Practical activity (6)

Simulated DNA Microarray Analysis

(Student version)

I. Background

The formation of cancer often involves uncontrolled cell growth and proliferation. Upon the complete sequencing of the human genome, scientists had a better understanding of the relationship between cancer and genes. In humans, cell growth is tightly regulated by different genes. Generally speaking, the expression of tumor suppressor genes prevents cancer formation, whereas the over-expression of oncogenes stimulates or accelerates the progression of cancer. Hence, the gene expression patterns in those cancerous cells are very different from those in the normal cells.

DNA microarray is a novel and powerful technology that allows us to perform large-scale screenings of gene variations, which can be used as a diagnostic tool in cancer study. In fact, the DNA microarray technology works through depositing many (tens of thousands) different short DNA sequences onto a small surface of a specialised glass slide, which is often regarded as a "chip". One single microarray chip (also known as gene chip or DNA chip, Fig. 1) contains tens of thousands of tiny fluorescent spots arranged in rows and columns. Different known sequences of DNA are immobilised at the designated spots, and hence the identity of each DNA fragment is specified through its location on the array. These short DNA sequences are usually called DNA probes or gene probes.

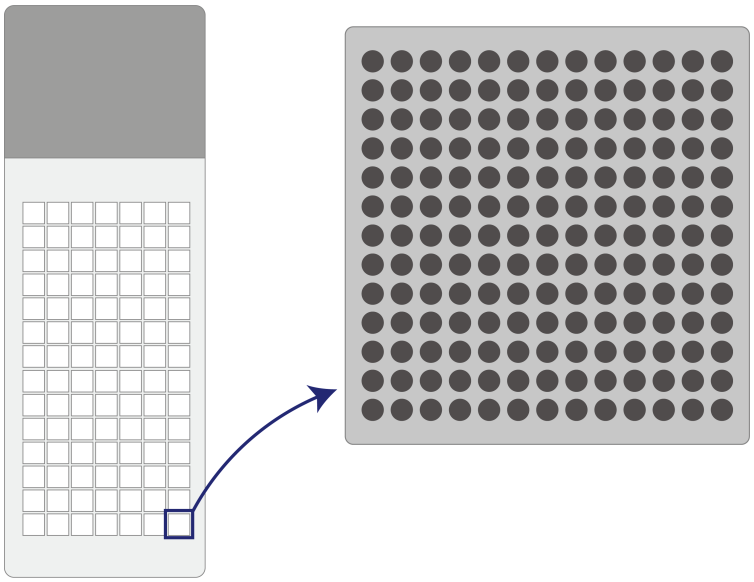


Fig. 1 Diagram of DNA microarray chip

Messenger RNA (mRNA) is an intermediate molecule that conveys genetic information from the cell nucleus to the cytoplasm for protein synthesis. Whenever some genes are being expressed, many copies of mRNA corresponding to the particular genes are produced by a process called transcription. These mRNAs are then used for the synthesis of corresponding proteins by a process termed translation. Hence, we may know what genes are being expressed in cells indirectly by assessing the expression of a particular sequence of mRNAs. In other words, mRNA acts as a surrogate marker.

To investigate the different patterns of gene expression in cancerous tissue and normal tissue, we first need to isolate mRNAs from the two kinds of tissue. Since mRNA gets degraded easily, it is necessary to convert it into a more stable form, i.e. complementary DNA (cDNA), before further experimental procedures. cDNAs can be easily obtained upon the reverse transcription of mRNAs. To distinguish the cDNA fragments from mRNAs, we often tagged them with fluorescent labels (Fig. 2).

