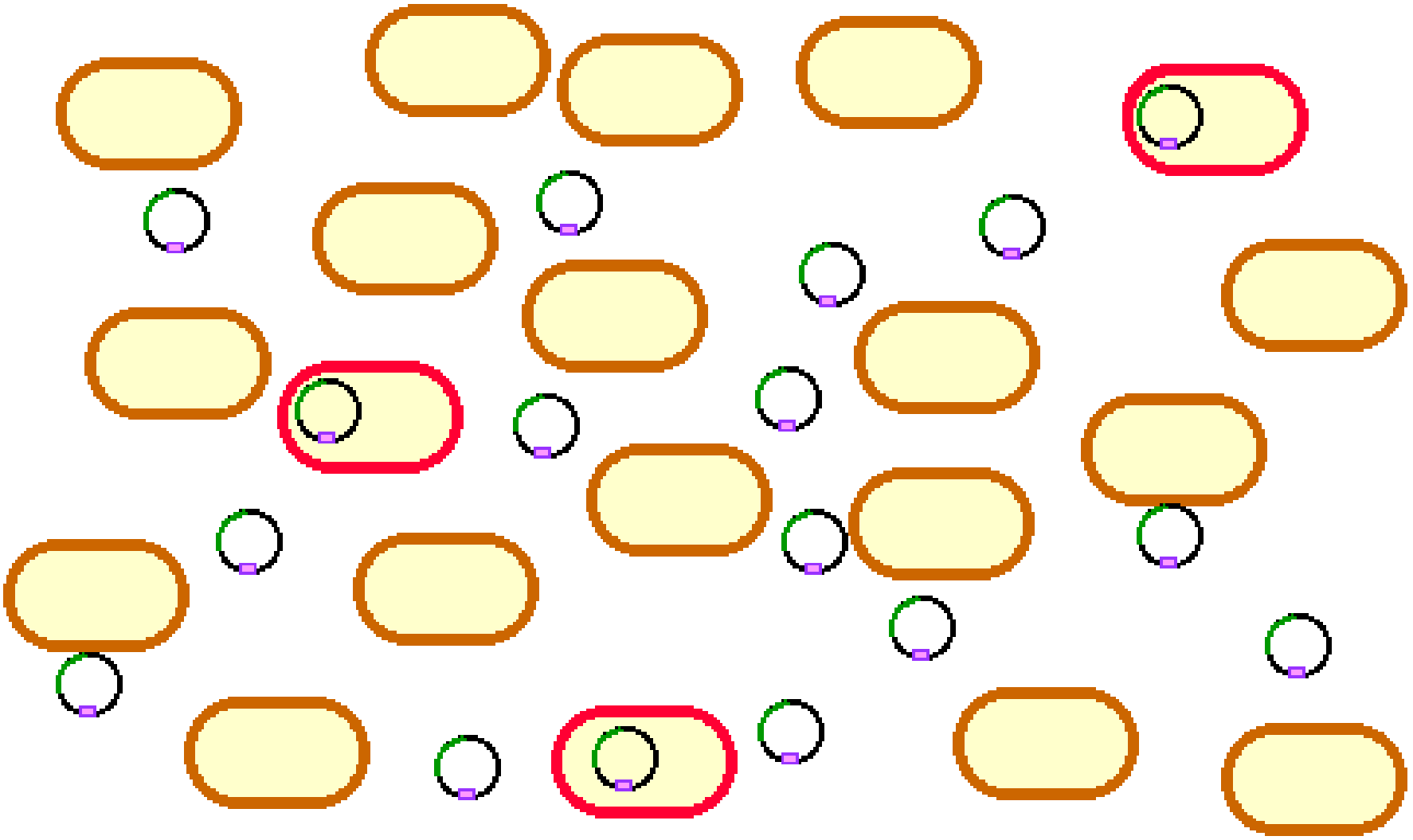
# DETECTION OF YOUR CLONING AND PICK UP YOUR RIGHT CLONE

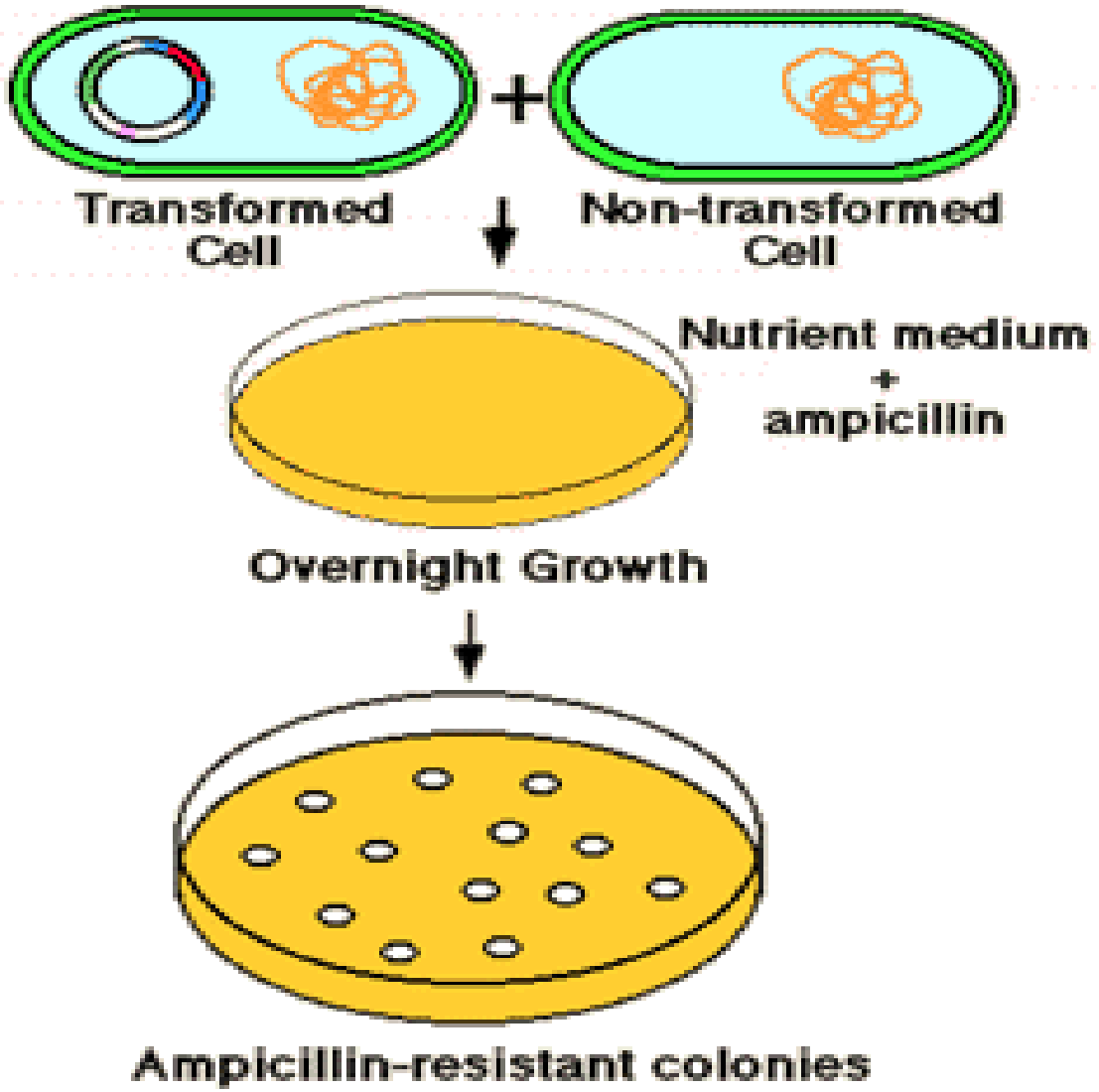
# SELECTION OF THE CELLS WITH GENE OF INTEREST

# Introduction

- **Once recombinant plasmid is constructed → 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

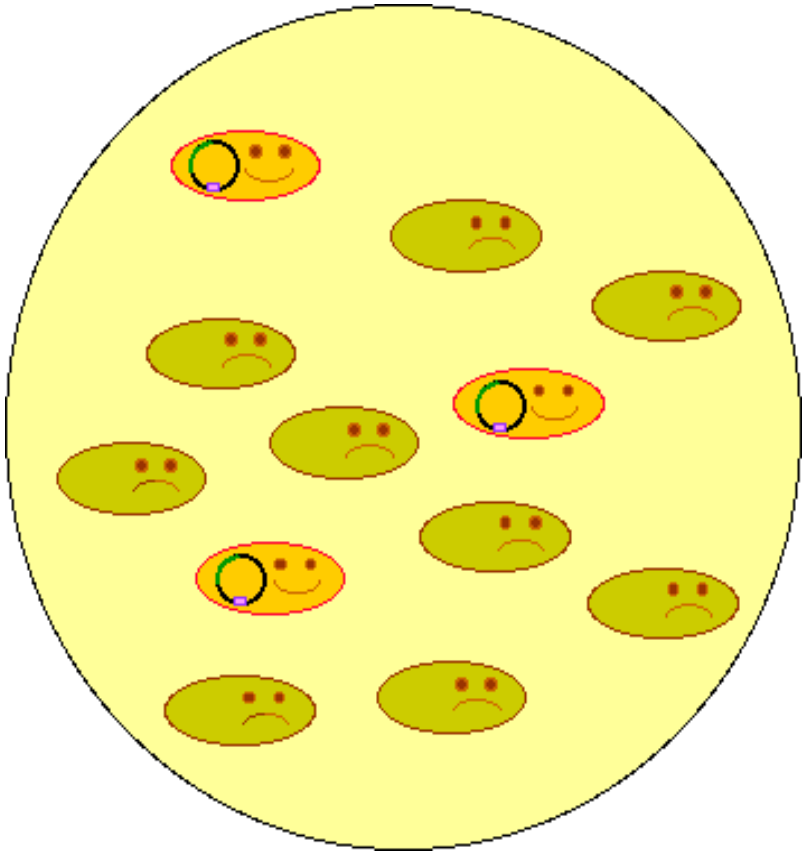



Transformed Cell
Non-transformed Cell
Nutrient medium + ampicillin
Overnight Growth
Ampicillin-resistant colonies

