Don't see everyone's flaws
Don't listen to everything you're told
Don't speak if it's not kind

Always look for the good in people.
Not everything is truth.
Only speak words of kindness

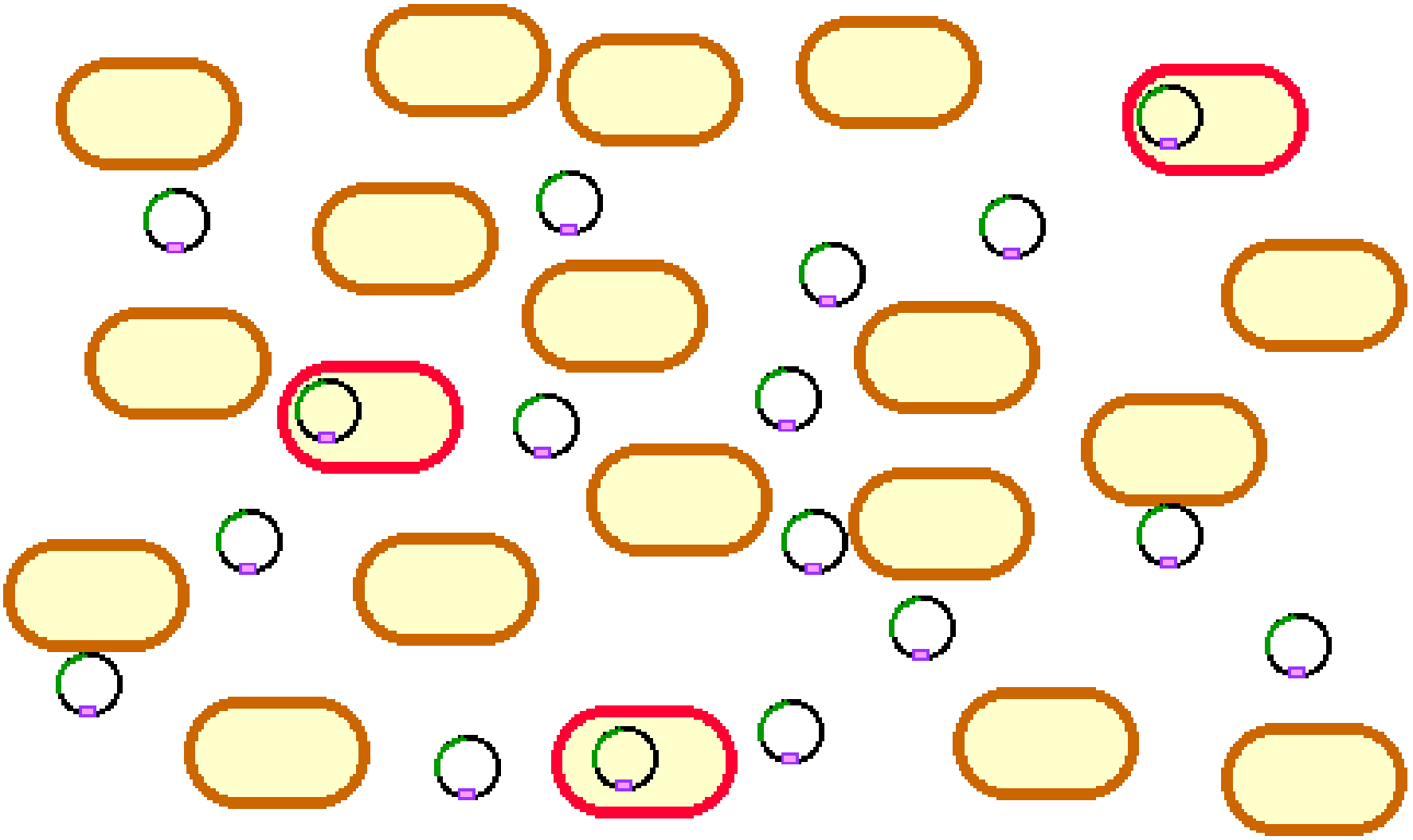
Live Life Positively

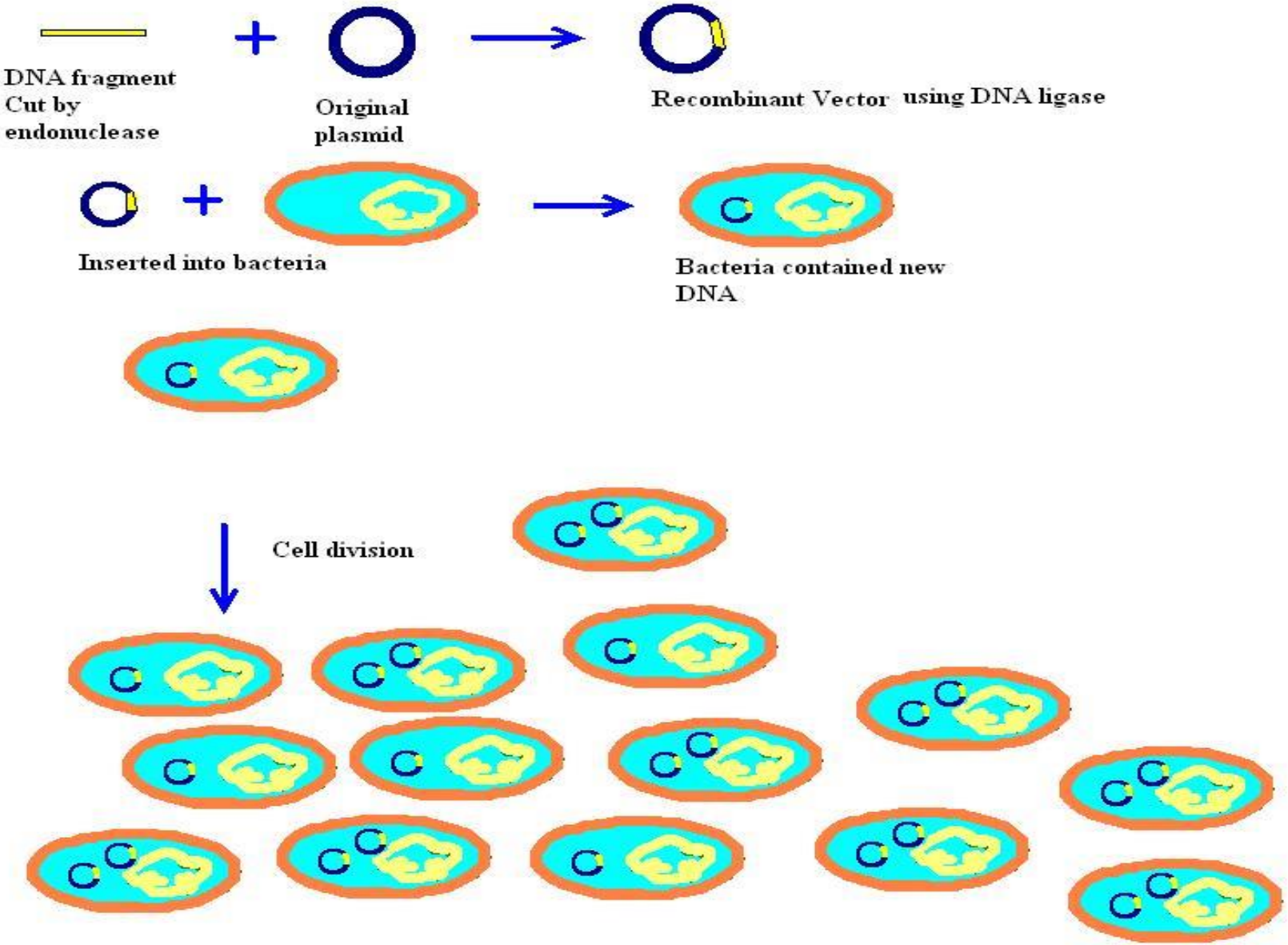
DETECTION OF YOUR CLONING AND PICK UP YOUR RIGHT CLONE

Introduction

- Once recombinant plasmid is constructed, it is introduced into recipient cells.
- Introduction of recombinant DNA into recipient cells is called transformation: ***introduction of foreign DNA changes (transforms) properties of the organism.***
- Special treatment makes ***cells competent*** - capable of accepting foreign DNA.
- Usually, these treatments make cell membrane more permeable for a DNA molecule.
- When competent cells are mixed with DNA some cells (actually, very few) become transformed.

