BHARATHIDASAN UNIVERSITY

Program: M.Sc., Biomedical Science

Course Code : 18BMS47C10

Course Title : Genetic Engineering

Colony Hybridization

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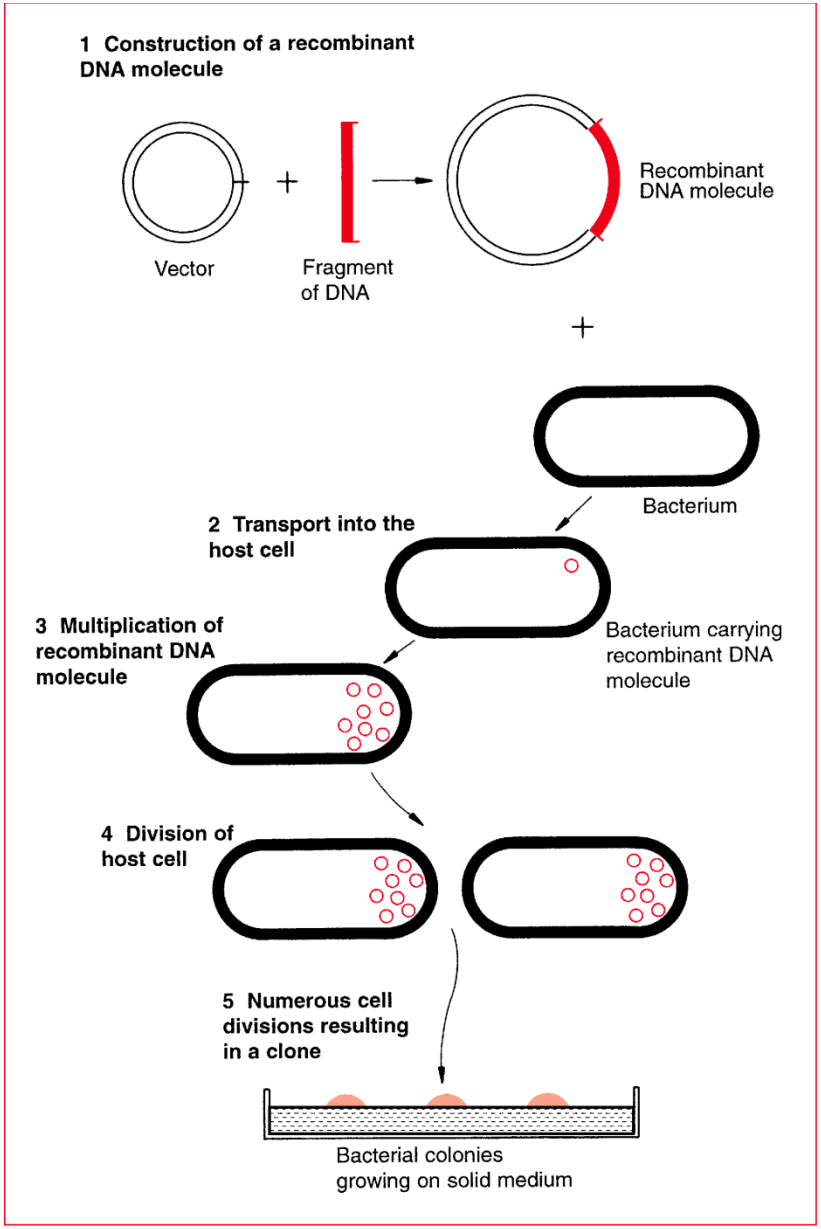
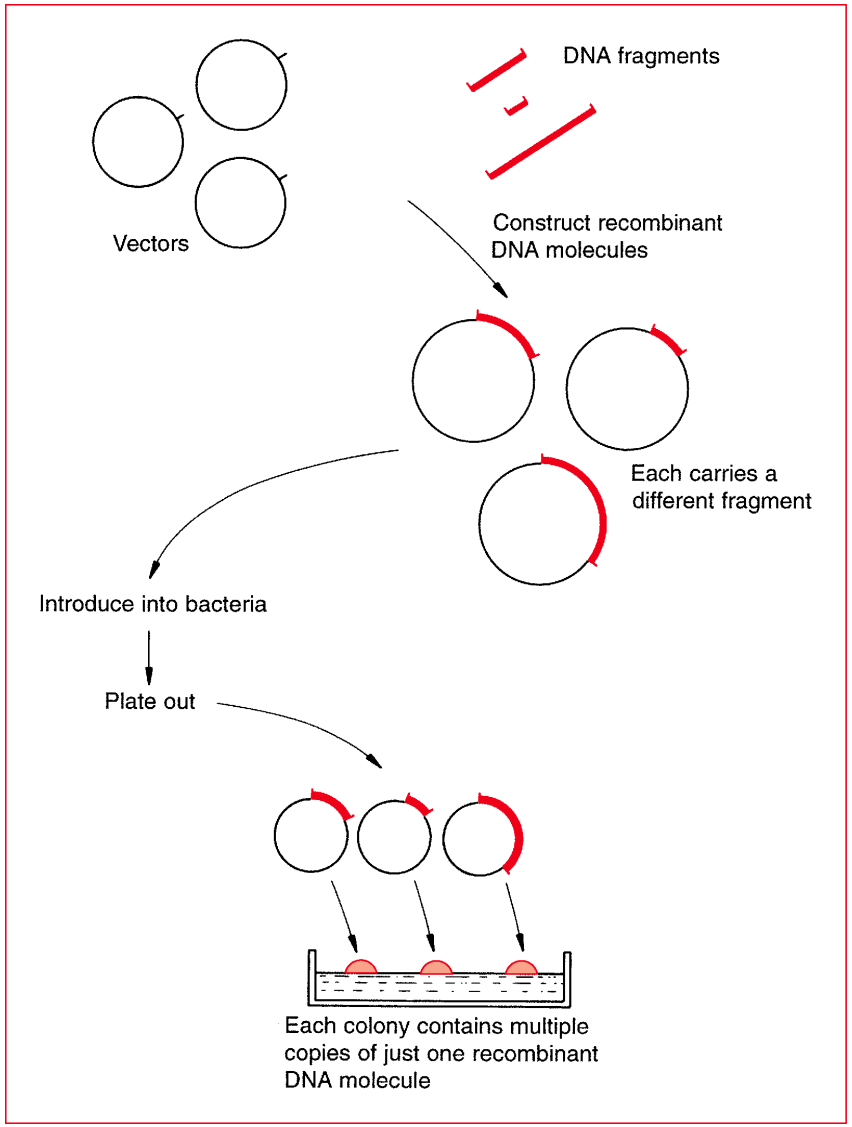
Dept. of Biomedical Science



