

IN THE CLASSROOM

1. The magic mirror: a simple story approach to introduce microarrays in the classroom.
2. The flow of information from DNA to proteins
3. Gene expression analysis
4. How to explain hybridisation
5. Practical advice for the microarray activity
6. Clustering
7. Microarray exercise

1. The magic mirror

It was 8:30 am on a grey Monday morning. My alarm clock was RINGING. I reluctantly stretch my hand out from underneath the warm bed covers and turn it off. With that awful Monday morning feeling, I slowly got out of bed, made my way to the bathroom, grabbed my tooth brush and started brushing my teeth wondering what my sister was doing..... It had been quite a long time since I'd last spoken to her. As I glanced up at the mirror there she was right in front of my very eyes. She was driving to work and didn't appear as happy as usual (maybe this had to do with it being Monday morning!). My first thought was that the additional glass of red wine that I'd had the night before was having revenge on me. "Although, come to think of it, I hadn't drunk that much!! Or could it be that I was dreaming? How could I have possibly seen my sister because she lives in Greece. Perhaps the mirror was magical?"

So I decided to take another look to see if it was really magical, or just a figment of my imagination. Maybe, if I thought of another person and stared at the mirror long enough, I could conjure up their image too. So I tried again! I thought of my mother, who was also living in Greece, and all of a sudden, an image of my mother at work appeared in the mirror. "OK", I said to myself. "This cannot be happening". I called my mother immediately and asked her where she was. "In my office", she replied. "I always wake up early on a Monday. Why do you ask?" I didn't have the courage to tell her what was going on, so I hung up.

After that, I began to examine the mirror very carefully. I checked to see if there was a camera of some sort hidden behind it. I stared at it to see if there was anything strange about it. Then I started to examine the whole bathroom. You see, I was expecting that sooner or later somebody would

jump out and say: "Smile, you're on Candy Camera." But there was no body around, and my friends certainly wouldn't have played such a joke on me.

I decided to challenge the mirror again. I concentrated my thoughts on Maria a friend of mine who lives in Mexico. And sure enough an image of Maria immediately appeared in the mirror. She was in bed, sleeping. I rushed into the living room and tried to find a map showing the time differences between countries around the world. "It was true!" At 9:15 am in Germany, it is 2:15 am in Mexico. "That's why she was asleep!" I thought. "I'm not mad after all!" "How could I possibly have known what she was doing? I didn't even know that it was night in Mexico."

I went back to the mirror and I mused, "What would happen if I thought of a person that I had not met before? How about a famous person, a celebrity? That would be quite a challenge for the mirror." So, I started to think of famous people: Madonna, David Beckham, Rowan Atkinson, Gerard Depardieu, Johnny Depp, J. K. Rowling. As many people as I could! The president of the United States! (Sorry I should not disclose this information. I may get into trouble with the intelligence services).

All I had to do was to think of a person and their image appeared right in front of my eyes! Amazing! It didn't matter who it was. It didn't matter where they were. It was a true! The mirror was magical!

And then without thinking, I suddenly became curious about what my boss was doing. And his image appeared right in front of my eyes. Oh, no! He was in the lab. "Isn't that MY lab bench he's hovering over? Aren't those MY samples in front of him? Oh my God! It's already 10:15! I am late and my boss is waiting for me to start the experiment!"

I grabbed my bag, glanced quickly at the mirror and headed for the door. I decided not to tell anyone about the mirror. They wouldn't believe me anyway.

On the way to work.....

On my way up to EMBL, I started to think about all the cities in the world and how they were connected to one another. Roads connect cities in the same continent, and ports and airports connect cities in different continents. Take any person in any city in the world. They can travel to any other city in the world if they want to.

Because of the earth's rotation around its axis, some cities experience daylight while others are in complete darkness.

What people are doing, at any one time, in a city depends if it is day or night. In most cities in the world, people wake up in the morning and go to work

until early afternoon. After that, most people remain active before going to bed when it gets dark. This was also the case for the people I saw in the magic mirror: my mother, my sister, my friend.