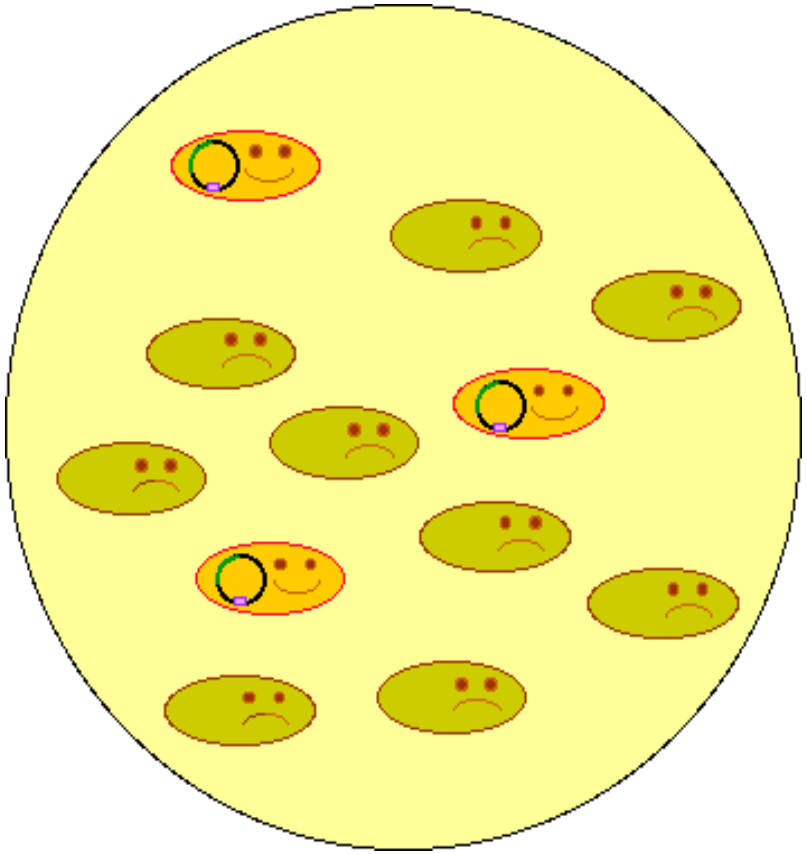
Competent cells transformation



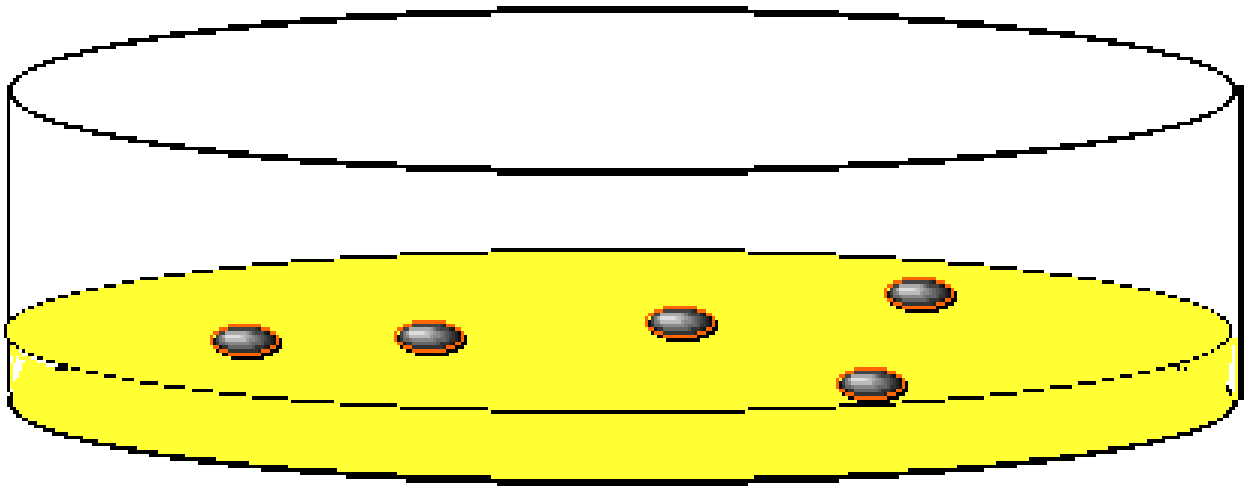

DNA fragment
Cut by
endonuclease
Original
plasmid
Recombinant Vector using DNA ligase
Inserted into bacteria
Bacteria contained new
DNA
Cell division

Competent cells culture:

After transformation, cells are plated onto agar medium that contains selective antibiotic: only transformed cells, will survive and form colonies. All the untransformed cells will die.