The basic steps in gene cloning.

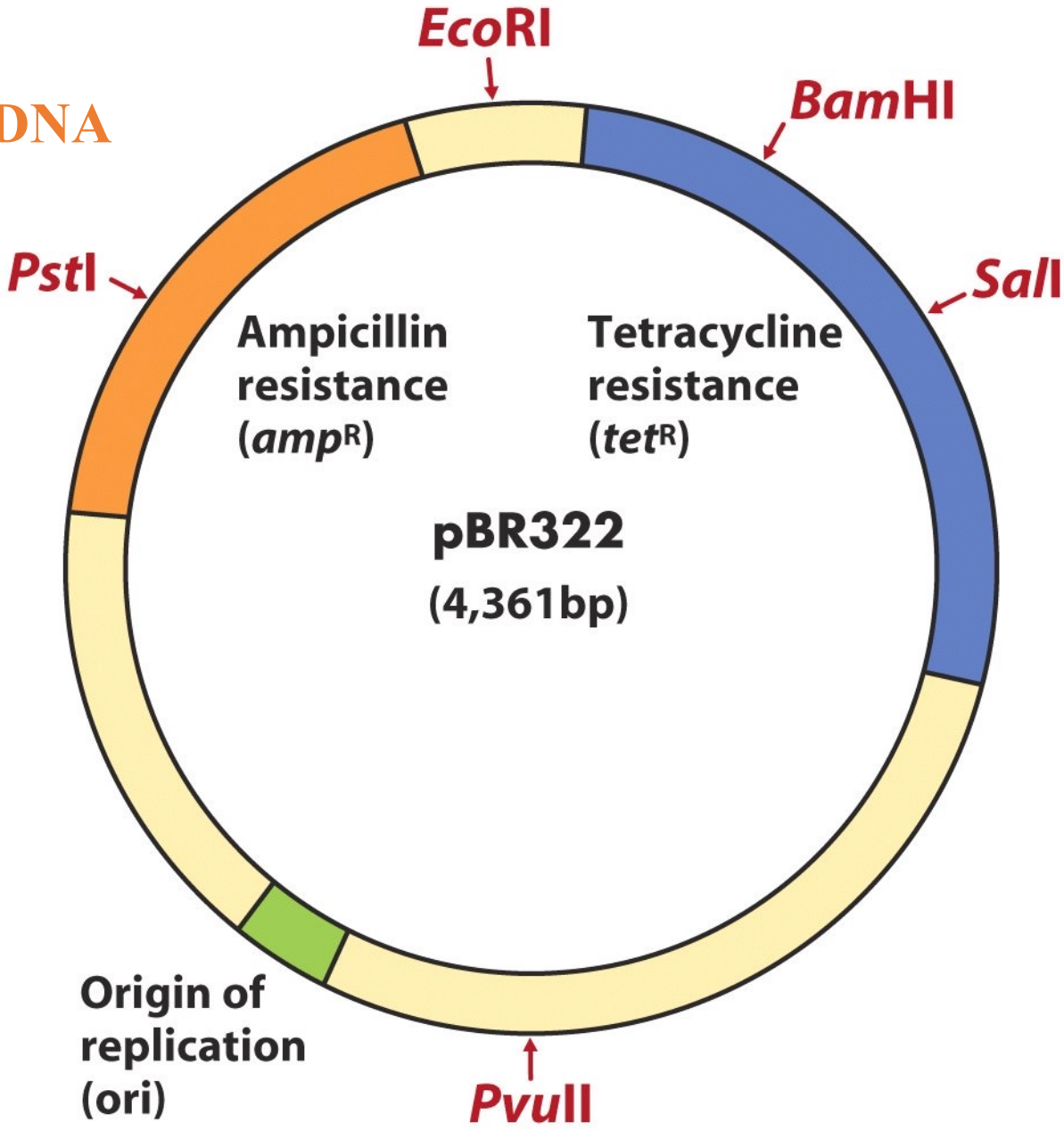


Cloning allows individual fragments of DNA to be purified.

