Cell Culture, MTT assay, and DNA Microarray Technology

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What is cell culture?

Cell culture refers to the removal of cells from an animal, and their subsequent growth in a favorable artificial environment.

The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

Acquiring Cell Lines:

- You may establish your own culture from primary cells, or you may choose to buy established cell cultures from commercial or non-profit suppliers (i.e., cell banks);
- Reputable suppliers provide high quality cell lines that are carefully tested for their integrity and to ensure that the culture is free from contaminants;
-It can be advise against borrowing cultures from other laboratories because they carry a high risk of contamination.
- Regardless of their source, make sure that all new cell lines are tested for mycoplasma contamination before you begin to use them.

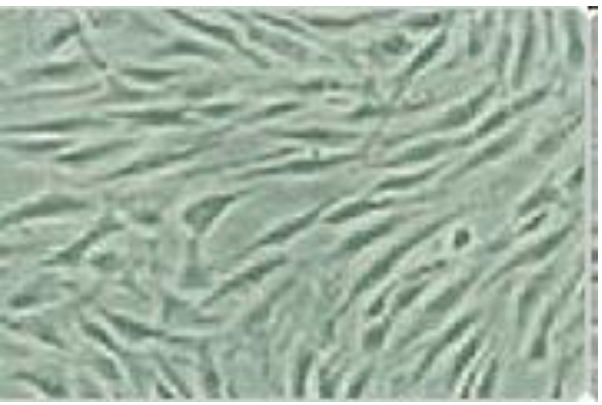
Mammalian Cell Morphology

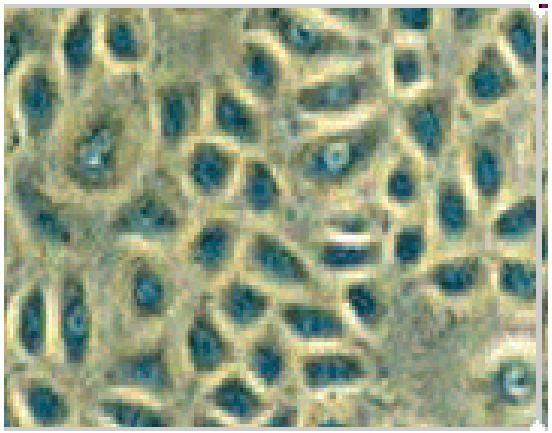
Most mammalian cells in culture can be divided in to three basic categories based on their morphology:

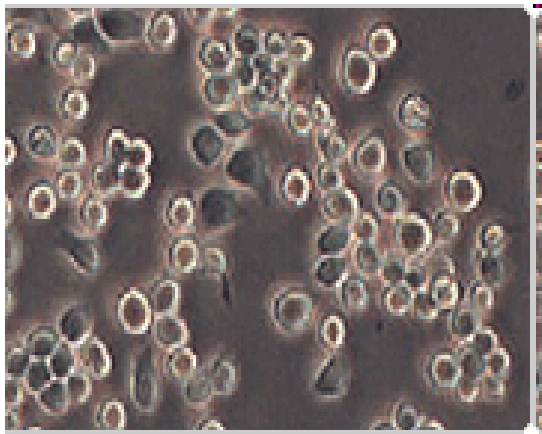
1) Fibroblastic (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate;

2) Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches;

3) Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface.







Selecting the Appropriate Cell Line

A- Generally,Consider the following criteria for selecting the appropriate cell line:

1- Species: Non-human and non-primate cell lines usually have fewer biosafety restrictions, but ultimately your experiments will dictate whether to use species-specific cultures or not;

2- Functional characteristics: What is the purpose of your experiments? For example, liver- and kidney-derived cell lines may be more suitable for toxicity testing;

3- Finite or continuous: While choosing from finite cell lines may give you more options to express the correct functions, continuous cell lines are often easier to clone and maintain;

4- Normal or transformed: Transformed cell lines usually have an increased growth rate and higher plating efficiency, are continuous, and require less serum in media, but they have undergone a permanent change in their phenotype through a genetic transformation;

Selecting the Appropriate Cell Line

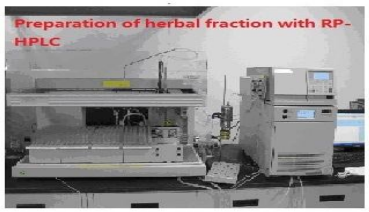
B- Consider the following criteria for selecting the appropriate cell line for your experiments:

1- Growth conditions & characteristics: What are your requirements with respect to growth rate, saturation density, cloning efficiency, and the ability to grow in suspension? For example, to express a recombinant protein in high yields, you might want to choose a cell line with a fast growth rate and an ability to grow in suspension.

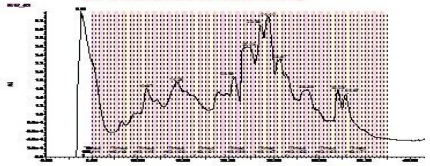
2- Other criteria: If you are using a finite cell line, are there sufficient stocks available? Is the cell line well-characterized, or do you have to perform the validation yourself? If you are using an abnormal cell line, do you have an equivalent normal cell line that you can use as a control? Is the cell line stable? If not, how easy it is to clone it and generate sufficient frozen stocks for your experiments?

