DNA Arrays
Affymetrix GeneChip System

hybridization

chip scanner

data analysis
...TGTGATGGGTCAGAAGGACTGTGGCTAGGCGCTGCC...

GGAATTGGGTCAGAAGGACTGTGGCTAG

GGAATTGGGTCACAGGACTGTGGCTAGGCGCTGCC

Perfect match oligo

Mismatch oligo

mRNA

Perfect match probe cells

Mismatch probe cells
**Prepare Sample**

Reference  
Test  

Label with Fluorescent Dyes  
Combine cDNAs  

Hybridize to microarray  
Scan

**Print Microarray**

cDNA Library or Oligo Probes  
Microarray slides
The benchtop microarray facility...

**Synthesis**

Microarray synthesis and hybridization is performed inside a three dimensional microchannel structure - the DNA processor™. It supports up to eight microarrays. An interface connects microfluidics and DNA processor™ with the reservoirs containing reagents, solvents, and buffers needed for synthesis and hybridization. The entire microfluidic system is kept in an inert argon atmosphere providing superior reaction conditions for oligonucleotide synthesis.

**Injection**

After amplification and labeling DNA samples are simply injected in a port using a syringe. Compared to other microarray technologies, which typically consume up to 500 µl of prepared sample, geniom® requires 30 µl per array only.

**Hybridization**

Defined and reproducible hybridization conditions are provided by a Peltier element. For optimum hybridization results time and temperature are easily programmable. In addition, different wash and hybridization buffers can be used.

**Detection**

Different filters facilitate the use of all standard fluorescence dyes. Detection of dual labeling is possible by simply switching filters via software. Hybridization of the fluorescence labeled DNA is detected by a CCD camera. Therefore, experimental results are available as digital files within seconds for immediate downstream analysis.
... delivering real time microarrays

The DNA processor™

Central element of the geniom® technology is the DNA processor™, a unique 3-D microchannel structure. It is subdivided into eight segments with individual fluid control resulting in eight independent microarrays.

Microarrays are build up by \textit{in situ} oligonucleotide synthesis inside the DNA processor™. A digital projector and proprietary phosphoramidite chemistry allow maskless light activated synthesis. This results in spots of defined oligonucleotides with a size of 34 µm x 34 µm.

The transparent reactive surface is about 1 cm² in total and allows parallel synthesis of up to eight arrays with a minimum of 6,000 oligonucleotide probes each.

The disposable DNA processor™ is held in a cartridge for easy handling and auto alignment in the instrument’s fluid system and optical path. A memory module in the cartridge assures correct identification and history tracking.

Adjust throughput or content

The DNA processor™ provides flexibility in sample throughput. Hybridization may be performed with eight different samples in parallel against microarrays of at least 6,000 features each.

If higher content is required more than one array of the DNA processor™ can be used. Thus, defining a complete DNA processor™ as one array, a high density array of at least 48,000 probes results.

Choose optimum probe length

geniom® one offers flexibility in oligonucleotide probe length – even within probes of one array.

- Start with validated probes of fixed length and increase specificity by reduction of probe length
- Compensate for different hybridization temperature optimum of individual probes by adjusting individual probe length
SAGE, Serial Analysis of Gene Expression

Biotinylated cDNA

Cleave with anchoring enzyme (AE) and bind to streptaviding beads

Divide in half and ligate to linkers A and B

Cleave with tagging enzyme (TE)

Ligate and amplify with primers A and B

Ditag

Cleave with anchoring enzyme
Isolate ditags
Concatenate and clone

CTAGXXXXXXXXXXXXXXXXYYYYYYYYYCTACXXXXXXXXXXXXXXXXYYYYYYYYYYYYCTAGGATCGATCXXXXXXXXXXXXXXXXYYYYYYYYYGGATCGATCXXXXXXXXXXXXXXXXYYYYYYYYYGGATC

Ditag

Ditag

Ditag
Detecting Effects of HIV-1 on Host Cell Transcription

Human T cell

RNA extraction

Quantitative RT PCR

Reverse transcription

cDNA

Cut with TaqI, label radioactively and run on gel

Differential Display

Label green and hybridize to spotted array

In Vitro Transcription

cRNA

Fragment and label, hybridize to array

Affymetrix GeneChip

Human T cell

RNA extraction

RNA

Reverse transcription

cDNA

Cut with TaqI, label radioactively and run on gel

Label red and hybridize to spotted array

In Vitro Transcription

cRNA

Fragment and label, hybridize to array

Affymetrix GeneChip
Image Analysis

1. Gridding: identify spots
   (manual, semi-automatic, automatic)

2. Segmentation: Separate spots from background
   (fixed circle (B), adaptive circle (C), adaptive shape (D), histogram)

3. Intensity extraction: Derive one number for spot and one number for background. (mean or median of pixels)

4. Subtract background: local or global background?

Choices of methods depend on image and array quality