Microarray: measuring gene expression

spice
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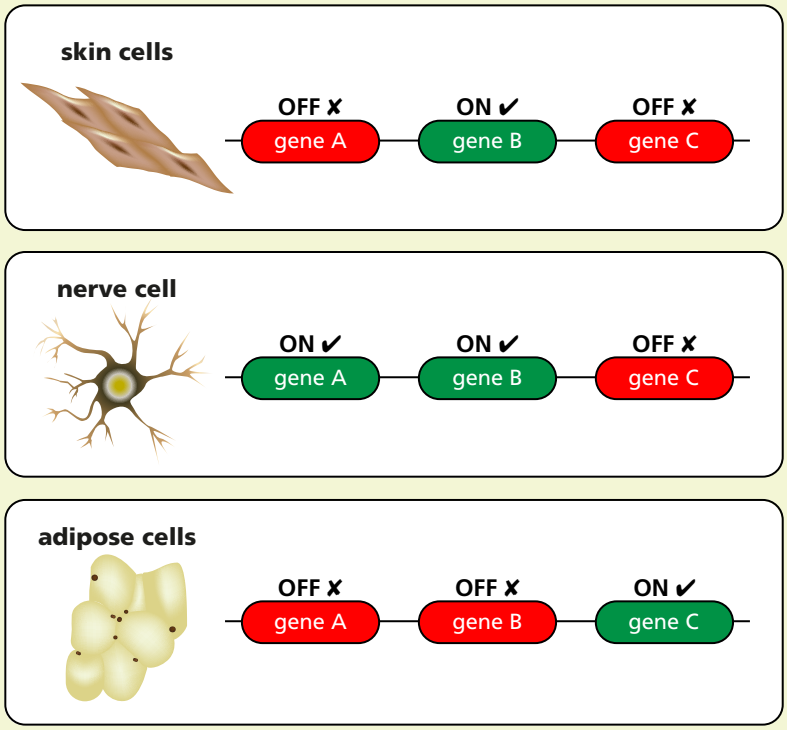
Microarray: measuring gene expression

Almost every cell in our body contains the same genetic information. Inside the nucleus of every cell is the same set of chromosomes and genes; only gametes and red blood cells are unique. Yet there are many different cell types, for example: skin cells, muscle cells, nerve cells … So, if the genetic information is the same, why are there so many different cell types?

The reason for these differences is gene expression, which means a gene is switched 'on' or 'off'.

Gene expression refers to activity levels of genes within a cell. Some genes are expressed (switched on) while others aren't. For instance, only 70% of genes in an adult liver cell are expressed. Gene expression explains why structure and function of a liver cell is different from a nerve cell: different genes are switched on.



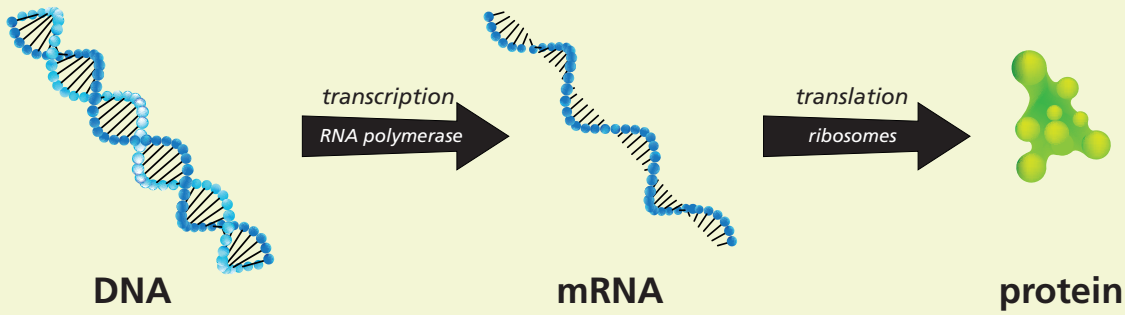
Different cell types are the result of different patterns of gene expression.

Some genes are expressed in all cells. These genes are called housekeeping genes as they're usually associated with essential cell functions, such as cellular respiration.

When are genes expressed?

A gene is expressed when its nucleotide sequence is transcribed into messenger RNA (mRNA). Different mRNAs make different products, usually proteins, but sometimes other molecules.