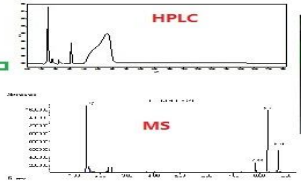
500 herbs

Pretreatment
Centrifugation
Filtration



80 fractions for each herb



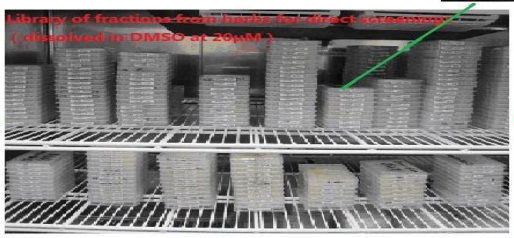


Quality control

<20 compounds per fraction

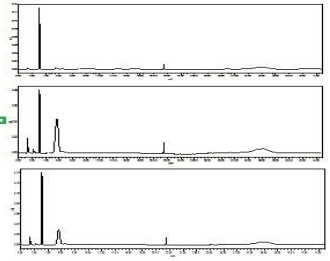
Transferation

Library of dried fractions from herbs stored at -20°C



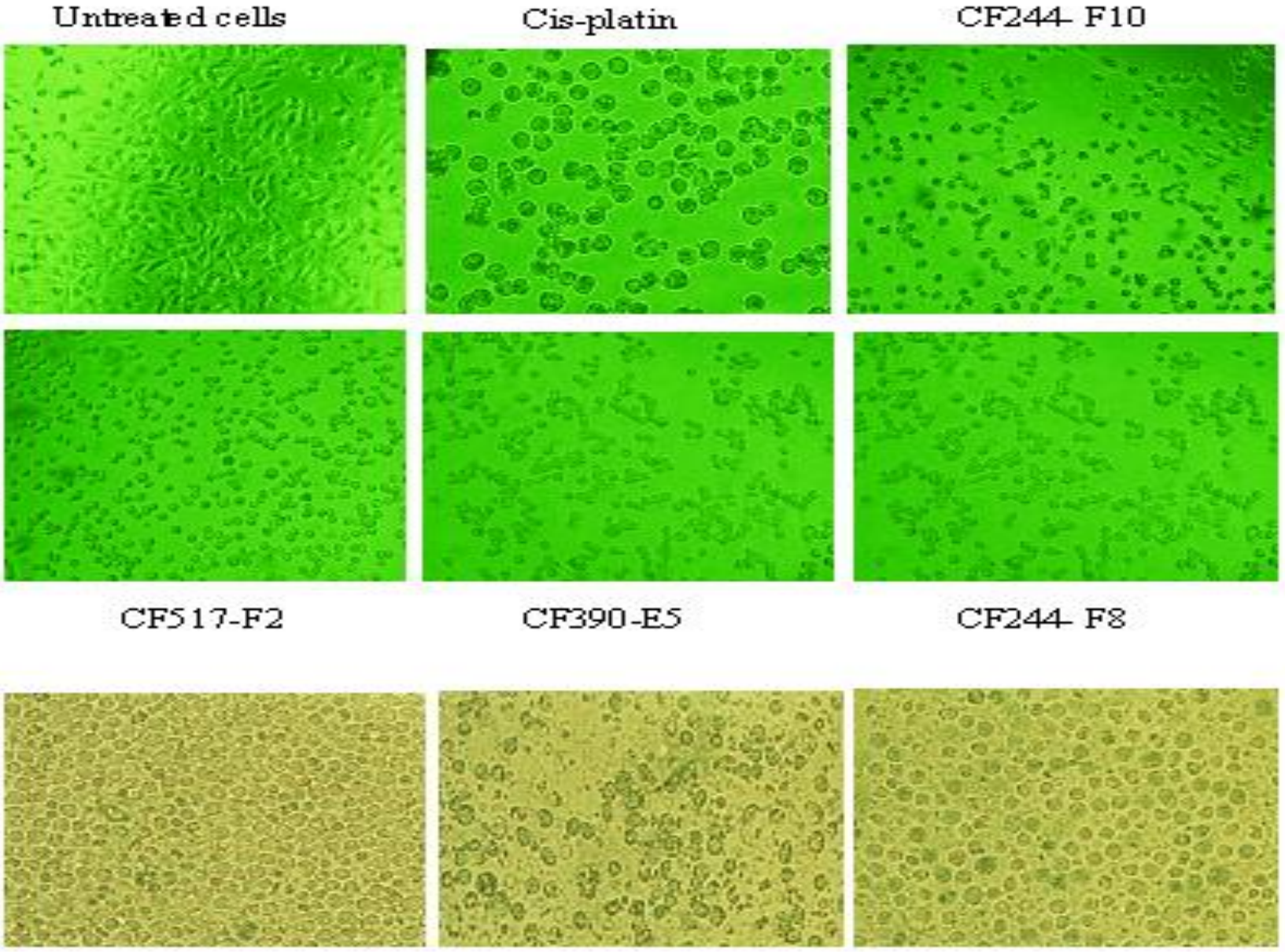
Activity test on melanoma cells

Identification of target by analytical HPLC



Enrichment of the target

Further purification of the target


Untreated cells
Cis-platin
CF244- F10
CF517-F2
CF390-E5
CF244- F8
Splenocytes- control
Splenocytes treated with cis-platin
Splenocytes-treated with CF244- F10

MTT assay

Principle of assay:

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(MTT)

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
(Formazan)

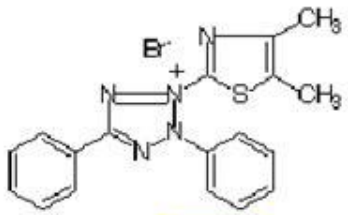
Procedure:

cells/well were seeded in a 96-well plate and treated with 0, 5, 10, 12.5, 20, 25, 35, 40, and 100 µM for 24 h. Following treatment, MTT reagent (500 µg/ml) was added and cells were incubated at 37˚C for a further 4 h. DMSO (150 µl) was subsequently added to dissolve the formazan crystals and the absorbance was read at 570 nm using a microplate reader (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Data were expressed as the percentage viability, assuming that the absorbance of untreated control cells was 100%. The percentage cell viability was calculated using the following formula:

Cell viability (%) = [(A570, sample - A570, blank) / (A570, control-A570, blank)] x100.

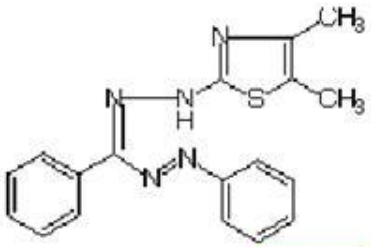
Non-cytotoxic dose determined via MTT ASSAY

MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide



MTT **yellow**

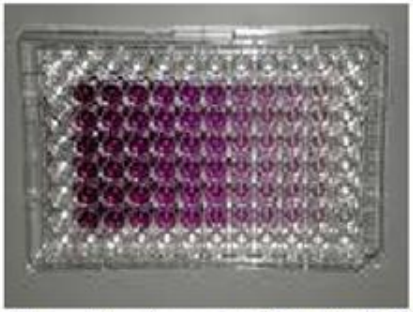
Live cells → Mitochondrial reductase present → converts



MTT formazan **violet**

www.phs.osaka-u.ac.jp

Absorbance read at 690 nm and subtract background at 570 nm.



http://en.wikipedia.org/wiki/File:MTT_Plate.jpg

MTT test at different concentrations

The low non-cytotoxic dose can be determined.

