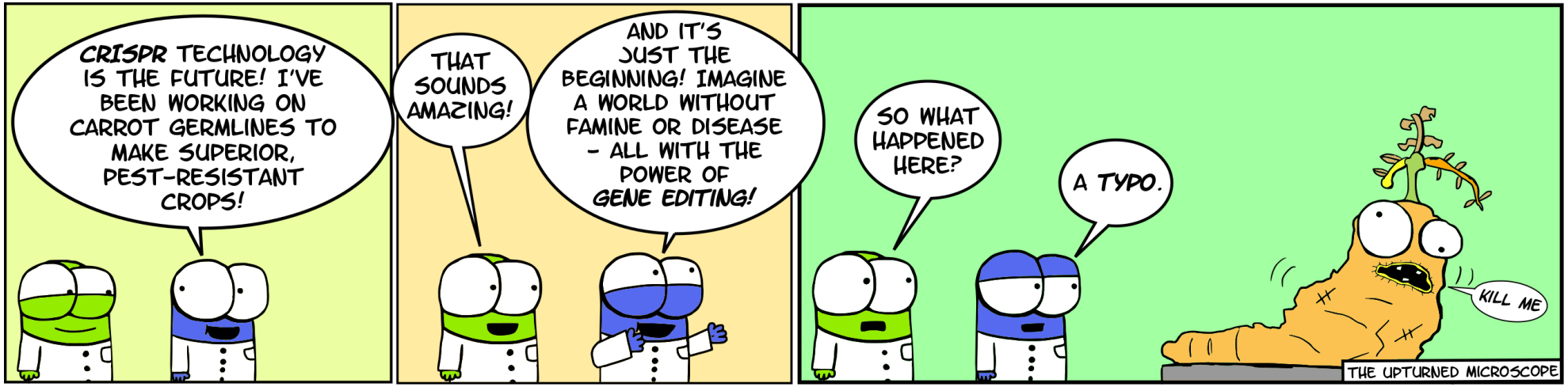
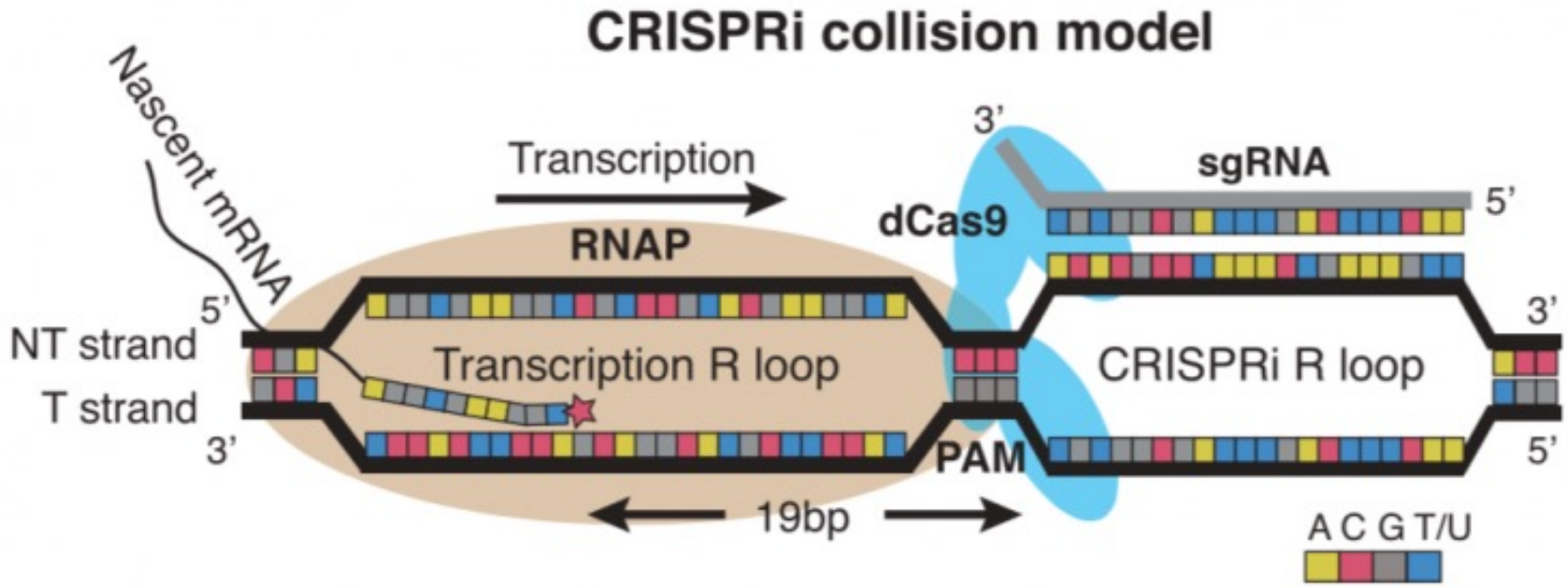
M2D2: Design gRNA sequence for CRISPRi system

