

Generation of Transcription Factor Constructs for Mammalian Transfection

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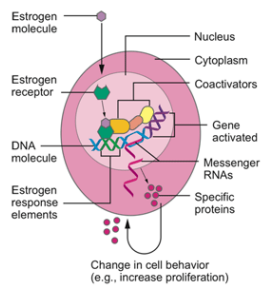
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INTRODUCTION

17 β -estradiol (E_2) is an ovarian steroid and regulates a number of target tissues, including the breast and uterus. The physiological effects of E_2 require binding to an estrogen receptor (ER), also a transcription factor (nuclear protein). Subsequent binding of the E_2 -ER complex to an estrogen response element (ERE) in a target gene allows recruitment of various other transcription factors and co-regulator proteins, which collectively serve to enhance or suppress gene expression. These actions ultimately regulate the production of proteins, the functional molecules that change the behavior of a target cell.



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Our research focus concerns the mechanisms by which environmental estrogens (EEs) affect growth of cancer and pituitary cells. We explicitly accomplish this by introducing specific genes in various cell types, treating those cells with environmental estrogens, and measuring the degree of gene activation.

Reliable results require use of high quality gene (DNA) constructs. Therefore, the goals of the current study are:

1. **Produce, isolate and purify genes of interest related to this study**
2. **Introduce those gene constructs into mammalian cells**

MATERIALS AND METHODS

- ✓ **Bacterial transformation**—Introduces gene constructs of interest into bacterial cells. The cells are plated on culture plates to form single colonies.
- ✓ **Construct Isolation/Purification**—Single bacterial colonies were picked from the agar plates and cultured in nutritive broth containing an antibiotic. The rapid growth of bacteria under ideal conditions amplifies the numbers of genes. Then bacteria were lysed under alkaline conditions. The gene of interest was isolated, purified, and quantified.

CURRENT RESULTS

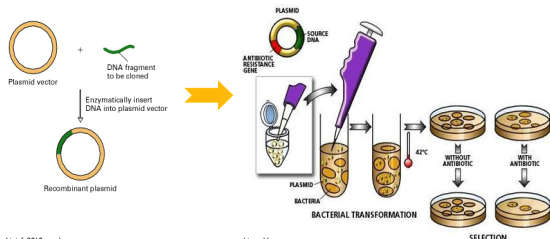
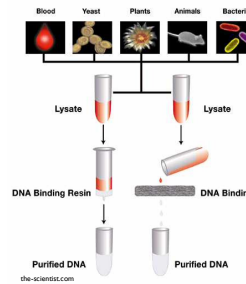


Figure 1. A plasmid is a circular form of bacterial DNA that contains genes conferring antibiotic resistance. Plasmid DNA can also be used as a vector, allowing genes of interest to be introduced into eukaryotic cells. All of the genes used in this study had been previously introduced into plasmid DNA containing an ampicillin resistant gene. The plasma membrane of XLI Blue cells was disrupted with heat. The ER, Vit-6, pcDNA, and pGL3 plasmids were taken up by the bacterial cells. Following a brief incubation at 37°C, 100 μ l of cells were added to agar plates containing ampicillin. Therefore, only the bacterial cells that had taken up the plasmid would grow and form colonies on the plate because the plasmid confers ampicillin resistance! **We were able to successfully transform bacterial cells using all plasmids tested and grow those cells as single colonies.**



| Plasmid | Restriction Enzymes | Expected Fragment Size (base pairs) |
|-------------|---------------------|-------------------------------------|
| ER α | Xba I | 900 |
| ER β | Xba I | 1700 |
| Vit-6 | Eco R I | 600, 2900 |
| pGL3 | Sph I | 700, 1420 |
| pcDNA | Pst I | 962, 2300 |

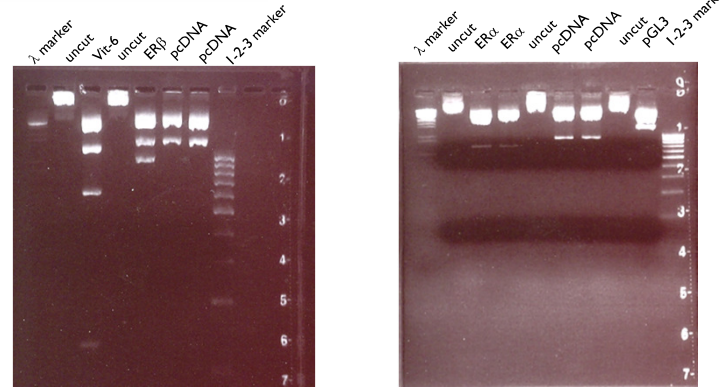
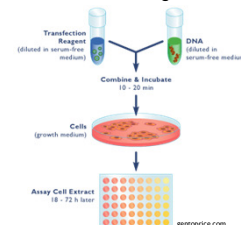


Figure 2. Single colonies were used to inoculate 50ml of Luria Broth (a nutritive medium) containing ampicillin. The cells were incubated overnight at 37°C. The plasmid DNA in bacterial cells replicates independently and at a higher rate than genomic DNA, so growth of cells is correlated to high amplification of the genes in our plasmids. The next day, bacterial cells were lysed under alkaline conditions and the plasmid DNA attached to a filter containing a binding resin. The DNA was washed with an ethanol solution and eluted with water. The plasmid DNA was precipitated once more using sodium chloride and ethanol to concentrate the sample.

- ✓ **Restriction digest analysis verified the presence of the correct genes in our preparation**
- ✓ **Spectrophotometric analysis demonstrated**
 - ✓ **A high yield of all samples (4.2-6.5 μ g/ml)**
 - ✓ **High purity of all samples ($A_{260/280}$ ratio = 1.7-1.8)**

FUTURE STUDIES

We are currently in the process of culturing JEG-3 cells. This estrogen sensitive human placental cell line expresses ER and is thus a suitable model for studying gene activation. The genes that we have isolated and purified will be introduced into these cells and the functionality of the proteins they encode will be measured via reporter assay. Briefly, lipid reagents will be used to disrupt the plasma membranes of these placental cells, which then take up the plasmid constructs. When cells are treated with EEs, the compounds bind to the estrogen receptor. The subsequent hormone receptor complex should bind the reporter gene (Vit-6 or pGL3) that contains an ERE. The cells will then be lysed. Since the reporter genes we use also contain a gene for the enzyme luciferase, the addition of luciferin (the substrate for luciferase) should result in the production of light. Light intensity can be measured by an instrument called a luminometer. Thus, the amount of light produced is directly correlated with the amount of gene activation.



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