

## Introduction

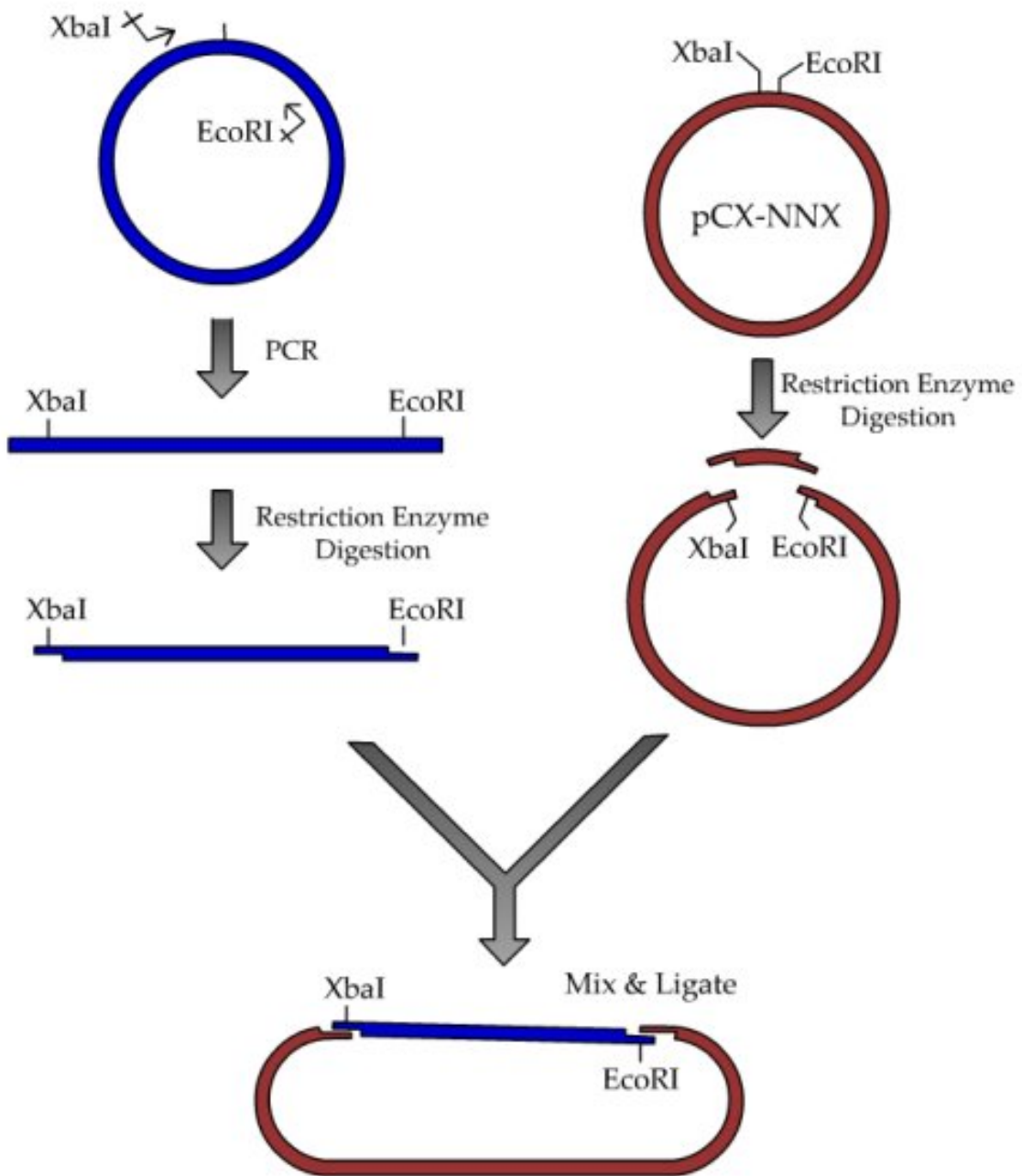
As you heard about in lecture, you'll be starting a project to study homologous recombination and in the process you'll be learning some fundamental tools and techniques of molecular biology. One major goal we have for this module is to establish good habits for documentation of your work. By documenting your work according to the exercises done today, you will

- Be better research students (in 20.109 as well as any research lab you may join)
- Be better writers since a clear record of what you've done will improve your data analysis
- Be better scientists, since you'll eventually train others to document things this way too

Today's lab has five parts. First, you will follow a four-part exercise to design a pair of PCR primers and to generate a primer record, and then later today you will use the primers you designed to set up a PCR. Next time you'll start cloning the PCR product.