However, not all people work during the day. Take people that work at an airport, for example, they work day and night to ensure that airplanes can take off and land safely. Firemen don't work all the time, but are ready to work, whenever duty calls. Policemen are another example of people who work around the clock. They patrol in towns and in the countryside making the world a safer place to live in.

But what happens when there is some kind of danger during the night? What happens when there is a fire or a crime in a city? According to the extent of the fire or crime, a number of policemen and firemen will wake up and rush to the spot to provide immediate help, while the rest of the population continues to work or sleep. But if something major happens in a city, let's say an earthquake, things are different. Most people will wake up, and as many firemen, policemen and doctors as possible will rush to the scene of the disaster to help out. Sometimes, there are not sufficient resources in the affected city. In such cases, help from other cities is brought in. Slowly, the city will recover and start functioning again at its normal pace.

I realized that with the magic mirror, I could see what people all around the world were doing at any one time. It was like having a magical camera, taking snapshots around the world.

I had almost arrived at EMBL, when it dawned on me that a city works in exactly the same way as cells in an organism. Most organisms are composed of organs, and these organs are made up of cells, like cities around the world. The way a cell works depends on its identity, what kind of job it is destined to do: muscle cells of the heart work continuously in order to pump oxygen, whereas cells of the retina work only when we see. But what do we mean when we say that cells *are working*? We mean that these cells can make proteins that they will use for the different functions in the cell. However, the instructions to make the proteins are written in the genes of the cells, in their DNA. A small part of the DNA encodes information on how to make a particular protein. This information is first copied into another molecule, called mRNA, which will be then used to make protein. Ultimately, when we say a gene is working, we say that it is being *transcribed* into mRNA, and this mRNA is then used to make protein.

Just like airports, some genes work all the time. Consider, for example, the fact that a cell needs energy all the time. Some genes, called *housekeeping genes*, work all the time to make the necessary proteins to ensure that there will always be energy available for the cell. However, what happens to the cell when it faces danger of some kind? If, for example, there is lack of oxygen, a virus has entered, or the cell has become cancerous, then there will always be changes in the genes that are working. Some genes may wake up, while others increase their workload. There is also the possibility that

some genes may stop working altogether, because they are not needed anymore. In other words, some genes are **switched on**, others are **switched off**. The size of the change (the number of genes involved) depends on the challenge.

And then it dawned on me why had I seen those strange things in the mirror. On that very day my boss was waiting for me in the lab to do DNA microarray experiments. And DNA microarrays, also called DNA chips, are exactly like the magic mirror. They allow scientists to see what all the genes of an organism are doing at one particular time: if they are switched on or off. And when conditions change, they allow scientists to see which genes are active or inactive (**expressed**).

The magic mirror was just a prelude to what was going to happen in the lab. This of course didn't explain why the mirror had showed me these things in the first place, but at least I had an explanation of why it had happened. And with this in mind, I managed to find a parking spot, grabbed my bag and headed for the lab to start my microarray experiment. Another full day was on the cards...

The following allegories and metaphors can be used to illustrate:

2. The flow of information from DNA to proteins

Imagine that you want to bake a cake in your kitchen, but the instructions on how to make it are written in a recipe book in the living room. Normally, all you would have to do is go to the living room, find the book, bring it back to the kitchen, and start preparing the cake. But what would you do if we told you that it was strictly forbidden to take the book out of the living room?

Well, it might be forbidden to take the book out of the living room, but it is not forbidden to make a copy of the recipe (instructions). So you can take a piece of paper, write everything down, and then carry the paper to the kitchen. This is exactly what cells do to make proteins.

DNA (the recipe book) holds the instructions (recipe) to make proteins but cannot leave the nucleus (living room), so it makes a copy of itself (mRNA) which can move out of the nucleus into the cytoplasm where the factories for protein production (ribosomes) are located.

3. Gene expression analysis

When we say that a gene is active it means that the gene makes mRNA, which passes into the cytoplasm to make protein. How much mRNA is made depends on how much protein is needed. Almost all organisms have DNA to make the proteins that they need to survive. But this does not necessarily mean that all this information is used. Remember that DNA is like a cookbook. A cookbook contains many recipes, but we choose to make only a few of them. Cells also choose which proteins they want to make, and the way they look and behave very much depends on the proteins made.