- Prelab discussion
- Diagnostic digest electrophoresis
- Design gRNA primer to target (and inhibit) gene in the metabolic pathway of E. Coli



Using CRISPRi as an experimental tool



- sgRNA binds to region of gene of interest
  - contains a dCas9 handle

- dCas9 uses sgRNA as guide and sits on DNA without cleaving it
  - blocks transcription

Mod2 Overview

Research goal: Increase the yield of commercially valuable byproducts in *E.coli* using CRIPSRi technology to target genes involved in mixed-acid fermentation pathway.

Last Lab:

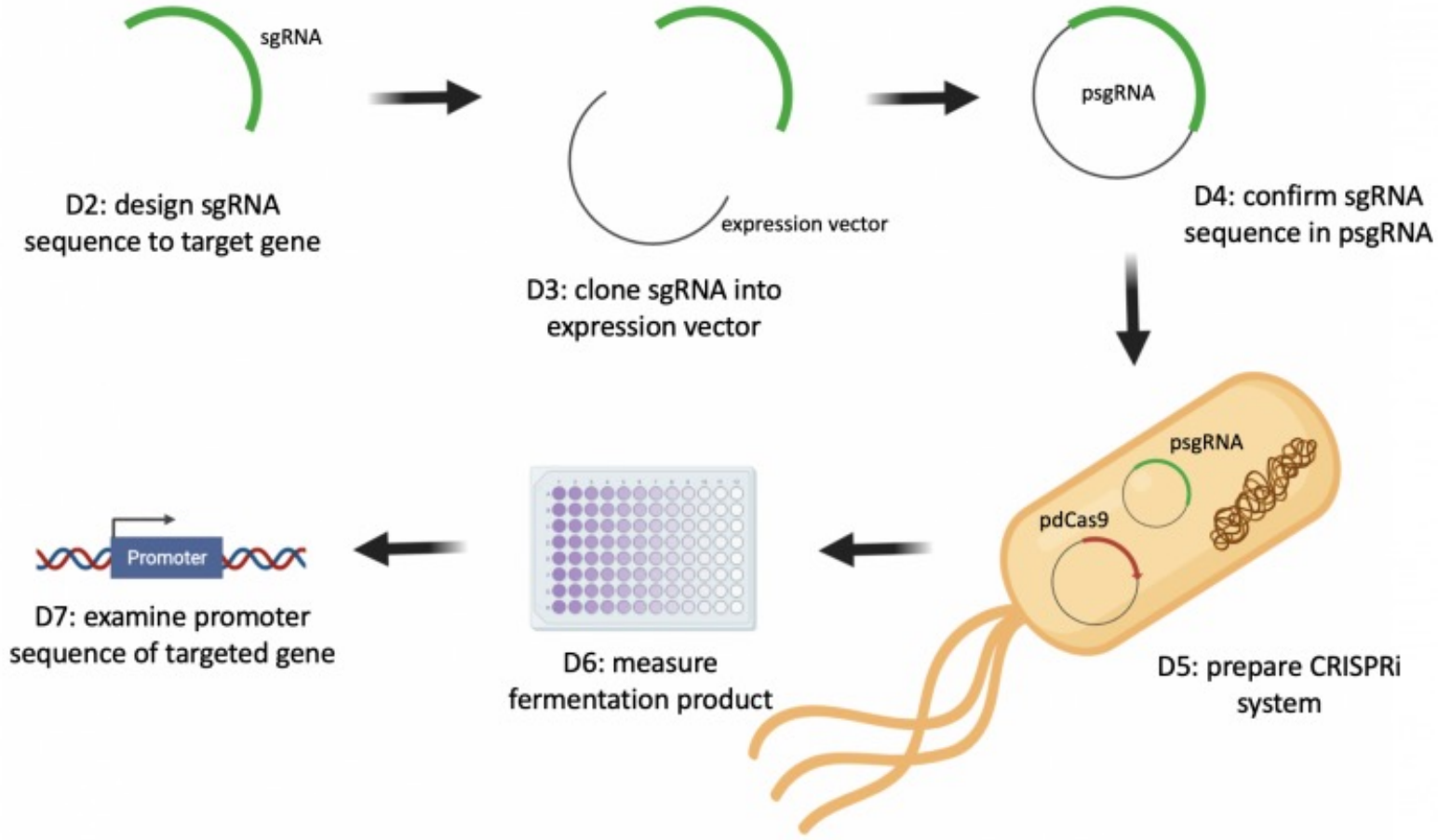
Cloned pdCas9 construct to generate 1st component of CRISPRi system