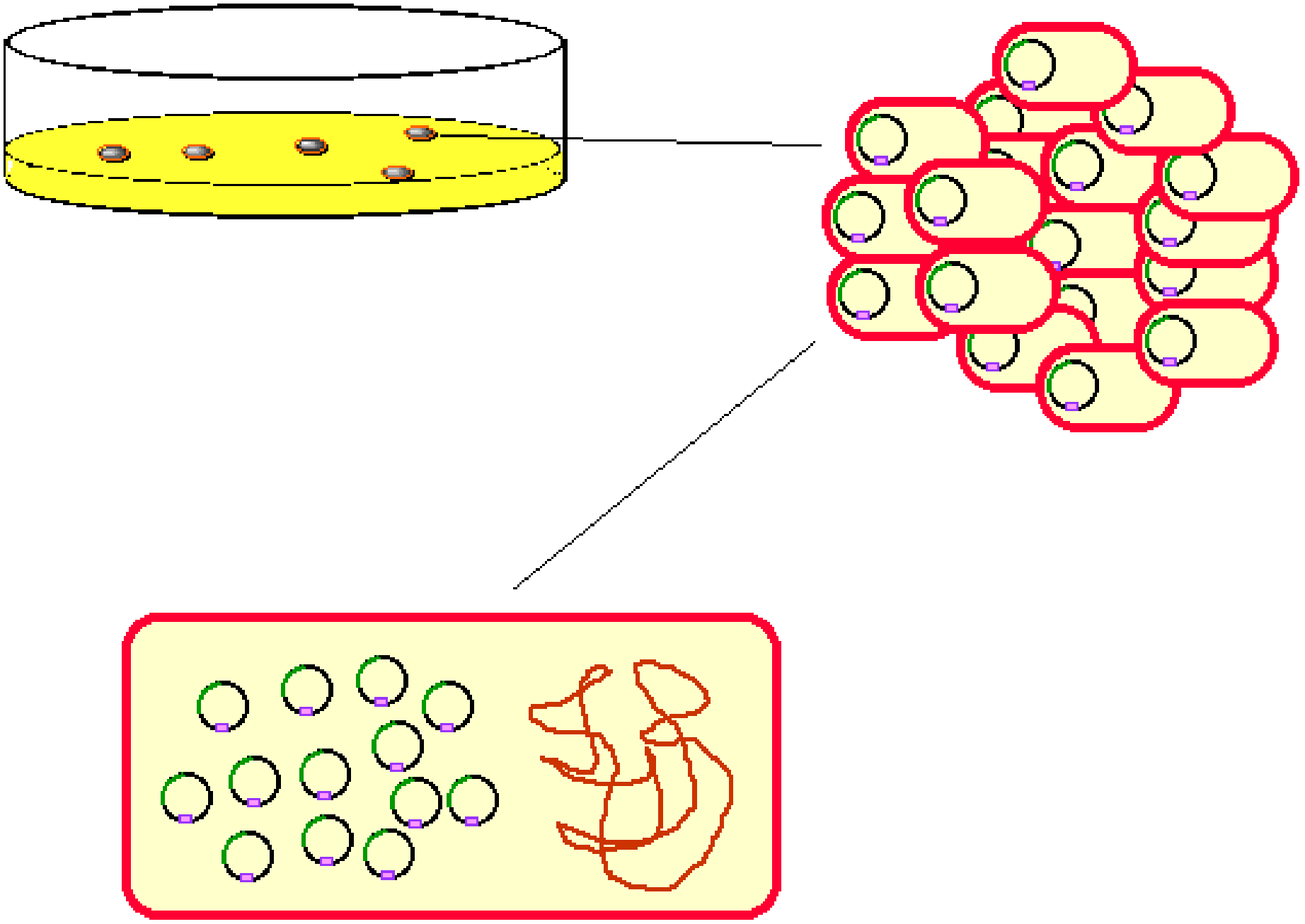


In each colony formed on the agar plate, all cells are descendants of one transformed cell.



agar medium with antibiotic

All cells in the clone are genetically identical and contain the same recombinant vector



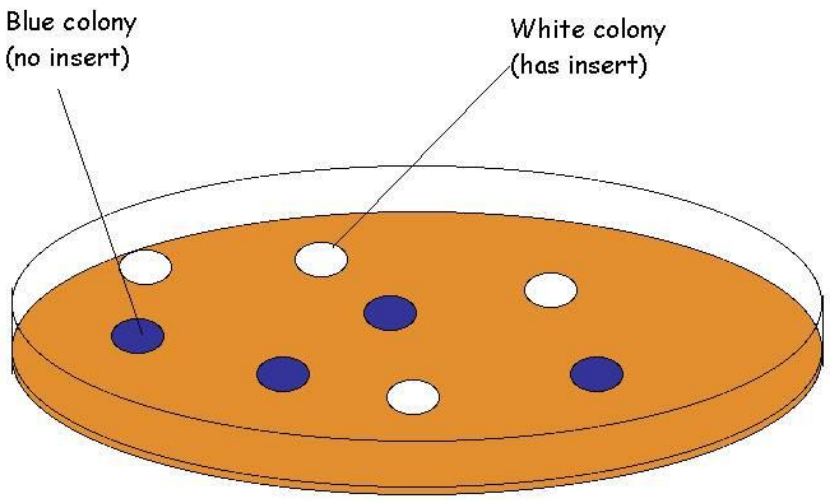
How to Find and Pick Up the Right Clone?

The most common methods include:

1. **Phenotypic screening.**
2. **Screening with antibodies.**
3. **DNA hybridization.**

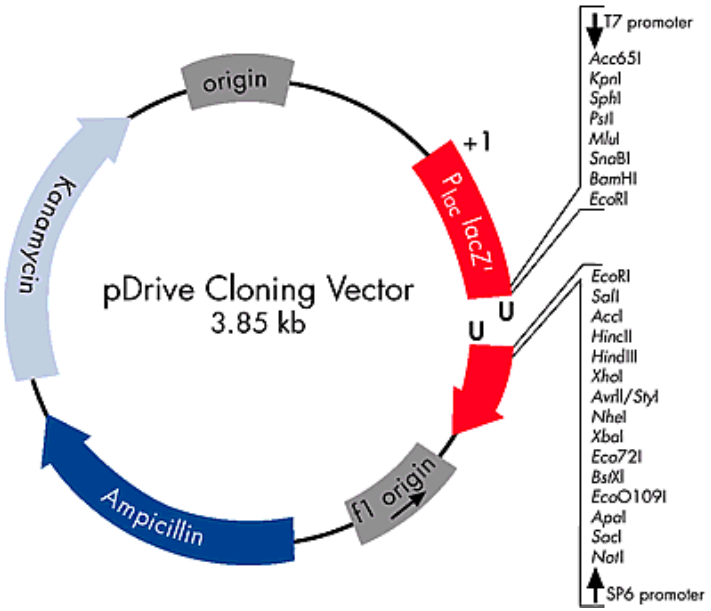
Phenotypic screening

Phenotypic screening is used when cloned gene is expressed and changes properties of the cell in an "obvious way". E.g. **Blue-White Screening**



Blue-White Screening

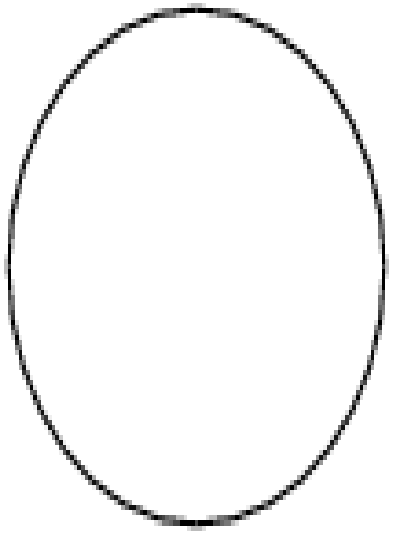
pDrive expresses LacZ α-peptide→ Provide β-galactosidase activity. X-gal is a colourless analog of lactose cleaved by β-galactosidase to form a bright blue insoluble pigment.



- No insert → intact lacZ →Blue
- Insert → disrupted lacZ →White

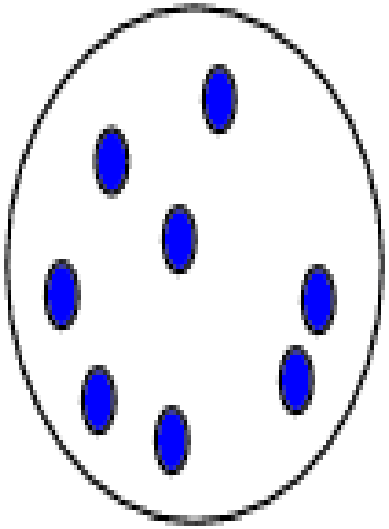
BLUE – WHITE SCEENING

A)



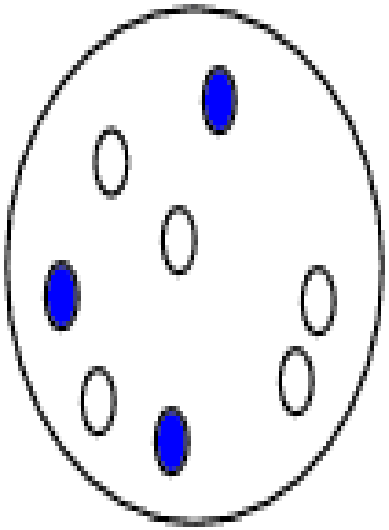
Agar plate
+ ampicillin
+bacteria
+**no plasmid**

B)



Agar plate
+ampicillin
+bacteria
+**plasmid**

C)