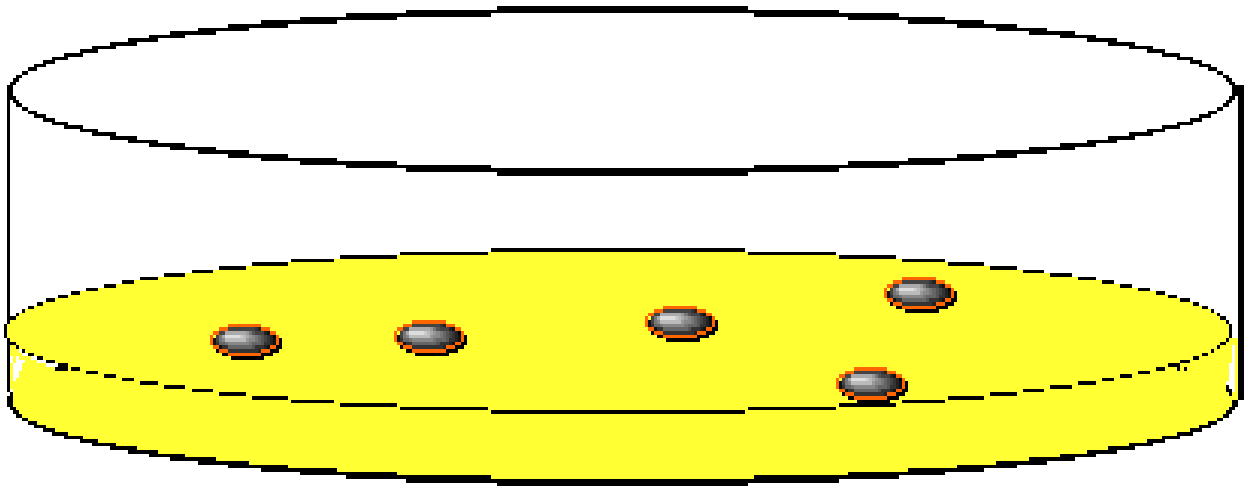
# Competent cells culture:

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.

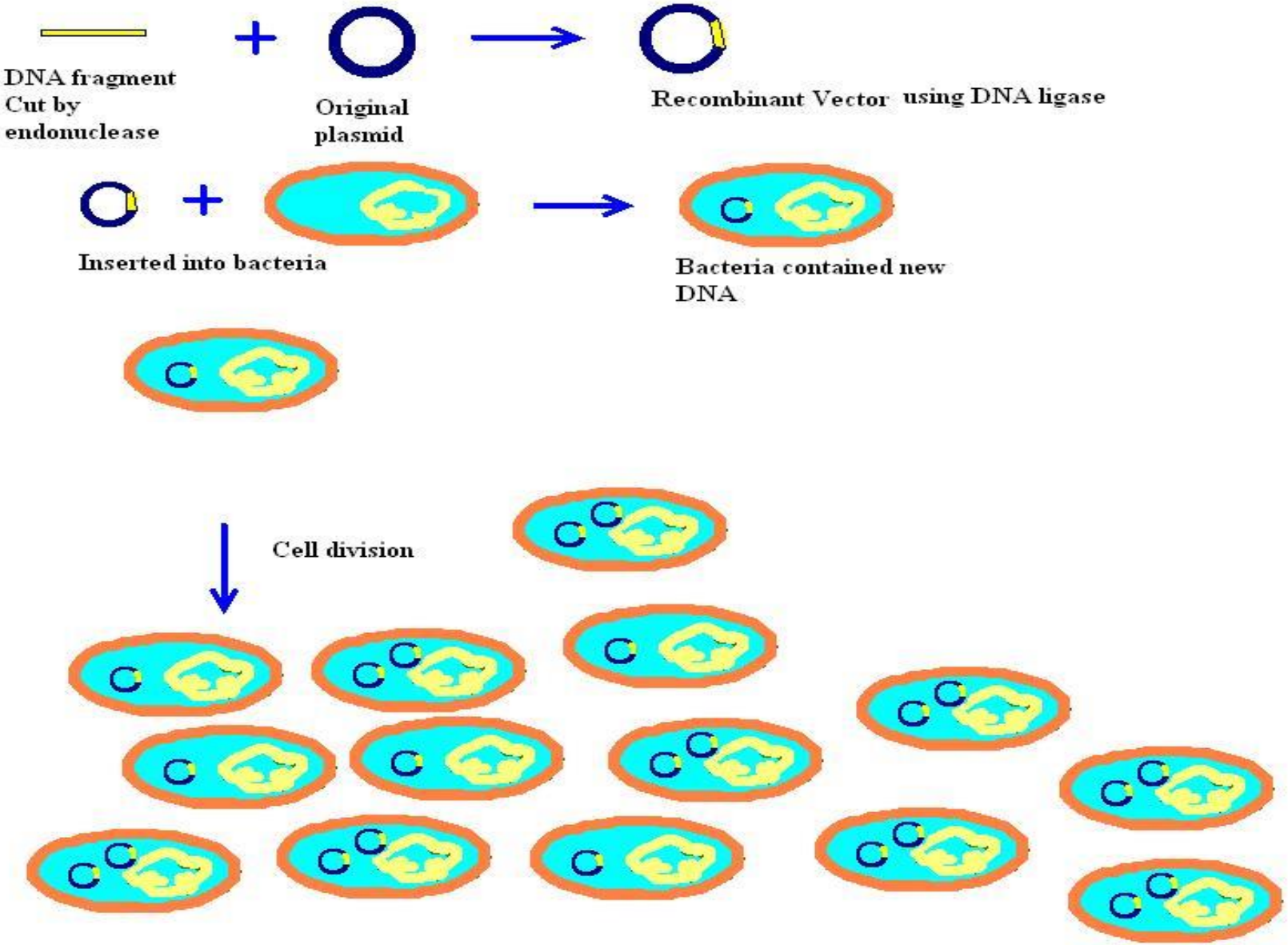


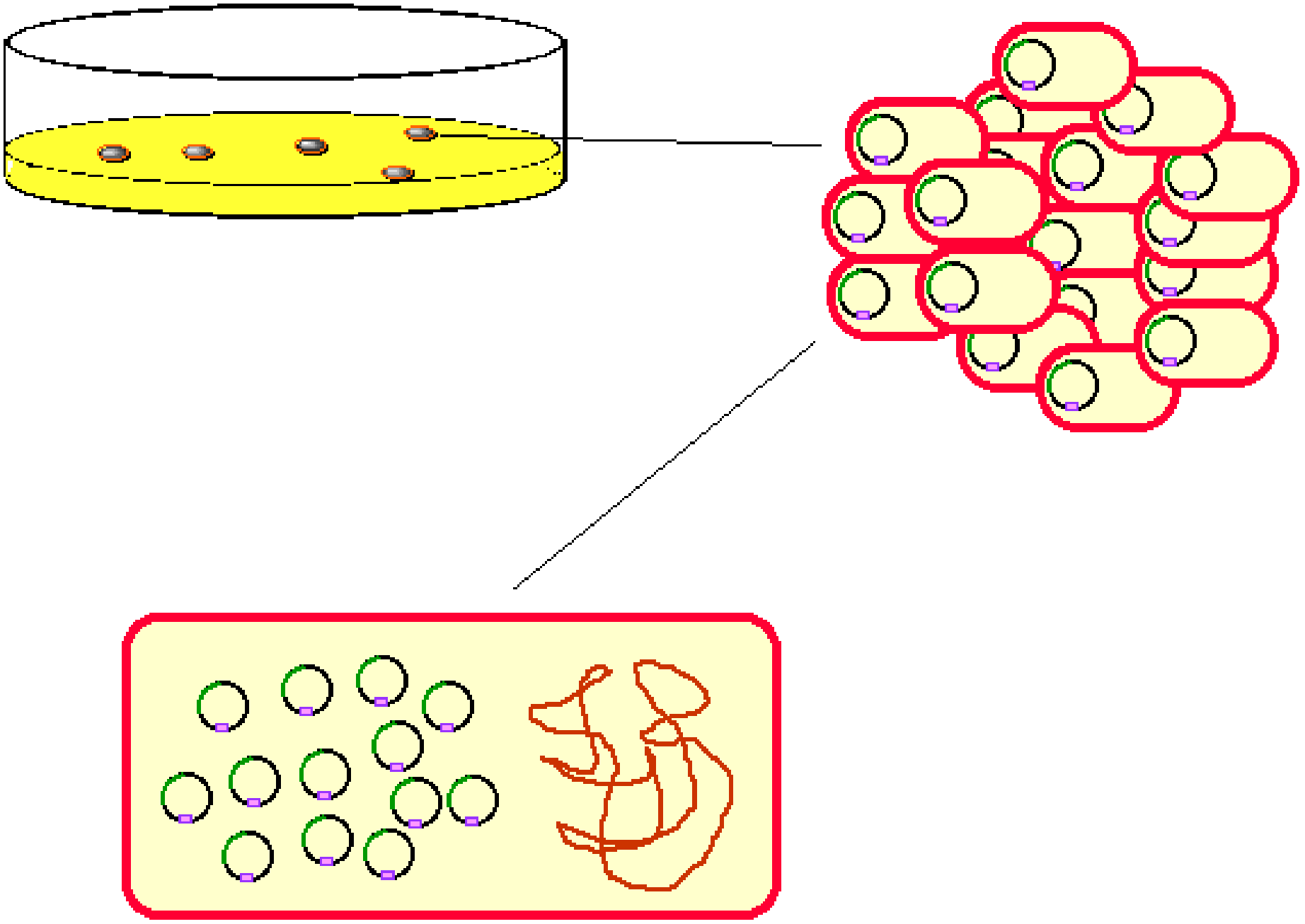
**In each colony formed on the agar plate, all cells are descendants of one transformed cell.**



agar medium with antibiotic

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


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



# How to Find and Pick Up the Right Clone?

The most common methods include:

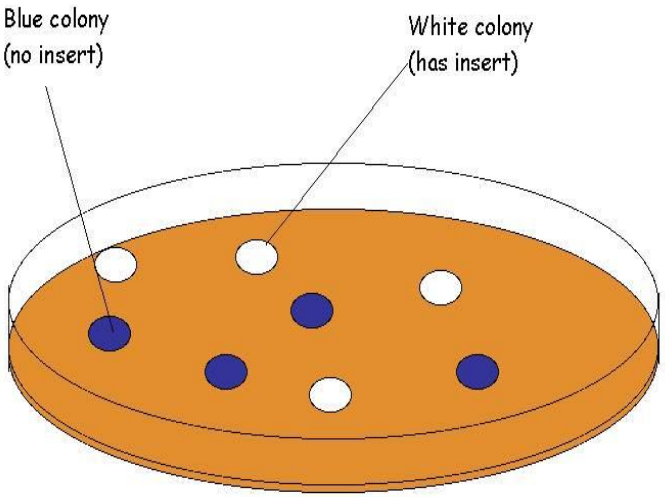
1. **Phenotypic screening.**
2. **Screening with antibodies.**
3. **DNA hybridization.**

# 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**

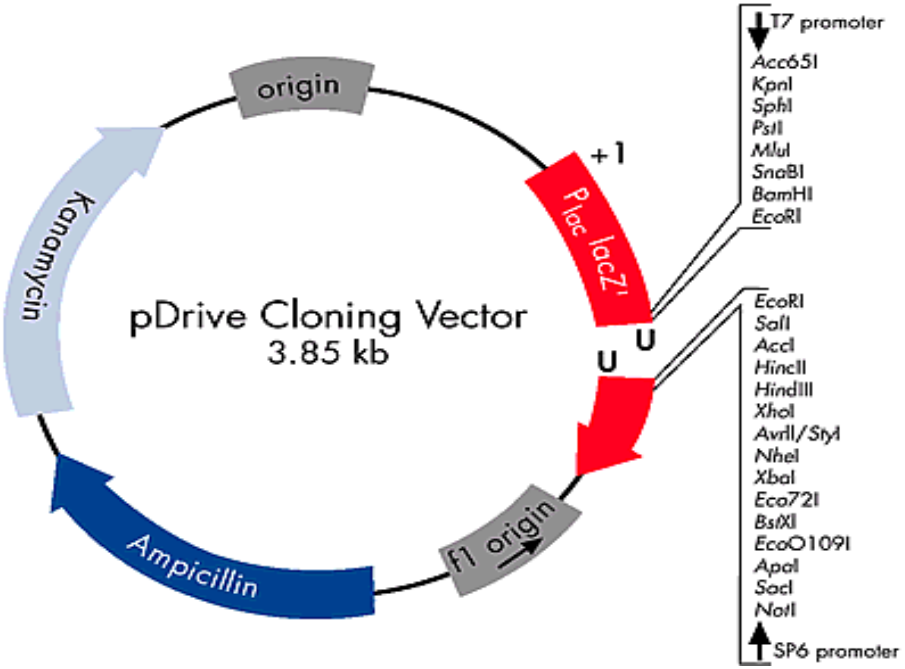
**Insertional mutagenesis**

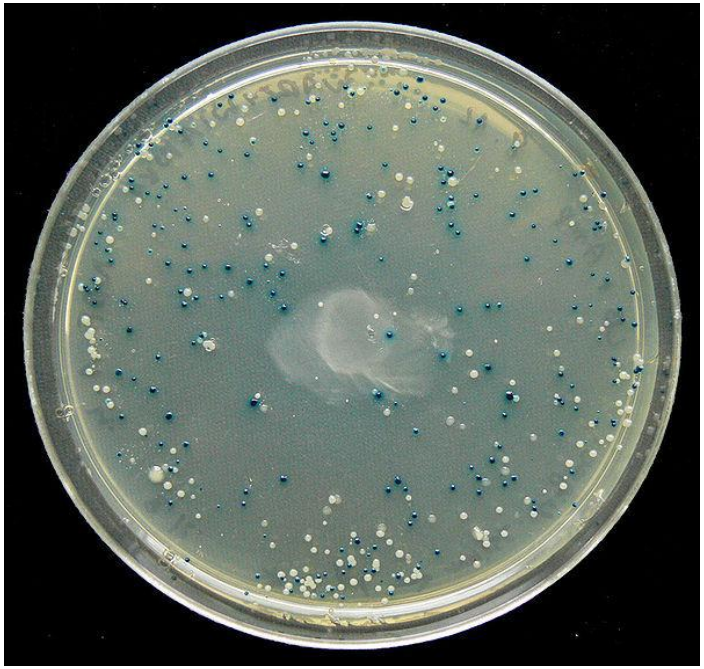


# Blue-White Screening

**LacZ α → Provide β-galactosidase activity.**

**X-gal is a colorless analog of lactose cleaved by β-galactosidase to form a bright blue insoluble pigment.**

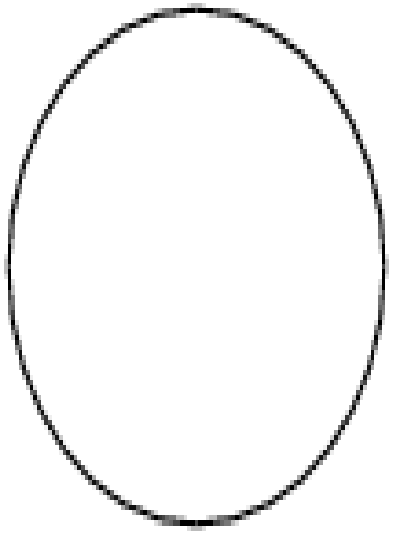


- No insert → intact lacZ → Blue
- Insert → disrupted lacZ → White

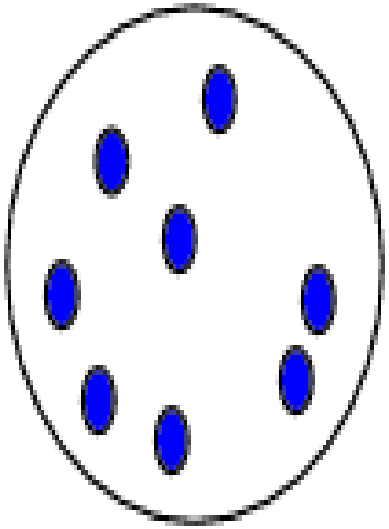
# BLUE – WHITE SCEENING

A)



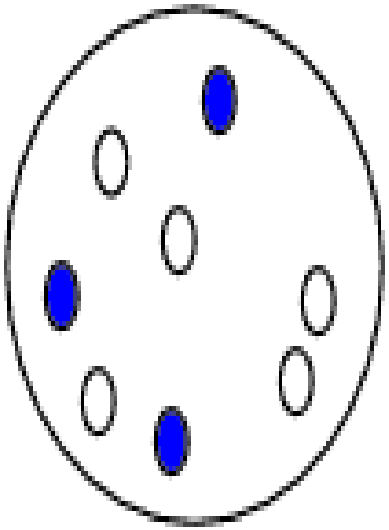
Agar plate
+ ampicillin
+bacteria
+**no plasmid**

B)



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

C)

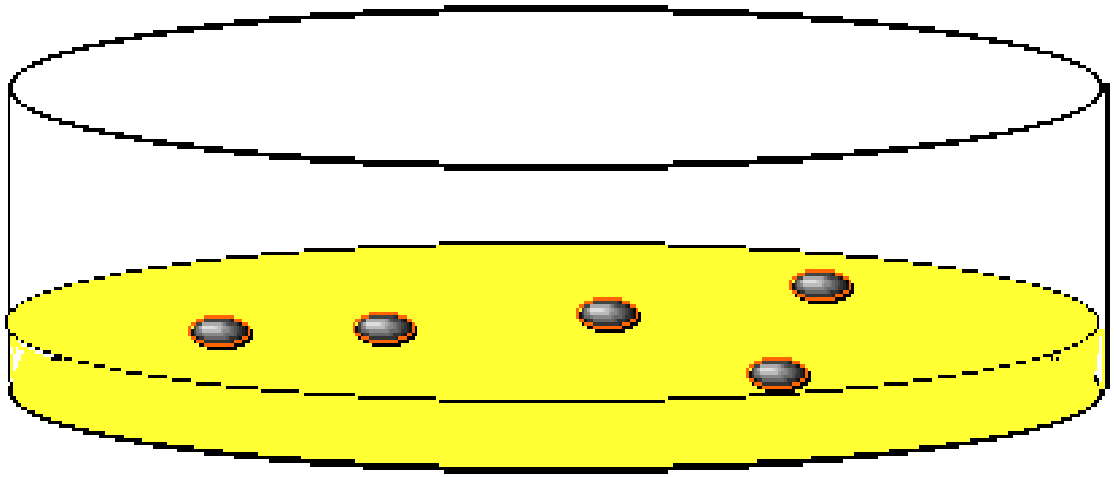


Agar plate +
ampicillin +
bacteria +
**recombinant plasmid**

# BLUE – WHITE SCEENING

# Screening with antibodies

→(Antibiotic Resistance).



