
# UG Diploma in Biotechnology
# Paper III Unit-1
# Transformation in *E. coli* & its different host strains

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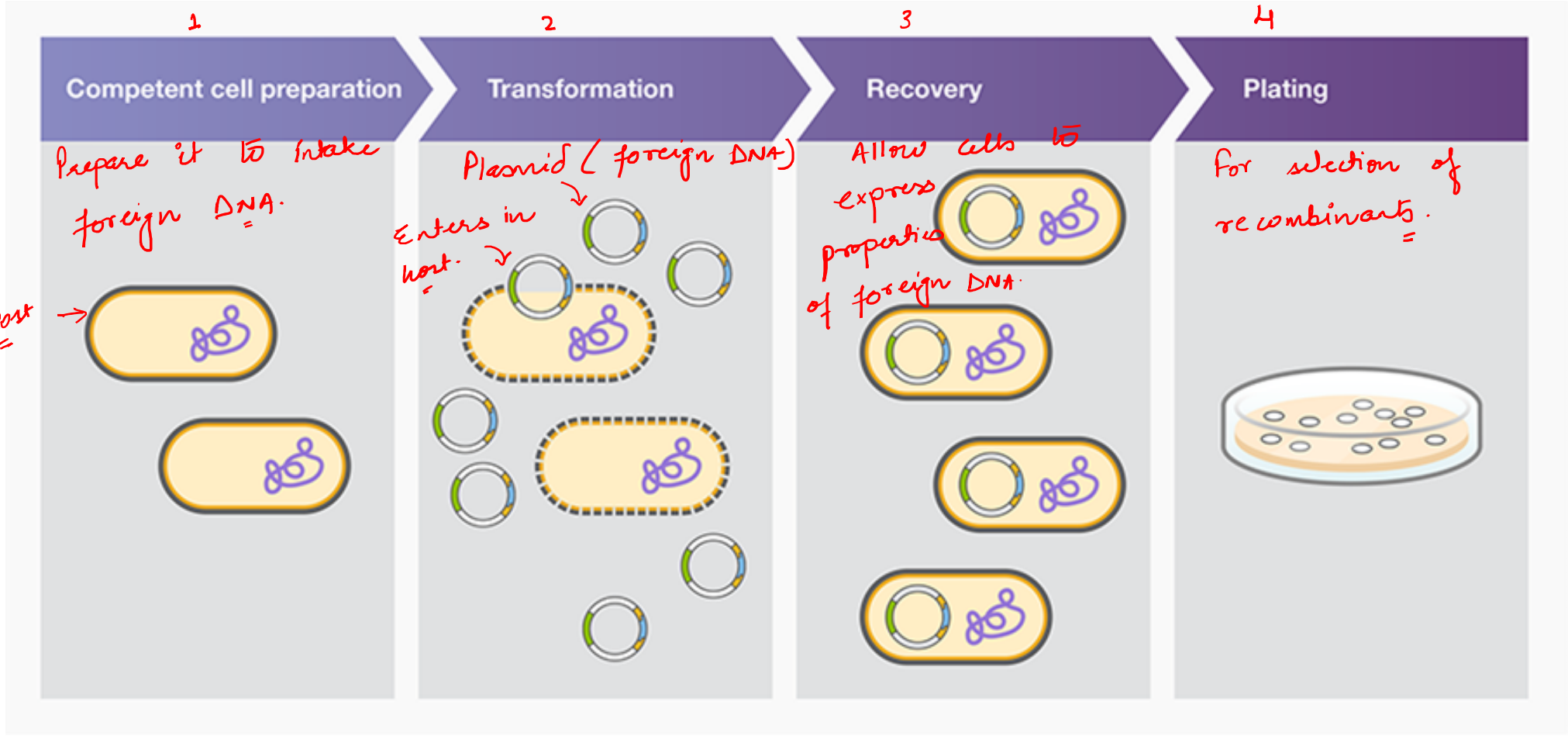
- **Transformation:** → especially used for bacterial host.
- It refers to entry of the foreign DNA such as plasmids into a host.
- Generally, this term is used in context of bacterial host.
- But during discussion we also loosely use the word transformation to convey that a recombinant DNA has been inserted in to a host cell of any kind.