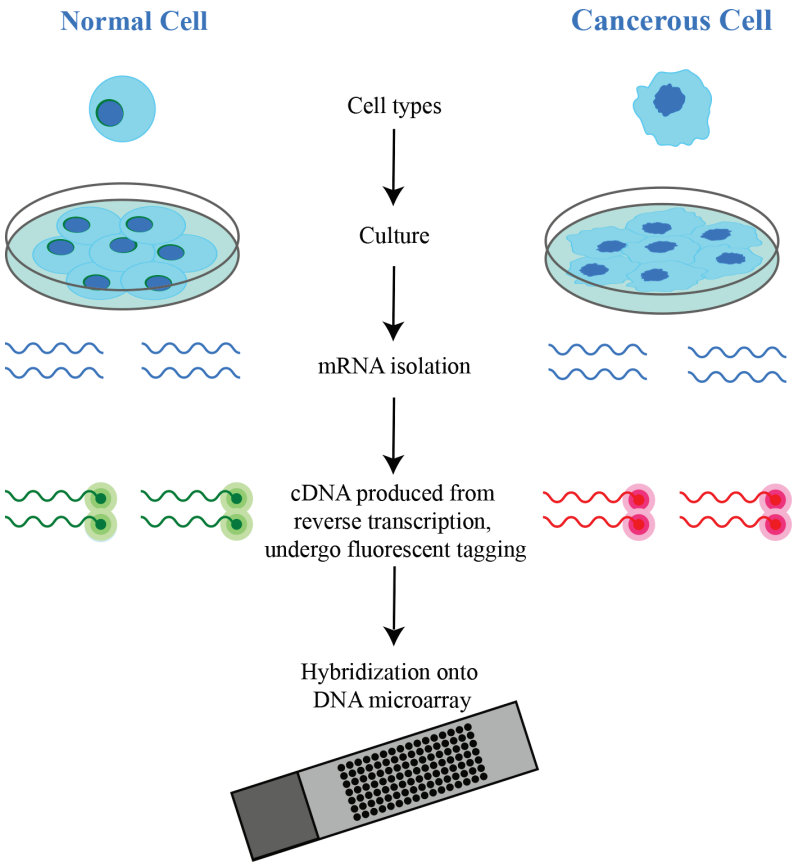


Fig. 2 Principle of DNA microarray

In DNA microarray experiments for comparing the gene expression pattern of normal and cancerous tissues, the cDNAs derived from cancerous tissues can be tagged with a red fluorescent dye. Those derived from normal tissues can be tagged with a green fluorescent dye. The two groups of tagged cDNA samples are then mixed and loaded onto a DNA chip. Then sufficient time will be allowed for nucleic acid hybridisation (formation of a double-stranded molecule by the combination of complementary nucleotide chains) between the cDNAs and the DNA probes printed on the chip. If these cDNA samples are complementary

to the DNA probes, they stick firmly to the probes at specific spots. The unbound cDNAs (no hybridization) are washed away during the rinsing processes. Ultimately, the microarray chip is subjected to scanning with a laser beam for the detection of any fluorescent signals from the successful hybridizations. A microarray computer program can be used to record the pattern of fluorescence emission and identify the DNA sequences of specific spots.

According to the labelling design as mentioned above, a red fluorescent spot on the DNA chip represents a gene that is expressed in cancerous tissues or at a level higher than in the normal tissues (Fig. 3). A green spot represents a gene that is expressed in normal tissues or at a level higher than in cancerous tissues. A yellow spot indicates that the gene is expressed equally in both types of tissues. If the gene is not expressed in either of the tissues, no fluorescent spot can be detected. Since each spot on a DNA microarray contains a known DNA sequence corresponding to a known gene, the identity of any hybridised signal can be determined. Based on the data from DNA microarray, investigators can easily distinguish which genes are related to the development of cancer. These cancer-related genes are the targets of cancer treatment-related research.

In this task, you will carry out a simulated microarray experiment. By comparing the relative level of expression of some genes in normal and cancerous cells using the **simulated microarray technique**, the genes related to a particular kind of cancer can be identified.

[Note: in this simulated microarray experiment, the coloured signals derived from the normal and cancerous cells are different from those seen in the authentic DNA microarray.]

