

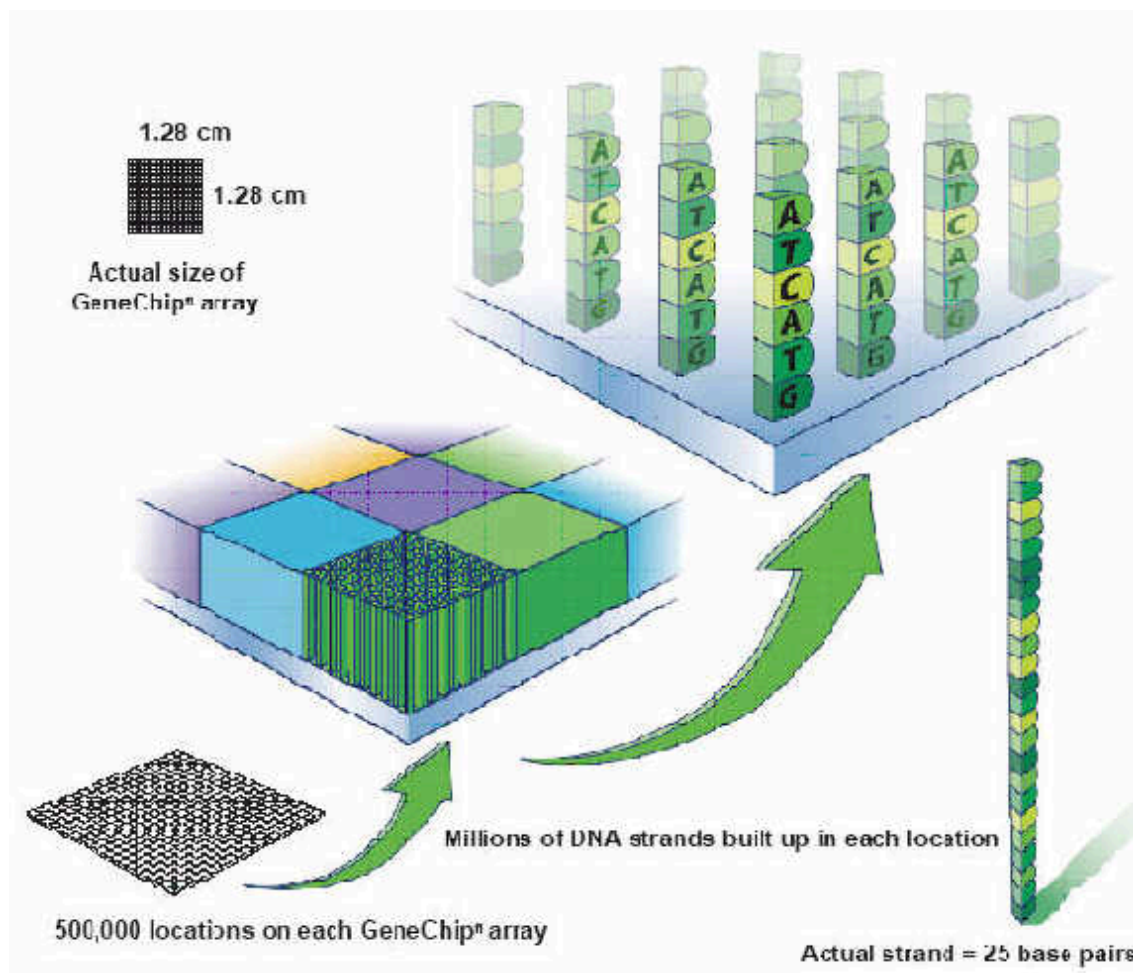
Genstat Knowledge Base 2024

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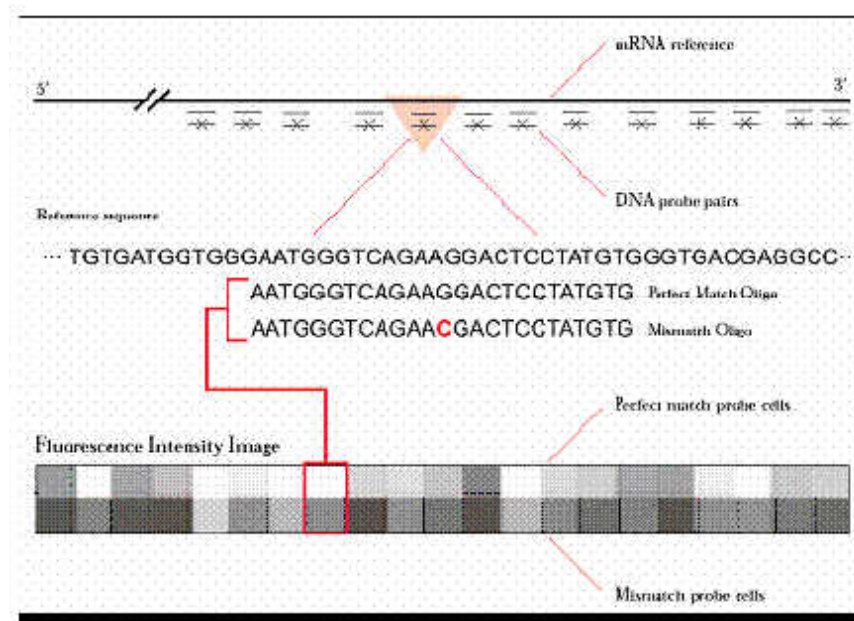
Affymetrix GeneChips are built by laying down sequences of bases using a combination of semiconductor-based photolithography and solid phase chemical synthesis technologies.

Oligonucleotides (oligos), usually 25-mers (25 bases long), are directly synthesized onto a glass wafer. Each array contains up to 900,000 different oligos and each oligo is present in millions of copies. Since oligonucleotide probes are synthesized in known locations on the array, the hybridization patterns and signal intensities can be interpreted in terms of gene identity and relative expression levels by Affymetrix GeneChip Operating Software. The following image gives a schematic of this process.

