Real and Virtual Microarrays: Step by Step

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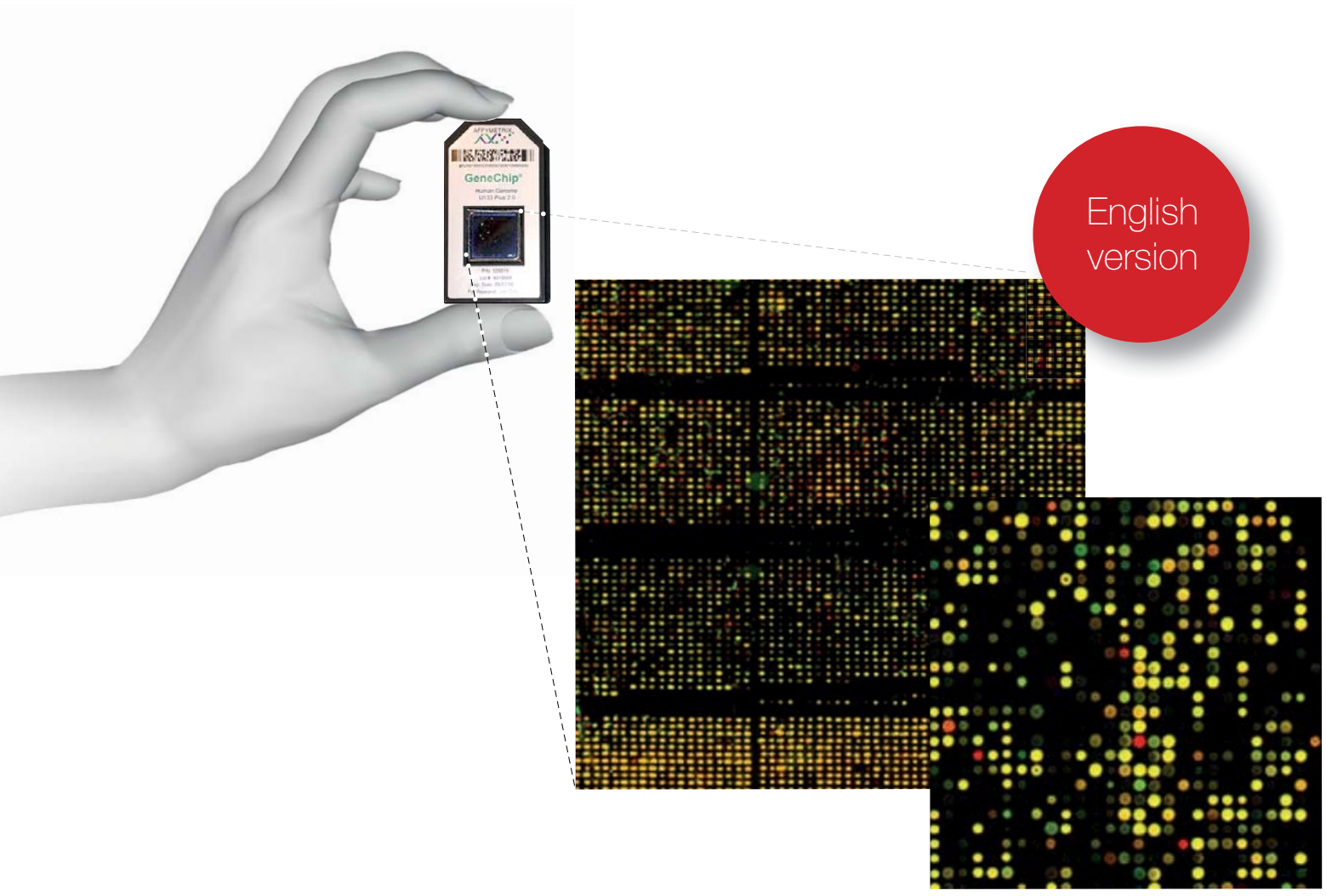
Version 2.3



English version

ELLS – European Learning Laboratory for the Life Sciences

EMBL

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Real and Virtual Microarrays

DNA microarrays: step by step

Production of DNA probes

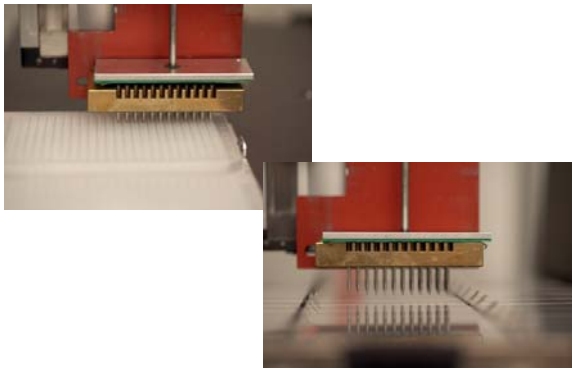
Scientists can produce “homemade” DNA microarrays by:

•using the polymerase chain reaction (PCR) which generates thousands of small pieces of DNA in double helix form

•or if they know the nucleotide sequence of a gene, they can ask a biotech company to make small, single chains of nucleotides with the same sequence.

