




Gene expression 3: Measuring gene expression

Components

	NAME	DESCRIPTION	AUDIENCE
	<i>Measuring gene expression</i> teacher guide	This guide provides information about a laboratory activity that introduces students to microarray, including technical notes for preparation of materials required.	teachers
	<i>Introducing microarray</i> presentation	This presentation (with presenter notes for teachers) provides background information on microarray, and an overview of the classroom activity.	students
	<i>Microarray: measuring gene expression</i> workbook	This student workbook contains background information on microarray and a laboratory procedure that simulates measuring gene expression with microarray.	students

Purpose

Students develop an understanding of a biotechnological technique, microarray, used to measure gene expression in cells by measuring amounts of mRNA.

Outcomes

Students understand that:

- microarray is a laboratory-based technique that allows scientists to measure gene expression by measuring the presence (and relative amount) of mRNA in a cell;
- microarray technique is based on complementary base pairing;
- microarray has application in areas of science such as cancer research; for example classification of cancer types based on patterns of gene expression.

Activity summary

The workbook, *Microarray: measuring gene expression*, provides students with background information (pp 2 – 6), a procedural guide to the laboratory activity (pp 7 – 9) and worksheet questions (p10).

ACTIVITY	POSSIBLE STRATEGY
Show presentation, <i>Introducing microarray</i> , and discuss with the class. We recommend the final two slides of the presentation be used to review lab results. Presenter notes (in the presentation and copied below) provide extra information.	whole class
Students read background information about the microarray lab activity (pp 2 – 6 of the workbook).	individually or as a class
Complete simulated microarray lab activity (pp 7 – 9) and worksheet questions (p 10).	small groups

Technical requirements

The teacher guide and workbook require Adobe Reader (version 5 or later), which is a free download from www.adobe.com. The presentation requires Microsoft PowerPoint version 8 or later.

Teacher notes

Microarray laboratory activity

The microarray laboratory activity is set within a melanoma context used throughout the resource package, *Gene expression*.

The aim of the activity is to simulate microarray technology to determine if gene expression differs between normal skin cells and melanoma cells. Colour changes, resulting from the use of two pH indicators, are used to simulate visualisation of gene expression.

Appendix 1 of this guide contains technical notes on preparation for this activity.

During the activity, students:

- prepare a microarray slide (depression tile), labelling wells and group number;
- prepare microarray slides with simulated DNA probes by adding samples from stock solutions into wells on microarray slide;
- collect simulated cDNA from prepared stock samples: normal skin cells and melanoma cells;
- mix together simulated cDNA from normal skin cells with cDNA from cancer cells;
- load mixed simulated cDNA sample into DNA probe wells on microarray slide;
- observe and record colour changes; and
- complete an accompanying worksheet.

The following teacher notes are also included in the PowerPoint presentation, *Introducing microarray*.

SLIDE	NOTES
2	<p>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, muscle and nerve cells.</p> <p>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'.</p>
3	<p>When a gene is transcribed messenger RNA (mRNA) is produced, and the gene is expressed. This happens in response to extracellular or intracellular signals. Signals from inside or outside a cell trigger gene expression.</p> <p>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.</p>
4	<p>The amount of mRNA in a cell provides a measure of gene expression. All these techniques allow researchers to identify which genes are expressed in a cell at a particular time, and in what amounts.</p> <p>Northern blot</p> <p>This technique detects specific mRNA present in a sample. Researchers isolate mRNA from cells and separate different sized fragments of mRNA using gel electrophoresis. These fragments are transferred to a blotting membrane; radioactively labelled hybridisation probes are added, which bind with the mRNA.</p> <p>Reverse transcription polymerase chain reaction (RT-PCR)</p> <p>This technique allows for amplification of mRNA samples and quantification. RT-PCR uses an enzyme (reverse transcriptase) to convert mRNA into complementary DNA (cDNA). cDNA is then amplified using PCR and the amounts quantified, allowing expression of one gene at a time.</p>
5	<p>Microarray technology is used to investigate gene expression in cells by measuring the amount of mRNA present in many samples at once. Microarray provides a 'snapshot' of which genes are expressed in a cell at a particular moment in time, and in what amounts.</p> <p>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.</p> <p>DNA strands embedded on microarray slides are from known genes and are manufactured artificially. Gene chips can contain tens of thousands of genes, and are used to study expression of thousands of genes simultaneously.</p>
6	<p>Cancer is a genetic disease which results in abnormal gene expression. Cancer can lead to gene expression being overexpressed, underexpressed, and in some cases, not expressed at all.</p> <p>Scientists look for differences in gene expression patterns between cancerous cells and normal cells, for instance, between normal skin cells and melanoma cells.</p>