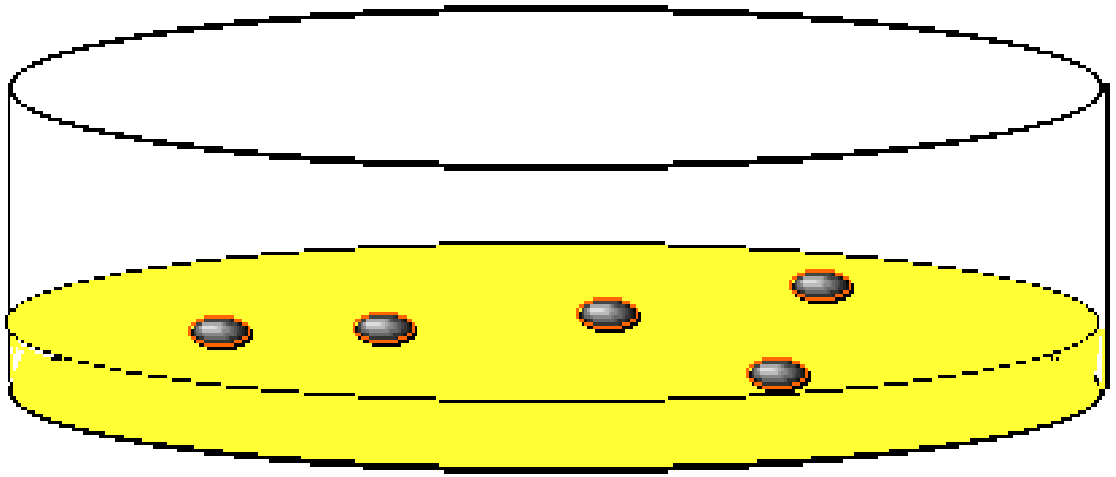
Agar plate +
ampicillin +
bacteria +
recombinant plasmid

BLUE – WHITE SCEENING

Screening with antibodies

Screening with antibodies is used when cloned gene is expressed and antibodies recognizing the encoded protein are available

→**(Antibiotic Resistance)**.

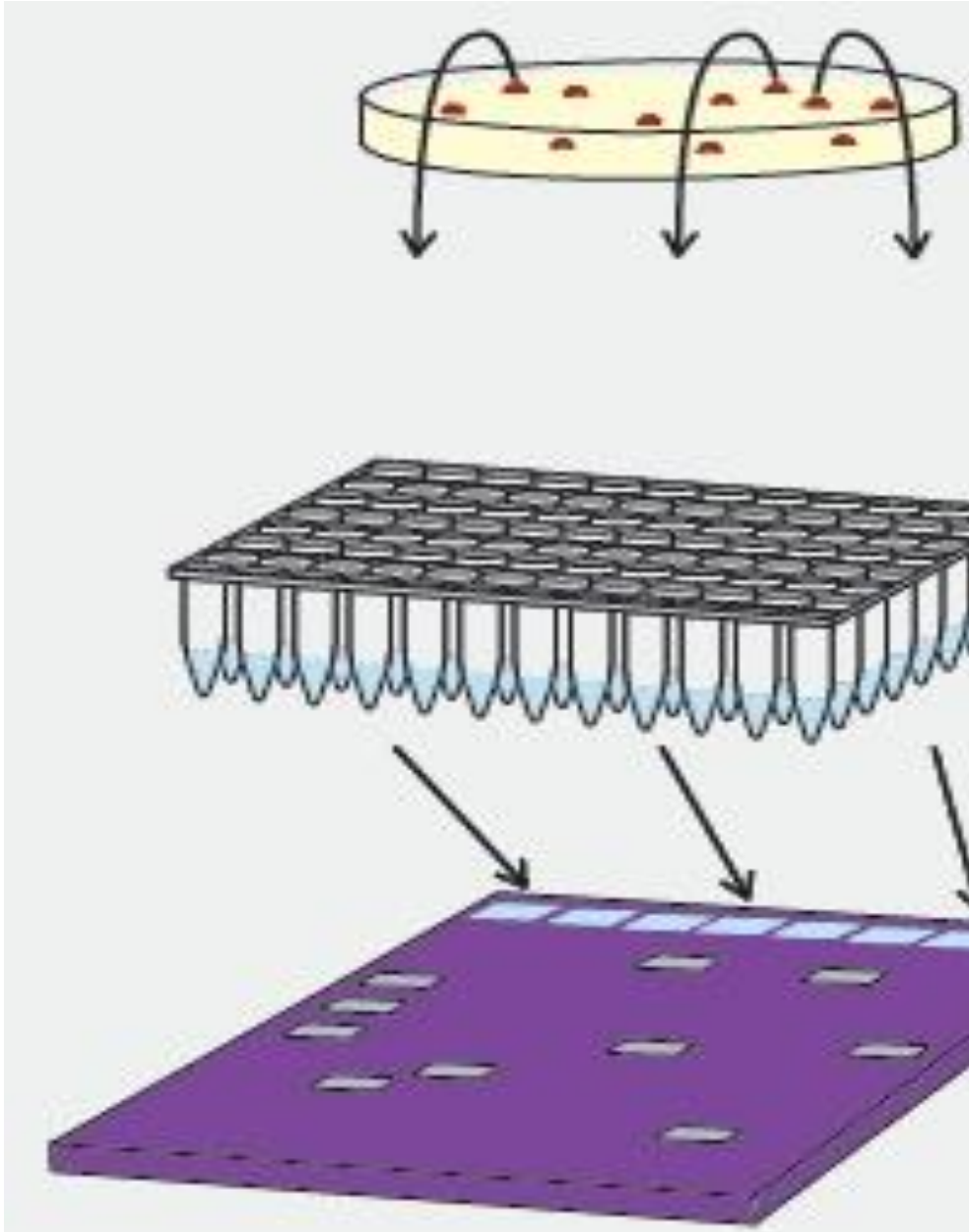


agar medium with antibiotic

Confirmation

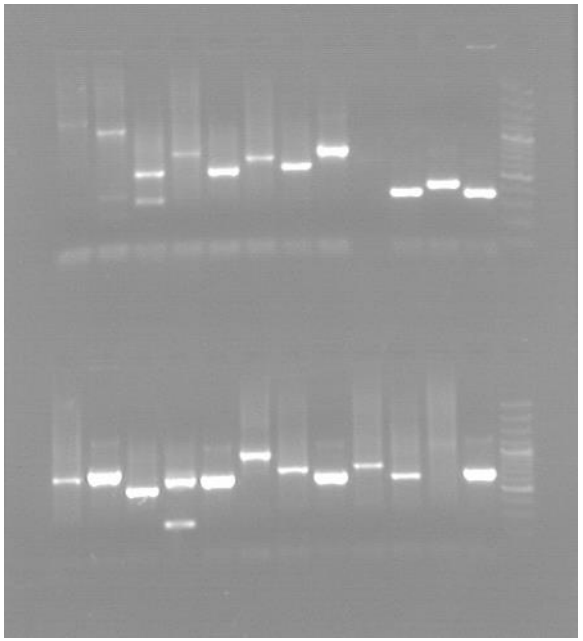
By using PCR:

The same primer to detect the insert	Specific primer for vector detection

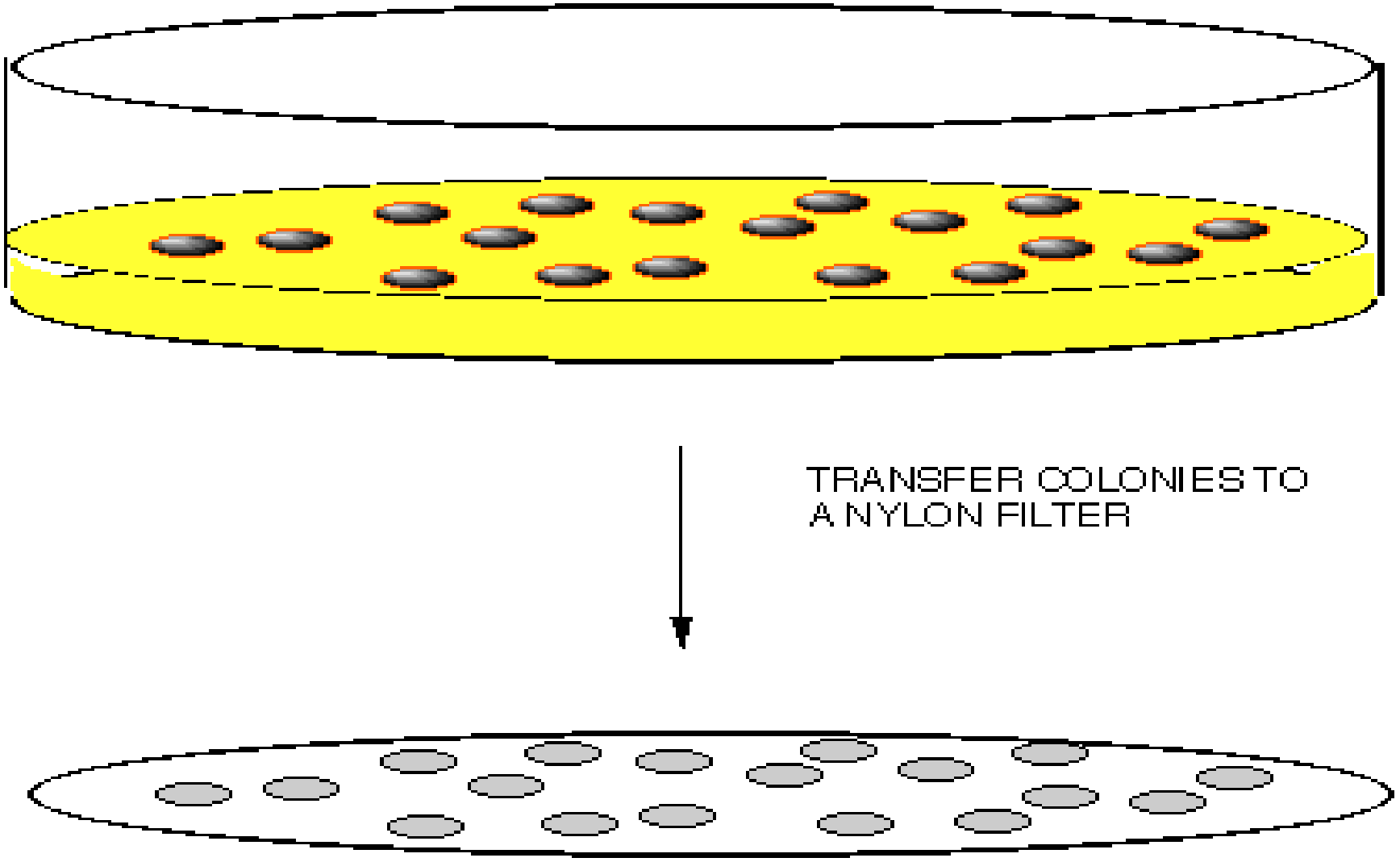


1. Pick colony into a microcentrifuge tube or microtiter well.
2. Add PCRLyse™ Solution, vortex, heat 5 min at 99°C.
3. Perform PCR using an aliquot of the lysed cells.
4. Analyze by gel electrophoresis.

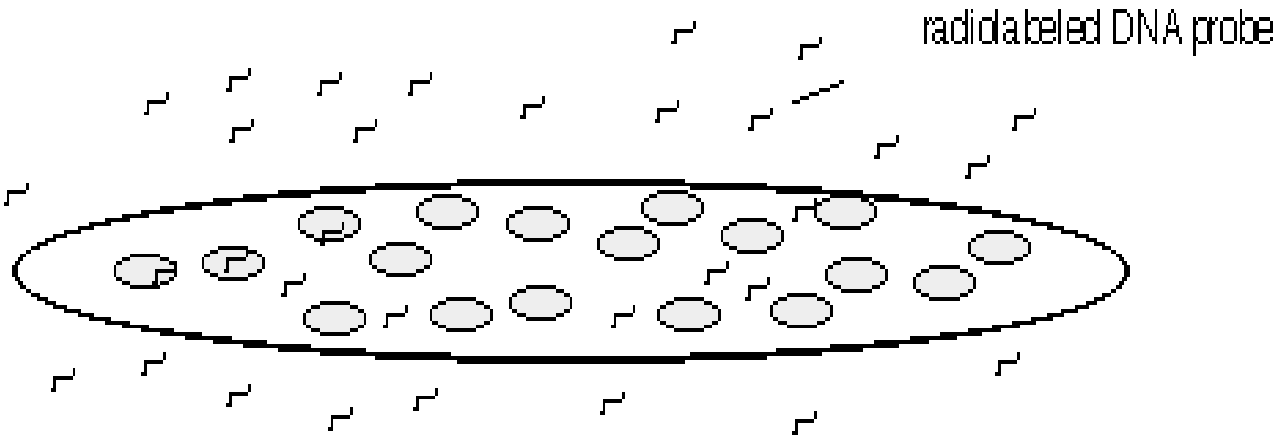
PCR for detection of the vector which carry the insert:



DNA hybridization

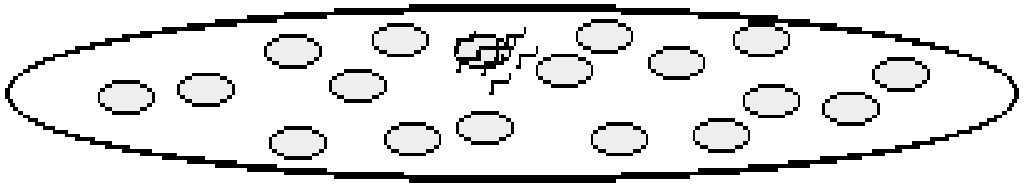


DNA hybridization

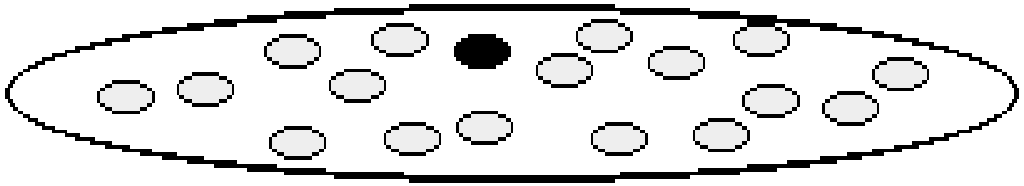


1. Synthesize a DNA fragment complementary to any strand of "our" gene.
2. Radiolabel the probe.
3. Lyse cells on the filter and denature DNA.
4. Hybridize the probe with the filter.
5. Wash out excess of the probe.