How we do the dose response IC50 (MTT assay):-

First, we let cells grow for 12 hrs then follow bellow:-

1- Select at least 5-8 concentrations for each drug which has effect on the cancer cells.

2- Use 96-well white drug plate for dose response (before using it, sterilizing it by UV light at least 15mins).

3- For each drug we use 8 wells in 96-well white drug plate to make 8 concentrations.

4- Add 250ul of MF or M to the first well and the other 7 wells add only 125ul of MF or M.

5- If we want to prepare the first concentration 400uM then we take 1ul of the drug (100mM) and dilute it in the first well which has 250ul of MF or M, then we mix it at least 10 times, then we take 125ul and add to the second well and mix it and then we do the same work to other wells, finally we leave the last well 250ul.

6- After prepare the concentrations, we aspirate the old medium and then take 100ul of each concentration of drug (we use 8 concentrations for one drug) and add them to the cells.

7- Observe the effect of drugs after 24hrs.

If the concentration of the drug is 100mM, then we add 1ul of the drug to 250ul of medium, then the concentration of 8 wells should be like follows:

µM		µl
400	→	1
200	→	0.5
100	→	0.25
50	→	0.125
25	→	0.0625
12.5	→	0.0312
6.25	→	0.0156
3.125	→	0.00781

For example we use 1 ul of the drug:

A- 250 ul of MF+ mix 1 ul of the drug (100mM) at least 10 times Take 125 ul and add it to the next well and mix at least 10 times

B- 125 ul (volume of the drug 0.5 ul) Take 125 ul and add it to the next well and mix at least 10 times

C- 125 ul (volume of the drug 0.25 ul) Take 125 ul and add it to the next well and mix at least 10 times

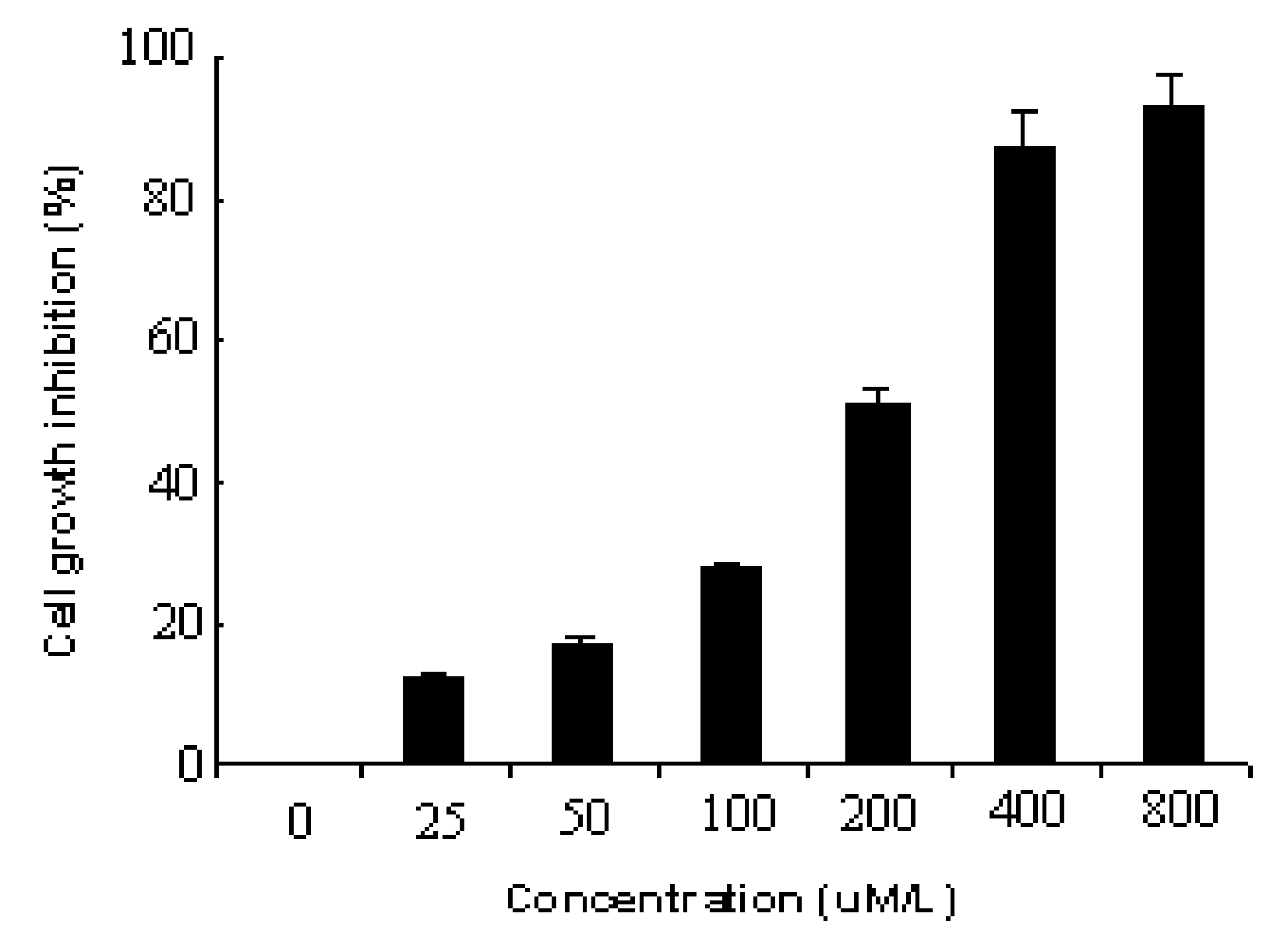
D- 125 ul (volume of the drug 0.125 ul) Take 125 ul and add it to the next well and mix at least 10 times