SLIDE	NOTES
7	<p>In this simulation you will investigate differences in gene expression between normal skin cells and melanoma cells.</p> <p>In microarray experiments there are five main steps.</p> <p>Step 1</p> <p>A microarray slide is embedded with manufactured single-stranded DNA segments from known genes.</p>
8	<p>Step 2</p> <p>Normal skin cells and melanoma cells are collected from each patient and mRNA isolated.</p>
9	<p>Step 3</p> <p>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.</p>
10	<p>Step 4</p> <p>Microarray works on the principle of complementary base pairing, where guanine binds with cytosine, and thymine with adenine.</p> <p>After cDNA has been added the microarray slide is washed to remove any cDNA that didn't bind with the embedded DNA.</p>
11	<p>Step 5</p> <p>The colour pattern of the scanned image is analysed to determine which genes are expressed in each cell type.</p> <p>Green dot: cDNA from normal skin cells has hybridised with embedded DNA. This gene is expressed in normal cells but not melanoma cells.</p> <p>Red dot: cDNA from melanoma cells has hybridised with embedded DNA. This gene is expressed in melanoma cells but not normal cells.</p> <p>Yellow dot: cDNA from both normal skin cells and melanoma cells have hybridised with embedded DNA. Yellow results when both green and red cDNA bind to the same site.</p> <p>Black dot: cDNA from neither sample hybridised with embedded DNA. This means the gene is not expressed in either normal skin cells or melanoma cells.</p> <p>The intensity of fluorescence provides further information about gene expression; bright red spots indicate higher levels of expression in melanoma cells but not normal skin cells, and bright green spots indicate higher levels of expression in normal skin cells but not melanoma cells.</p>
12	<p>Powerful computational programmes are required to interpret data generated by microarray experiments. Colour spots appearing on a microarray are analysed using statistical methods.</p> <p>Each coloured spot on a microarray slide provides information about gene expression, and helps scientists determine which genes are suitable for investigating in terms of disease, such as melanoma.</p> <p>Red and green coloured spots are genes that might be investigated, while yellow and black spots are probably not involved in cells becoming cancerous.</p> <p>The range of colours observed in microarray results reflects the relative amount of mRNA present, or the level of gene expression.</p>
13	<p>You'll follow the same steps observed in laboratory protocols.</p> <p>At the end of the experiment you'll observe colour spot changes on your microarray slide, and interpret these results.</p> <p>As this is a simulation, using indicators, observed colour changes will differ from those usually seen in microarray. Rather than red, green and yellow colour spots, you will observe pink, blue, and purple colour spots.</p> <p>Differences in the intensity of these colour changes reflect the relative amount of mRNA in each cell, or the level of gene expression.</p>

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Associated SPICE resources

Gene expression 3: Measuring gene expression may be used in conjunction with related SPICE resources to address the broader topic of gene expression and regulation.