- So unless mentioned otherwise, transformation refers to entry of plasmid or other recombinant DNA in a bacterial host and the DNA should be naked. → Not be in any envelope / No covering around DNA.
  - viral capsid
  - liposome

- Bacterial transformation has 4 steps:

1. Preparation of competent cells
2. Transformation
3. Cell recovery
4. Cell plating

# Steps in bacterial transformation



- **Preparation of competent cells:**

- *E. coli* is the most common bacterial species used in the transformation step of a cloning workflow.
- Since the natural competency of *E. coli* is very low or even nonexistent, the cells need to be made competent for transformation by heat shock or by electroporation.
- We will be discussing heat shock method as it is most widely used.
- In this, we take a single fresh colony of the desired *E. coli* strain is from an agar plate and inoculated into liquid medium for a starter culture.

Method used for making competent cells is suitable for heat shock transformation

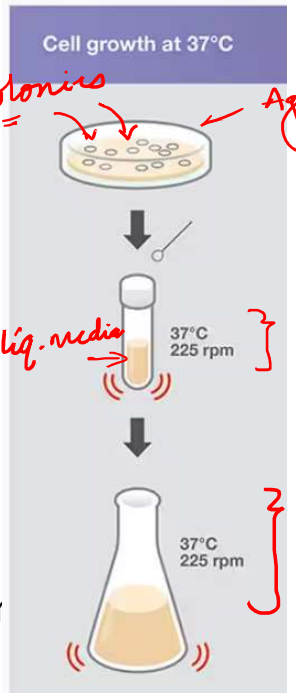
- This starter culture and the subsequent larger culture are carefully monitored for active growth by continually measuring optical density at 600 nm ($OD_{600}$).

spectrophotometer. mid log phase. $OD_{600}$

- To obtain high transformation efficiency, it is crucial that cell growth be in the mid-log phase at the time of harvest—which generally occurs at $OD_{600}$ between 0.4 and 0.9.

Generally = $OD_{600}$ = 0.5 to 0.6

- Harvest the cells and use process them to make competent cell suitable for the heat shock method.



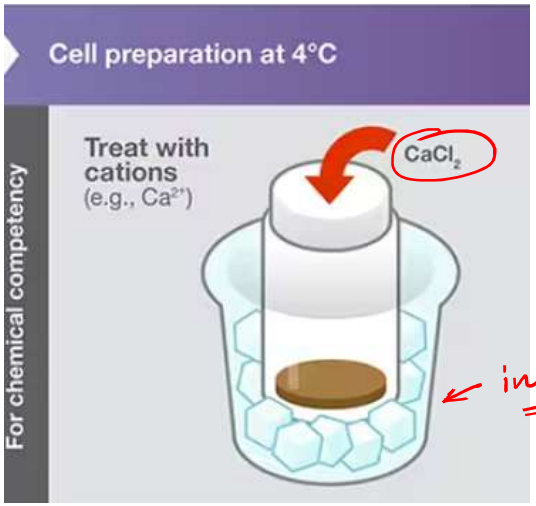

starter culture small scale

starter culture large scale.

- **Processing to make competent cells for Heat-shock transformation:**
- Competent cells are chemically prepared by incubating the cells in calcium chloride ($CaCl_2$) to make the cell membrane more permeable.
- To further improve competency, $Ca^{2+}$ may be supplemented or substituted with other cations and reagents, such as manganese ($Mn^{2+}$), potassium ($K^+$), cobalt ($[Co(NH_3)_6]^{3+}$), rubidium ($Rb^+$), dimethyl sulfoxide (DMSO), and/or dithiothreitol (DTT).