DNA hybridization



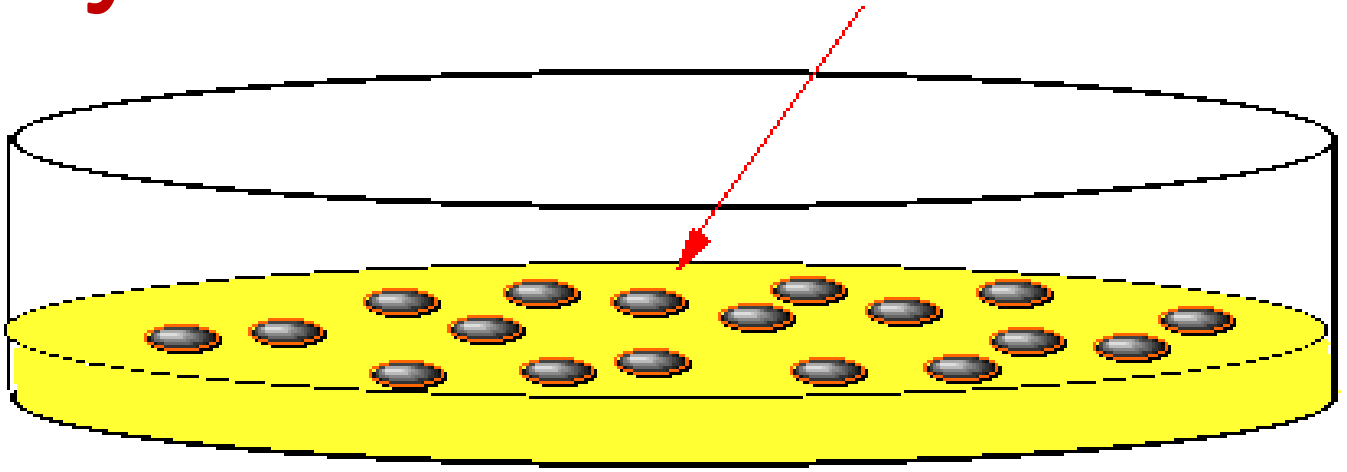
Only the colony that contains plasmids with “our gene” will hybridize with the probe and become radiolabeled.



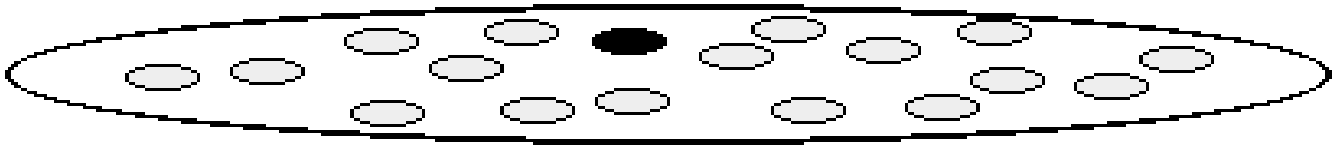
Expose the filter to the X-ray film: a black spot will appear on the place of a colony with “our gene”.