In order to find out which genes are active in a cell, all we have to do is extract the mRNAs from the cell, read them one by one and find out from which genes they come from. Sounds easy, but in fact it is almost impossible, unless you want to spend the rest of your life working 24/7 in the lab. Scientist can't wait that long, so they have found a way to solve this problem.

Imagine that you want to go fishing and catch a particular kind of fish but the lake is full of different fish. Because you are an experienced angler, you know that certain fish prefer certain bait. So you choose your bait carefully, attach it to the hook, cast your line and wait...Voila! You've caught a fish! If you do this over and over, with different type of bait you can catch all the different kind of fish you want to catch.

4. How to explain hybridization

This is exactly what scientists do, they “fish out” mRNAs of single genes that are contained in solution. All they have to do is to decide what the proper bait is to lure the mRNA. Now think of complementarity! All they have to do is to add the chain of DNA (bait) that is complementary to the mRNA (fish) they want to catch. The two chains will find each other and stick together (**hybridize**).

But what would you do if I told you that you have to catch all the different fish at once, and sort them out? You can forget the fishing rod. It'll take too long, especially if there are thousands of different types of fish. But you are clever. First you catch all the fish in the lake, then go to an aquarium and selectively cast thousands of lines in the water: in one area you attach one type of bait, in another different bait and so on. Now, you simply throw all the fish from the lake in the aquarium, wait a bit for all the fish to find their respective bait, and then haul them out. Each fish is now attached to a specific hook.

In the case of mRNAs, scientists do something similar. Let's consider a simple example first. Suppose that you have a small grid onto which you have managed to fix four different nucleotides (bait), in different areas:

A	C
G	T

*What would happen if you put a solution that contains guanine (G) onto this grid? Which area of the grid would it stick to (hybridize with)? If you remember, **G** always sticks to **C**, so the answer is that it will stick in the upper left-hand corner. But how are you going to see that G has stuck to C? These molecules are so small that it is impossible to see them, even with a microscope. To solve this problem scientists mark the Gs in the solution with a fluorescent marker that glows in the dark (this procedure is called **labelling**).*

Now instead of putting single nucleotides on a grid, you can replace them with entire chains of DNA that belong to a particular gene. If you now apply a solution that has fluorescent mRNAs, only those mRNAs that stick to the DNA will glow in the dark. In this case, you will have found a way to “fish out” the genes.

And finally microarrays

Imagine a small glass surface that can fit in the palm of your hand. Imagine that you have put not 1, not 2, but 20,000 different DNA chains that belong to all the different genes of an organism onto the glass surface. Each gene takes up a very small space on the surface, typically a dot that is many times smaller than the full stop at the end of this sentence. On this small area, you essentially have hooks to “fish out” all the different genes of an organism, as you have bait (DNA sequences) in the aquarium for all the fish (mRNA). In other words, you have a way to find out which genes in an organism are active. You have a DNA microarray!

5. Practical advice for the microarray activity

This activity has been designed to simulate the different steps of a DNA microarray experiment in the classroom. Before performing the activity, you should read this section carefully and familiarize yourself with the basic principles of microarrays.

PLEASE send us any feedback on interesting facts, intriguing aspects or even difficulties that you have experienced while performing this activity in the classroom. Your comments and suggestions will be highly appreciated and will contribute to further improvements of the activity. Please send your input to ells@embl.de. We hope that you will have as much fun performing the virtual microarray in the classroom as we have had creating it here at ELLS!!

How should you begin?

Look at the different sections of this activity and try to decide which are going to be interesting for your class. If you have time, try to involve your pupils in the pre-activity preparations (labelling the torches, etc). However, if time is limited, you can prepare everything in advance and just perform the hybridization step in class.