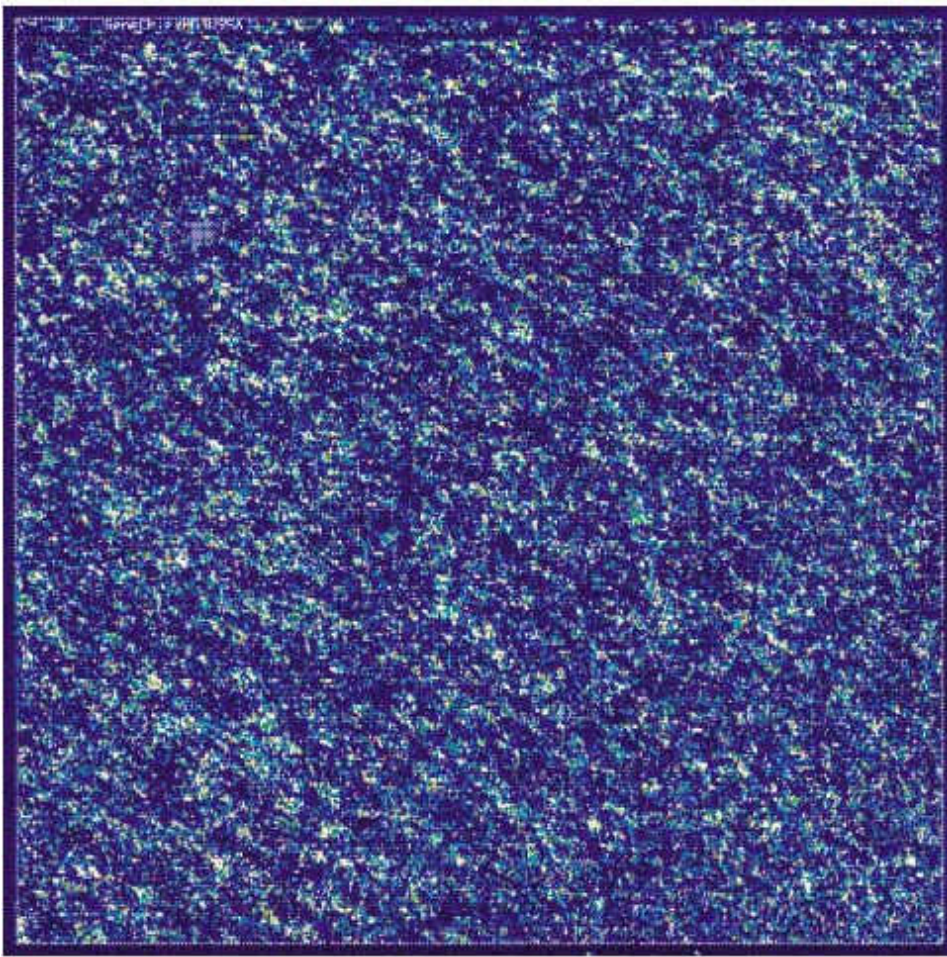


Each gene is represented on the array by a series of different oligonucleotide probes. Each probe pair consists of a perfect match oligonucleotide and a mismatch oligonucleotide. The perfect

match probe has a sequence exactly complementary to the particular gene and thus measures the expression of the gene. The mismatch probe differs from the perfect match probe by a single base substitution at the centre base position, disturbing the binding of the target gene transcript. This helps to determine the background and nonspecific hybridization that contributes to the signal measured for the perfect match oligo. The GeneChip Operating Software MAS algorithm subtracts the hybridization intensities of the mismatch probes from those of the perfect match probes to determine the absolute or specific intensity value for each probe set. Probes are chosen based on current information from Genbank and other nucleotide repositories. The sequences on the expression arrays are believed to recognize unique regions of the 3' end of the gene. The following diagram shows a schematic of a probe set:



The slide is then hybridized with RNA from the selected target. After hybridization, the chip is stained with a fluorescent molecule (streptavidin-phycoerythrin) that binds to biotin. The staining protocol includes a signal amplification step that employs anti-Streptavidin antibody (goat) and biotinylated goat IgG antibody (The series of washes and stains with aforementioned reagents binds the biotin and provides an amplified flour that emits light when the chip is then scanned with a confocal laser and the distribution pattern of signal in the array is recorded. The following image shows an Affymetrix GeneChip that has been hybridized.



Terms used in Affymetrix GeneChips

Target

Fragmented, biotinylated anti-sense cRNA prepared from mRNA to be analysed. Target molecules are hybridized to the probe array and the levels of hybridization are measured with the GeneArray scanner after the array is stained with biotin – streptavidin-phycoerythrin (SAPE).

