Rapid colony transformation of e - coli with plasmid dna - lab report example

Health & Medicine

Rapid Colony Transformation of E -Coli with Plasmid DNA

2. Incubation: The DNA is added and the cell suspension is kept at 0C. the cations are thought to neutralize negatively charged phosphates in the DNA and the cell membrane.

3. Heat Shock: The cell +DNA suspension is briefly incubated at 42C and then returned to 0C. The rapid temperature change creates a heat imbalance on either side of the E-Coli membrane and is supposed to create a wave that sweeps plasmids into the cell.

4. Recovery: LB broth is added to the DNA/cell suspension and incubated at 37C before being put on plates with different selected antibiotic-resistant markers. Transformed cells recover from the treatment, amplify the transformed plasmid, and begin to express the antibiotic-resistant strain.

Method: Samples of E-coli cells are taken from a nutrient agar plate (LB agar) and suspended in two tubes containing a solution of calcium chloride. Plasmid pAMP is added to one cell suspension. Both the tubes are then incubated at 0C for 15 minutes. After this, a brief heat shock is administered at 42 . the samples are cooled and LB broth is added. Samples of both the cell suspensions, one with just the E-coli and the other with E-Coli with added plasmids-are put on two plates with two types of growth agent, LB agar and LB agar ampicillin (LB /amp)

The plates are then incubated for 12 to 24hours at 37and then checked for bacterial growth. Only cells that have been transformed by absorbing the plasmid DNA with the ampicillin-resistant gene will grow on the /amp plate.

Results:

Subsequent division of an antibiotic-resistant cell will produce a colony of resistant clones. Thus, each colony on an ampicillin plate represents a single transformation event.

Ampicillin is the most practical antibiotic -resistance marker because of its capability to rapidly transform. Ampicillin interferes with the construction of the layer of the cell wall and restricts itself to killing only replicating cells that are creating new cell envelopes. On-replicating E-coli is untouched. Thus, cells can be put on plates with the ampicillin medium directly after heat shock, omitting the recovery step.

Conclusion: By itself-Coli cannot form ampicillin resistance, but the addition of plasmid DNA that contains an ampicillin-resistant gene (pAMP) produce a colony of resistant clones. Plates containing E-coli are used for the laboratory source. These cells are best used within 12-24 hours of incubation. However, it can be noted that good results are obtained when cells are also stored at room temperature for 1-2 additional days. This causes the E-coli colonies to proliferate and makes it easy to take samples.