Cloning vectors allow amplification of inserted DNA segments

E coli plasmid pBR 322

1. Origin of replication
2. Antibiotic resistance genes
3. Unique restriction sites
4. Small size



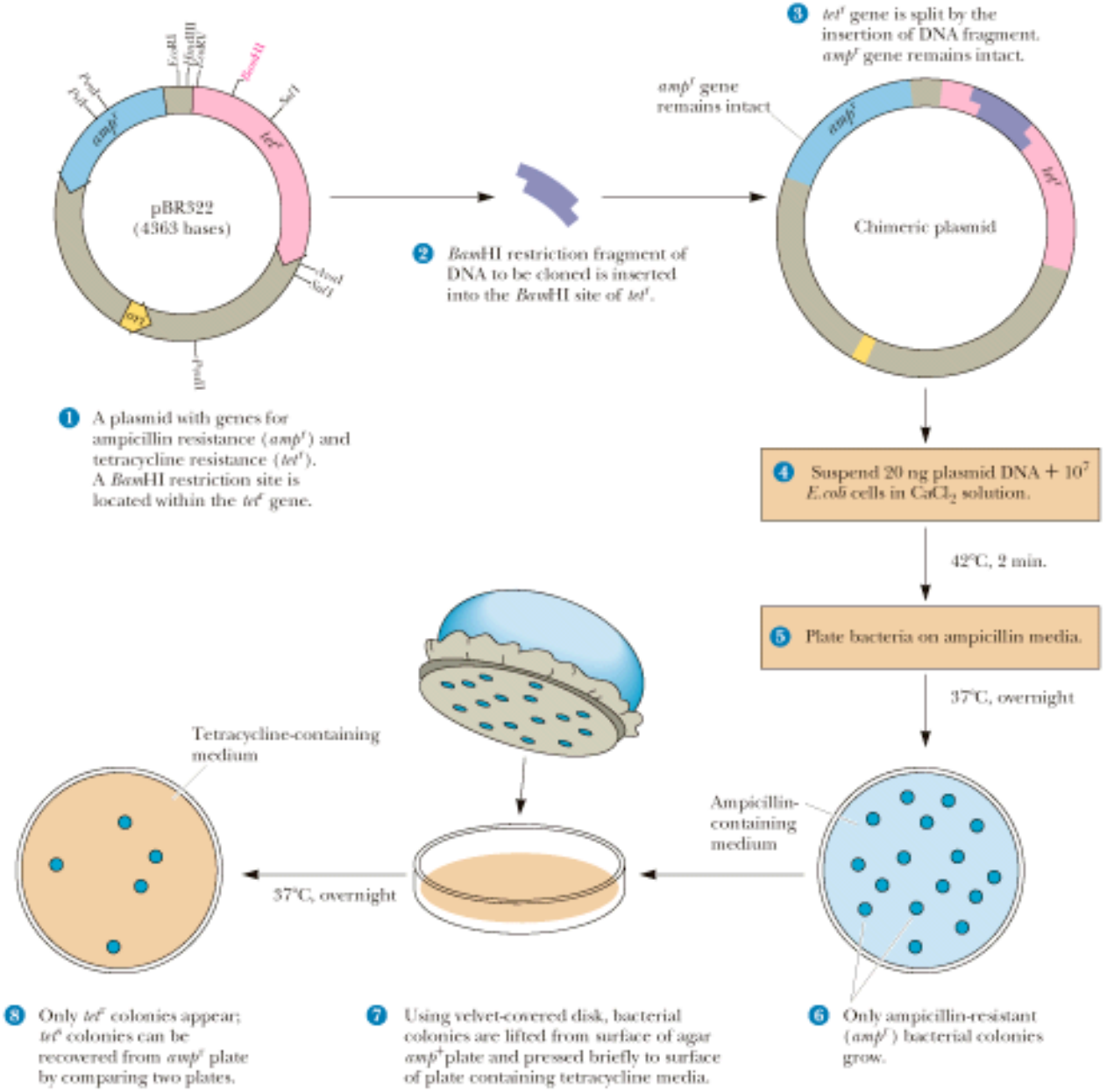
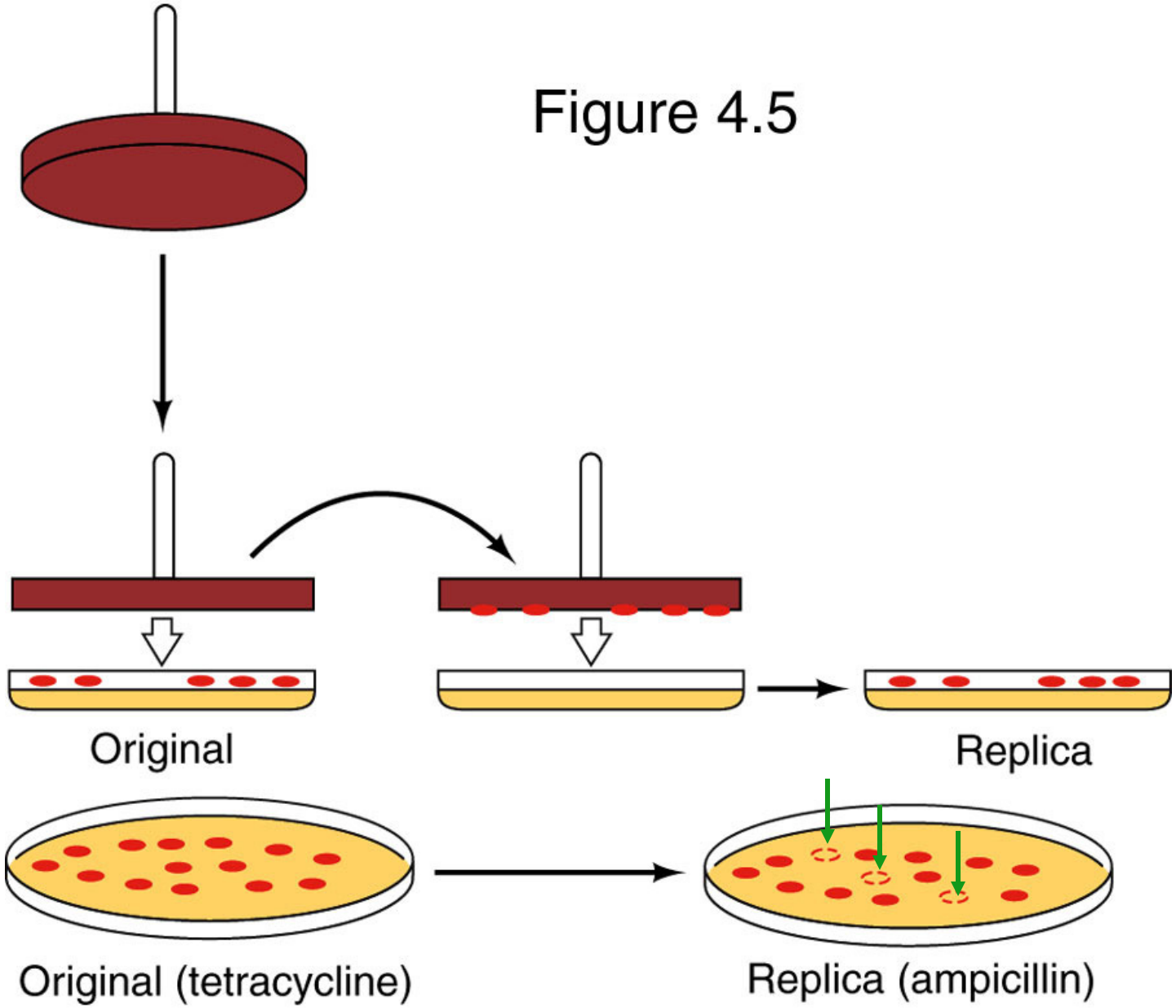
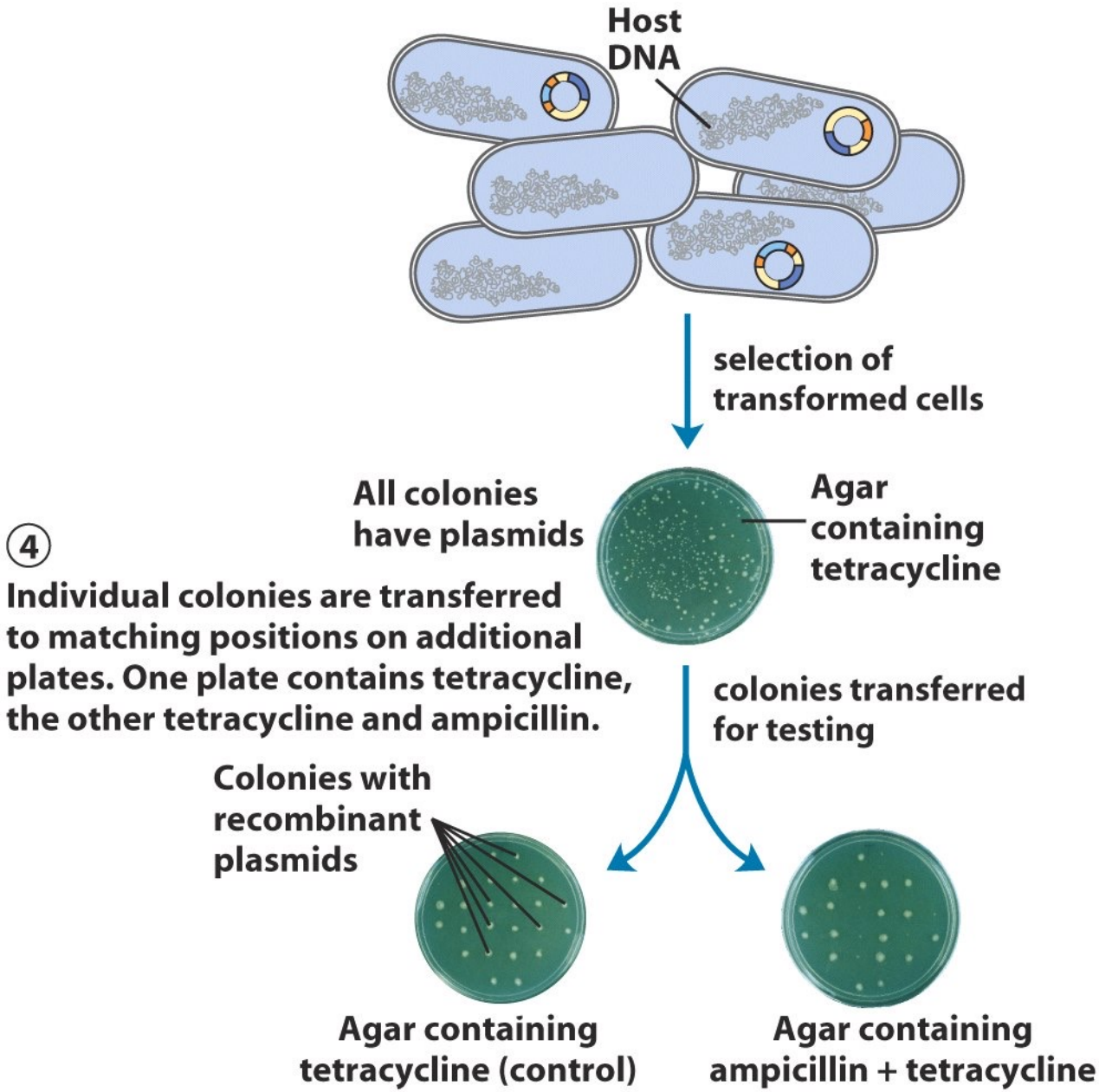