This Lab:

Choosing metabolic gene of interest and designing sgRNA to target it

Next Lab:

Will clone sgRNA into vector to create 2nd component of CRISPRi system



Today’s goal:

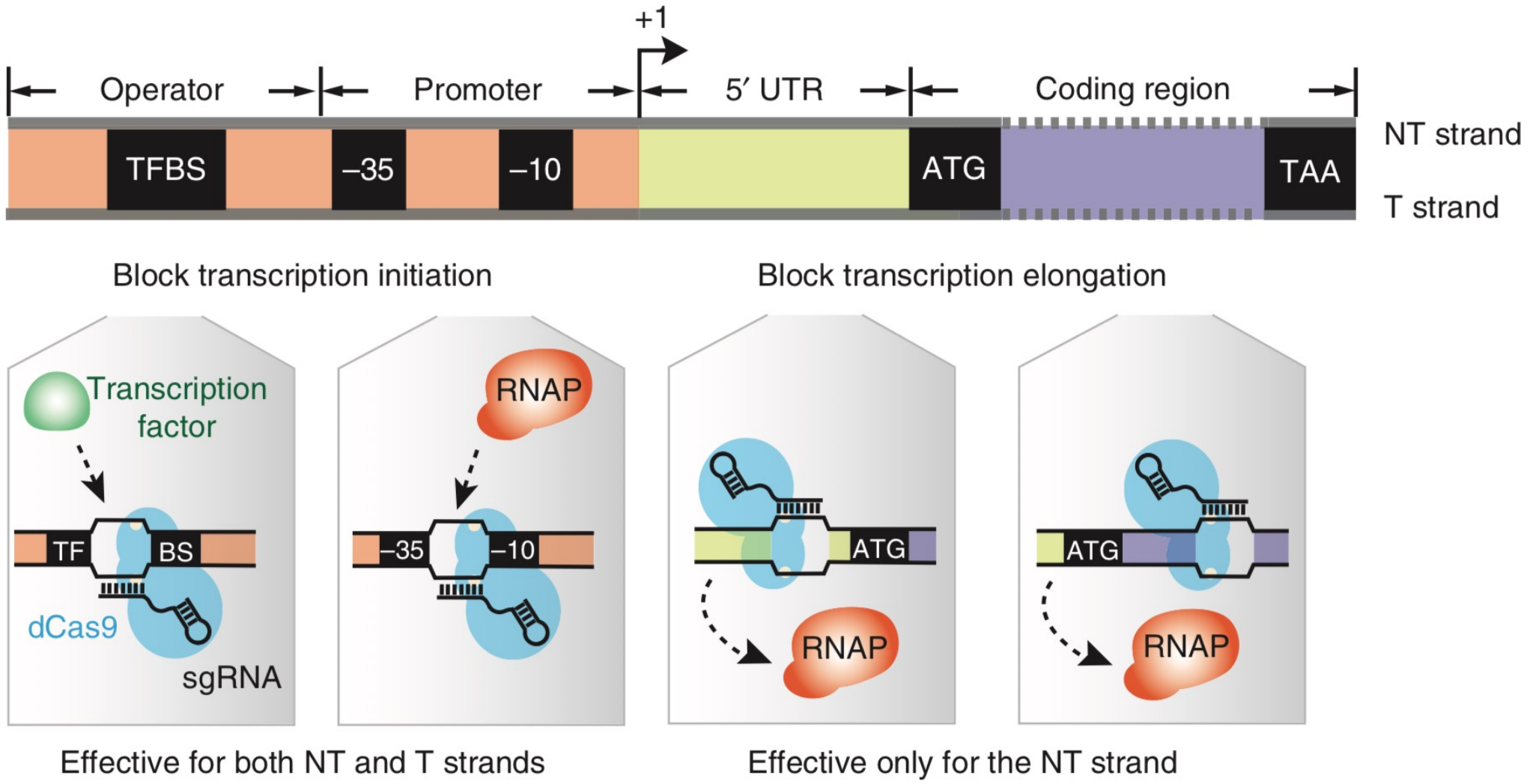
Design gRNA sequence to repress a gene, such that the **production of ethanol or acetate will increase.**