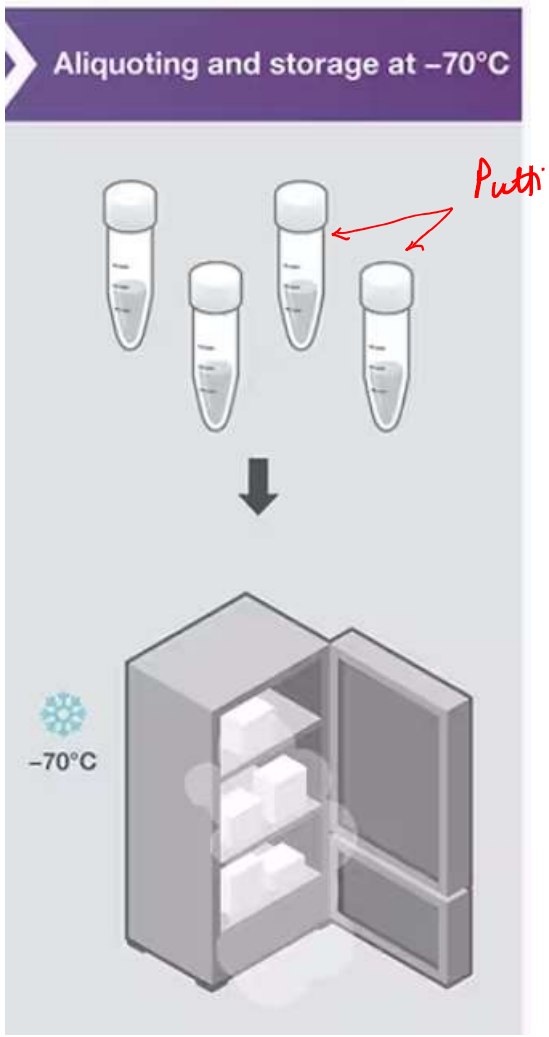
- Once prepared, competent cells should be evaluated for transformation efficiency.
- The **transformation efficiency** of competent cells is measured by the uptake of subsaturating amounts of a supercoiled intact plasmid (e.g., 10–500 pg of pUC DNA). → pUC plasmid.
- The results are expressed as the number of colonies formed (transformants), or colony forming units (CFU), per microgram of plasmid DNA used (CFU/µg).

Competent cell making →



- After this, competent cells are aliquoted to small volumes to minimize freeze/thaw cycles, and stored at -70° C to maintain viability.

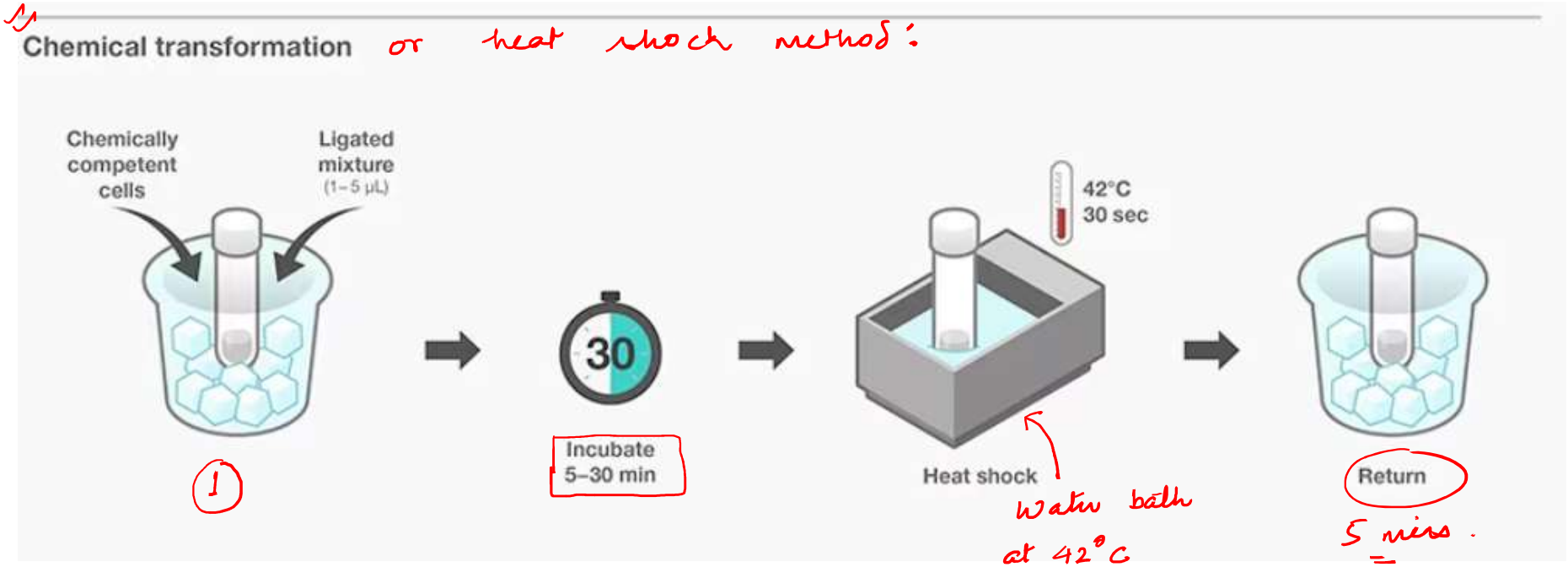
- Since the cells are made competent using chemical treatment, we also call them chemically competent cells.