Printing or “spotting”

DNA microarrays are very compact and can easily be produced in the lab using glass slides rather like the ones used for microscopy. As you can imagine, printing 20,000, minute spots of DNA (each spot containing billions of copies of DNA from a single gene) on such a small surface is a difficult task. Not only do the spots have to be exactly the same shape, they also have to be equidistant from one another. These problems have been solved by robotics.



The virtual microarray: step by step

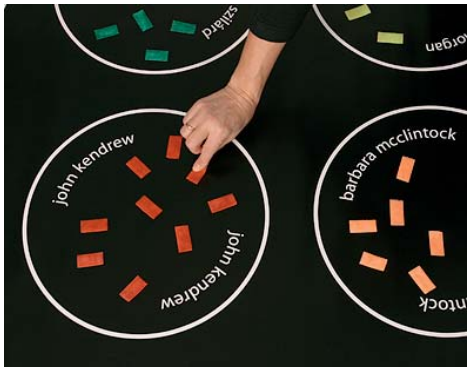
The mat

You can either make your own mat or you can write to ells@embl.de and order a custom-made, Lite version, mat (1x2.5 m) for 40 Euros (excluding postage).

Printing or “spotting”

In the virtual microarray a mat represents the glass slide with an array of 10 spots containing single-stranded DNA sequences derived from 10 genes: alexander fleming, jacques monod, thomas morgan, barbara mcclintock, leo szilárd, john kendrew, francis crick, rosalind franklin, maurice wilkins, james watson. The DNA molecules are represented by coloured Velcro, e.g. for the john kendrew gene the Velcro is red. *You will have to stick your own Velcro in the circles.*

A robotic arm guides a special set of 48 gold pins (capillaries) to reservoirs containing the DNA probes to be printed on the slides (because the pins are very delicate and extremely expensive, the machine is only equipped with 48, which are used over and over again). The robotic arm dips the pins into the samples, withdraws them, and moves to a position above a row of pre-treated slides, then gently presses the pins down for a split second onto the glass, "spotting" 200 nl of DNA from each pin tip onto the surface. After one line of spots has been printed on the first slide, the arm moves onto the next slide and so on, until all the slides have an equivalent row of spots. At the end of the row the arm washes the pins, dips them into a new set of samples, returns to the first slide, and prints a new series. This procedure is repeated over and over again until a series of slides is produced, each slide bearing a "microarray" of 20,000 dots. After printing the slides are heated to "fix" the DNA.



Extraction of mRNA

In order to perform a microarray experiment, scientists extract mRNA from the cells they want to study. This process is relatively easy to perform.

For each experiment, mRNA from two different cell types is needed: from control cells and the cell line under study. For example, if you were interested in studying cancer cells, you would take mRNA from normal (non-cancerous) cells as the control and mRNA from cancer cells of the same tissue.

Once extracted, the mRNAs need to be labelled

Extraction of mRNA

The mRNA is represented by a number of small, pocket-sized torches. Each torch corresponds to a single chain of mRNA from a particular gene (red Velcro for mRNA from john kendrew) and each has a nametag.

The exact number and names of torches will be discussed later. For now, it is sufficient to know that there are torches corresponding to the mRNA from control cells and to the cell line under study.

The torches need to be labelled with green or red transparent sticky paper representing the labelled mRNA from the corresponding cell types: red to

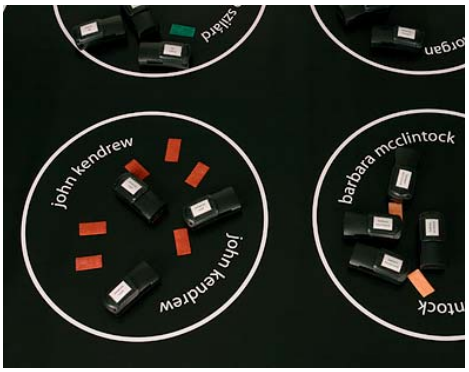
with fluorescent markers so that they can be detected later, on the surface of the microarray. mRNA of the control cells is usually labelled with green fluorescent marker, and mRNA of the cells under study with red fluorescent marker.

Hybridisation

In this step, the control mRNA (labelled green) is mixed with the test mRNA (labelled red). The mixture is then flooded over the surface of a slide, which is then incubated at 42°C, so that single strands of mRNA in the mixture hybridise with their complementary DNA on the microarray.

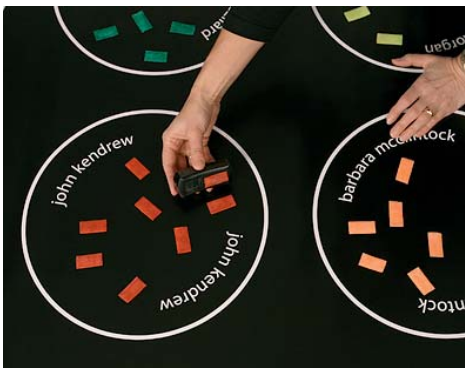
After 12 hours, the microarray is washed to remove any mRNAs that have not found their complementary target DNA. The microarray is now ready for scanning.

signify mRNA from cancer cells or green for mRNA from control cells.