pBR322
(4363 bases)
amp^r
tet^r
ori
PvuII
PstI
EcoRI
HindIII
SalI
BamHI
AccI
ClaI
PvuII
1 A plasmid with genes for ampicillin resistance (amp^r) and tetracycline resistance (tet^r). A BamHI restriction site is located within the tet^r gene.
2 BamHI restriction fragment of DNA to be cloned is inserted into the BamHI site of tet^r.
3 tet^r gene is split by the insertion of DNA fragment. amp^r gene remains intact.
amp^r gene remains intact
Chimeric plasmid
4 Suspend 20 ng plasmid DNA + 10^7 E.coli cells in $CaCl_2$ solution.
42°C, 2 min.
5 Plate bacteria on ampicillin media.
37°C, overnight
Ampicillin-containing medium
6 Only ampicillin-resistant (amp^r) bacterial colonies grow.
7 Using velvet-covered disk, bacterial colonies are lifted from surface of agar amp^+ plate and pressed briefly to surface of plate containing tetracycline media.
37°C, overnight
Tetracycline-containing medium
8 Only tet^r colonies appear; tet^s colonies can be recovered from amp^r plate by comparing two plates.

Figure 4.5


Original
Replica
Original (tetracycline)
Replica (ampicillin)

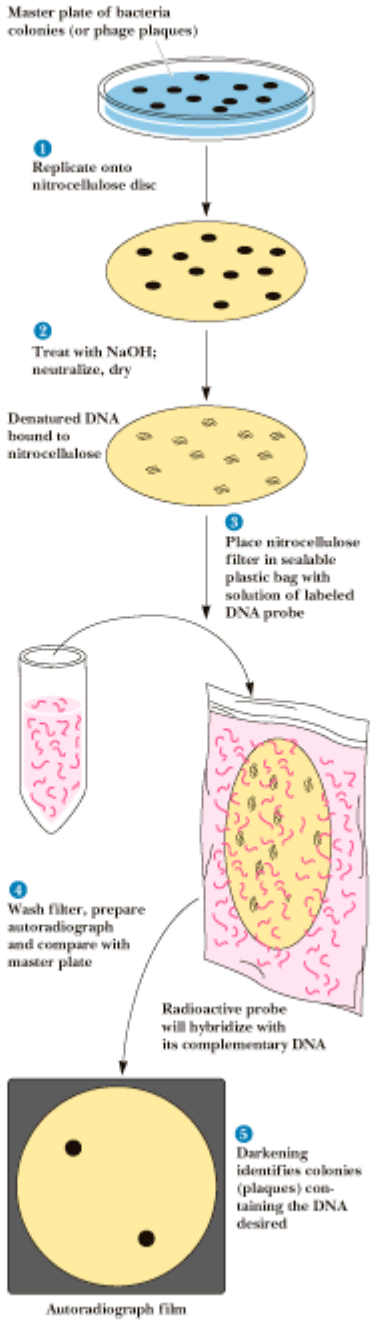

Host
DNA
selection of
transformed cells
All colonies
have plasmids
Agar
containing
tetracycline
④
Individual colonies are transferred
to matching positions on additional
plates. One plate contains tetracycline,
the other tetracycline and ampicillin.
colonies transferred
for testing
Colonies with
recombinant
plasmids
Agar containing
tetracycline (control)
Agar containing
ampicillin + tetracycline

Colony Hybridization

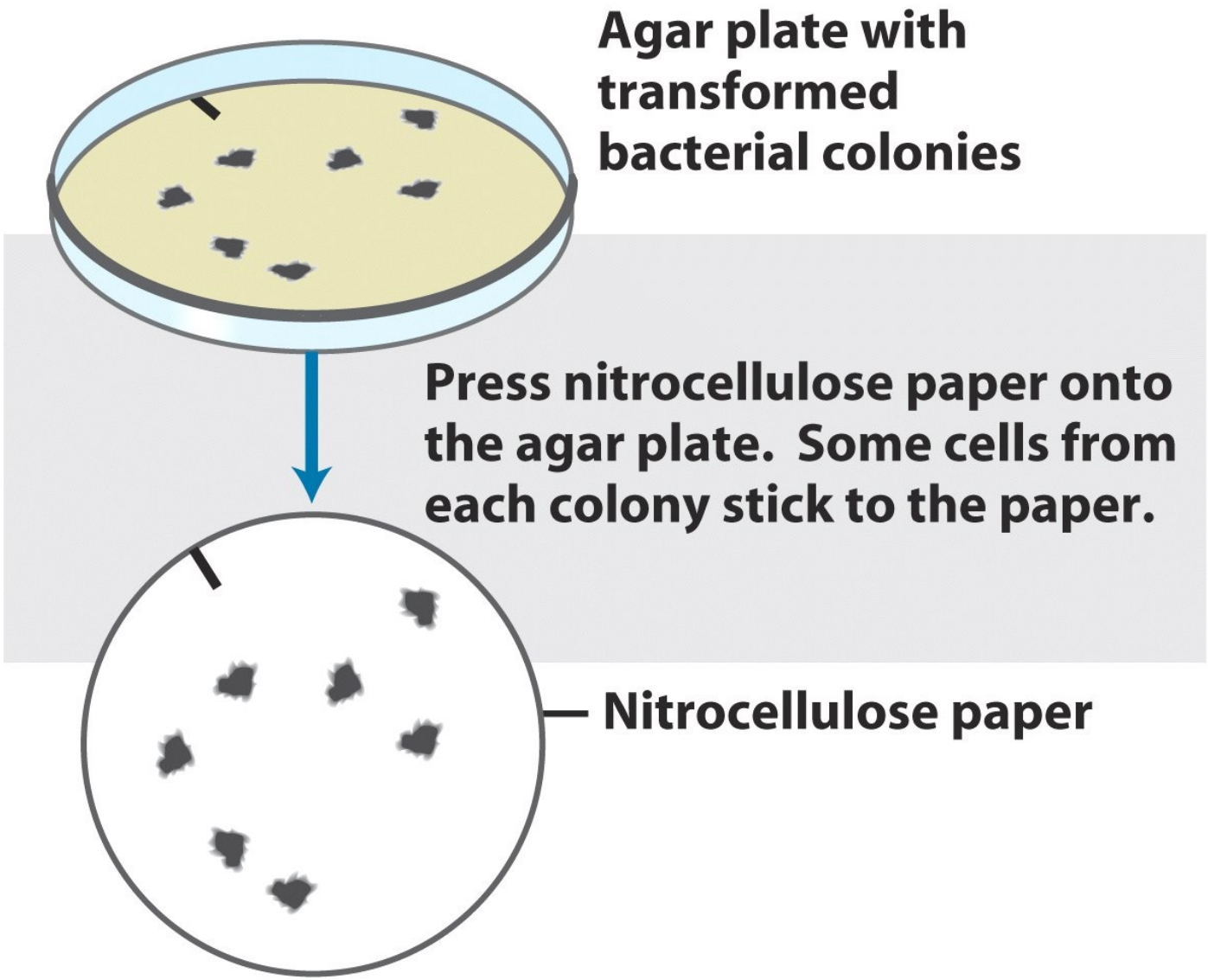
A way to screen plasmid-based genome libraries for a DNA fragment of interest