DESCRIPTION	LEARNING PURPOSE
<p><i>Gene expression (overview)</i></p> <p>This learning pathway shows how a number of SPICE resources can be combined to teach the topic: gene expression and regulation.</p> <p>All resources use a human disease context, melanoma, which helps students relate to advances in biotechnology and our understanding of molecular genetics.</p>	
<p><i>Gene expression 1: Melanoma risk factors</i></p> <p>Students use an interactive learning object to investigate risk factors associated with melanoma developing.</p>	Engage
<p><i>Gene expression 2: Polymerase chain reaction</i></p> <p>Students simulate polymerase chain reaction in the classroom.</p>	Explore
<p><i>BioDiscovery activity (optional)</i></p> <p>Students attend the LotteryWest Biodiscovery Centre at the Harry Perkins Institute of Medical Research to participate in a SPICE-developed PCR laboratory activity. See <i>Gene expression (overview)</i> for details.</p>	Explore
<p><i>Gene expression 3: Measuring gene expression</i></p> <p>Students measure gene expression via a microarray simulation conducted in the school laboratory.</p>	Explore
<p><i>Gene expression 4: Regulating gene expression</i></p> <p>An animation explains how gene expression is regulated by complex molecular interactions. These processes are important in increasing organism adaptability, flexibility and complexity.</p>	Explain
<p><i>Gene expression 5: Personalised medicine</i></p> <p>Students explore an interactive story to discover how increased understanding of molecular biology and advances in biotechnology have led to development of personalised medical treatments for melanoma patients.</p>	Elaborate

Appendix 1: Technical notes – microarray preparation

This simulation of gene expression requires preparation of six DNA probe solutions. Each DNA probe solution represents a gene. DNA probe solutions are made from pH indicators for this simulation. Whilst relatively stable, DNA probe solutions are best prepared on the day of use and stored at room temperature.

Simulated samples from normal skin cells and melanoma cells requires preparation of two cDNA samples. These simulated cDNA samples are made from NaOH.

Materials

- phenolphthalein pH indicator
- thymolphthalein pH indicator
- 0.1M NaOH (pH >10, must prepare fresh)

Thymolphthalein may not be an indicator usually stocked at your school. If your preferred supplier doesn't stock it, it's available from Perth Scientific Pty Ltd.

Preparation of DNA probe solutions

To create the six DNA probe solutions prepare two indicator stock solutions of 100 mL each.

- phenolphthalein pH indicator: Dissolve 0.05 g in 50 mL ethanol, then add 50 mL H₂O.
- thymolphthalein pH indicator: Dissolve 0.04 g in 50 mL ethanol, then add 50 mL H₂O.

Clearly label and wrap foil around pH indicators to protect from light.

Table 1 shows volumes of each indicator stock solution required to create six DNA probe solutions. These volumes are sufficient for an entire class.

Table 1: DNA probe solutions

TUBE	GENE	GENE ACTION	COLOUR	THYMOLPHTHALEIN SOLUTION	PHENOLPHTHALEIN SOLUTION	WATER	TOTAL VOLUME
1	XRCC1	DNA repair	blue	500 µL (10 drops)	0	1000 µL (20 drops)	1500 µL 1.5 mL
2	BUB1	mitosis	pink	0	500 µL (10 drops)	1000 µL (20 drops)	1500 µL 1.5 mL
3	FGFR2	apoptosis (cell death)	light blue	100 µL (2 drops)	0	1000 µL (20 drops)	1100 µL 1.1 mL
4	HBG1	haemoglobin production	colourless	0	0	1000 µL (20 drops)	1000 µL 1.0 mL
5	Hsp90	protein folding	purple	250 µL (5 drops)	250 µL (5 drops)	1000 µL (20 drops)	1500 µL 1.5 mL
6	VEGFA	blood vessel growth (angiogenesis)	pink	0	100 µL (2 drops)	1000 µL (20 drops)	1100 µL 1.1 mL

Each student group requires one drop (approximately 50 µL) of each DNA probe solution to place on their slide. (1.5 mL is enough for 30 samples.) Make an adequate volume, allowing for waste.

Preparation of cDNA samples

The two simulated cDNA samples are both composed of 0.1M NaOH.

Prepare NaOH solution and separate into two test tubes labelled **sample 1** (normal skin cells) and **sample 2** (melanoma cells).

Each group needs approximately: 2 drops/probe for 6 probes: 12 drops/group (approximately 600 µL).
10 mL of NaOH should serve the whole class with spare.