Probe

Single-stranded DNA oligonucleotide synthesized directly on the surface of the GeneChip array using photolithography and combinatorial chemistry. The 25 base oligonucleotide is designed to be complementary to a specific gene transcript.

Probe Cell

Single square-shaped feature on an array containing probes with a unique sequence. The size can vary depending on the array type, typically 20 µm or 18 µm. Each probe cell contains millions of probe molecules. **Perfect Match (PM)**

Probes that are designed to be complementary to a reference sequence.

Mismatch (MM)

Probes that are designed to be complementary to a reference sequence except for a homomeric mismatch at the central position (e.g., 13th position of 25 base probe, A->T or G->C). Mismatch probes serve as a control for cross-hybridization.

Probe Pair

Two probe cells, a PM and its corresponding MM. On the probe array, a probe pair is arranged with a PM cell directly above a MM cell.

Probe set

A set of probes designed to detect one transcript. A probe set usually consists of 11-20 probe pairs. For example, an 11 probe pair set is made up of 11 PM probes and 11 MM probes for a total of 22 probe cells. Newer array designs from Affymetrix, e.g., HG-U133, contain probe sets with 11 probe pairs. Older designs have average probe set numbers of 16 or 20 probe pairs.

Target Sequence

The portion of a transcript reference sequence that is interrogated by a probe set on the array. The target sequence extends from the first base of the most 5' probe to the last base of the most 3' probe.

See also

- [Microarray Menus](#)
- [Open Microarray Data Files](#)
- [Calculate Affymetrix Expression Values](#)
- [One channel Quantile Normalization](#)
- [One Channel ANOVA](#)
- [One Channel Regression](#)
- [Robust Means Analysis](#)

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