Hybridisation

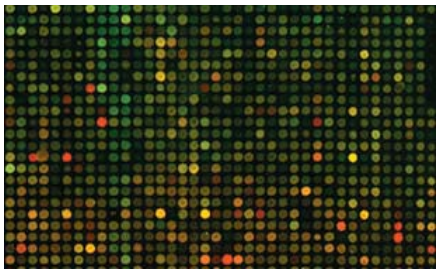
Now it is time to hybridise the labelled mRNAs (torches) with the DNA probes (Velcro) on the virtual microarray. The microarray is flooded with the mRNA mixture (torches) but only those torches which have the appropriate coloured Velcro attached will hybridise with the complementary single-stranded DNA on the microarray (mat), i.e. torches with red Velcro hybridise with red Velcro on the microarray, yellow with yellow, etc.



Torches bearing coloured Velcro which is not found on the mat will not bind. Multicoloured Velcro on torches represents the case where only a small sequence of the mRNA is complementary to the DNA and hybridisation is very weak. During the washing step these weakly bound mRNA molecules are washed away.

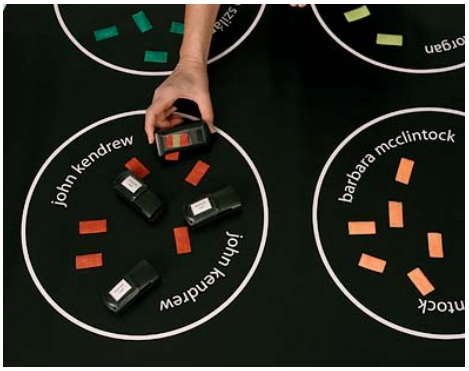
Scanning the microarray—acquiring an image

Now it is time to look at the results of the experiment to see which mRNA has hybridised with which target DNA. This is done with a laser scanner, which works in pretty much the same way as the scanner you use with a computer. The laser scans the slide and a computer combines the images and produces a picture similar to the one in the figure below.



But wait a minute there are not just red and green dots there are yellow and orange dots as well!

This can easily be explained. It is clear that the red spots contain mRNA from cancer cells and green spots mRNA from non-cancerous control cells. But what happens if equal amounts of mRNA from control and cancerous cell lines hybridise with the same target DNA? The green and red signals combine to give yellow!



So now, stick the torches on the appropriate Velcro in the circles on the microarray. The torches should be switched off at this point.

Scanning the microarray—acquiring an image

In order to “scan” the virtual slide, switch on all the torches lying on the microarray mat, taking care not to displace any of them. Switch off the lights in the room and look at the microarray.

Go through the array, spot by spot (circle by circle). What can you say about the colour and intensity of the lights? Note, in the virtual microarray it is not possible to reproduce yellow spots. However, red and green spots should be evident. Note down the numbers of red and green torches for each gene. The numbers will be needed later for the analysis.

As for the real microarray, the circles show a gradation of colours. In a circle that appears red, only mRNA from cancer cells (red torches) has

Remember mRNA hybridizes with its complementary DNA and one spot on the microarray represents billions of copies of DNA from ONE gene. In other words, when a spot is yellow, there are equal amounts of mRNA of the gene found in cancerous and control cells.

If a spot is orange, it means that there is more mRNA of the gene in cancerous cells than control cells. And black means that there is no mRNA of that gene either in the control or cancerous cells.

Normalisation

In a DNA microarray, the intensity of the spot does not always represent the true amount of hybridised mRNA, because the labelling of mRNA is influenced by the mRNA chain size and type of visual marker used.

A mathematical process known as normalisation corrects the intensities of the spots, so that they directly reflect the amount of mRNA present in the hybridised molecule. After the data has been normalised, the analysis can begin.

hybridised with the DNA molecules. And in the same way green circles correspond to exclusive hybridisation of control mRNA. Yellow circles correspond to equal amounts of cancer and control cell mRNA. This is very simplistic because if you go back to the real microarray again, you can see a huge variation in spot colouration (look at the “icon” on the web page!), which can only be deciphered by a laser scanner programme.

Normalisation

According to what you know so far, scientists can infer the amount of mRNA from the colour of a spot on a microarrray. As a parallel, you can guess the number of the torches per circle on the microarray from the intensity of the light.

But let us consider the following example. If there is one torch on one spot and on another there are two, and if all the torches have the same batteries, then you would expect the light from two torches to be as twice as strong as that from one torch.

But what if the batteries in the two torches are flat and the torches are running at half strength? Then they would emit the same amount of light as one torch. In other words, the amount of light that you see in the virtual microarray is not only related to how many torches you have, but also to the state of the batteries in each torch.

Analysis and Clustering

It would take years to analyse a microarray spot by spot. So scientists have devised a way of grouping genes that behave similarly into clusters. And as usual, complex computer programs have been written to perform these steps automatically.

Analysis and Clustering

This will be explained in “Clustering Exercises for the Classroom”.

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