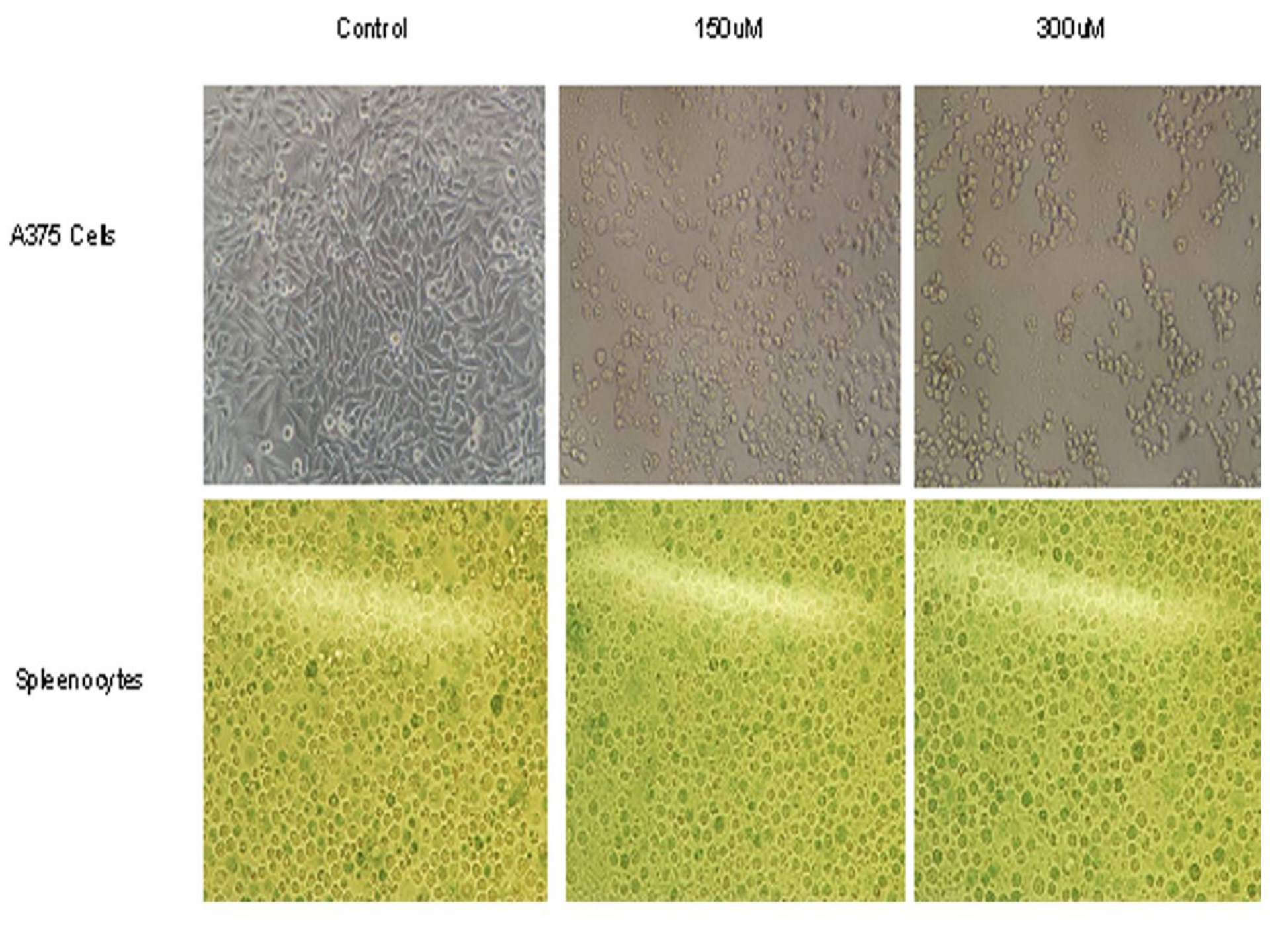
E- 125 ul (volume of the drug 0.0625 ul) Take 125 ul and add it to the next well and mix at least 10 times

F- 125 ul (volume of the drug 0.0312 ul) Take 125 ul and add it to the next well and mix at least 10 times

G- 125 ul (volume of the drug0.0156 ul) Take 125 ul and add it to the next well and mix at least 10 times

H- 125 ul + 125 ul (volume of the drug 0.0078125 ul) mix at least 10 times which take it from the first well to other wells (one by one)


100
80
60
40
20
0
Cell growth inhibition (%)
0
25
50
100
200
400
800
Concentration (uM/L)


Control
150uM
300uM
A375 Cells
Spleenocytes

DNA Microarray Technology

- Microarrays are extremely powerful ways to analyze gene expression.
- Using a microarray, it is possible to examine the expression level of thousands of genes in one experiment
- Microarrays can be used to compare the expression of many genes under different conditions (cancer cells vs. normal cells, skin cells vs. organ cells, rapidly growing cells vs. quiescent cells, etc.).

Types of Microarrays:

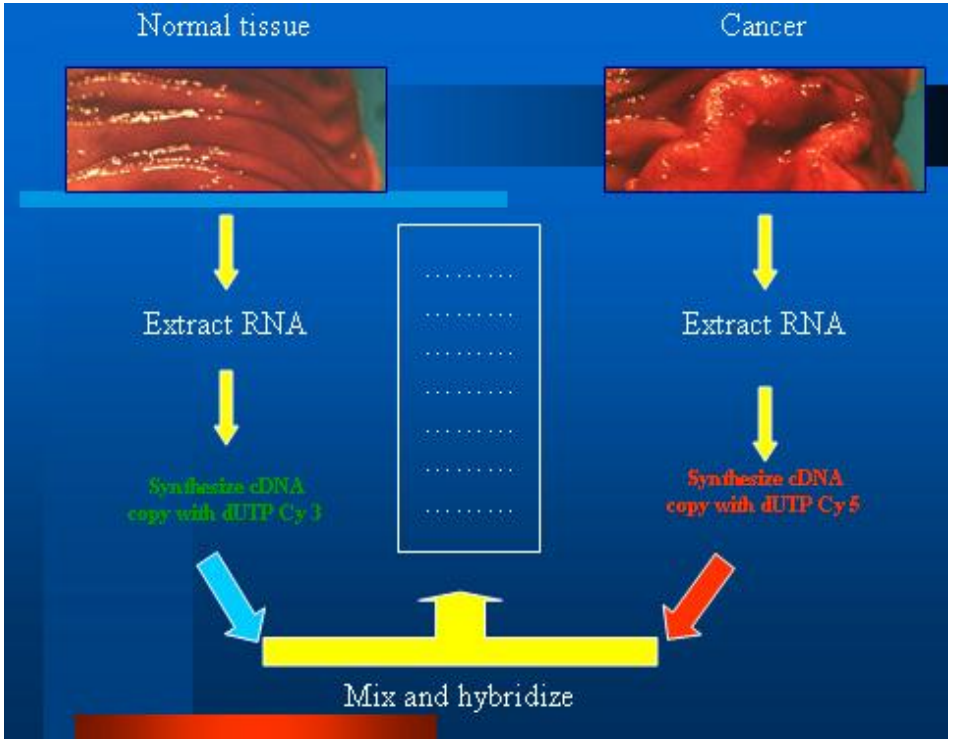
Microarrays can be classified into two general types:

1- cDNA arrays consist of cDNA copies of mRNA spotted onto a glass slide.

2- oligo arrays consist of strands of oligonucleotides either spotted onto a glass slide or lithographed onto a solid surface.

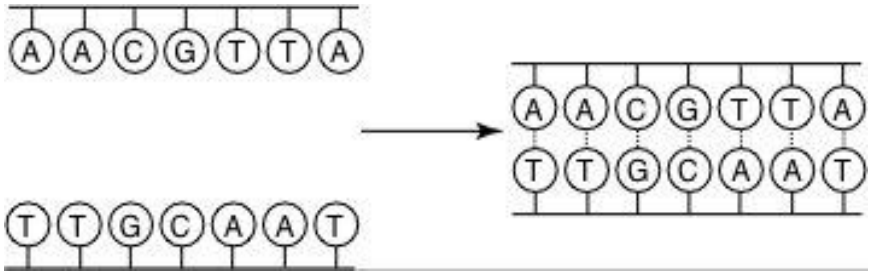
Microarray experiments

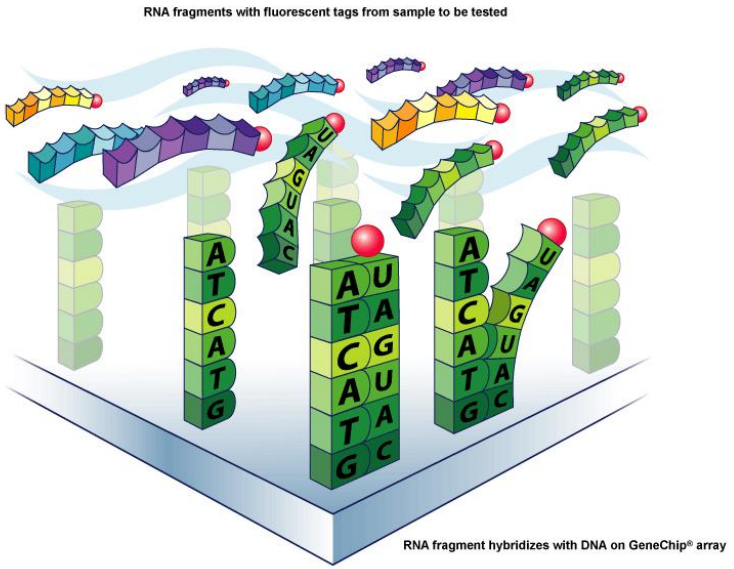
- A typical microarray experiment consists of extracting RNA from the cells or tissue being examined, converting the RNA to cDNA, labelling the cDNA with fluorescent dyes and allowing the labelled cDNA to hybridize with the material (cDNA or oligonucleotide) on the microarray slide.
- In some experiments, the control and subject RNAs are synthesized with different fluorescent dyes and mixed on the same slide. Other protocols use separate slides for the subject and control RNAs.



DNA Microarrays consist of 100 - 1 million DNA probes attached to a surface of 1 cm by 1 cm (chip).

By hybridisation, they can detect DNA or RNA:

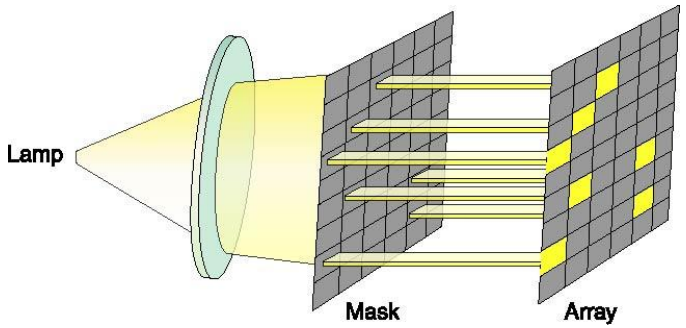




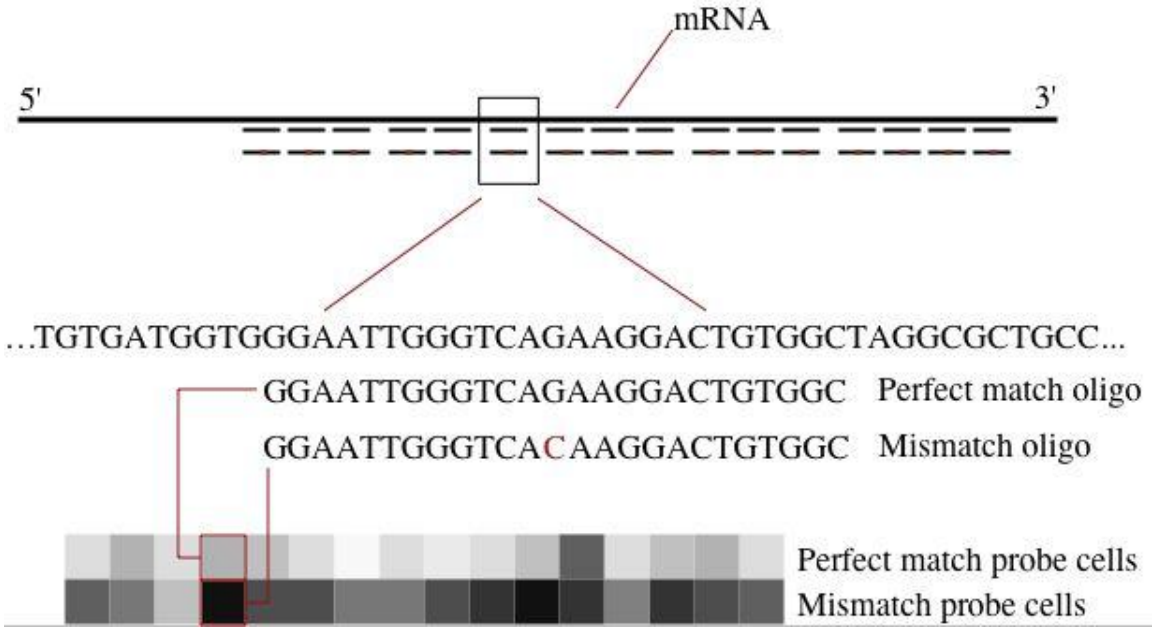
If the hybridised DNA or RNA is labelled fluorescently it can
be quantified by scanning of the chip.

DNA microarrays can be manufactured by:

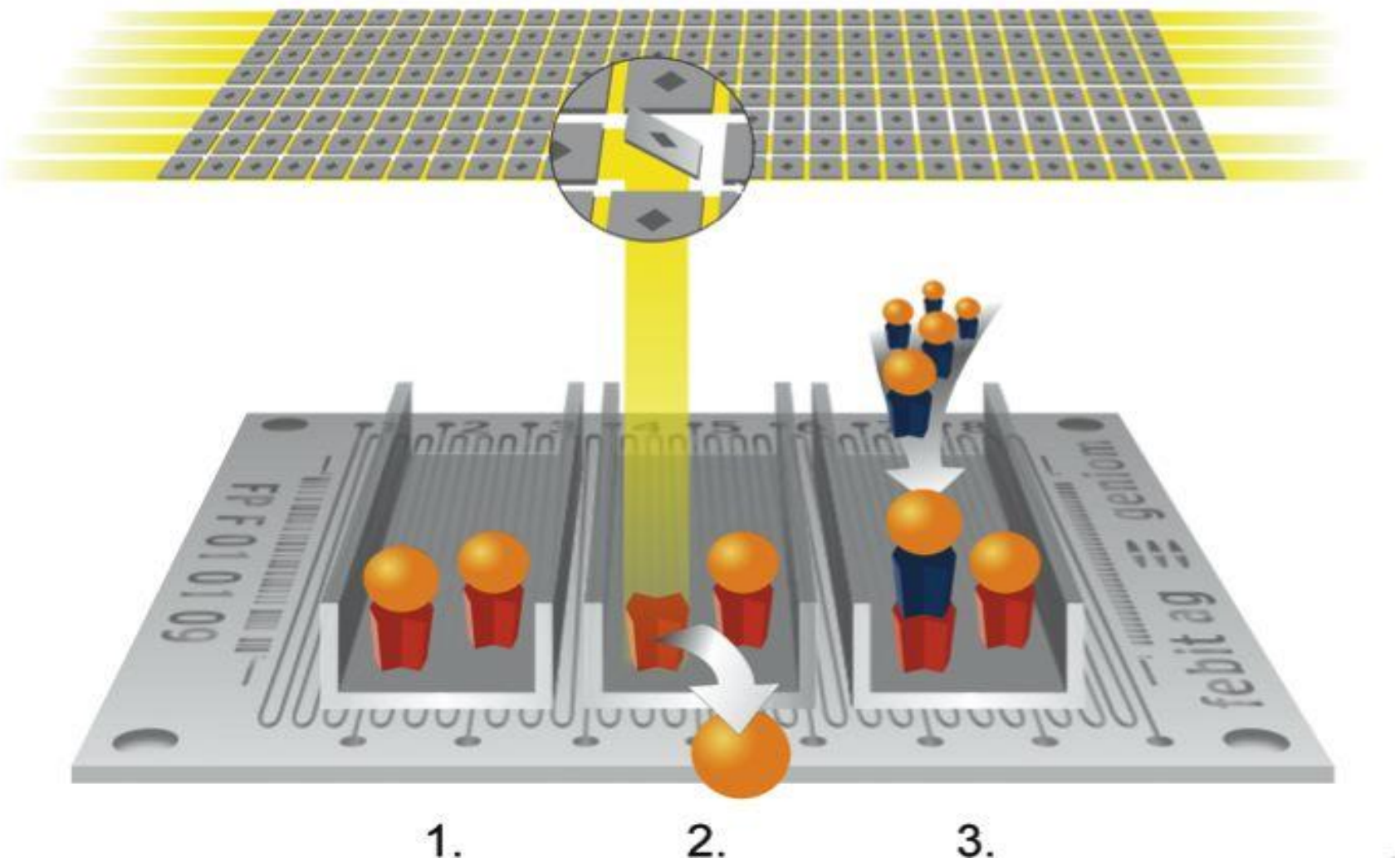
1- Affymetrix photolitography:



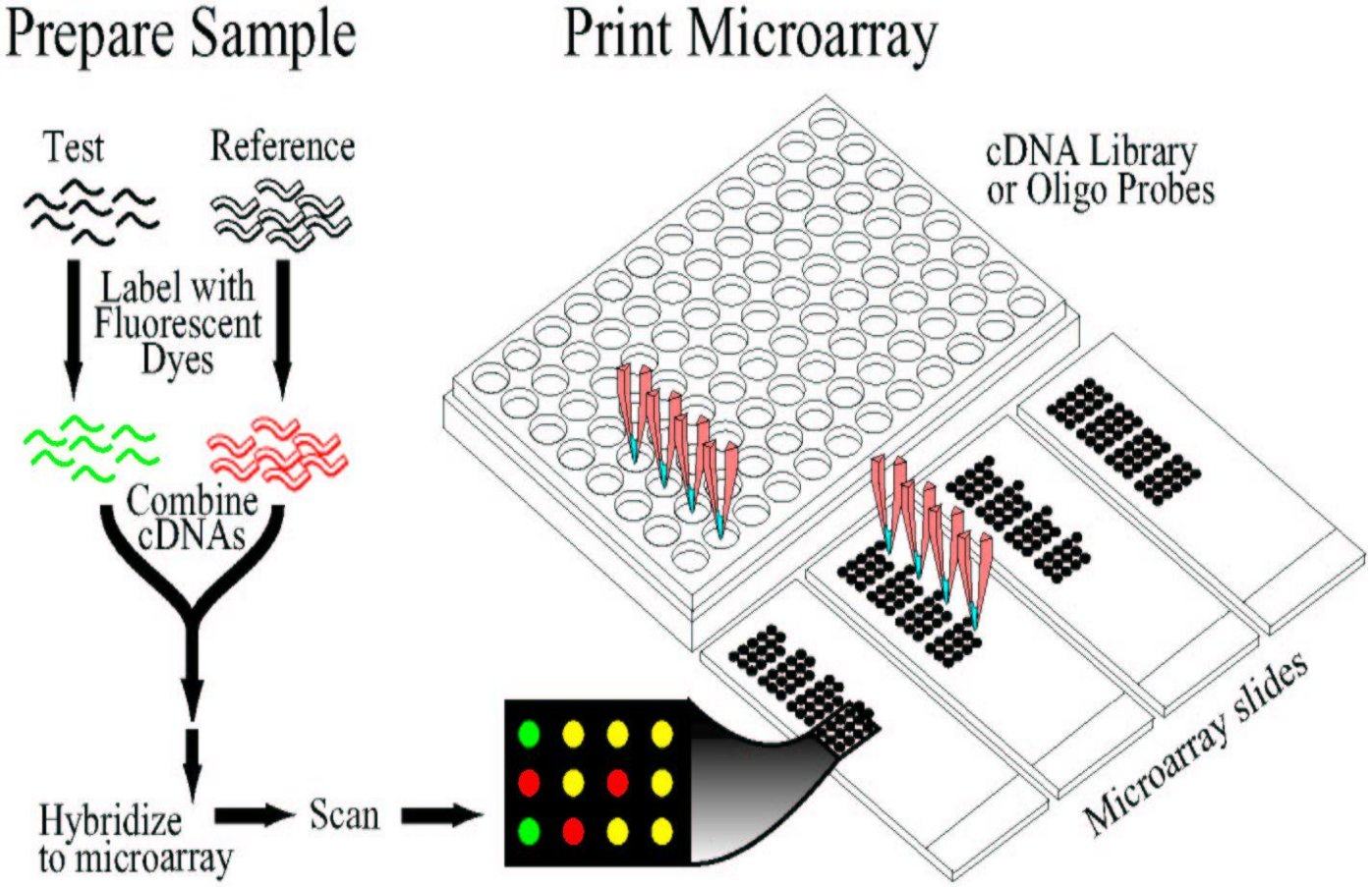
- Each probe 25 bp long
- 22-40 probes per gene
- Perfect Match (PM) as well as MisMatch (MM) probes