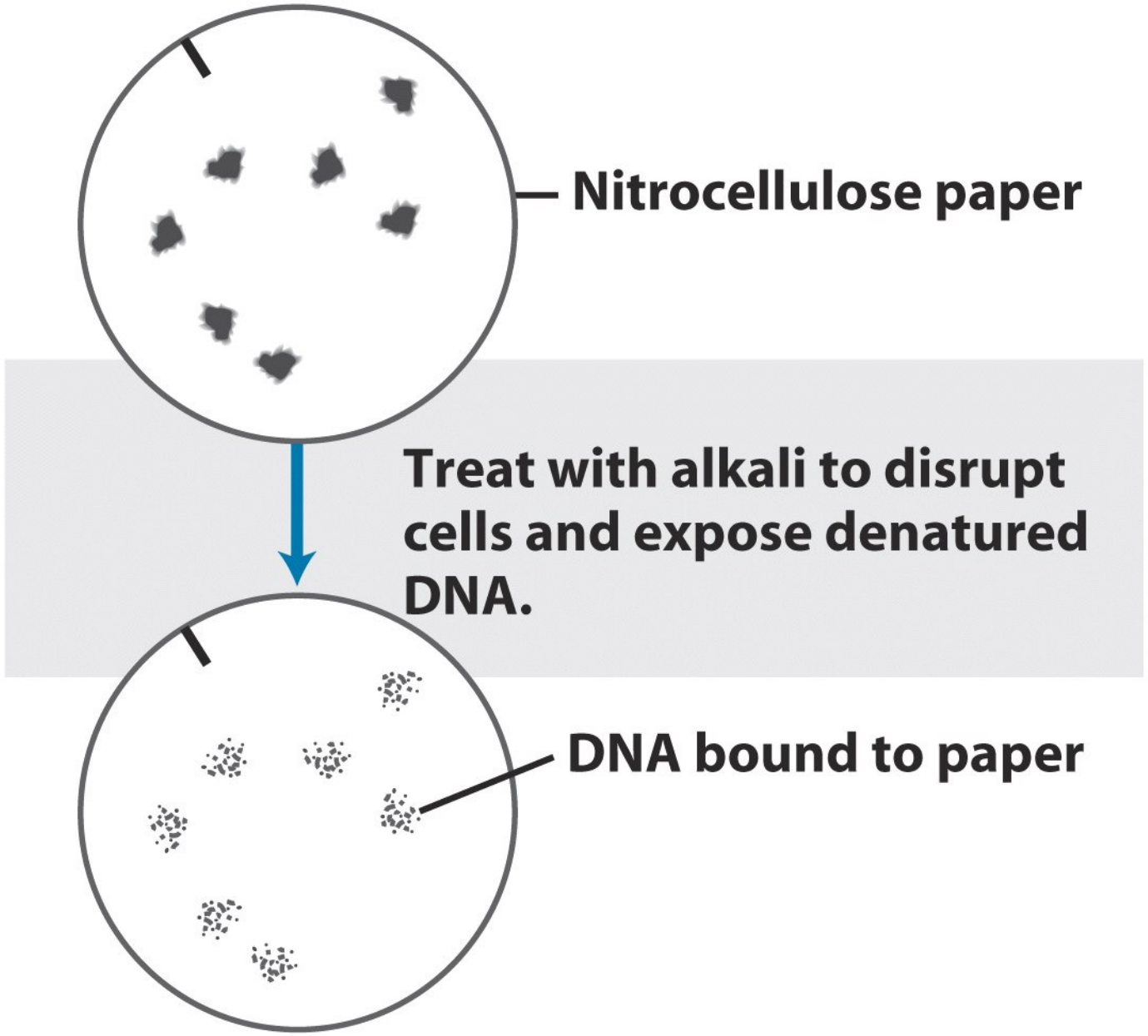
- Host bacteria containing a plasmid-based library of DNA fragments are plated on a petri dish and allowed to grow overnight to form colonies
- Replica of dish made with a nitrocellulose disk

