GeneChip® Probe Arrays

GeneChip® Probe Array

Hybridized Probe Cell

Image of Hybridized Probe Array

>200,000 different complementary probes

Single stranded, labeled RNA target

Oligonucleotide probe

Millions of copies of a specific oligonucleotide probe

>200,000 different complementary probes

GeneChip® Probe Array

Image of Hybridized Probe Array

Courtesy of: Mike Lelivelt

Damla D. Bilgin
Univ. of Illinois, Urbana

Photolithographic Synthesis

Lamp

Mask

Chip

Courtesy of: Mike Lelivelt

Damla D. Bilgin
Univ. of Illinois, Urbana
The GeneChip® Soybean Genome Array can be used to study gene expression of over 37,500 soybean (*Glycine max*) transcripts.

The array includes probe sets to detect approximately 15,800 transcripts for *Phytophthora sojae* as well as 7,500 *Heterodera glycines* (cyst nematode pathogen) transcripts.

Damla D. Bilgin
Univ. of Illinois, Urbana
Synthesis of Ordered Oligonucleotide Arrays

Courtesy of: Mike Lelievelt

GeneChip Probes (Affymetrix)

- 25-mer oligonucleotides synthesized directly on the glass
- 11-20 probe pairs are selected among all possible 25-mers to represent each transcript
- Perfect Match/Mismatch probe pair strategy

Courtesy of: Mike Lelievelt

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Probe – a single-stranded DNA oligonucleotide complementary to a specific sequence. Each probe cell consists of millions of the same probe molecules.

The intensity of each cell is an average of each of its scanned pixels.
Each pair represents a different subsequence of the gene.
Definitions

**Probe** – a single-stranded DNA oligonucleotide complementary to a specific sequence. Each probe cell consists of millions of probe molecules.

**Probe Array** – a collection of probes sets.

**Probe Set** – a set of probes designed to detect one transcript. 16-20 probe pairs. A 20 probe pair set is made up of 20 PM and 20 MM for a total of 40 probe cells.

**Probe Pair** – Two probe cells, a PM and its corresponding MM.

**Perfect Match (PM)** – probes that are designed to be complementary to the reference sequence.

**MisMatch (MM)** – probes that are designed to be complementary to the reference sequence except for 1 base.

**Target** – sequence from your sample.

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Procedures for Target Preparation

1. **Cells** → **Poly (A)/** → **Total RNA** → **cDNA** → **IVT** (Biotin-UTP, Biotin-CTP) → **Labeled transcript**
2. **Wash & Stain** → **Hybridize** (16 hours) → **Labeled fragments**

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Courtesy of: Mike Lelivelt

Damla D. Bilgin
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Argon laser 488nm

570nm

Scanner based on epifluorescence confocal microscopy

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Affymetrix Core Facility at W.M. Keck Center at Univ. of Illinois at Urbana-Champaign

Useful web sites

http://www.affymetrix.com/products/arrays/index.affx

http://www.biotech.uiuc.edu/centers/Keck/Functional_genomics/

http://www.iobion.com/support/support_GT_training.html

http://www.cropsci.uiuc.edu/faculty/clough/

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