Putting into small tubes = Aliquoting.

- **Heat shock method of transformation:**
- First, competent cells are incubated with plasmid DNA on ice for 5–30 minutes in a polypropylene tube.
- For successful chemical transformation, 50–100 µL of competent cells and 1–10 ng of DNA are recommended.
- **Heat shock** is performed at 37–42°C for 25–45 seconds as appropriate for the bacterial strain and DNA used.
- Heat-shocked cells are then returned to ice for ≥2 minutes.

DNA doesn't stick to sides of the tube.

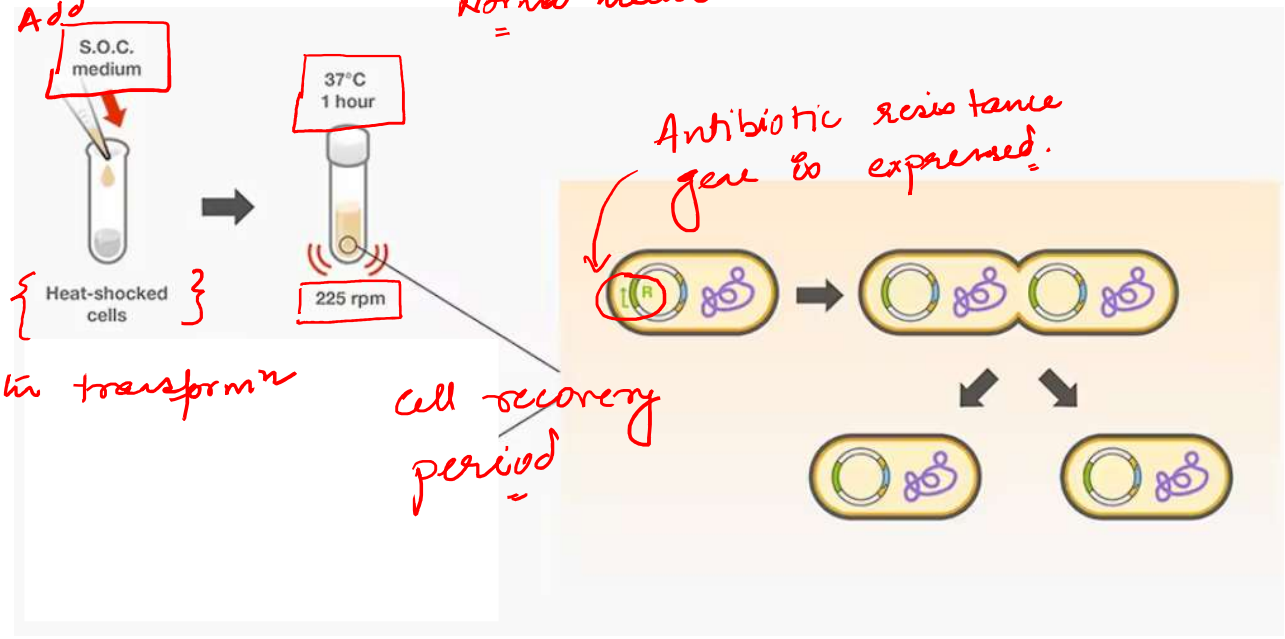



or heat shock method:

Water bath at 42°C

5 mins.

