Amplification of part of the *Lux A* gene

This procedure will be the first attempt to amplify the lux gene from known bioluminescent species. We will use the chromosomal DNA we purified in Ex 5. This will be a test to see if we can amplify the purified DNA that we know has the sequence of the lux primers. The primers for this amplification were designed by aligning known luxA genes from the public database and using areas with high homology for the primer binding sites. The area between the primers spans about 570 bp in *V. fischeri* and shares less sequence homology than the primer binding sites. Rather than adding individual components to the PCR we will use Fermentas 2X Ready Mix Buffer. This contains everything needed for PCR except the template and primers.

We will use three dilutions of each template we will try to amplify, so you will need to set up the following reactions:

1. 1:10 dilution of template
2. 1:100 dilution of template
3. 1:1000 dilution of template
4. Negative control with water as a template
5. Negative control with 1:10 dilution of template but no primers

Polymerase chain reaction

Make sure to use the PCR tubes, not the eppendorf tubes for this!!!

1. Each reaction will need the following reagents:
    a. 10 µl of Fermentas 2X PCR mix (this contains *Taq* polymerase, dNTPs, and buffers.
    b. 1 µl of **each** 10 mM primer (LuxA F and LuxA R)
        replace with water in tube 5
    c. 1 µl template
    d. Water to a total volume of 20 µl

Mix well. This ensures that a consistent mixture of reagents is in each tube. After

Finally, keep your reaction on ice (but don't freeze) until the whole class is ready, at which point we will place the reactions in the thermocycler and amplify with the following conditions