Considerations for successful gRNA primer design

Primer size range: 20-25bps

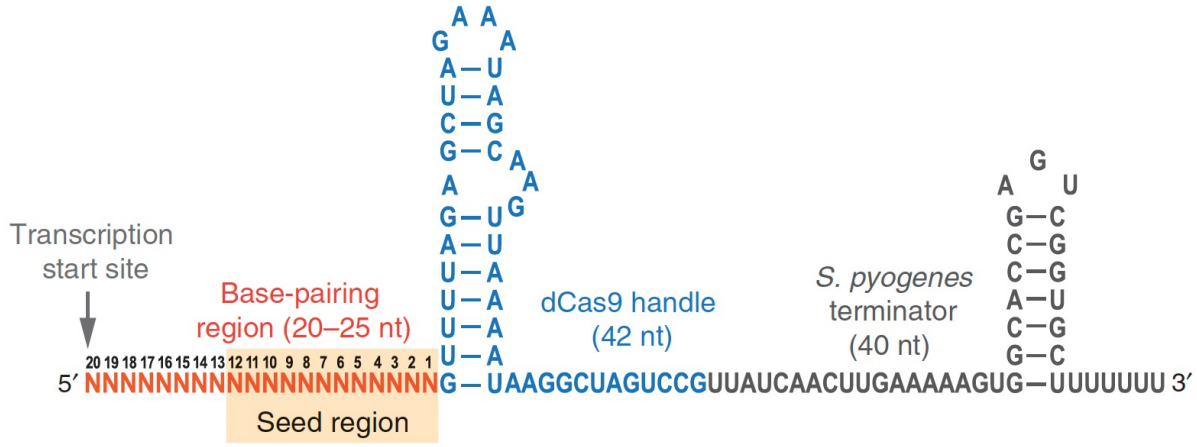
- Gene target choice
  - Location in the fermentation pathway
  - Location on the genome map
- Sequence specificity
  - BLAST
- Target strand
  - template vs nontemplate
- Sequence location
  - operator
  - promotor
  - 5’ UTR
  - coding

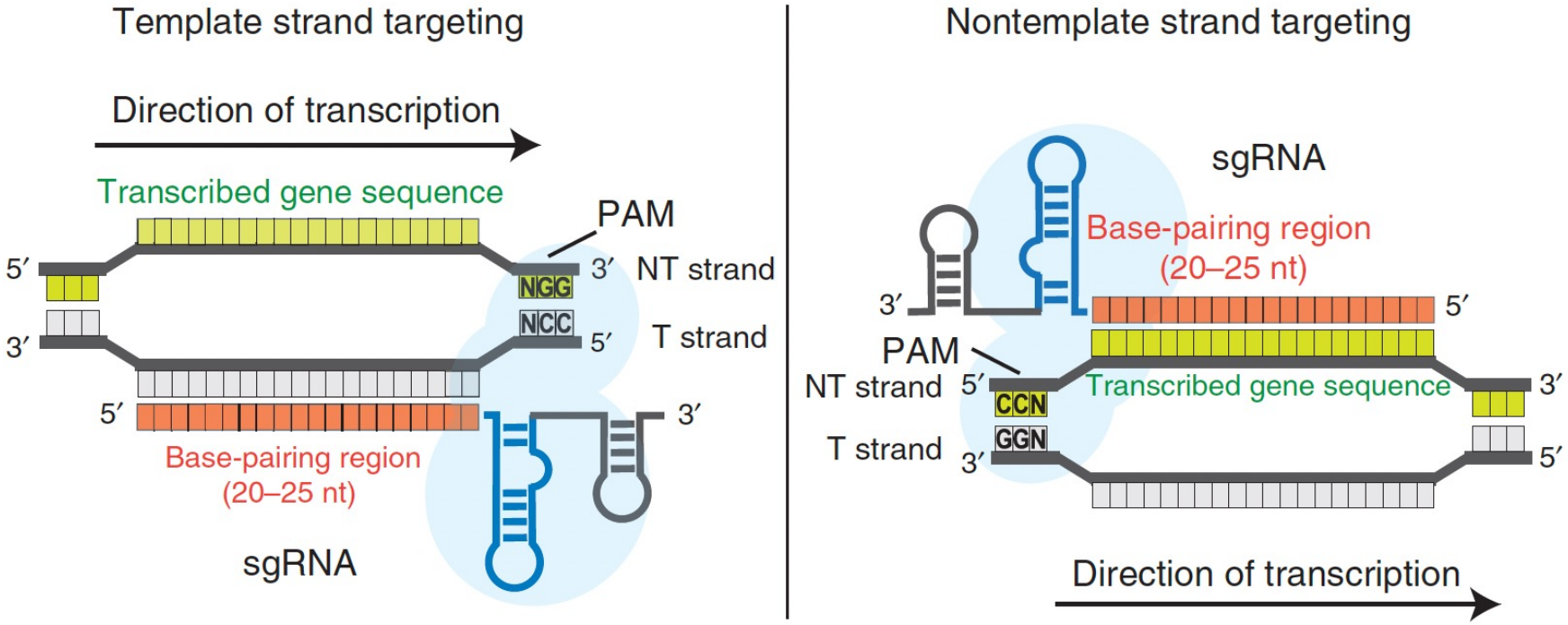
Which part of your selected gene is best to target?