DNA hybridization

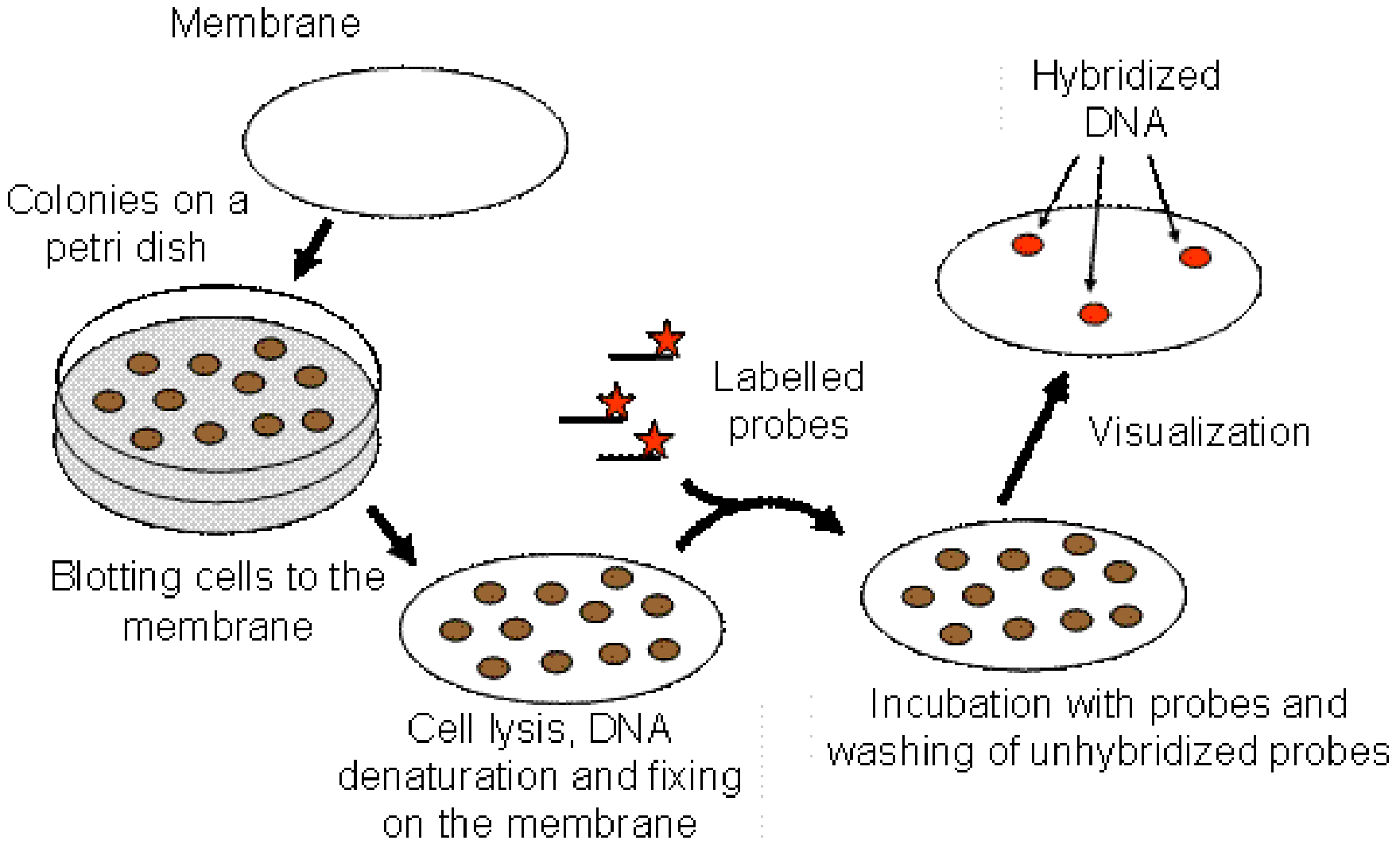
Master plate

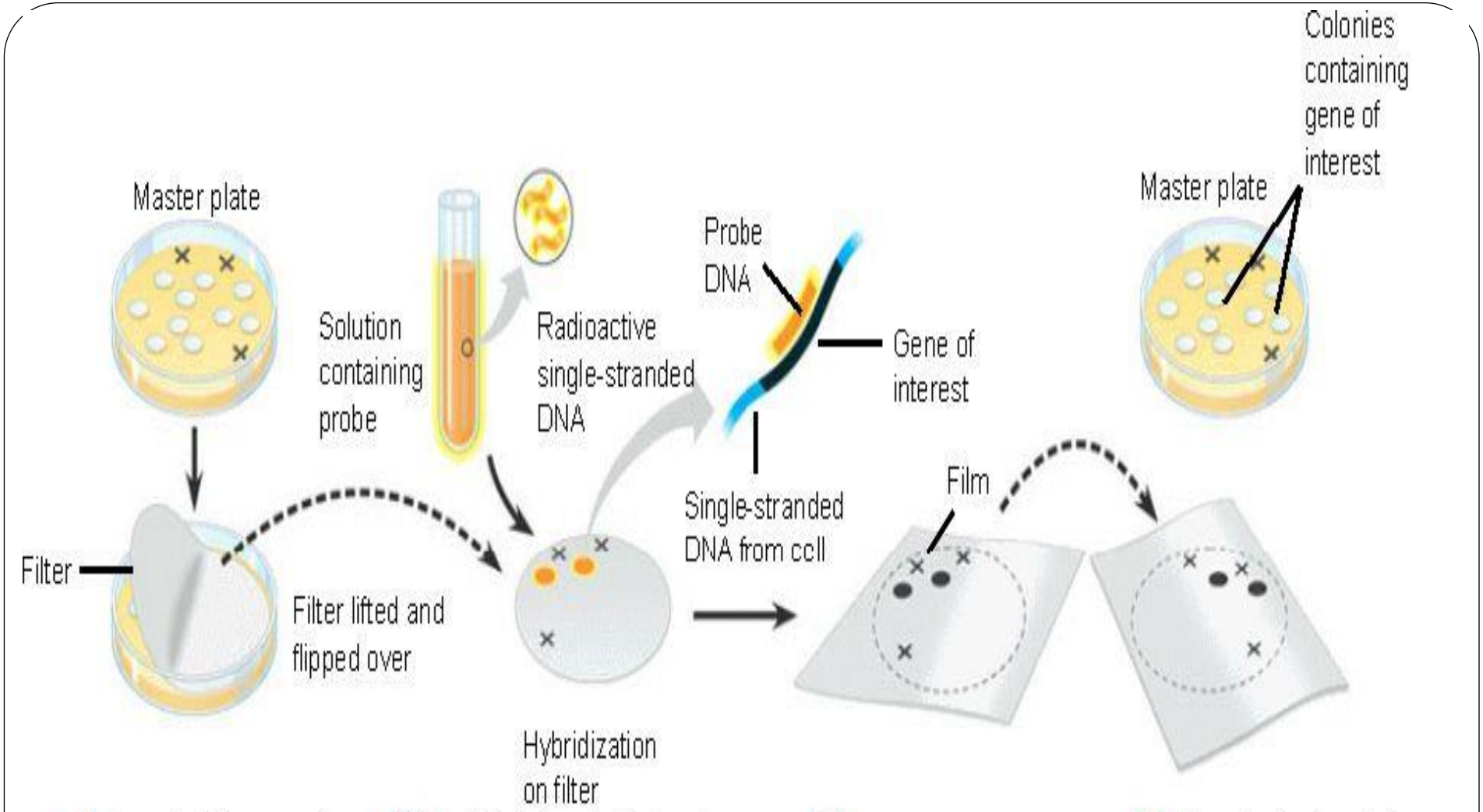


X-ray film

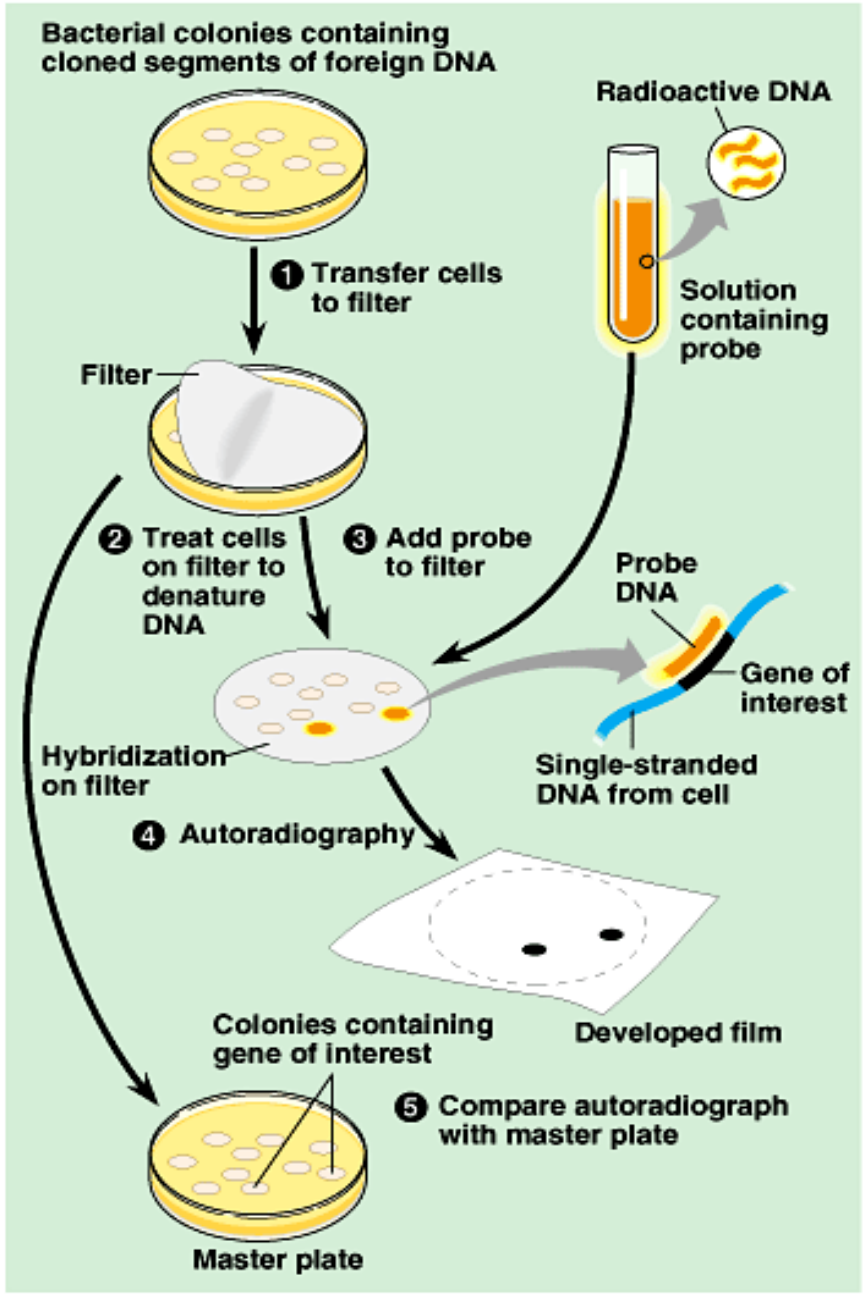


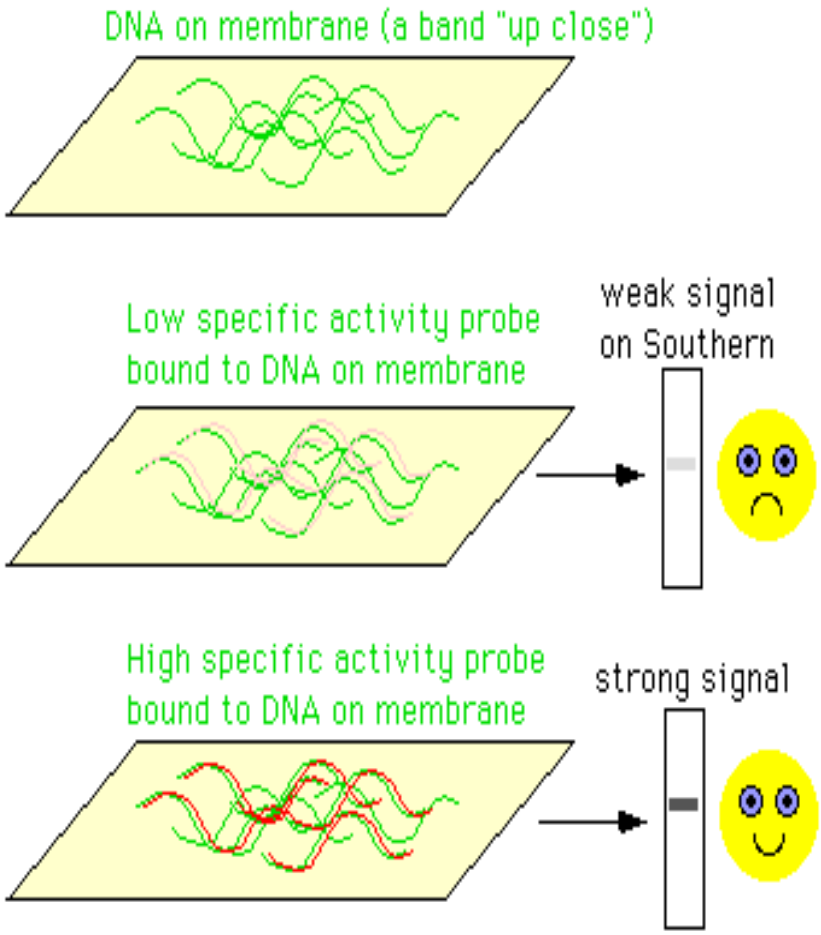
Find the colony on the master plate that contains the cloned gene.


