

Week 1st:

Get the vector with P_{sal} promoter and double digest it with X,P.

Get the insert of GFP gene and double digest it with S,P

Ligase the two part together in order to construct the plasmid of P_{sal} promoter and GFP,

Transfer the plasmid into trans 5a bacteria

Get the vector with P_{bad} promoter and double digest it with X,P.

Get the insert of GFP gene and double digest it with S,P

Ligase the two part together in order to construct the plasmid of P_{bad} promoter and GFP,

Transfer the plasmid into trans 5a bacteria

Week 2nd:

Get the vector with T7 promoter and double digest it with X,P.

Get the insert of GFP gene and double digest it with S,P

Ligase the two part together in order to construct the plasmid of T7 promoter and GFP,

Transfer the plasmid into trans 5a bacteria

Week 3rd:

Make the different types of protection media with gradient of trehalose PVP.

Week 4th:

Use IPTG to induce the fresh cell containing plasmid of T7 promoter and GFP gene to express GFP.]

Detect it with flow cytometry assay and enzyme-linked immunosorbent assay.

Determine the response curve of the cell, and the intensity of GFP expression level.

Week 5th:

Transfer the cells of T7 promoter and GFP, Mer promoter and GFP into protection media and dry them in the water pump. Store in fridge in 4° C.

Week 6th:

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Week 7th:

recover the stored cells and use cytometry assay and enzyme-linked immunosorbent assay, the same methods used in fresh cells to determine the same parameter.

Week 8th:

recover the stored cells and use cytometry assay and enzyme-linked immunosorbent assay, the same methods used in fresh cells to determine the same parameter.

Week 9th:

recover the stored cells and use cytometry assay and enzyme-linked immunosorbent assay, the same methods used in fresh cells to determine the same parameter.

Week 10th:

collect the figure and data achieved in the previous three weeks to see how the condition and vigor of cells change. Determine which kind of protection media is the best.