Larson et al. 2013

Design of the sgRNA





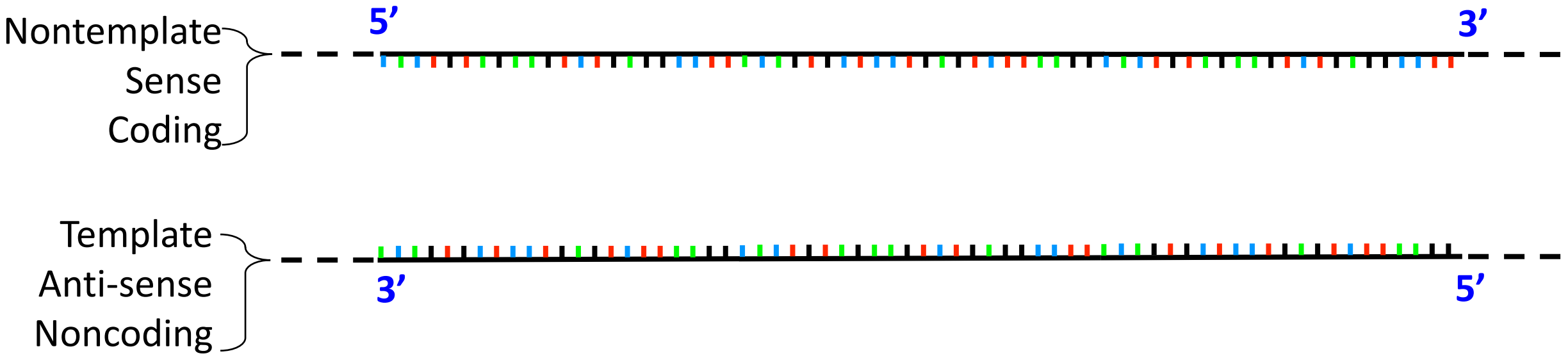
Design of gRNA for CRISPRi system

(1) Target the TEMPLATE DNA strand:

gRNA sequence will be the same as the transcribed (nontemplate) sequence.

(2) Target the NONTEMPLATE strand:

gRNA sequence will be the reverse-complement of the transcribed sequence.



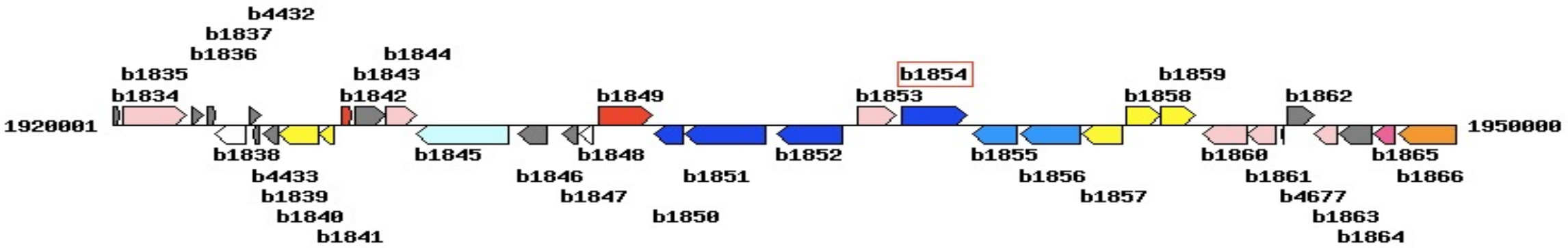
Use the KEGG database to find sequence information for your target gene