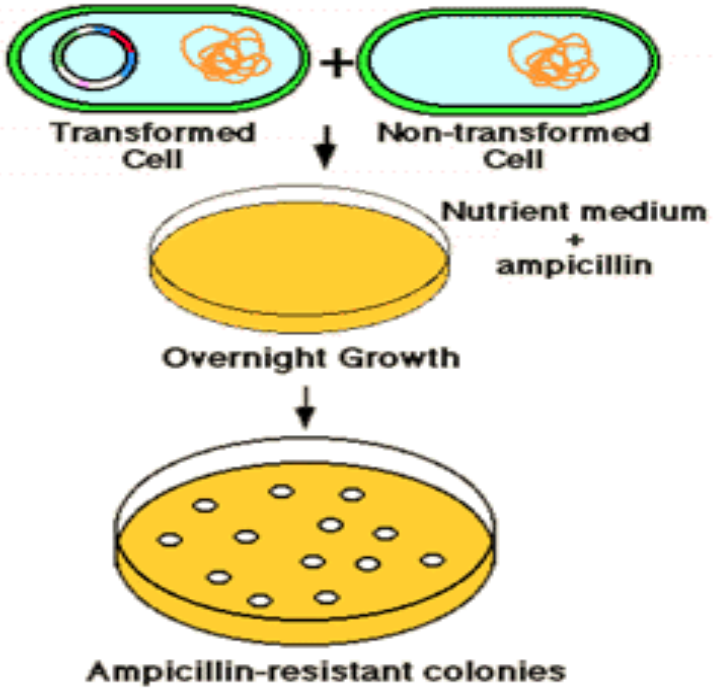
agar medium with antibiotic

Plasmid vector contains an ampicillin resistance gene making the cell resistant to ampicillin containing medium.

Growth of transformed cells (cells receiving the plasmid) can be identified on agar medium containing (e.g.) ampicillin.

Thus, the cells with recombinant vector can be selected.

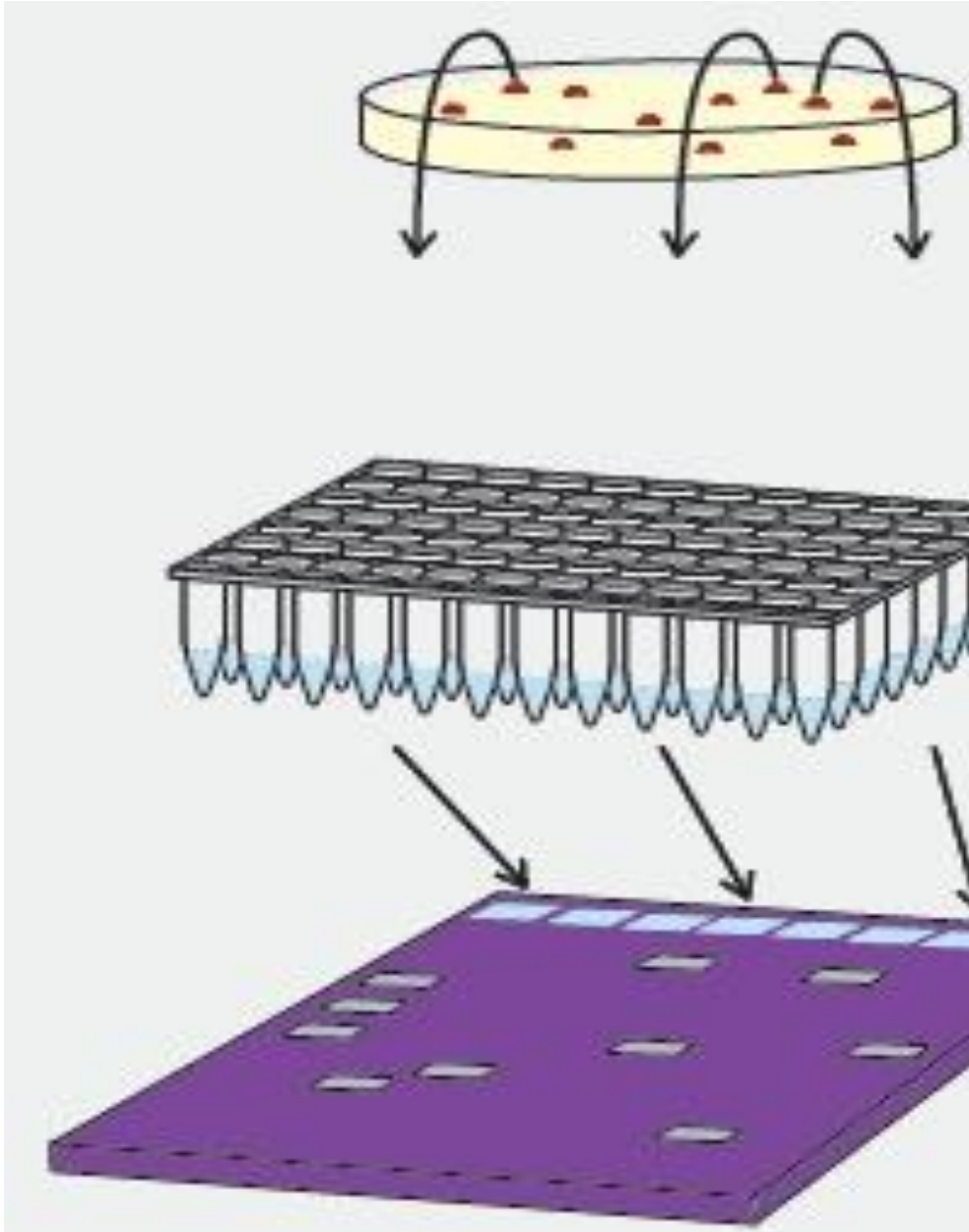
This is an direct selection procedure.



