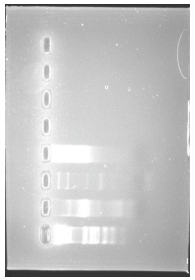
Qualitatively, it looked as if a number of scanned arrays showed a greater signal when the oligonucleotides were bound to the spotted plasmid DNA and not the PCR product. There was, however, a noticeably higher degree of smearing from the plasmid spots, possibly due to a higher number of molecules per spot.

At the very least, this experiment shows some promise for future examinations of the possibility of using plasmid DNA in DNA microarray experiments. To further probe this issue, an experiment designed to test the differences between plasmids and PCR product in regards to their ratio of red to green signal, will be performed.

Preparation of PCR DNA

After the yeast genes were cloned into *E. coli*, the DNA was isolated and amplified using Polymerase Chain Reaction.

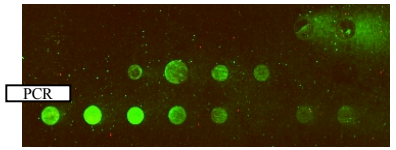
Although PCR is a very acceptable method for obtaining large quantities of DNA, an increase in steps increases the chance that mistakes could be made. Note the smearing and uncertainty in interpretation of the electrophoresis gel at right.

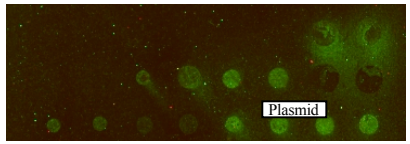


Courtesy Emily Oldham

Results

The results were mixed, with some of the six microarray slides showing strong oligonucleotide binding to the PCR product . . .





. . . and other slides showing strong binding to the spotted plasmid.

Future Experiment

The design of the future experiment will look at using specific oligonucleotides when probing eleven yeast genes in plasmids at three different concentrations. This will further test the plasmids for non-specific binding. Also, the reproducibility of the post-scanning red to green ratios will be further examined. A look at the set up of the grids is below:

GRID #1	rad 120	rad 120	rim 480	rim 480			FLY 480	FLY 480
	cat 480	cat 480	rad 240	rad 240	rim 120	rim 120		
			cat 120	cat 120	rad 480	rad 480	rim 240	rim 240
	FLY 240	FLY 240			cat 240	cat 240	FLY 120	FLY 120
GRID #2	upc 120	upc 120	idh1 480	idh1 480			mhp 480	mhp 480
	mdh 480	mdh 480	upc 240	upc 240	idh1 120	idh1 120		
			mdh 120	mdh 120	upc 480	upc 480	idh1 240	idh1 240
	mhp 240	mhp 240			mdh 240	mdh 240	mhp 120	mhp 120
GRID#3	SHY 120	SHY 120	dbr 480	dbr 480				
	idh2 480	idh2 480	SHY 240	SHY 240	dbr 120	dbr 120		
			idh2 120	idh2 120	SHY 480	SHY 480	dbr 240	dbr 240
					idh2 240	idh2 240		

Acknowledgements: Thanks to Dr. Campbell and Emily Oldham