Signals from inside or outside a cell trigger gene expression. Different genes are expressed at different times and in different amounts, depending on environmental conditions and a cell's needs. For instance, during wound healing, gene expression of macrophage inflammatory proteins increases to help clear away infectious material.



Gene expression: information from a gene is used to make a product, in this case, protein.

How do we measure gene expression?

The amount of mRNA in a cell provides a measure of gene expression. Different techniques are used to measure the mRNA, such as microarray.

These techniques allow researchers to identify which genes are expressed in a cell at a particular time, and in what amounts.

Gene expression and cancer

Cancer is a genetic disease, which results in abnormal gene expression. Cancer can lead to genes being overexpressed, underexpressed, and in some cases, not expressed at all.

There are a number of genes known to be involved in cancer.

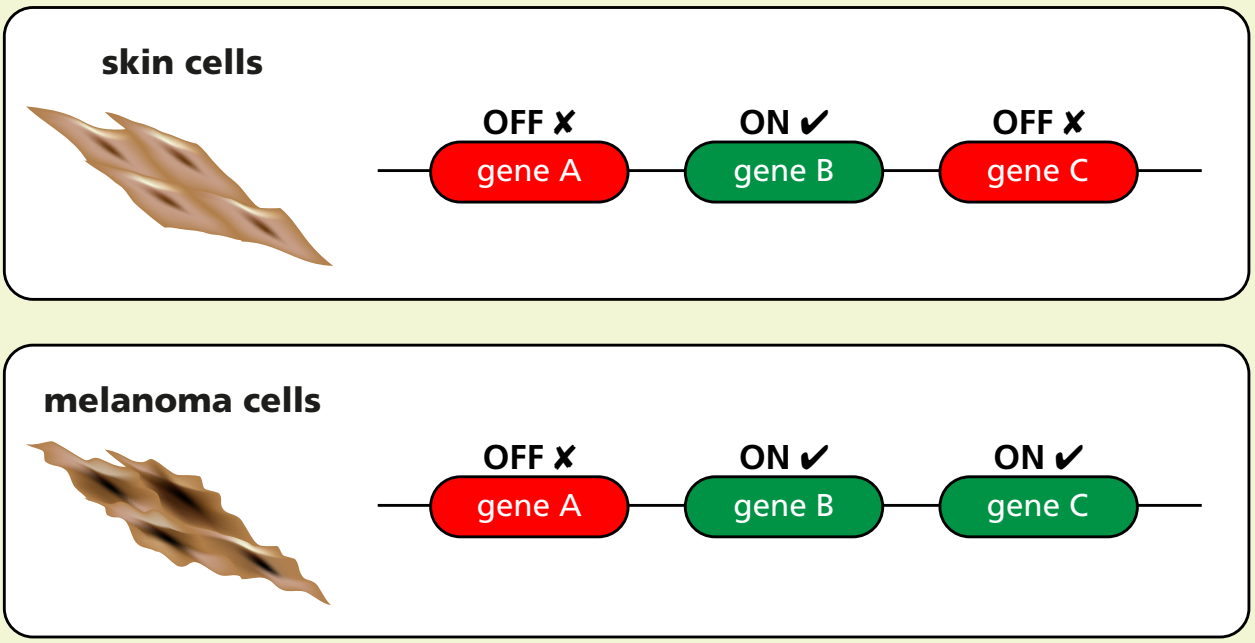
Genes with potential to cause cancer are called oncogenes. In many cases these genes cause cells to grow. In cancer, mutated oncogenes result in either accelerated cell division, or promote cell growth.

Tumour suppressor genes produce proteins that inhibit continuous cell growth. In cancer, these genes are often mutated and may be underexpressed or sometimes turned off altogether. This means loss of tumour-inhibiting function, within affected cells, and uncontrolled growth.

DNA repair genes produce proteins that monitor changes to DNA, and initiate repair. In cancer, these genes are also often mutated and may become underexpressed or are turned off, which means damage to DNA is unchecked.

All cells require blood vessels to deliver vital food and oxygen to ensure their survival. Normal cells initiate blood vessel growth (angiogenesis) during development and wound healing. But cancer cells can also signal normal cells to initiate blood vessel growth. This ensures they're continually provided with the nutrients and oxygen they need.

The main culprits in cancer appear to be oncogenes, tumour suppressor genes and DNA repair genes. However, mutations in cancer accumulate over time and usually involve multiple genes.



Gene expression patterns in cancer cells are different from those in normal cells.