# Confirmation

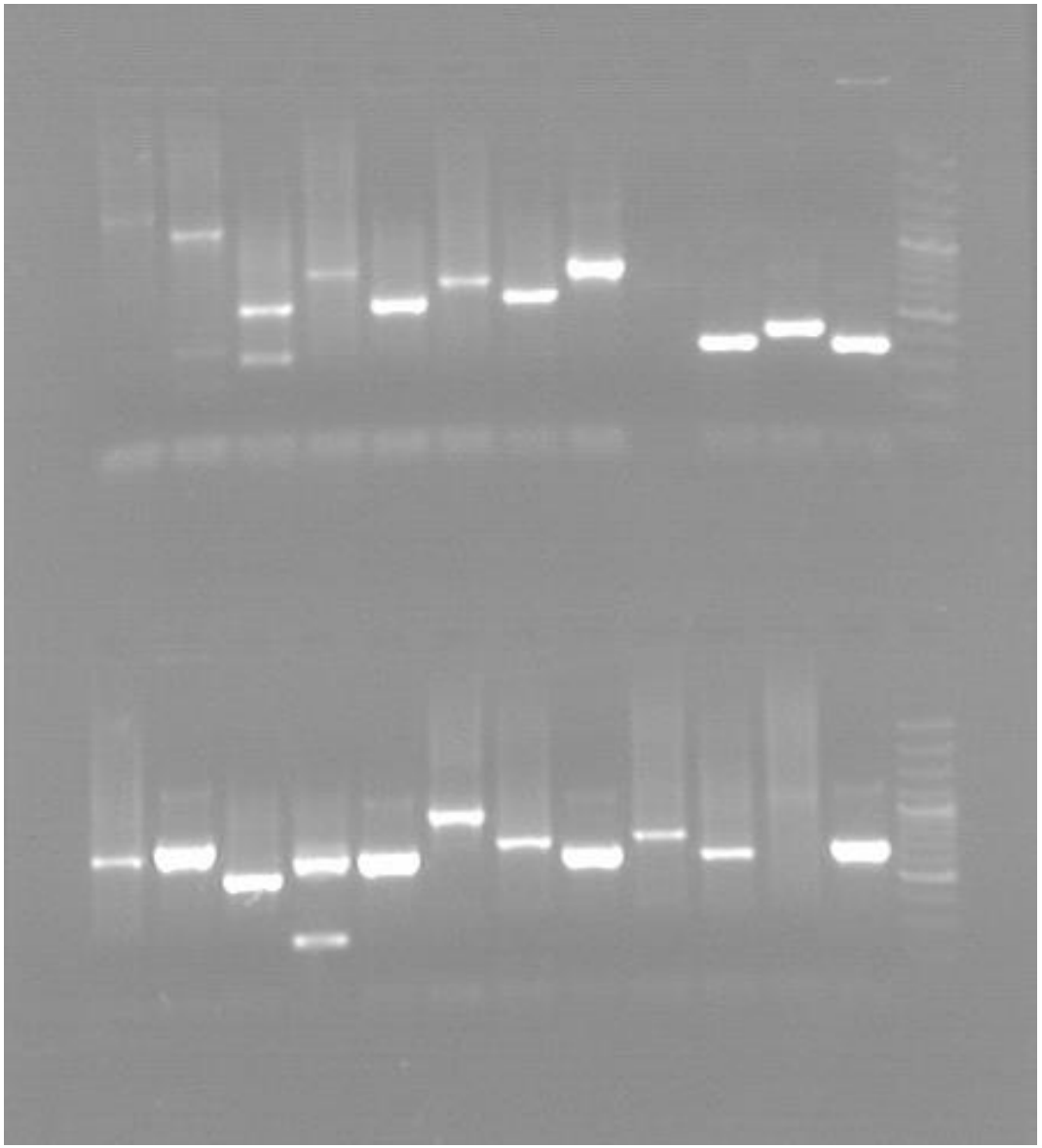
**By using PCR:**

**Specific primer for the Cloning Vector and the DNA insert**

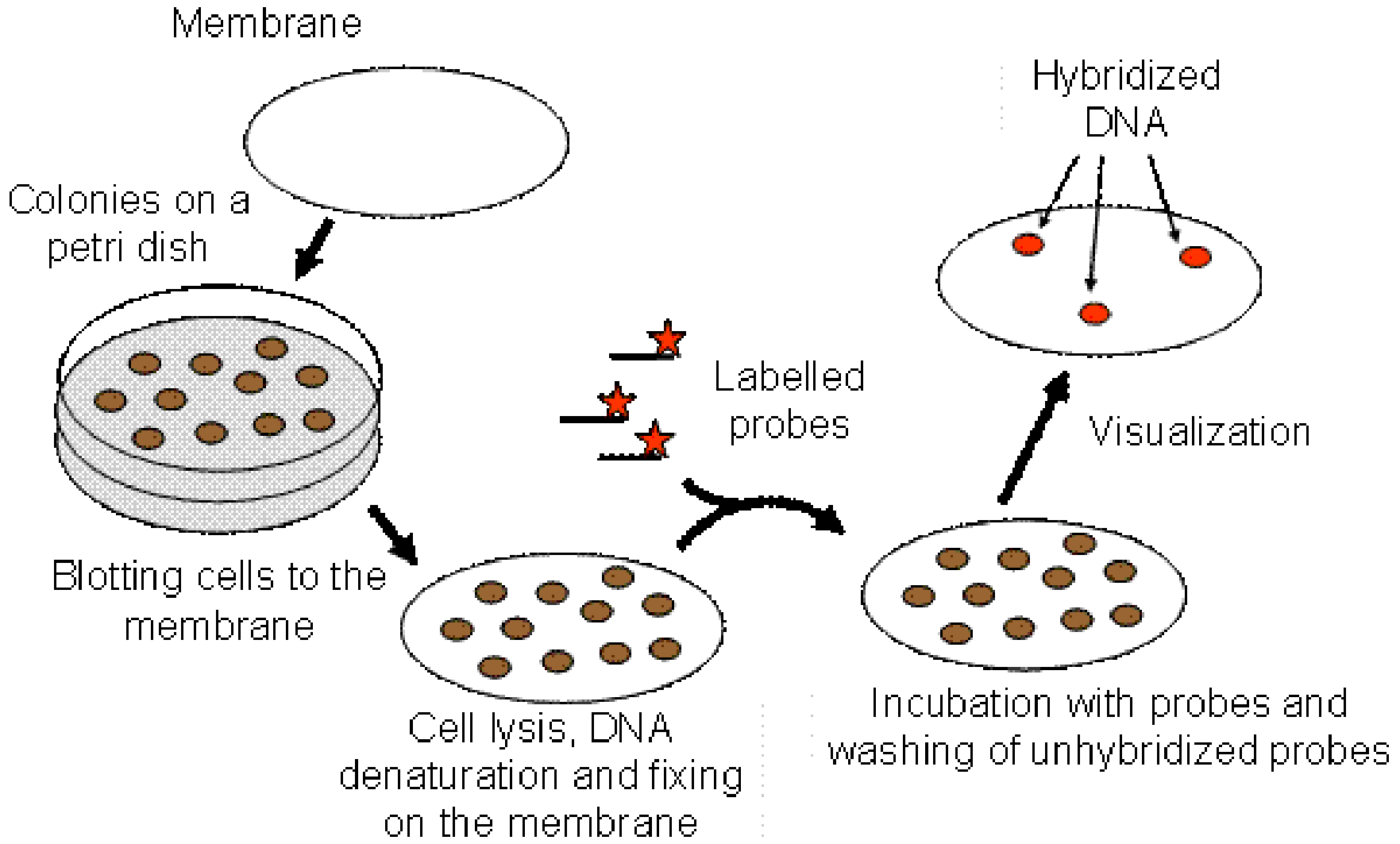


1. Pick colony into a microcentrifuge tube or microtiter well.
2. Add PCRLyse™ Solution, vortex, heat 5 min at 99°C.
3. Perform PCR using an aliquot of the lysed cells.
4. Analyze by gel electrophoresis.

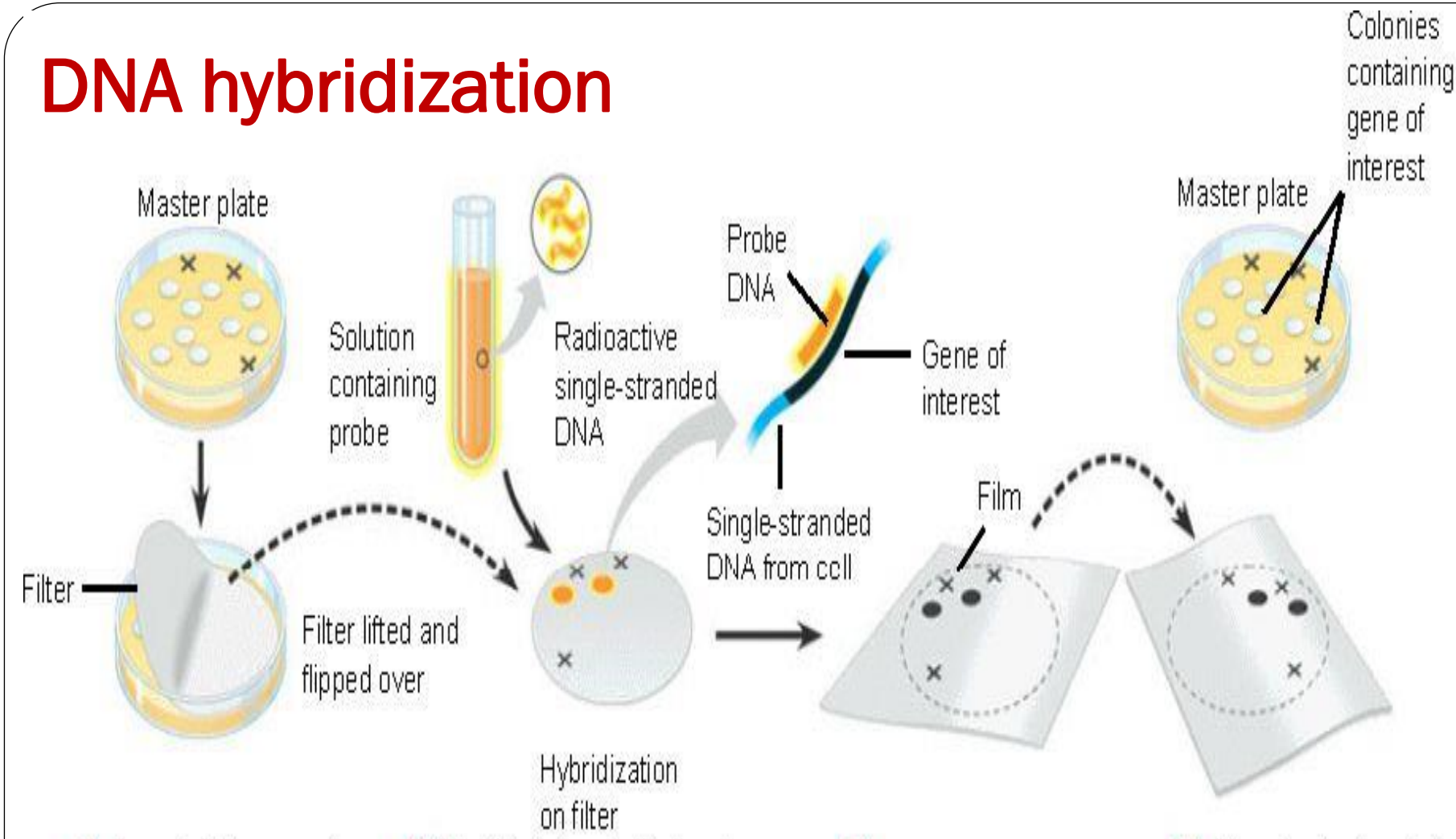
# PCR for detection of the vector which carry the insert:



