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Novel concept of microarray construction and their application in biology

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Introduction

In the advancement of high throughput methods to study biological systems, microarrays have demonstrated dramatic progress over the past decade. Microarrays provide the ability to measure the expression of thousands of genes in parallel even if only a small amount of biological material is available, which is the main reason, why DNA microarrays have become a powerful tool for studying gene expression and various aspects of genomics. However, traditional DNA microarray technology is not without its challenges and limitations and that is why this technique is constantly improved. For microarrays produced using spotting technologies, much attention has to be given to the development of slide surfaces, attachment chemistries, and spotting solutions. The main target are arrays with high sensitivity and low variability. Application of the optimal and reliable methods ensuring effective binding of nucleic acid probes with slide surface is one of the key factors warranting high quality results. Developments in the field of microarrays occur at a rapid pace and some novel approaches may offer suggestions of new strategies.

Methods of slides preparation

The quality of microarray data depends directly on the quality of starting reagents, especially for printed microarray slides. This fact makes surface chemistry the a crucial factor in microarray production. In order to obtain the best possible results we tested several methods of slides preparation. One promising method involves coating with GTMS epoxide. The results show that there is no significant difference between comercial and our slides.

A B

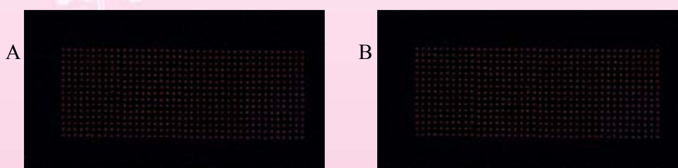


Fig.1 Results of hybridization Q-670 labeled random 9-mer oligo on homemade epoxide slide (A) and Epoxide Corning® slide (B).

Peptide/protein microarrays

Microarrays of protein or peptide molecules are different in their chemistry and use than nucleic acid ones. There are 20 natural amino acids compared to only four natural DNA or RNA nucleotides. This is the reason, why the chemistry of peptide synthesis is much more complicated. Moreover, peptides and proteins have multifaceted properties, while nucleic acids are distinct in their backbone of negative charges. The interactions of peptides or proteins with other molecules are mainly dominated with/by molecular forces of different nature. Because of diverse chemical properties, peptides and proteins play role as receptors, fusion mediators, structural stabilizers, and regulatory factors, in many biochemical processes. Peptide or protein microarrays find key applications for therapeutic target identification, protein function assay, drug discovery, and diagnostics. It is crucial to develop peptide and protein microarrays that are easy to produce at minimal cost and time consumption. They also should be flexible enough to suit a wide range of needs, specifity and sensitivity for target detection, reliable reproducibility and stability under the conditions of assays and storage.

Tab.1 Application of various microarray types.

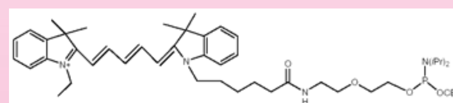
Type of microarray			
Protein	**Peptide**	**Antibody**	**Antigen**
•Antibody detection •Protein-protein interactions •Protein-DNA/RNA interactions •Ligand-binding screening (substrates, drug candidates, biomarkers)	•Epitope screaning •Drug discovery •Protein function assay •Protein recognition •Protein-peptide interactions •Peptide-DNA/RNA interactions •Biomarkers •Biosensors	•Protein profiling •Affinity binding to generate protein microarrays •Cell surface antigen analysis (cell arrays)	•Antibody detection •Target identification

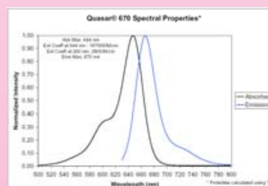
Quality assesment with Quasar-670

The most reliable microarray quality assesment can be achieved through hybridization with a fluorescent-labeled oligo. The reaction with q-670 reagent is easy way to confirm presence of probes, proper spots morphology and finally functional ability of immobilized probes to hybridize. To determine the most accurate combination of slide-surface chemistry and nucleic acid probe design, we used q-670 reagent (a set of random nonamers labeled with Quasar-670 on the 5'-end). This reagent was syntesised with Quasar-670 phosphoramidite produced by Bioserch Technologies. Solution containing q-670 and print buffer was spotted on both commercially available and homemade epoxy surfaces. Hybridization with complementary oligomers showed accurate signal intensity, optimal spot morphology and lowest background for the 0,5 μmolar concentration.