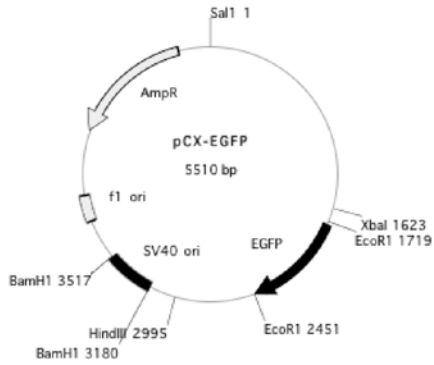
A general roadmap for the plasmid construction part of this experiment is below.

## Roadmap for Plasmid Construction



### Experiment roadmap

Courtesy of Justin Lo. Used with permission.



## Map of pCX-EGFP

Courtesy of Dr. Natalie Kuldell. Used with permission.

## The sequence of pCX-EGFP

```

GTCGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA
GTTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCC
GCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTAT
GTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCC
AGTACATGACCTTATGGGACTTCCTACTTGGCAGTACATCTACGTATTAGTCA
TCGCTATTACCATGGGTTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCAT
CTCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTTTAATTATTTGT
GCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGC
GGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGGCGGCAGCCAAT
CAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGGCGGGCGGC
GGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGTTGCCTTC
GCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGA
CCGCGTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTG
TAATTAGCGCTTGGTTTAAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTAAA
GCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGG
GTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCCGCGCTGCC
GGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGT
GCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCGGTGCGGGGGGGCTGC
GAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGG
GGTGTGGGCGCGGCGGTTCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGA
GTTGCTGAGCACGGCCCCGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCG
CGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCG
GGGCGGGGCCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCC
CCGGAGCGCCGGCGGCTGTTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTA
TGGTAATCGTGCAGAGGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGA
GCCGAAATCTGGGAGGCGCCGCCGCACCCCTCTAGCGGGCGCGGGCGAAG

```

CGGTGCGGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTTCG  
CCGCGCCCGCCGTCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGA  
CGGCTGCCTTCGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTG  
ACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTA  
CAGCTCCTGGGCAACGTGCTGGTTGTTGTGCTGTCTCATCATTTTGGCAAAGA  
ATTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCC  
ATCCTGGTCGAGCTGGACGGCGACGTAACGGCCACAAGTTCAGCGTGTCCG  
GCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTG  
CACCACCGGCAAGCTGCCCCTGCCCTGGCCACCCTCGTGACCACCCTGACC  
TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACT  
TCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC  
AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGGCGAC  
ACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGC  
AACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATA  
TCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCC  
ACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACA  
CCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCAC  
CCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTG  
CTGGAGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACA  
AGTAAGAATTCACCTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGG  
TGTGGCCAATGCCCTGGCTCACAATACTACTGAGATCTTTTTCCCTCTGCCA  
AAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA  
AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACT  
CGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTG  
GTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGG  
CTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCTTATT  
CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTTATATTTTGTGTTTGTGTT  
ATTTTTTTCTTTAACATCCCTAAAATTTTCTTACATGTTTTACTAGCCAGATT  
TTTCTCTCTCTCTGACTACTCCAGTCATAGCTGTCCCTCTTCTCTTATGAAG  
ATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCT  
GTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCA  
TAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGC  
GTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCC  
GCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGC  
CCCTAACTCCGCCAGTTCGGCCATTCTCCGCCCCATGGCTGACTAATTTTTT  
TTATTTATGCAGAGGCCGAGGCCGCTCGGCCCTCTGAGCTATTCCAGAAGTA  
GTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTAT  
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAATTCACAAAT  
AAAGCATTTTTTCTACTGCATTCTAGTTGTGGTTTTGTCCAACTCATCAATGTA  
TCTTATCATGTCTGGATCCGCTGCATTAATGAATCGGCCAACGCGCGGGGAG  
AGGCGGTTTTGCGTATTGGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGC  
GCTCGGTCGTTCCGGCTGCGGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAAT  
ACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAA  
AGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTTC  
CATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGA  
GGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG

CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCG  
CCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTAT  
CTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCC  
CGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC  
CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAG  
CAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC  
TACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG  
TTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGC  
TGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAA  
GGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAA  
CGAAAACACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTC  
ACCTAGATCCTTTTAAATTA AAAATGAAGTTTTAAATCAATCTAAAGTATATA  
TGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT  
CAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAG  
ATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATAC  
CGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGC  
CGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAG  
TCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTT  
GCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTG  
GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCC  
CCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAG  
AAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATT  
CTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA  
ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCCG  
CGTCAATACGGGATAATACCGCGCCACATAGCAGA ACTTTAAAAGTGCTCAT  
CATTGGAAAACGTTCTTCGGGGCGAAA ACTCTCAAGGATCTTACCGCTGTTG  
AGATCCAGTTCGATGTAACCCACTCGTGCACCCA ACTGATCTTCAGCATCTTT  
TACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA  
AAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCCTTT  
TTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATA  
TTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCC  
GAAAAGTGCCACCTG

end of the sequence -