- **Cell recovery period :**
- Following heat shock, transformed cells are cultured in antibiotic-free liquid medium for a short period to allow expression of antibiotic resistance gene(s) from the acquired plasmid to begin.
- This step improves cell viability and cloning efficiency.
- In this step, transformed cells are cultured in 1 mL of prewarmed S.O.C. medium at 37°C with shaking at 225 rpm for 1 hour.
- S.O.C. medium, which contains glucose and $MgCl_2$, is recommended to maximize transformation efficiency.
- Use of S.O.C. medium, instead of Lennox L Broth (LB Broth), can increase formation of transformed colonies 2- to 3-fold.



- After growing in S.O.C. medium, the cells are plated on LB agar with appropriate antibiotic(s) or other agents for identification and recovery of successful transformants.
- For example, if blue/white screening is to be performed, X-Gal and IPTG must be included in the agar plate.

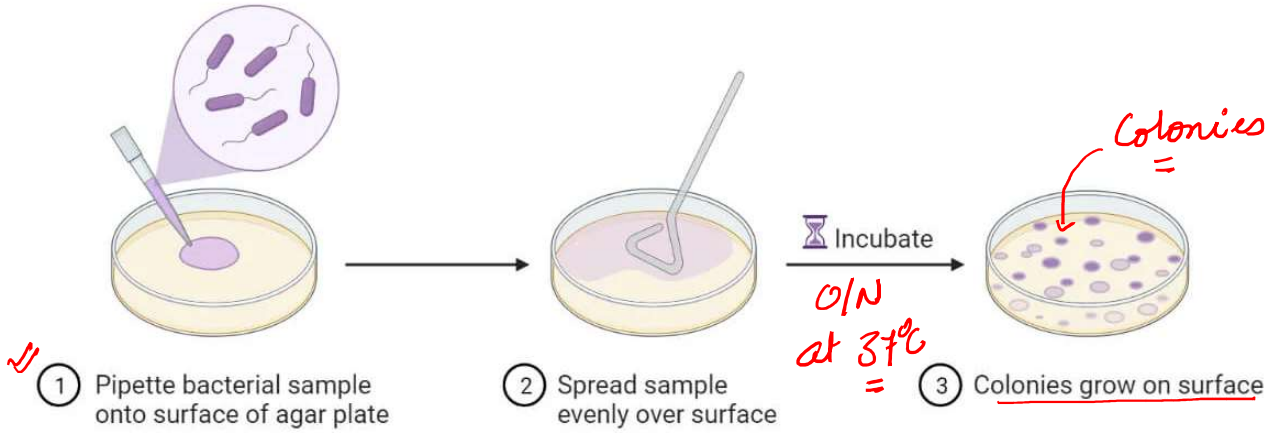
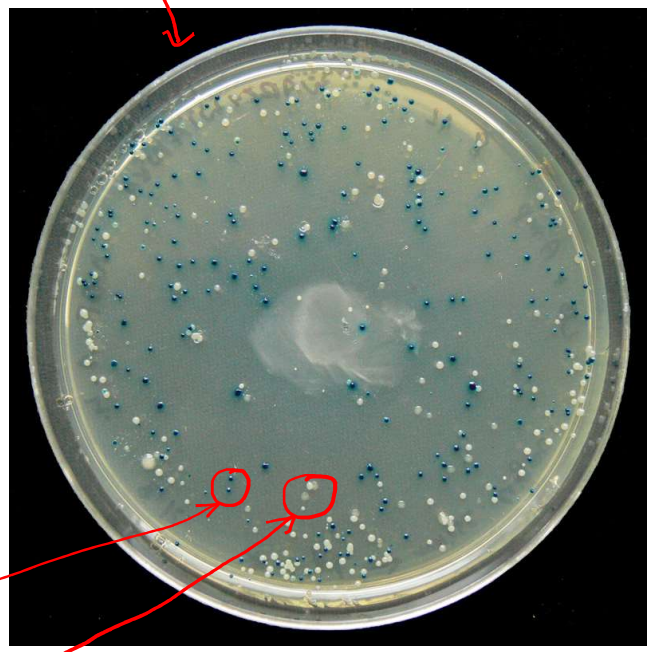



Plate showing blue - white screening



No insert

Insert present.