# DNA hybridization

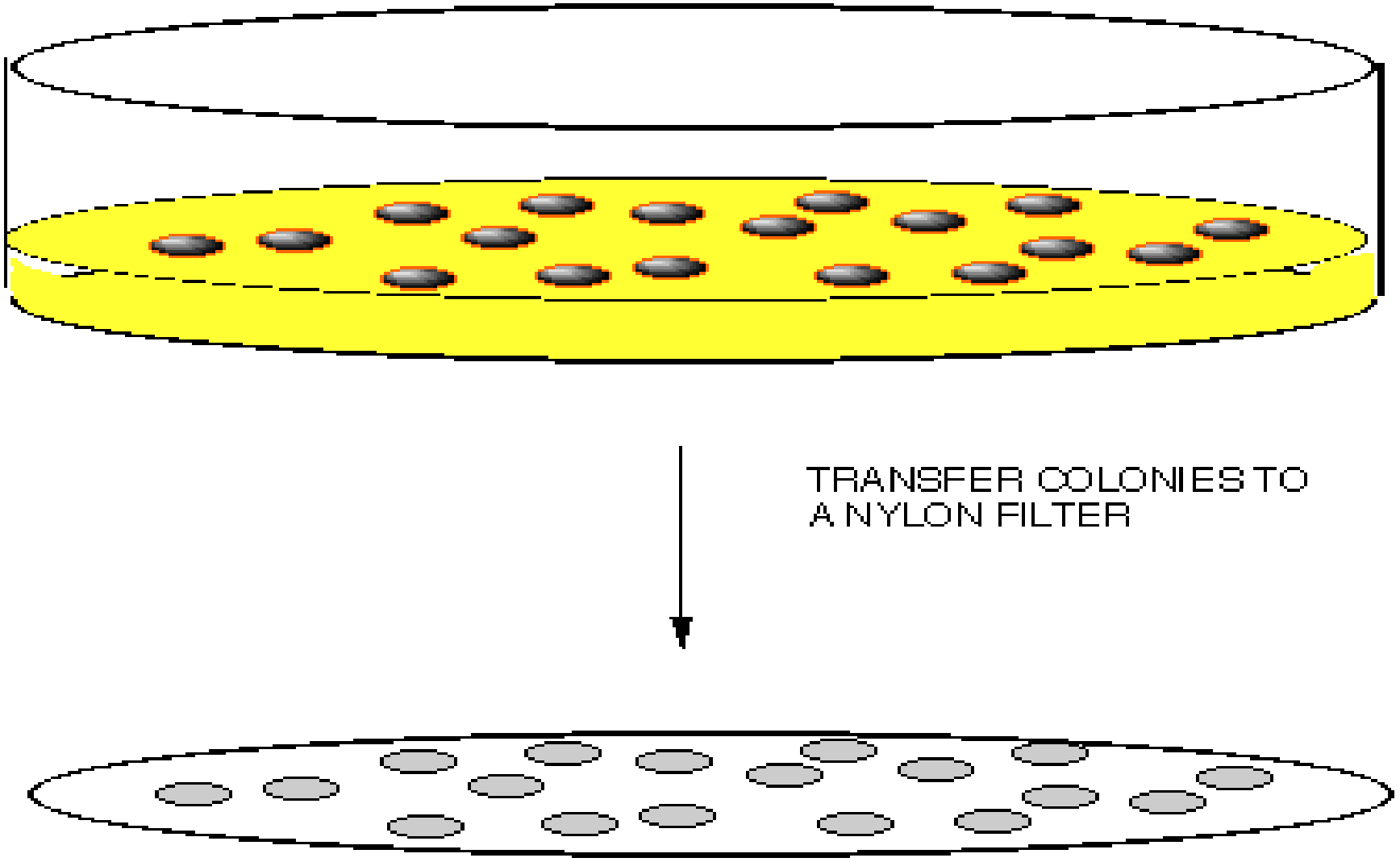


# DNA hybridization

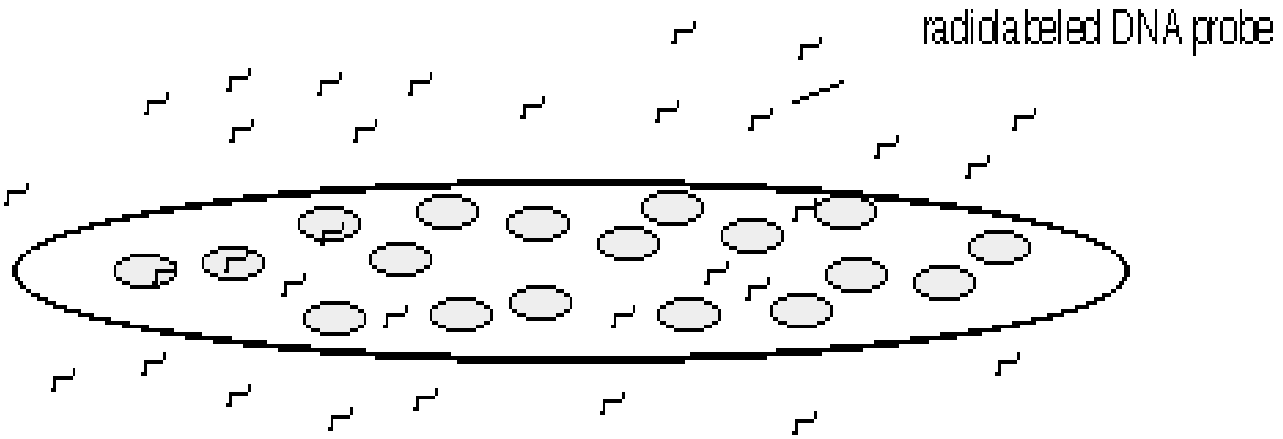



1. A special filter paper is pressed against the master plate, transferring cells to the bottom side of the filter.
2. The filter is treated to break open the cells and denature their DNA; the resulting single-stranded DNA molecules are treated so that they stick to the filter.
3. The filter is laid 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

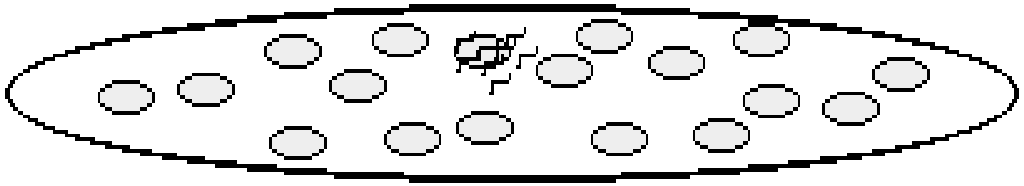


# DNA hybridization

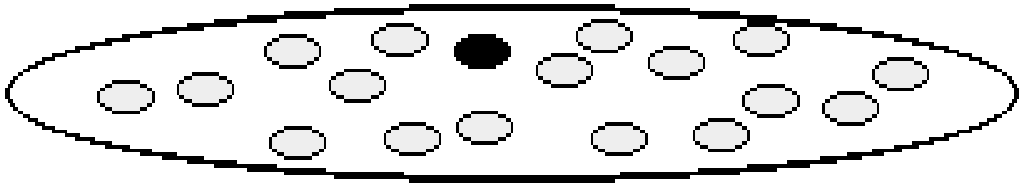



1. Synthesize a DNA fragment complementary to any strand of "our" gene.
2. Radiolabel the probe.
3. Lyse cells on the filter and denature DNA.
4. Hybridize the probe with the filter.
5. Wash out excess of the probe.

# DNA hybridization



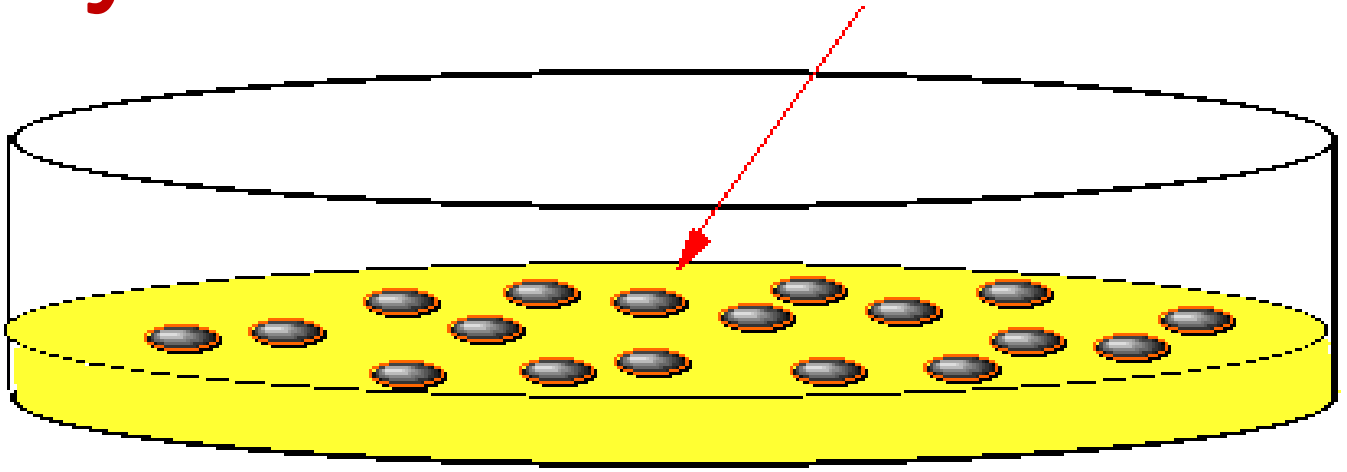
Only the colony that contains plasmids with “our gene” will hybridize with the probe and become radiolabeled.



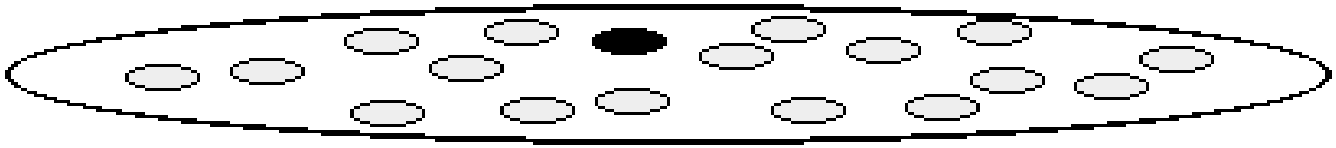
Expose the filter to the X-ray film: a black spot will appear on the place of a colony with “our gene”.