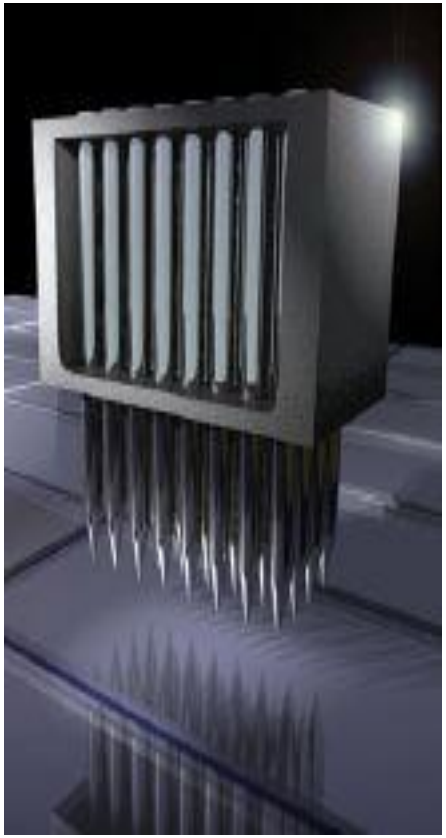


Febit/NimbleGen photolitography

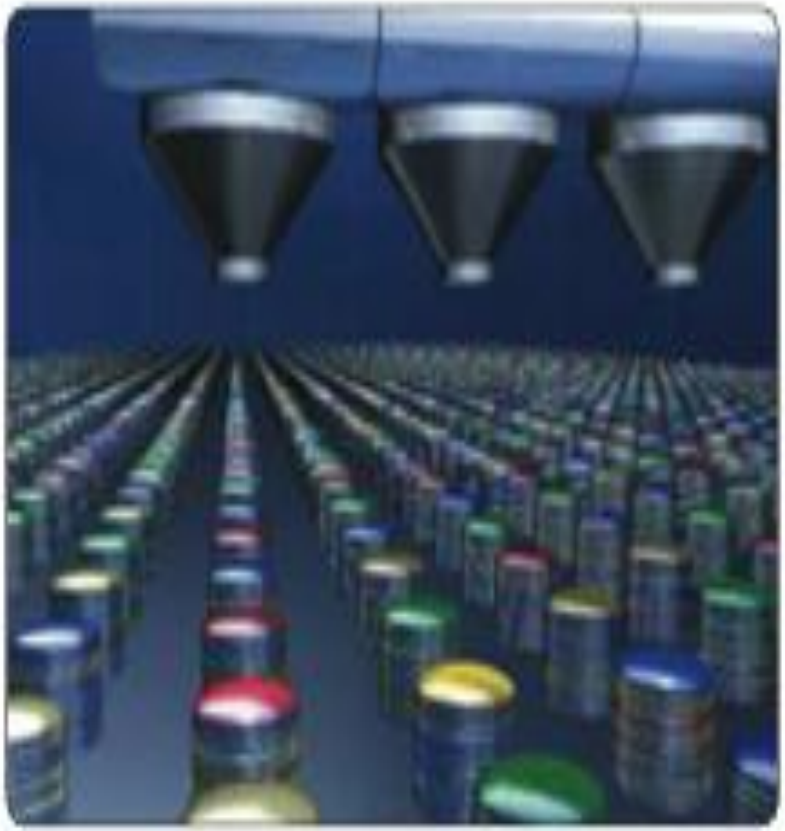


2- Robot Spotting





3- InkJet (HP/Canon) technology



Summary

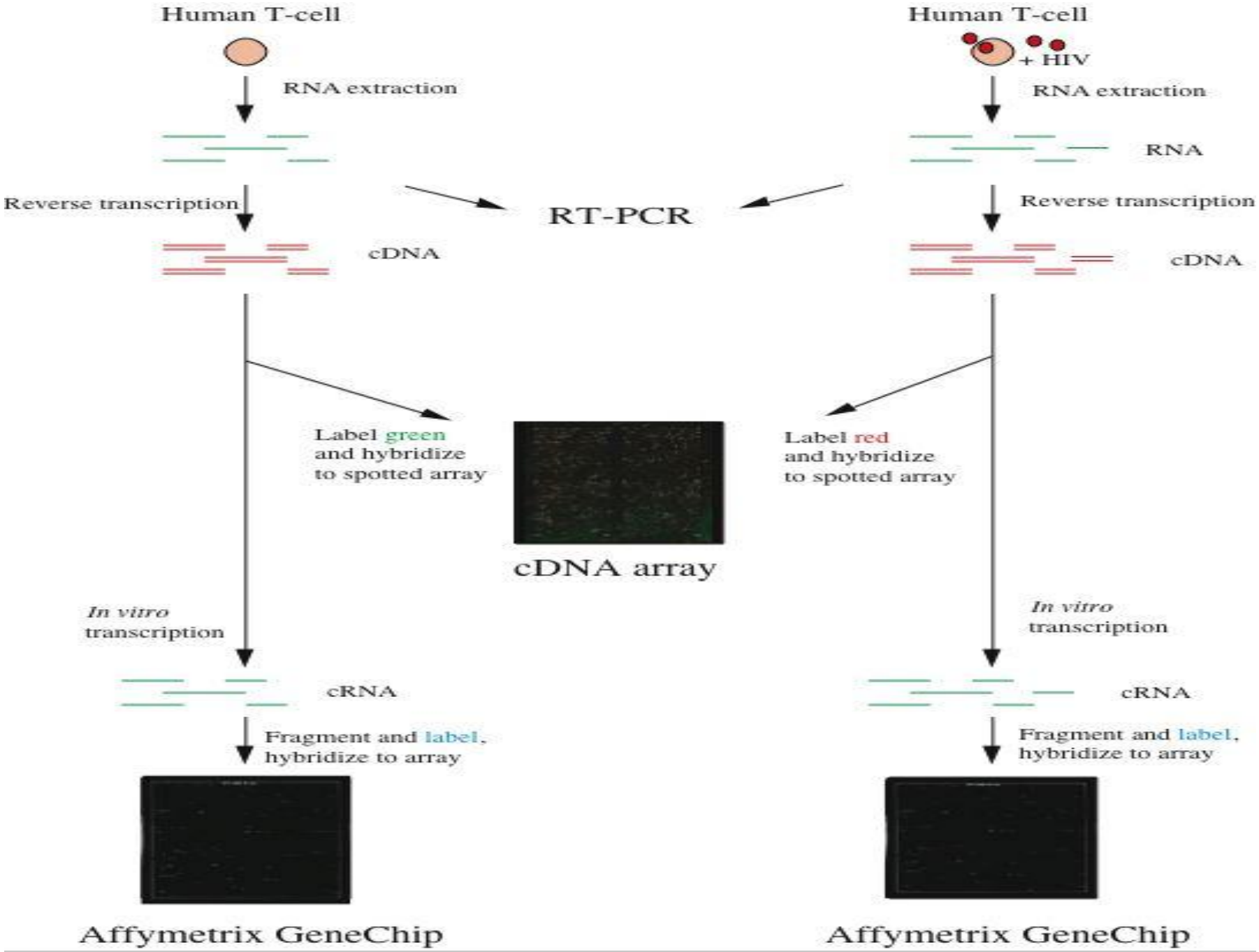
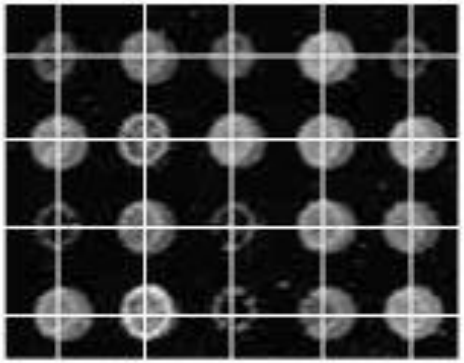

Human T-cell
RNA extraction
Reverse transcription
cDNA
Human T-cell
+ HIV
RNA extraction
RNA
Reverse transcription
cDNA
RT-PCR
Label green
and hybridize
to spotted array
cDNA array
Label red
and hybridize
to spotted array
In vitro
transcription
cRNA
Fragment and label,
hybridize to array
Affymetrix GeneChip
In vitro
transcription
cRNA
Fragment and label,
hybridize to array
Affymetrix GeneChip

Image Analysis



1. Gridding: identify spots (automatic, semiautomatic, manual)
2. Segmentation: separate spots from background. Fixed circle (B), Adaptive circle C, Adaptive shape (D), Histogram
3. Intensity extraction: mean or median of pixels in spot
4. Background correction: local or global