Before you begin:

Make sure that you can find a room or classroom that has sufficient lighting, but that can also be darkened (with curtains or blinds). You should make enough space in the middle of the room to lay the mat on the floor and allow the pupils to lie in a circle around it. (It is very important to get away from the classroom atmosphere, so avoid sitting on chairs). Moreover, you can ask the pupils to stand on desks pushed up against the wall, so that they can have a bird’s eye view of the carpet when it is lit up.

What you will need:

Make sure that you have enough torches. We have based our activity on 67 small torches, called “promotional flashlights” that can be purchased relatively cheaply for promotional purposes.

However, if your budget can’t stretch that far, ask your students to bring torches (ones that work!!) from home. In this way, the pupils will have contributed material for the activity.

*It might be sensible to restrict the number of students to 12. Given the fact that most of the students will bring 1-2 flashlights, it might be very hard to collect 67 torches. In this case, you can modify the number of flashlights that are used for each gene. Take care, though, not to alter the regulation (**up regulation** or **down regulation**) of each gene. For example, the genes *maurice wilkins*, *rosalind franklin* and *francis crick* have the same number of red and green flashlights. You may change the number, but you must always have equal numbers of green and red. In the case of genes that are up regulated, that is they have more red than green torches, you may also change the numbers but make sure that they have more red than green torches, and so on.*

*A word of caution! While it may be sensible to change the number of flashlights, try to avoid having genes with exactly the same number of flashlights. For example, try to avoid having one green and one red flashlight for **Cluster 1**, *maurice wilkins*, *francis crick*, *rosalind franklin* and *james Watson*. In this way, when it’s time for the students to do the clustering, they will understand that genes should be clustered together because they have the same behaviour (in our example because they have identical regulation) and not because they have the same number of flashlights.*

There is something else about the flashlights that you may wish to consider. The ones we use can be laid flat on the ground with the beam pointing up: it is very important that the light is emitted from the floor to the ceiling. However, most torches are cylindrical. So if the pupils bring their own torches, ask them to bring something to stand them in as well: a small mug or pencil holder will do.

It might be useful to collect all the flashlights some days in advance, so that you can solve any problems that might arise.

Labelling and hybridisation:

For the experimental step, divide your class into two groups: the control cell line and the cancerous one. Give each student a box containing all the name-tagged flashlights of their cell type. If you have time, ask each team to label the flashlights with the appropriate colour: green for the control, red for the cancerous cell line. Depending on the transparency of the paper, it might be necessary to stick more than one layer on the glass to produce an intense colour. Finally, ask the students to place the flashlights into the proper position in the virtual microarray matt.

*Alternatively, if you have not managed to find the sufficient amount of flashlights, you can still perform the activity with just 10, one for each gene. Instead of having the colours for each molecule of mRNA, you may provide the colour of the spots as a whole. Such as, in the case of the genes maurice wilkins, rosalind franklin, frances crick and james watson in which you have equal amounts of mRNA in both samples, you may choose to put YELLOW transparent sticky paper on the flashlights, one for every gene. In the case of alexander fleming, thomas morgan, barbara mcclintock (the “**up regulated genes**”) the colour should be red, and in the case of leo szilárd, jacques monod and john kendrew the colour should be green. Be prepared, however, to explain to them that the yellow colour you see in a microarray is artificial and comes from the merging of green and red.*

Scanning of the array:

During the scanning process, when all the flashlights are lit and the room is dark, encourage your students to talk about what they see for each gene. Try to make them draw conclusions about each gene, rather than you telling them what’s going on in each example!!!

6. Clustering

During the clustering process, it is important to get the students to understand that the criteria to group genes together is their same behaviour in the microarray.

(See Teacher’s guide to clustering exercises [PDF], Clustering exercises for the classroom [PDF])

*Discuss different ways of **clustering** the genes, and mention to them that there is no wrong way or right way, but that the conclusions drawn from each cluster will be different. Ultimately, show them the clustering that is discussed in the earlier section, and make them search for things that the “genes” might share.*

7. Microarray exercise

If you have enough time, you can try to do the exercise in the classroom. Alternatively, you can set the exercise for homework, and then discuss the results in the next class.

(See Teacher’s guide to microarray exercise [PDF], Microarray exercise for the classroom [PDF])