# DNA hybridization

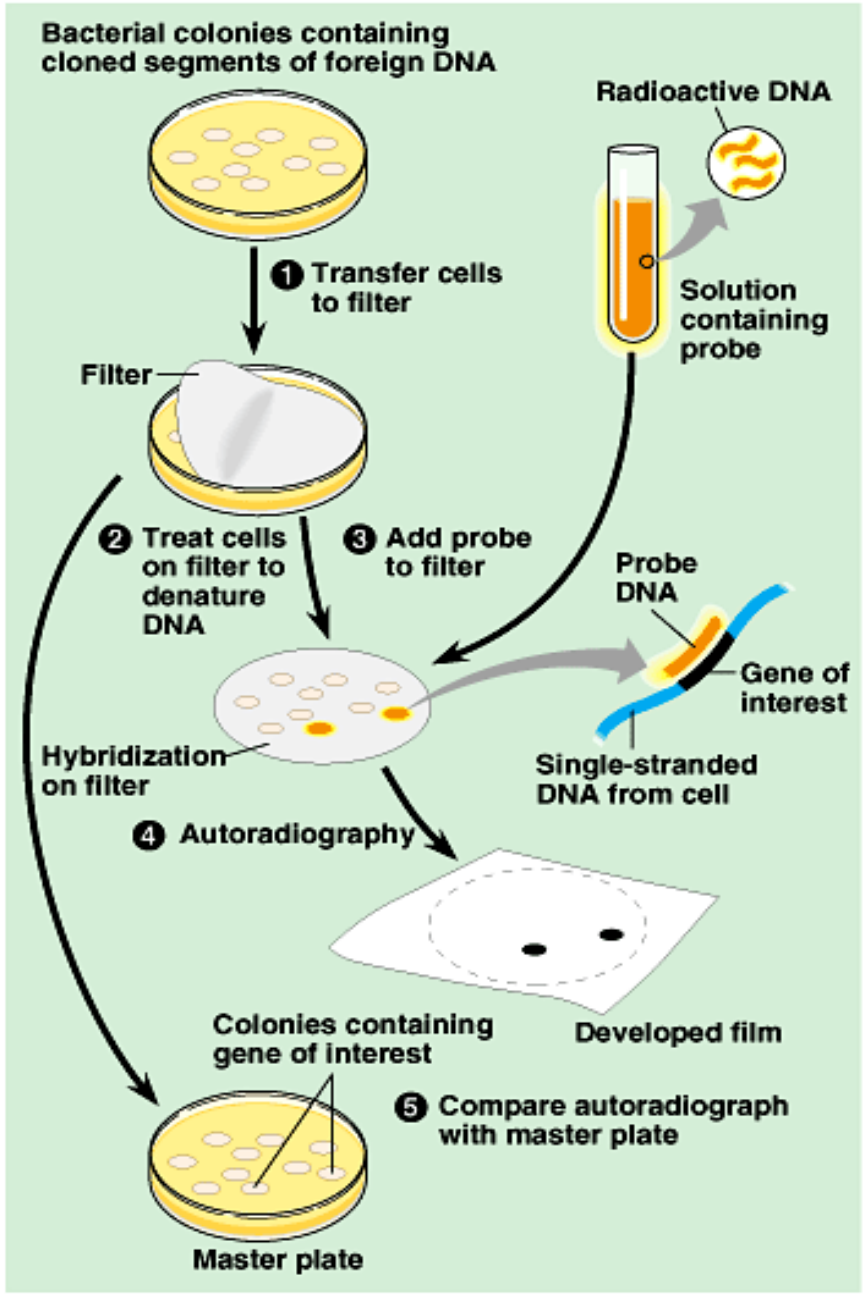
Master plate



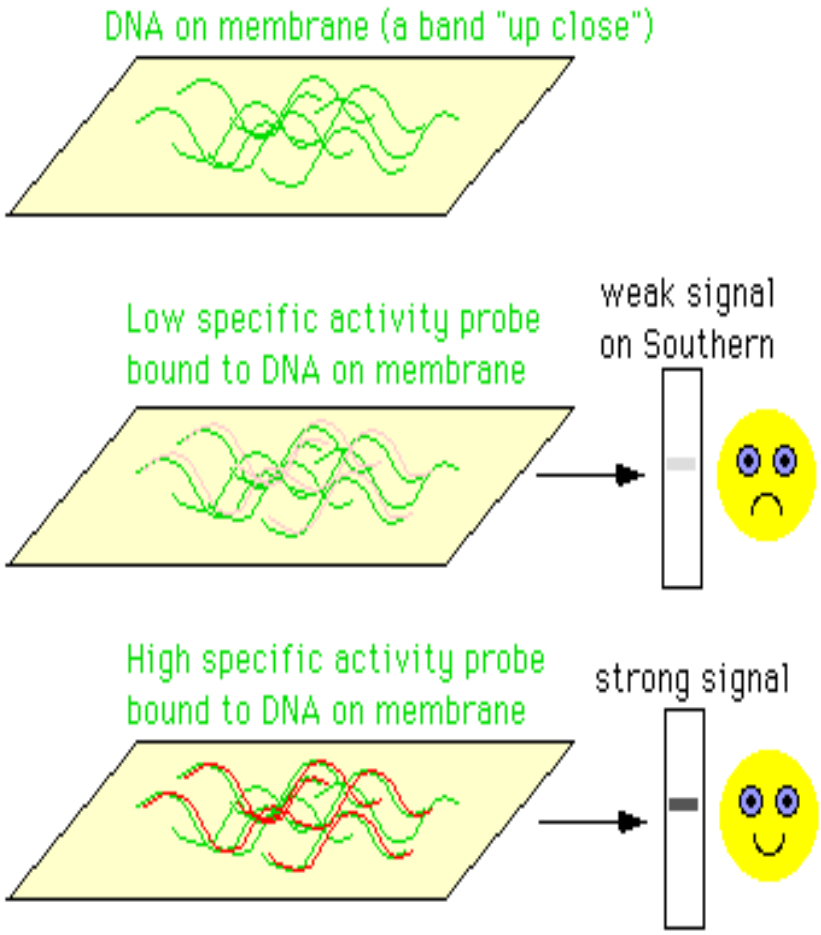
X-ray film



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





# DNA hybridization

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

```
3'CTGTCAGTCAGTCAGTCA 5'
+
5' GATCGTCGATTACCAAATGCAGTCAGACAGTCAGTCAGTGAGTCCAGTCCCCATTCAG
```

Incubate DNA fragments together

```
     3'CTGTCAGTCAGTCAGTCA 5'
       | |  | |   | |
5' GATCGTCGATTACCAAATGCAGTCAGACAGTCAGTCAGTGAGTCCAGTCCCCATTCAG
```