Melanoma and gene expression

Melanoma, like all cancers, is characterised by uncontrolled cell division. In melanoma, cells found in the skin (melanocytes) continually divide and grow, and are capable of spreading to other parts of the body, that is, they metastasise.

By studying changes to gene expression in melanoma, scientists can identify:

- different subtypes of the disease;
- genes that may be useful targets for personalised medical treatment; and
- treatments that are suitable for individual patients.

Microarray and gene expression

Microarray technology is used to investigate gene expression in cells by measuring the amount of mRNA present. Microarray provides a 'snapshot' of which genes are expressed in a cell at a particular moment in time, and in what amounts.

Microarray is carried out using a gene chip or microarray slide. A gene chip is a solid surface, usually a glass plate, into which thousands of short strands of single-stranded DNA are embedded. These DNA strands, known as probes, are from known genes and are manufactured artificially. Each probe has a unique nucleotide sequence (typically 25 – 60 nucleotides long) that is complementary to a target mRNA sequence. Gene chips can contain tens of thousands of genes, and are used to study expression of thousands of genes simultaneously.

Microarray is often used to study disease, particularly cancer. Scientists look for differences in gene expression between cancerous cells and normal cells, for instance, between normal skin cells and melanoma cells.

Affymetrix Genechip
Image courtesy of Affymetrix Inc

Analysing melanoma gene expression with microarray

In microarray experiments there are five main steps. You'll conduct a laboratory simulation activity following four of these steps.

Step 1: Prepare the microarray slide.

A microarray slide is embedded with DNA of interest: manufactured single-stranded DNA segments, or DNA probes.

Step 2: Collect patient samples.

To compare differences in gene expression between melanoma cells and normal skin cells, samples are taken from a single patient, and mRNA from these two cell types isolated.

Step 3: Prepare cDNA samples.

mRNA deteriorates easily and isn't reliable in microarray experiments. To avoid this problem, an enzyme called reverse transcriptase is used to convert mRNA into complementary DNA (cDNA). This cDNA is more stable and less likely to degrade during microarray experiments.

The two types of cDNA collected from the patient are labelled with fluorescent dyes to distinguish them from each other: cDNA from normal skin cells with green dye, and cDNA from melanoma cells with red. Once labelled, the two cDNA samples are mixed together.

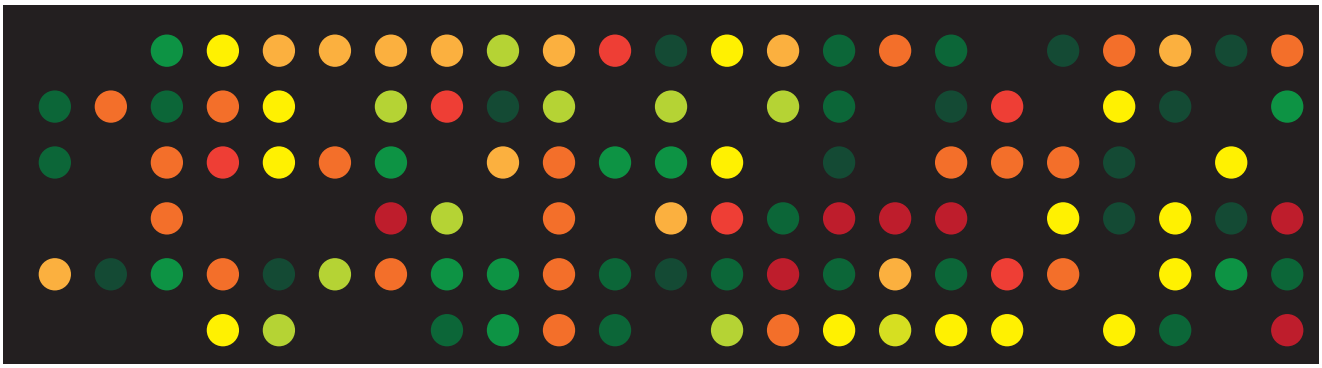
Step 4: Add cDNA to microarray slide.

The cDNA mixture is added to the microarray slide. Microarray works on the principle of complementary base pairing, where guanine binds with cytosine, and thymine with adenine. When the cDNA sample is added to the microarray slide it will hybridise (bind) with single-stranded DNA probes embedded in the slide. cDNA will only form a strong bond if there's a close match to the DNA probe. The slide is then washed to remove any cDNA that didn't bind with the DNA probes.