- **Different host strains and their uses:** ( E. coli )

- *E. coli* are gram-negative, rod shaped bacteria that were named after Dr. Theodor Escherich, the scientist who first described them in 1885.

- *E. coli* are mainly found in the intestinal tract of animals.

- There are many different naturally occurring strains of *E. coli*, some of which are deadly to humans.
- The majority of all common, commercial lab strains of *E. coli* used today are descended from two individual isolates, the K-12 strain and the B strain.

- K-12 was isolated from a patient in 1920 and eventually led to the common lab strains MG1655 and its derivatives DH5alpha and DH10b (also known as TOP10).

- The history of B strain is complex but it was likely isolated in 1918 but was first referred to as "B strain" in 1942.

- The BL21 strain and its derivatives are the most common examples of the *E. coli* B strain descendants.

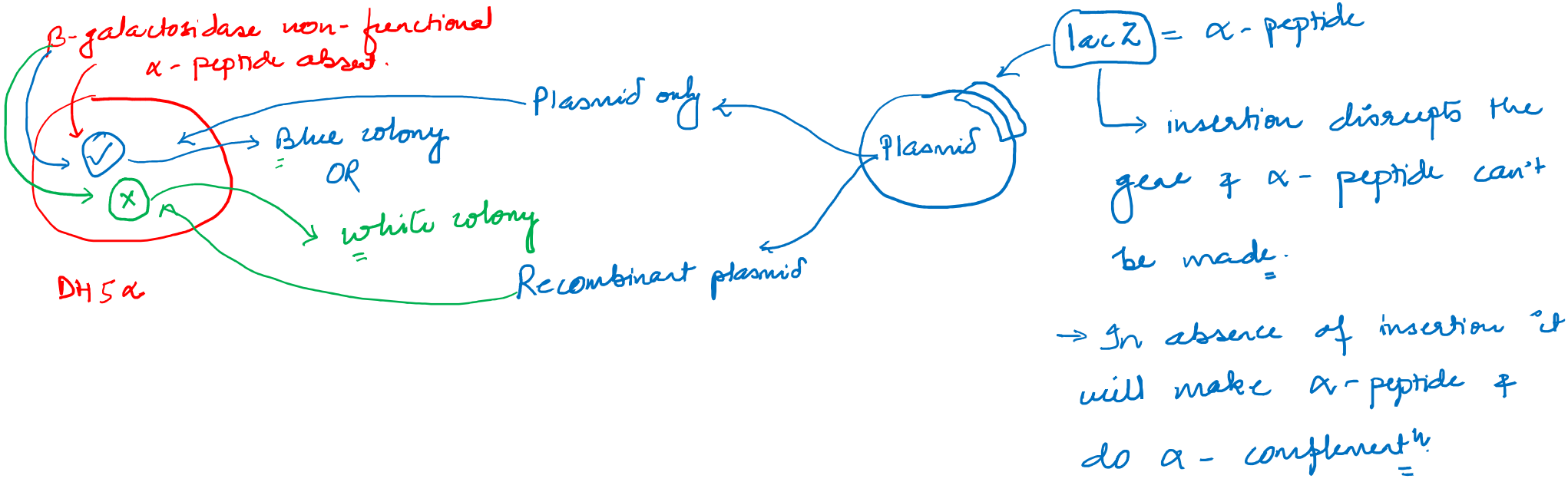
- **DH5α:**
- It is used for general cloning and sub-cloning applications. (Not used for protein expression)
→ Its genotype facilitates cloning but not expression of the gene.
- Major features are:
  - Blue/white color screening with *lac*ZΔM15 ← host's having this mutn show non-functional β-galactosidase due to absence of functional α-peptide.
  - High insert stability due to *rec*A1 mutation
  - High yield and quality of DNA due to *end*A mutation

- DH5 Alpha *E. coli* cells are genetically engineered by American biologist Douglas Hanahan to maximize the efficiency of transformation.

Imp recA protein = Recombinase which could remove the insert from plasmid.

- recA1 is a single point mutation that replaces glycine 160 with and an aspartic acid residue in the recA polypeptide. This mutation disables the activity of the recombinases and inactivates homologous recombination.
(*)
↳ non-functional. leads to high insert stability.