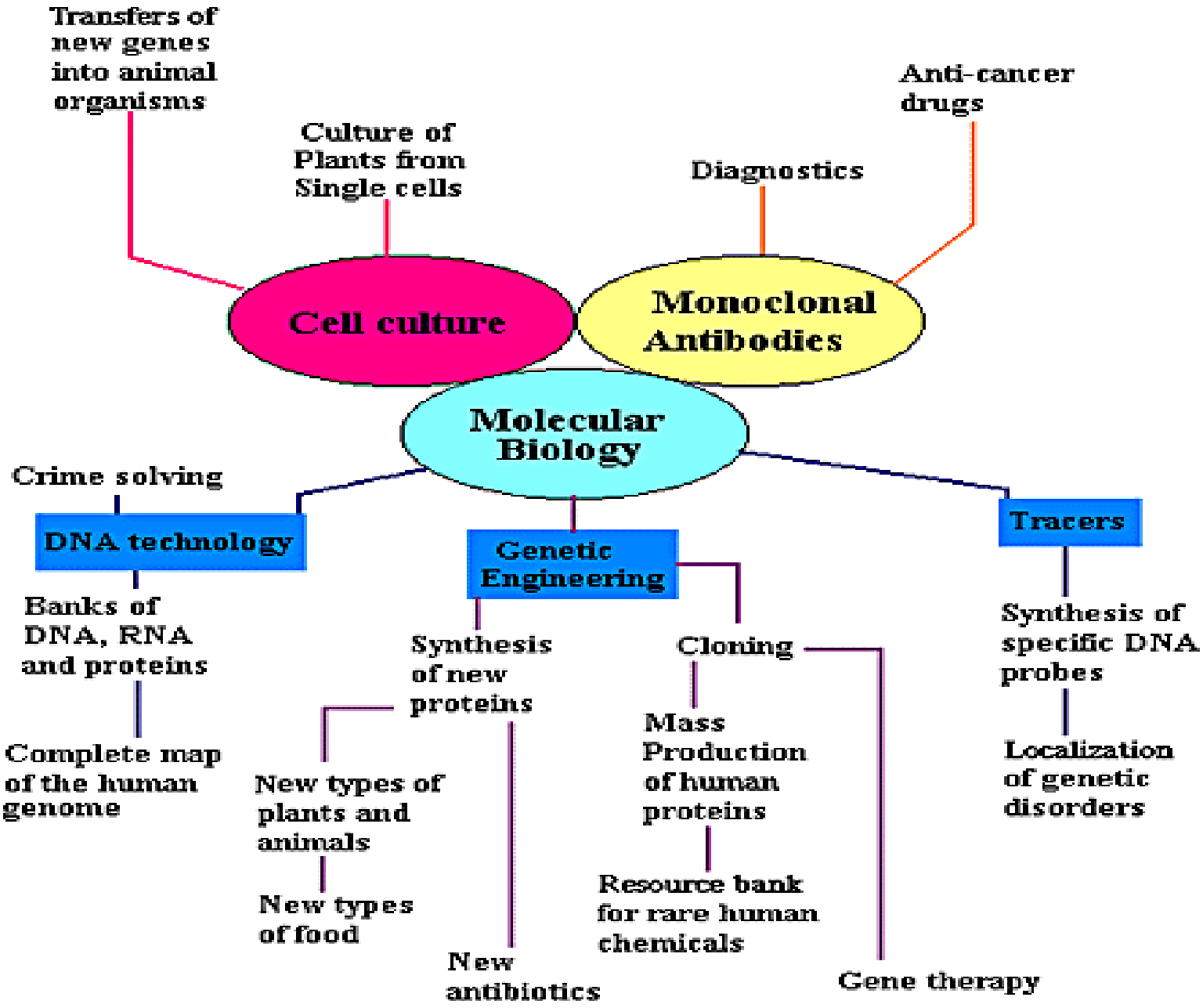
unstable complex

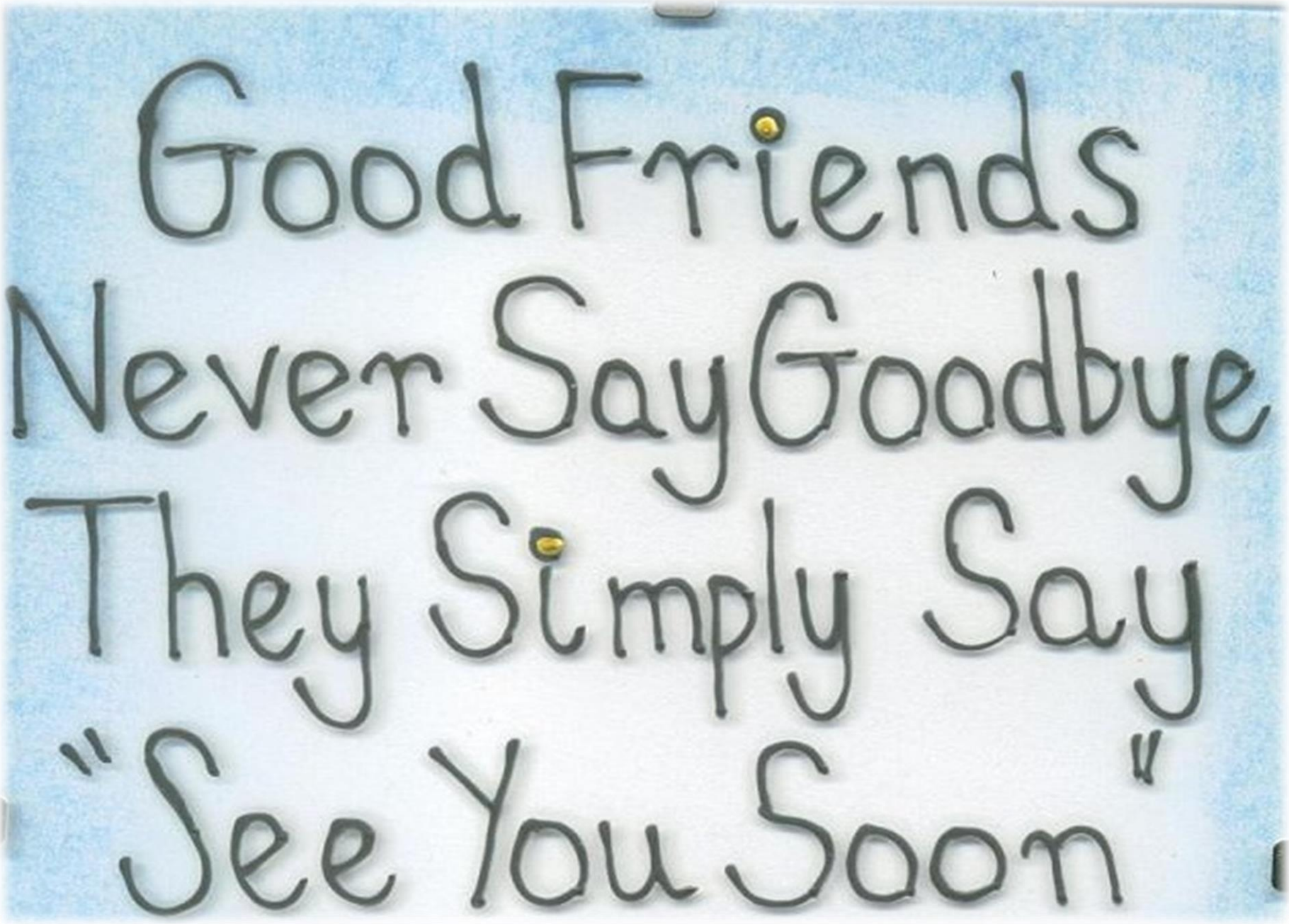
```
                                3'CTGTCAGTCAGTCAGTCA
                                  | ||||  ||
5' GATCGTCGATTACCAAATGCAGTCAGACAGTCAGTCAGTGAGTCCAGTCCCCATTCAG
```

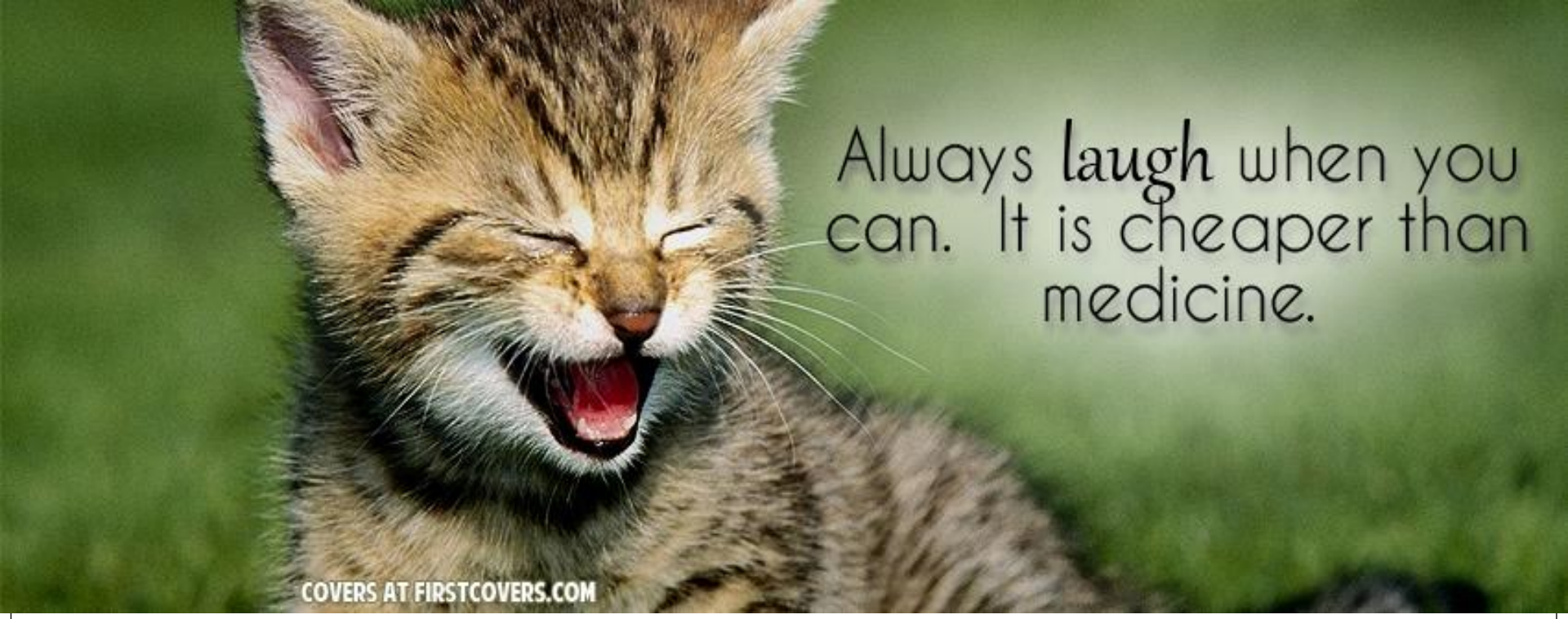
unstable complex

```
                              3'CTGTCAGTCAGTCAGTCA !
                                ||||||||||||||||
5' GATCGTCGATTACCAAATGCAGTCAGACAGTCAGTCAGTGAGTCCAGTCCCCATTCAG
```

stable complex


Transfers of new genes into animal organisms
Culture of Plants from Single cells
Diagnostics
Anti-cancer drugs
Cell culture
Monoclonal Antibodies
Molecular Biology
Crime solving
DNA technology
Genetic Engineering
Tracers
Banks of DNA, RNA and proteins
Complete map of the human genome
Synthesis of new proteins
New types of plants and animals
New types of food
New antibiotics
Cloning
Mass Production of human proteins
Resource bank for rare human chemicals
Gene therapy
Synthesis of specific DNA probes
Localization of genetic disorders


Good Friends
Never Say Goodbye
They Simply Say
"See You Soon"




# Thanks a lot

with my Best Regards and My Best wishes

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