- Disk is treated with base or heated to convert dsDNA to ssDNA and incubated with probes
- Colonies that bind probe (with P-32) hold the fragment of interest



Specific sequences are detectable by hybridization




Nitrocellulose paper
Treat with alkali to disrupt cells and expose denatured DNA.
DNA bound to paper

45°C is enough to denature this

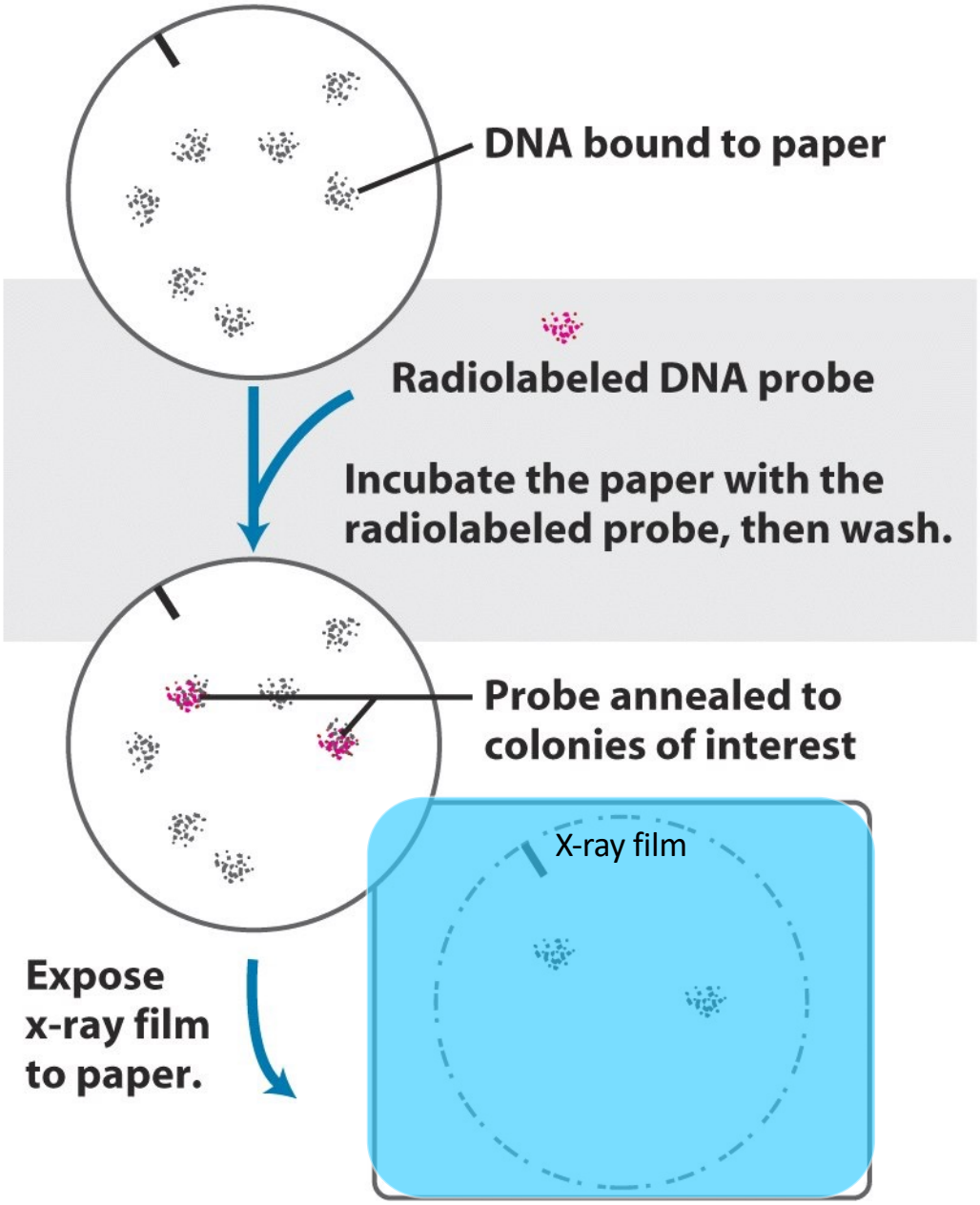
```
TGACGCATGCCCCCC CACTGCTAGCTGCACGAACCGGAATGCTGTGCATGCTT
|||||||||||    |  |     ||||||||||||       ||       |||||
ACTGCGTACGTCCGTGACGTGATCGACGTGCAACCGGTTAGCTGACGTACGTT
```

60°C is need to denature this

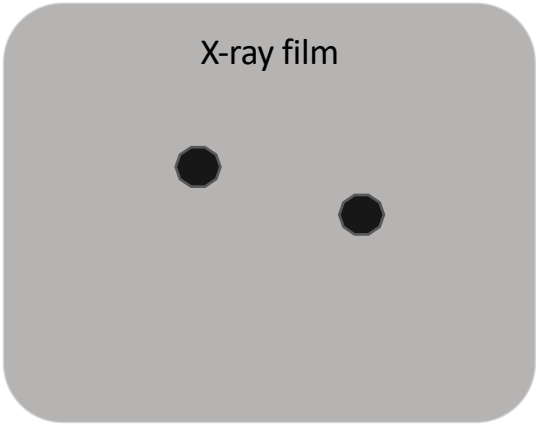
```
TGACGCATGCACCCACTGCACTAGCTGCTCGTTGGCCAATCGACTCGATGCAA
|||||||||||    |||||||||||||||||||||||||||||||||||  ||||||
ACTGCGTACGTCCGTGACGTGATCGACGTGCAACCGGTTAGCTGACGTACGTT
```

