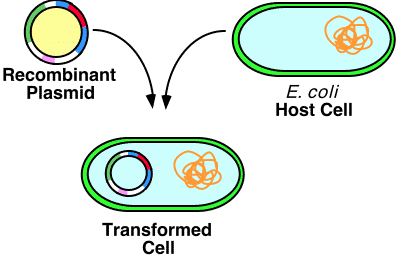
**Introduction to pGLO**

Tomorrow we will be completing a genetic transformation of pGLO. In order to be successful in lab, we need to understand the procedure and why it is important!

GFP_wheel1.jpg                                                 00016E3F
KSD Server                     B471509A:

**What is a plasmid?**

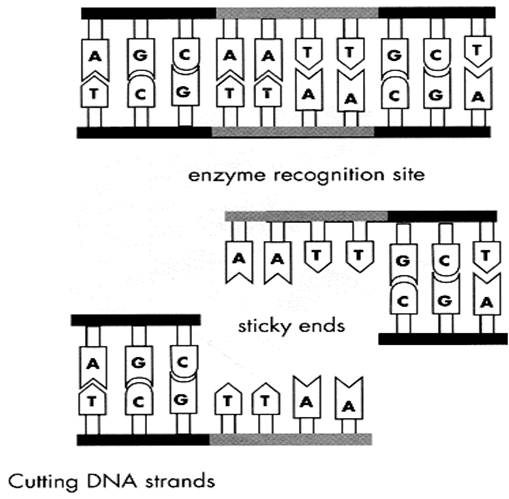
* Provides beneficial Genes such as **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
* Useful for DNA technology: splice in gene of interest, put back into bacteria to grow…VOILA…lots of copies of the gene!

**What is the gene of interest for this lab?**

**Where does the gene come from?**

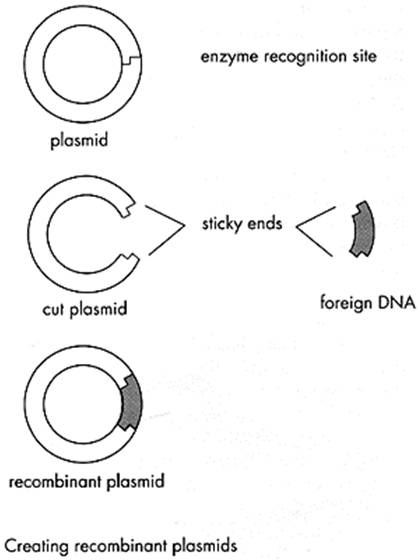
**What are we going to do in lab tomorrow?**

We are going to put the \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ plasmid into \_\_\_\_\_\_\_ bacteria!

**Why is the GFP so cool?!**

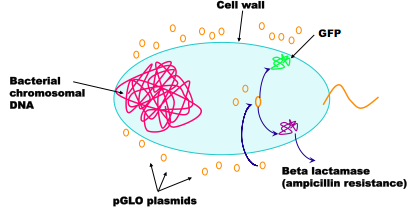
**SPLICING DNA**

First, the pGLO plasmid and the GFP gene (from the jellyfish) had to be cut by the same **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**. This produced \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

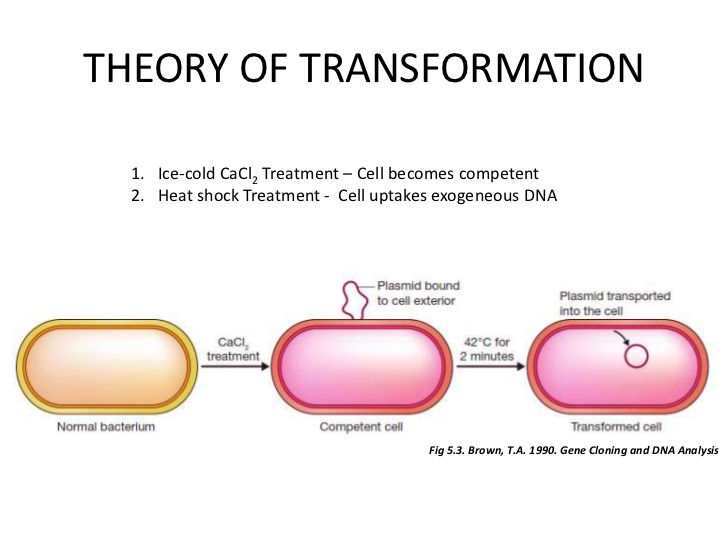
****Second, the sticky ends of the isolated GFP gene and plasmid were matched up. Now it is a *recombinant* plasmid

**Recombinant**

GFP_wheel2.jpg                                                 00016E3F
KSD Server                     B471509A:**KNOWN GENES ON pGLO PLASMID**

**The Goal of Bacterial Transformation**

**What do we need to make Bacterial Transformation Successful?**

****

Transformation Procedure

****

Cell membrane becomes \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ - plasmid can “sneak in through holes

|  |  |
| --- | --- |
| **Calcium Chloride** | **Heat Shock** |
|  |  |

* Suspend *E. coli* starter culture in Transformation solution (CaCl2)
* Add pGLO plasmid DNA
* Place tubes in ice
* Heat-shock at 42°C and place on ice
* Incubate with nutrient broth
* Streak plates
* Grow overnight in incubator