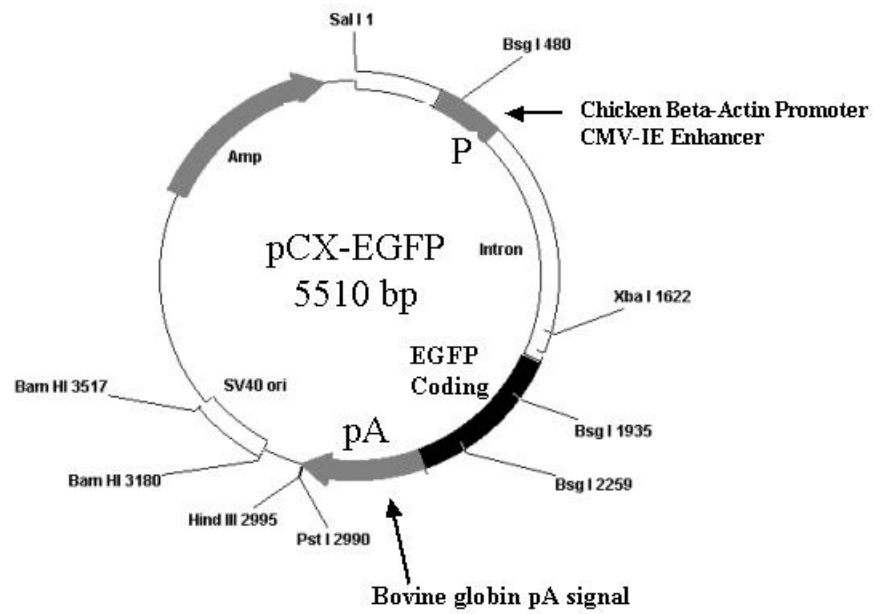


For next time

1. Sketch the expected product from the PCR you performed, clearly indicating the 5' and 3' end. Include the restriction sites that you have introduced and the expected length of the product.
2. Read the introduction to Module 1 Day 2 then consider the following experiment: You would like to express EGFP in yeast. The plasmid you have, pCX-EGFP, has an SV40 origin of replication that won't work in yeast. BamHI sites flank the origin.
You have used PCR to amplify a 500 bp yeast origin, called "CEN/ARS." The PCR primers were designed to introduce BglII sites on each side of the product. You would like to replace the SV40 origin with the CEN/ARS one you just amplified. See figure below.
 - o What are the recognition sites for BamHI and BglII? Use the [New England Biolabs website](#) or your NEB catalog to help you. From the web site, just type the name of your enzyme into the search box of the main homepage.
 - o What size fragments do you expect from pCX-EGFP when it is completely digested with BamHI? What about if it were "partially" digested, i.e. cut at one site only?
 - o What is the 6 base-sequence that results when the overhangs of a BamHI and BglII site anneal? Will either enzyme digest the recombinant site?
3. Read pages 232-233 from the 02-03 NEB catalog (or find it online: NEB's "[Setting up a Restriction Endonuclease Reaction](#)").
4. *Advanced Preparation for your Lab Report:* You will be required to describe in your lab report what the basic design elements are for your primers. This description should be sufficiently informative such that a classmate would have the necessary information to make their own primers that serve the same purpose (even if the primers are not identical). You now have the option of drafting this part of your final report.

For basic information on homologous recombination, read the excellent review by Tomas Helleday.



pCX-EGFP vector map