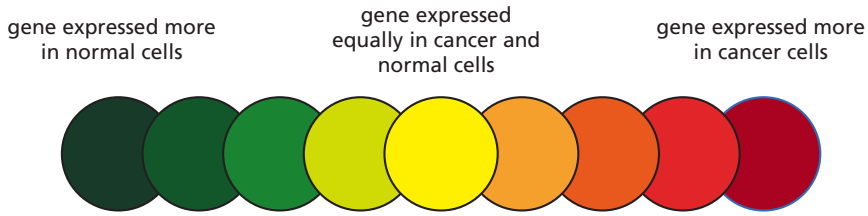
Step 5: Interpreting microarray results

Using laser microscopy the microarray slide is scanned revealing different coloured spots on the slide: red, green, yellow and black. Different colours indicate differences in gene expression between melanoma and normal skin cells.

The colour pattern of the scanned image is analysed to determine which genes are expressed in each cell type.



Microarray colour pattern after scanning.



Microarray results show a range of colours which reflect the relative amount of mRNA present, or the level of gene expression.

Green dot: cDNA from normal skin cells has hybridised with DNA probes. This gene is expressed in normal cells but not melanoma cells. This difference in gene expression is worth investigating to further understand melanoma.

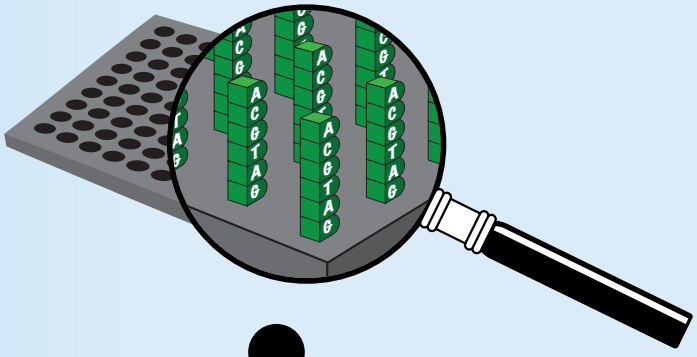
Red dot: cDNA from melanoma cells has hybridised with DNA probes. This gene is expressed in melanoma cells but not normal cells. This difference in gene expression is worth investigating to further understand melanoma.

Yellow dot: cDNA from both normal skin cells and melanoma cells have hybridised with DNA probes. Yellow results when both green and red cDNA bind to the same site. Genes expressed in both normal and melanoma cells are probably not involved in cells becoming cancerous.

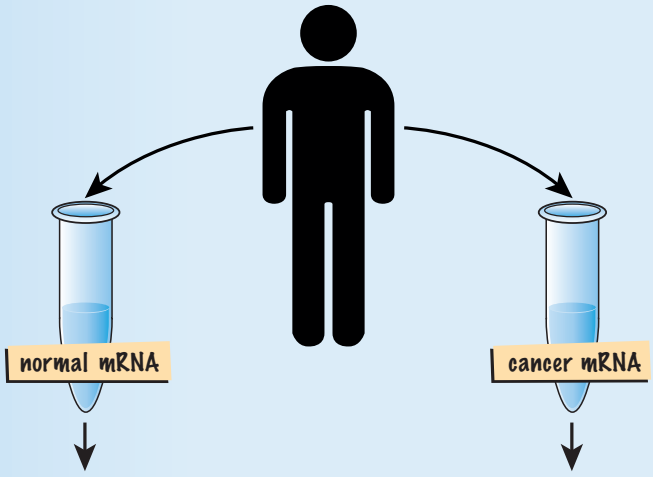
Black dot: cDNA from neither sample hybridised with DNA probes. This means the gene is not expressed in either normal skin cells or melanoma cells. Not all genes are active in every cell, and this gene would not be of interest in the investigation of melanoma.

How does microarray work?

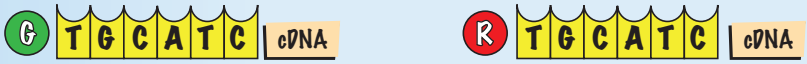
1. Embed manufactured probes (single-stranded segments of DNA) on the microarray slide.