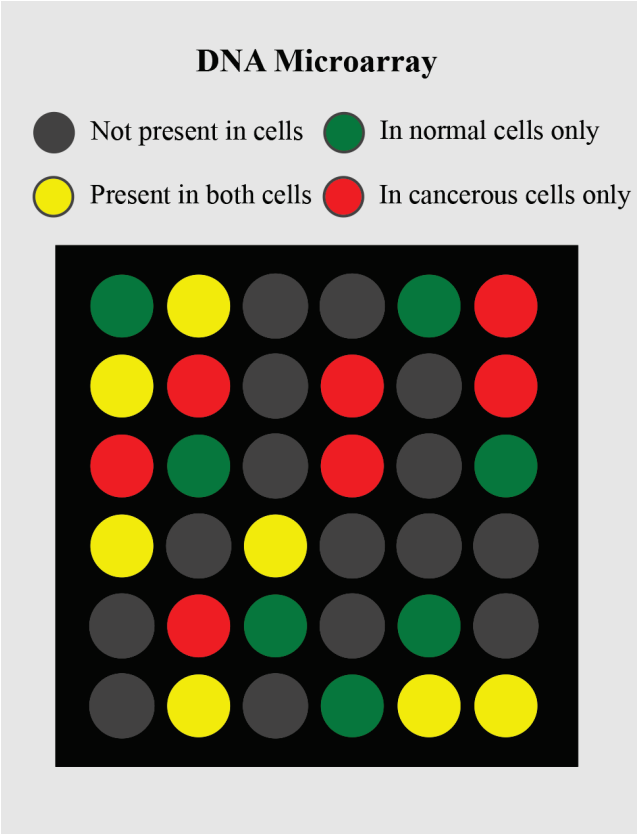


Fig. 3

II. Guiding questions about the design of the experiment

1. When a normal cell develops into a cancerous cell, the expression level of

   (a) the tumor suppressor gene (cancer-preventing gene) will _____.

   (b) the oncogene (cancer-promoting gene) will _____.

2. How can the differences in gene expression patterns in normal and cancerous cells be reflected in a DNA microarray experiment?

III. Objectives of the experiment

1. To carry out a simulated DNA microarray experiment; and
2. To differentiate the gene expression patterns in normal and in cancerous cells.

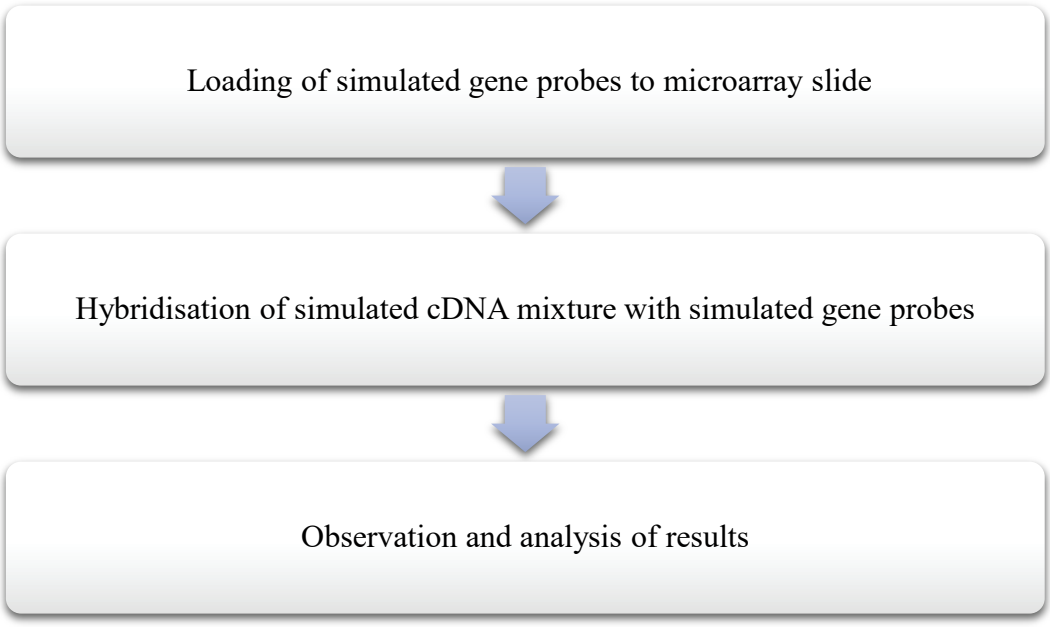
IV. Expected Learning Outcomes

Upon the completion of the activities, students should be able to:

1. recognise the basic principle of DNA microarray;
2. briefly describe the differences in gene expression in normal and in cancerous cells; and
3. explain the results observed from the simulated microarray experiment.