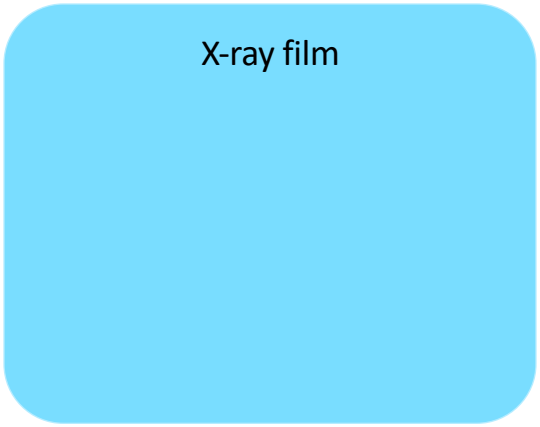
100°C is need to denature this

```
TGACGCATGCAGGCACTGCACTAGCTGCACGTTGGCCAATCGACTGCATGCAA
|||||||||||||||||||||||||||||||||||||||||||||||||||||
ACTGCGTACGTCCGTGACGTGATCGACGTGCAACCGGTTAGCTGACGTACGTT
```


DNA bound to paper
Radiolabeled DNA probe
Incubate the paper with the radiolabeled probe, then wash.
Probe annealed to colonies of interest
X-ray film
Expose x-ray film to paper.

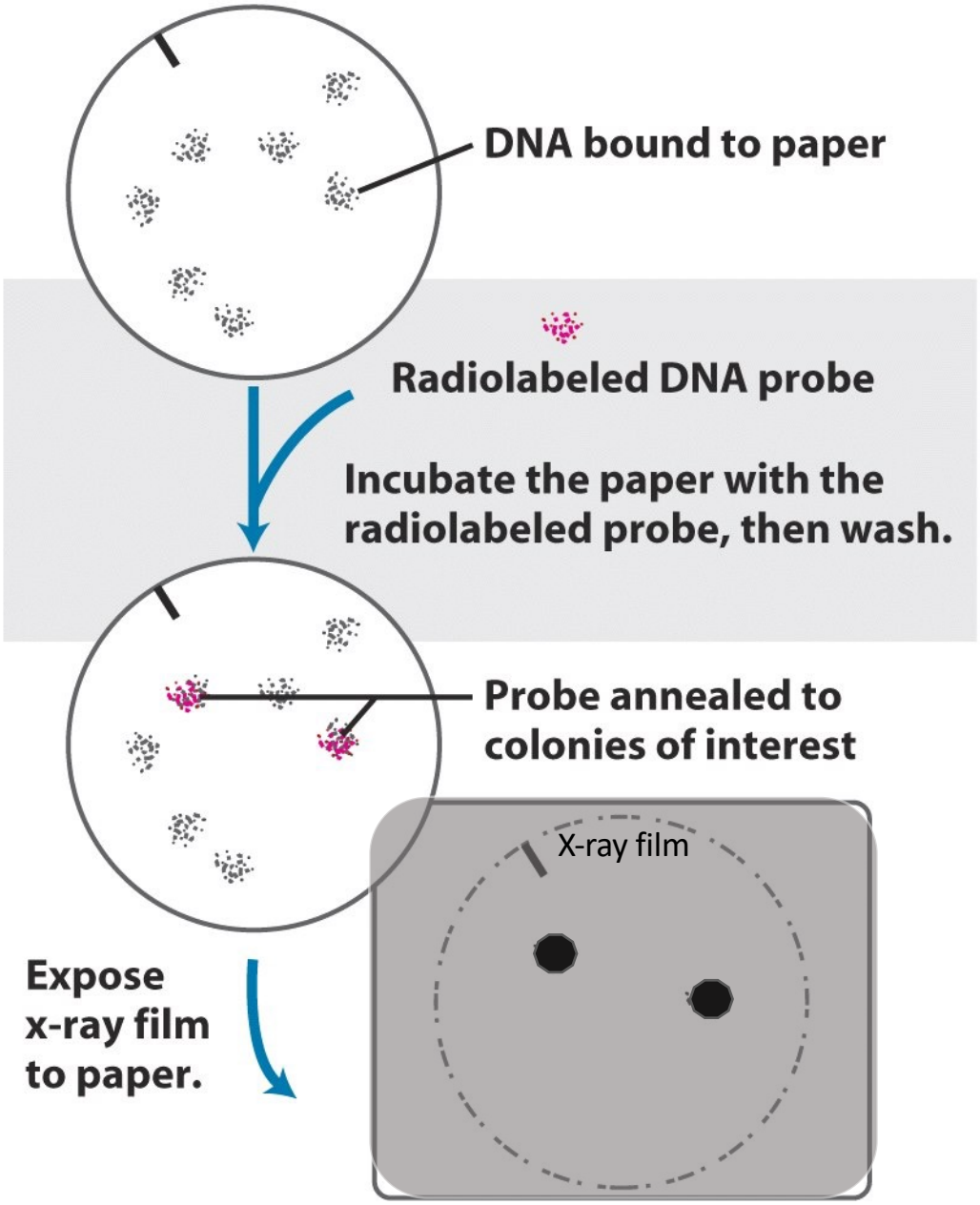
Super imposition of blot and X-ray film





Photographic Processing of X-ray film

Exposure of blot to X-ray film for 2hr to overnight


DNA bound to paper
Radiolabeled DNA probe
Incubate the paper with the radiolabeled probe, then wash.
Probe annealed to colonies of interest
X-ray film
Expose x-ray film to paper.