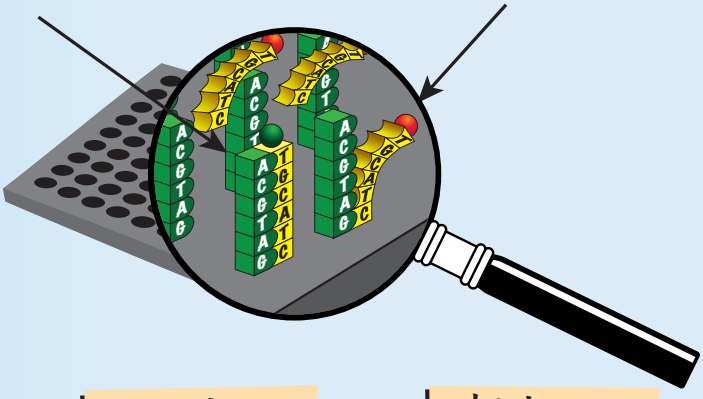
2. Collect patient samples from normal cells and cancer cells and isolate mRNA.



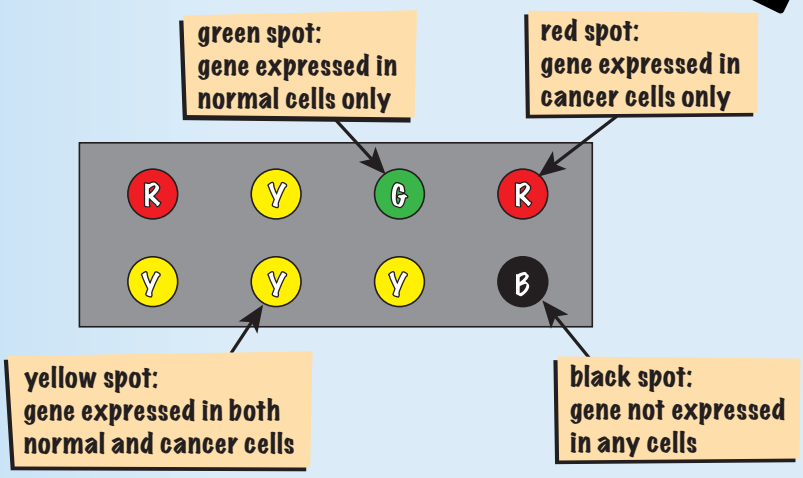
3. mRNA breaks down easily, so is converted into cDNA (complementary DNA) which is stable. The cDNA is labelled with red and green fluorescent tags and then mixed.



4. Add the cDNA mix to the microarray slide. Complementary base pairing binds cDNA and embedded probes.



5. Scan the microarray slide and analyse the pattern.



Microarray simulation activity

Aim

To discover, through laboratory simulation, how microarray technology is used to measure gene expression. Using this technique you'll detect differences in gene expression between simulated normal skin cells and melanoma cells.

In this activity you will:

- prepare a microarray slide with DNA probes from six genes of interest;

- mix together labelled cDNA from normal skin cells and melanoma cells to form a cDNA sample mix;

- add the cDNA sample mix to the microarray slide; and

- observe, record and interpret any colour changes.

Materials for microarray

DNA probes

In this experiment you'll investigate gene expression of six genes found in both normal skin cells and melanoma cells. You'll have access to six individual test tubes containing DNA probes (short segments of single-stranded DNA) for the genes listed below.

Table 1: Genes

TEST TUBE NUMBER	GENE NAME	GENE ACTION
1	XRCC1	DNA repair
2	BUB1	mitosis
3	FGFR2	apoptosis (cell death)
4	HBG1	haemoglobin production
5	Hsp90	protein folding
6	VEGFA	blood vessel growth (angiogenesis)

cDNA samples

You'll be provided two cDNA samples for this activity. Sample 1 contains cDNA from normal skin cells and has been tagged with blue dye. Sample 2 contains cDNA from melanoma cells and has been tagged with pink dye. These samples appear colourless; you will only see colour changes at the end of the experiment.

Colour interpretation

If cDNA binds (hybridises) to complementary base pair sequences in DNA probes on your microarray slide, there will be a colour change in that well.

Genes that are expressed in normal skin cells will appear blue, and genes expressed in melanoma cells will appear pink. The intensity of colour reflects the relative amount of mRNA in the cell, or level of gene expression.