Tab.2 Comparison of Q-670 efficiency on different commercialy and non-commercialy slides.

Slide type	Dye	Intensities(pxs)	
		foreground	background
Epoxide Corning®	Q-670	4626	221
Homemade Epoxide	Q-670	4590	209





PROPERTIES

Chemical Name: Quasar 670 Amidite
Formula: C46H67N5O4P+
Molecular Weight: 785.03
Appearance: blue solid

Absorption Maximum (Lambda Max): 644 nm
Extinction Coefficient at Lambda max: 187000/M/cm
Extinction Coefficient at 260 nm: 2800/M/cm
Fluorescence Maximum: 670 nm

Fig.2 Quasar 670 phosphoramidite and its fluorescence properties

Biological applications of microarrays

Microarrays can be applied for nucleic acid enrichment in two opposite strategies. First approach enables selective hybridization and recovery of RNA/DNA of interest. This is often a crucial step and can be especially useful in miRNA enrichment, as currently available methods are not highly effective. By designing set of DNA probes complementary to particular miRNAs and optimal hybridization conditions, it is possible to "catch" all desired molecules; they can be later separated from non hybridizing RNAs by proper washing and recovery strategies.
In the opposite strategy, microarrays can also serve as "purification filters", which remove all redundant nucleic acid from particular solution. Microarrays can be applied also to PCR reaction.

V. Trevino, F. Falciani, H.A. Barrera-Saldaña, *DNA microarrays: a powerful genomic tool for biomedical and clinical research*, Mol. Med. 13, 527-541 (2007).

Conceptual „Heat-Driven" approach to the synthesis of DNA oligonucleotides on microarrays

Thermolytic groups have been engineered for 5'-hydroxyl, P (III) protection and N-protection of deoxyribonucleoside phosphoramidites. The deprotection mechanism of these groups proceeds through an intramolecular cyclodeestrification or a cyclodecarbonation reaction depending on whether the grouos serve as phosphate or 5'-hydroxyl protecting groups and nucleobase, respectively.

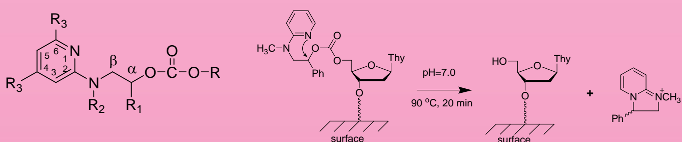


Fig.3 Structure of 2-pirydyl thermolabile protecting groups and their modifications

Fig.4 Mechanism of 5' thermodeprotecion of thermolabile protecting group

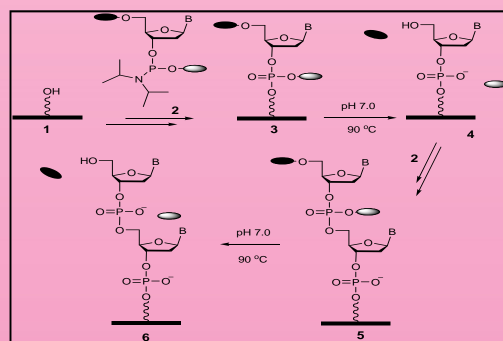


Fig.5 Conceptual approach to the synthesis of DNA oligonuclotides on micrarrays using thermolytic groups.

Chmielewski M.K., Marchán V., Cieślak J., Grajkowski A., Livengood V., Müchen U., Wilk A., Beaucage S.L., „Thermolytic carbonates for potencial 5'-hydroxyl protection of deoxyribonucleosides" *J.Org.Chem.*, **68**, 10003-10012 (2004)
Beaucage, S. L., Chmielewski M.K., „Thermolabile hydroxyl protecting group and method of use" PCT Int. Appl. WO2004101582 (2004)

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