V. The experiment

A. Overview



B. Experiment: Performing stimulated DNA microarray experiment

a) Equipment and materials (per group)

Equipment

- Hot water bath (70°C) × 1 (per class)
- Float rack × 1
- Micropipettes (P20) and sterile tips

Materials

- Simulated gene probe 1 (25 µl, tube 1) × 1
- Simulated gene probe 2 (25 µl, tube 2) × 1
- Simulated gene probe 3 (25 µl, tube 3) × 1
- Simulated gene probe 4 (25 µl, tube 4) × 1
- Simulated gene probe 5 (25 µl, tube 5) × 1
- Simulated gene probe 6 (25 µl, tube 6) × 1
- Simulated cDNA mixture (150 µl) × 1
- Special glass slide × 1

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

b) Safety precautions

- Wear a laboratory gown.
- Tie up long hair.
- Wear goggles and gloves during the experiment.
- Handle the hot samples with care to avoid the burning of the skin.
- Avoid the spilling of simulated cDNA mixture on eyes or bare skin. If spilling occurs, rinse the affected body part with plenty of water. Seek medical consultation when necessary.
- Pipette the solutions gently.
- Wash hands to remove all possible contamination before and after the experiment.

c) Procedure

1. Obtain 1 special glass slide from the teacher or laboratory technician. Mark on the labelling area with your group number* (Fig. 4).

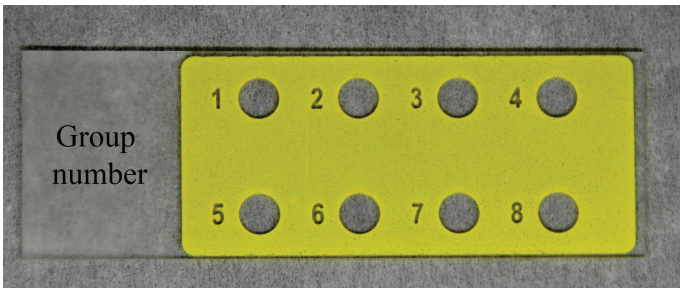


Fig. 4

> *Do not touch the surface of the glass slide. Only hold the edges when necessary.

2. Obtain tube 1 from the hot water bath#. Using P20 micropipette and sterile tips^, transfer 20 µl simulated gene probe 1 to Spot 1 of the glass slide (Fig. 5).

> **#Caution: The temperature of the water bath is about 70°C. Handle the samples with care, to avoid burning the skin.**

> ^Note: Use a new pipette tip when adding different gene probes.

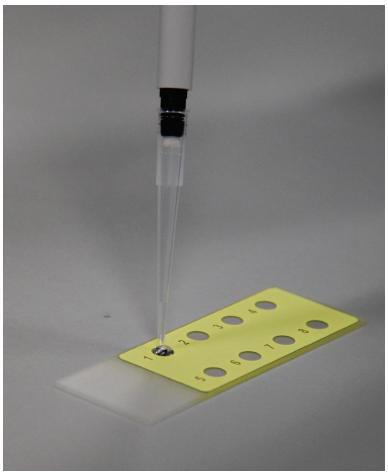


Fig. 5

3. Repeat Step 2 for transferring simulated gene probes~ 2−6 to their corresponding spots on the slide.

> ~If the simulated gene probes start clogging before transferring, warm the tubes further in the water bath for a while.

4. As soon as all simulated gene probes on the glass slide become clogged, proceed to step 5.

5. Add 20 µl simulated cDNA mixture to each of the 6 wells containing the clogged simulated gene probes*.

***Caution: Avoid spilling the mixture on eyes or bare skin.**

6. Record the colour change of each spot.

7. Discard all used tips in the designated disposal container with 10% chlorine bleach (no autoclave processing required) or in a biohazard bag for autoclave processing afterwards.

8. After observation and analysis, return the special glass slide to the teacher / laboratory technician.

VI. Results

1. Paste the photo or draw a picture of the microarray glass slide in the box below.

2. Record the results of the experiment in the following table.

Spot	Gene probe	Original colour	Colour after simulated cDNA mixture added
1			
2			
3			
4			
5			
6			

VII. Discussion

1. Describe the development of fluorescent signals from normal and cancerous cells in the authentic DNA microarray experiments.

2. In the current simulated DNA microarray experiment, the coloured signals are different from those seen in the authentic DNA microarray experiments. Here, the genes expressed in cancerous cells appeared pink, and the genes expressed in normal cells appeared blue. Interpret your results using the following table.

Gene probe	Colour after simulated cDNA mixture added	The implicated expression level of the corresponding gene probe in normal cells and cancerous cells
1		
2		
3		
4		
5		
6		