Membrane
Colonies on a petri dish
Blotting cells to the membrane
Cell lysis, DNA denaturation and fixing on the membrane
Labelled probes
Incubation with probes and washing of unhybridized probes
Visualization
Hybridized DNA



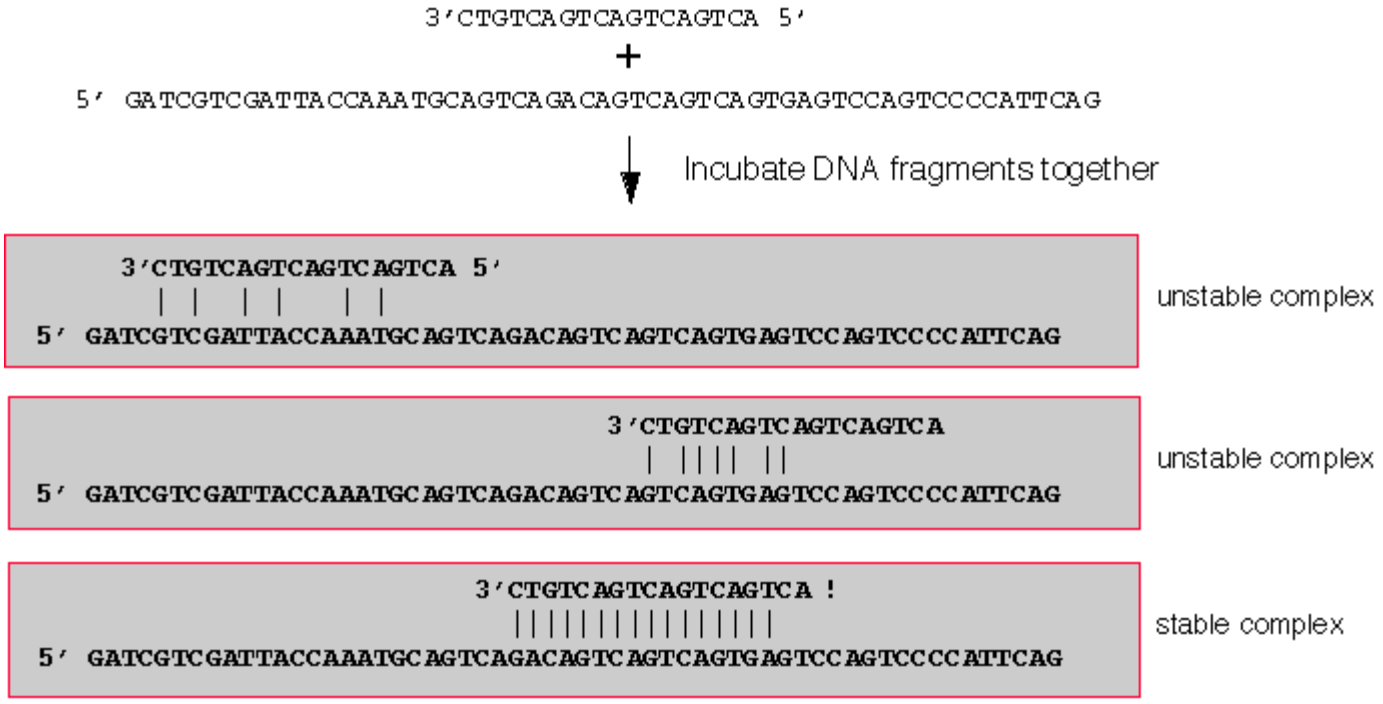
1. A special filter paper is pressed against the master plate, transferring cells to the bottom side of the filter.
2. The filter is treated to break open the cells and denature their DNA; the resulting single-stranded DNA molecules are treated so that they stick to the filter.
3. The filter is laid under photographic film, allowing any radioactive areas to expose the film (autoradiography).
4. After the developed film is flipped over, the reference marks on the film and master plate are aligned to locate colonies carrying the gene of interest.

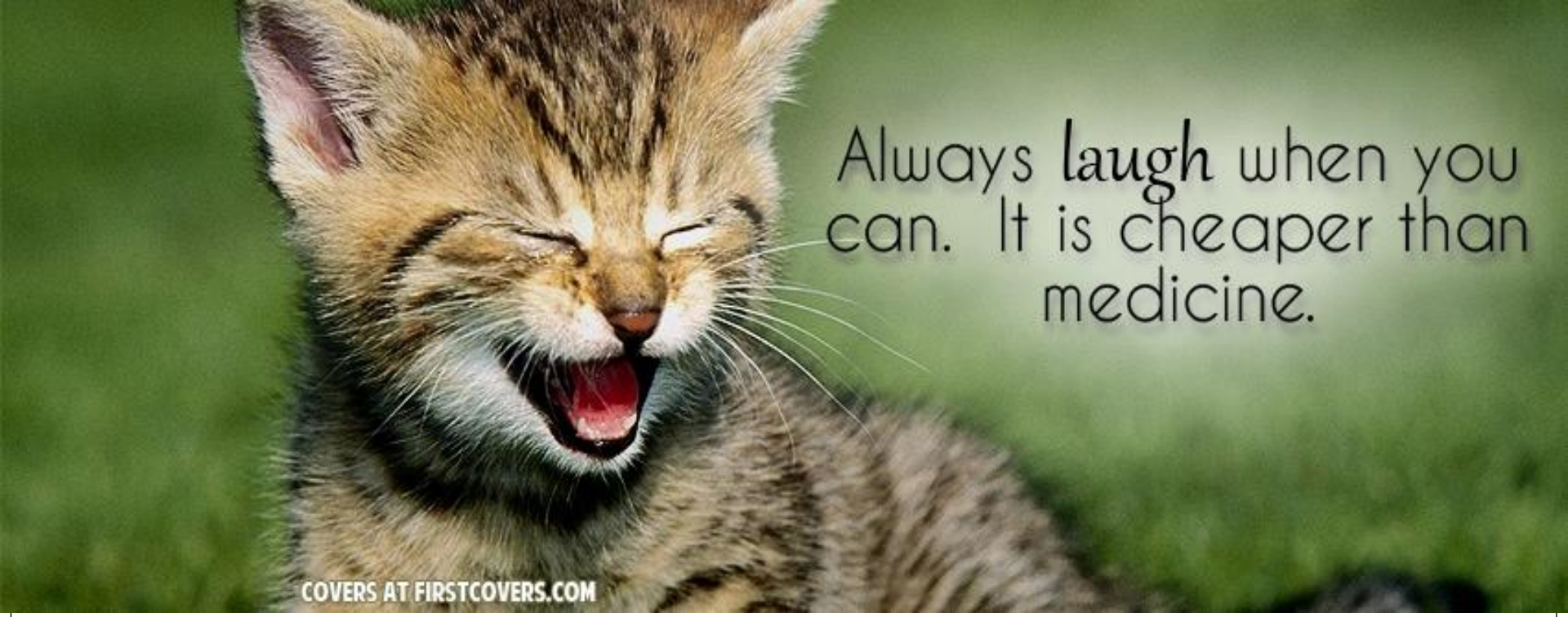




DNA hybridization

If two single stranded DNA molecules have complementary nucleotide sequences they can hybridize: form a stable double stranded complex





Thanks a lot

with my Best Regards and My Best wishes

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