Table 2: Meaning of colours

MICROARRAY COLOUR	GENE EXPRESSION
pink	Gene expressed in melanoma cells but not normal skin cells.
blue	Gene expressed in normal skin cells but not melanoma cells.
purple	Gene expressed in both cell types.
colourless	Gene not expressed in either cell type.

Microarray materials per group

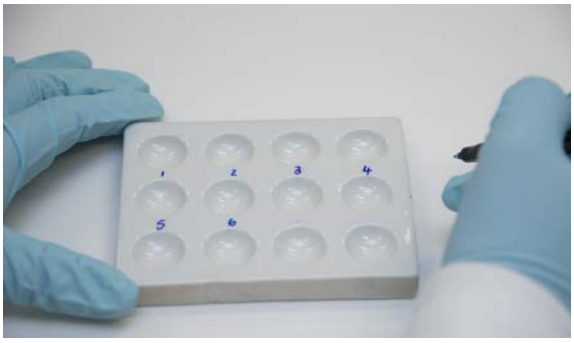
- microarray slide (depression tile)
- 1 pipette
- mini test tube
- marker pen
- safety glasses and gloves
- waste beaker at student bench
- waste beaker at cDNA mix station

Stock solutions (provided by teacher)

- 6 DNA probes labelled 1 – 6, each containing its own pipette
- cDNA sample 1 – from normal skin cells, containing its own pipette
- cDNA sample 2 – from melanoma cells, containing its own pipette

Method

Step 1: Prepare your microarray slide

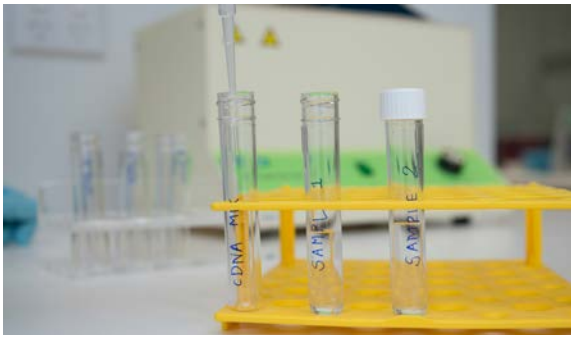


- Put on gloves and safety glasses.
- Use marker pen to label wells on the microarray slide from 1 to 6.
- Write your group name at one end of the microarray slide.

- Use the pipette to place one drop of DNA probe 1 into well number 1 on your microarray slide. Make sure you don't mix pipettes.
- Repeat for DNA probes 2 to 6.

Step 2: Prepare cDNA sample mix

Mix the 2 cDNA samples, sample 1 and sample 2, to form a cDNA sample mix.



- Label mini test tube: **cDNA sample mix** and also your **group name**.
- Use pipette provided in cDNA sample 1 and add 8 drops to your cDNA sample mix tube.
- Use pipette provided in cDNA sample 2 and add 8 drops to same cDNA sample mix tube.

- Secure tube's lid and invert a few times to mix thoroughly. If there's no lid swirl gently.
- Place cDNA sample mix on your bench.

Step 3: Add cDNA mix to microarray slide

- Use a new pipette to place two drops of cDNA sample mix into each DNA probe on your microarray slide, using the same pipette each time.
- *Make sure* the pipette tip doesn't touch the DNA probes.

Step 4: Visualise microarray results

cDNA may hybridise (bind) with a complementary base pair sequence in each DNA probe on the microarray slide. If hybridisation occurs colour changes will result.

- Wait a few minutes for all colour changes to occur.

Step 5: Record microarray results.

- Record any observed colour changes to DNA probes in the table below.

Table 3: Colour changes observed

WELL NUMBER	GENE NAME	GENE ACTION	COLOUR CHANGE
1	XRCC1	DNA repair	
2	BUB1	mitosis	
3	FGFR2	apoptosis (cell death)	
4	HBG1	haemoglobin production	
5	Hsp90	protein folding	
6	VEGFA	blood vessel growth (angiogenesis)	

Step 6: Clean up!

- Keep your gloves on and tip liquid from the microarray slide into waste beaker provided.

Analysing microarray results

Q1. Which genes were expressed (transcribed) in melanoma cells? How do you know?

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Q2. Which genes were expressed (transcribed) in normal skin cells? How do you know?

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Q3. Were any genes not expressed in either cell type? Explain why.

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Q4. Were any genes expressed in both cell types? Explain what type of gene this might be.

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Q5. Which genes may play a role in melanoma? Explain your answer.

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