- endA1 mutation inactivates an intracellular endonuclease which prevents it from degrading the inserted plasmid.
- lacZΔM15 mutation enables blue-white screening of transformed cells.
- They are often used with calcium chloride transformation to insert the desired plasmid.



- **BL21 strain:**
- It is protease deficient strain which is mainly used for expression of foreign proteins in *E.coli* cells.
- BL21 was first described by Studier in 1986 after various modifications of B line.
- BL21 cells are deficient in the LON protease, which degrades many foreign proteins.
- It is also missing another gene that codes for the outer membrane protease (OmpT) whose function is to degrade extracellular proteins.
- Due to lack of these proteins triggers the successful production of foreign protein in *E. coli* BL21 cells.
- Furthermore, hsdSB mutation in these cells prevents foreign DNA methylation and disruption in the BL21 cells.

- **How we make different strains of *E.coli*??**
- These strains are made for different purposes such as fast growth, high-throughput cloning, routine cloning, cloning unstable DNA, preparing unmethylated DNA**.**

- But to achieve the desired set of features, the host cells must have certain modifications in them.
- These modifications are induced in them by way of mutations.
- The list of most common mutations induced is given on the next page.

| Gene(s) | Description | Functional Consequence |
|---|---|---|
| **dam** | DNA adenine methylase mutation (GATC) | Preparing unmethylated DNA, important when trying to cut with certain restriction enzymes (ex: ClaI or XbaI) |
| **dcm** | DNA cytosine methylase mutation (CCWGG) | Preparing unmethylated DNA, important when trying to cut with certain restriction enzymes that are methylation sensitive. |
| **dnaJ** | Mutation in a chaperonin gene | Increases the stability of certain expressed proteins |
| **endA, endA1** | Endonuclease I (nonspecific cleavage of dsDNA ) mutation | Improves plasmid yield |
| **F** | Host does (F') or does not (F-) contain the fertility plasmid. | A low copy-number plasmid, encodes proteins needed for bacterial conjugation. Genes listed on F´ are wild-type unless indicated otherwise |
| **fhuA (formerly tonA)** | ferric hydroxamate uptake, iron uptake receptor mutation. | T1/T5 Phage resistance |
| **gal** | Mutation in galactose metabolism pathway | Cells cannot grow on galactose only |
| **gyrA, gyrA96** | DNA gyrase mutation | Confers resistance to nalidixic acid |
| **hsdRMS** | hsdR(rk-, mk+)<br>hsdS(rk-,mk-) | Unmethylated DNA not degraded, cell still can methylate DNA<br>Unmethylated DNA not degraded, cell cannot methylate DNA |

| | | |
|---|---|---|
| **lac** | Lac operon mutations | Blue/white screening of clones |
| | lacIq | lac repressor overproduced, expression from pLac repressed more |
| | LacZ | β-galactosidase activity abolished |
| | lacY | Lactose permease inactivated, lactose cannot be taken up by cell |
| **mcrA, mcrBC** | Inactivation of pathway that cleaves methylated cytosine DNA | Allows for uptake of foreign (methylated) DNA |
| **mrr, Δ(mcrC-mrr)** | Inactivation of pathway that cleaves methylated adenine or cytosine DNA | Allows for uptake of foreign (methylated) DNA |
| **recA, recA1, recA13** | Mutation in a DNA-dependent ATPase that is essential for recombination and general DNA repair | Reduces plasmid recombination, increases plasmid stability |
| **recBCD** | Exonuclease V activity abolished | Increased plasmid stability, reduced recombination |
| **relA or relA1** | Relaxed phenotype, mutation eliminating stringent factor | Allows RNA synthesis in absence of protein synthesis |
| **Ptrc-ccdA** | | Propagation of ccdB-containing plasmids |
| **Hte** | | High transformation efficiency |
| **deoR** | constitutive expression of genes for deoxyribose synthesis | Allows uptake of large plasmids |
| **hee** | | High electroporation efficiency |
| **supE44 (glnV44)** | | Suppression of the amber (UAG) stop codon by glutamine insertion |