Position	1937649..1939091 Genome map
AA seq	480 aa AA seq DB search MSRRLRRTKIVTTLGPATDRDNNLEKVIAAGANVVRMNFSHGSPEDHKMRADKVREIAAK LGRHVAILGDLQGPKIRVSTFKEGKVFLNIGDKFLLDANLGKGEGDKEKVGIDYKGLPAD VVPGDILLLDDGRVQLKVLEVQGMKVFTEVTVGGPLSNNKGINKLGGGLSAEALTEKDKA DIKTAALIGVDYLAVSFPRCGEDLNYARRLARDAGCDAKIVAKVERAEAVCSQDAMDDII LASDVVMVARGDLGVEIGDPELVGIQKALIRRARQLNRAVITATQMMESMITNPMPTRAE VMDVANAVLDGTDAVMLSAETAAGQYPSETVAAMARVCLGAEKIPSINVSKHRLDVQFDN VEEAIAMSAMYAANHLKGVTAIITMTESGRTALMTSRISSGLPIFAMSRHERTLNLTALY RGVTPVHFDSANDGVAAASEAVNLLRDKGYLMSGDLVIVTQGDVMSTVGSTNTTRILTVE
NT seq	1443 nt NT seq +upstream 0 nt +downstream 0 nt atgtccagaaggcttcgcagaacaaaaatcgttaccacgttaggcccagcaacagatcgc gataataatcttgaaaaagttatcgcggcgggtgccaacgttgtacgtatgaacttttct cacggctcgcctgaagatcacaaaatgcgcgcggataaagttcgtgagattgccgcaaaa ctggggcgtcatgtggctattctgggtgacctccaggggcccaaaatccgtgtatccacc tttaaagaaggcaaagttttcctcaatattggggataaattcctgctcgacgccaacctg ggtaaaggtgaaggcgacaaagaaaaagtcggtatcgactacaaaggcctgcctgctgac gtcgtgcctggtgacatcctgctgctggacgatggtcgcgtccagttaaaagtactggaa gttcagggcatgaaagtgttcaccgaagtcaccgtcggtggtccccctctccaacaataaa ggtatcaacaaacttggcggcggtttgtcggctgaagcgctgaccgaaaaagacaaagca gacattaagactgcggcgttgattggcgtagattacctggctgtctccttcccacgctgt ggcgaagatctgaactatgcccgtcgcctggcacgcgatgcaggatgtgatgcgaaaatt gttgccaaggttgaacgtgcggaagccgtttgcagccaggatgcaatggatgacatcatc ctcgcctctgacgtggtaatggttgcacgtggcgacctcggtgtggaaattggcgacccg gaactggtcggcattcagaaagcgttgatccgtcgtgcgcgtcagctaaaccgagcggta atcacggcgacccagatgatggagtcaatgattactaacccgatgccgacgcgtgcagaa gtcatggacgtagcaaacgccgttctggatggtactgacgctgtgatgctgtctgcagaa actgccgctgggcagtatccgtcagaaaccgttgcagccatggcgcgcgtttgcctgggt gcggaaaaaatcccgagcatcaacgtttctaaacaccgtctggacgttcagttcgacaat gtggaagaagctattgccatgtcagcaatgtacgcagctaaccacctgaaaggcgttacg gcgatcatcaccatgaccgaatcgggtcgtaccgcgctgatgacctcccgtatcagctct ggtctgccaattttcgccatgtcgcgccatgaacgtacgctgaacctgactgctctctat cgtggcgttacgccggtgcactttgatagcgctaatgacggcgtagcagctgccagcgaa gcggttaatctgctgcgcgataaaggttacttgatgtctggtgacctggtgattgtcacc cagggcgacgtgatgagtaccgtgggttctactaataccacgcgtattttaacggtagag taa

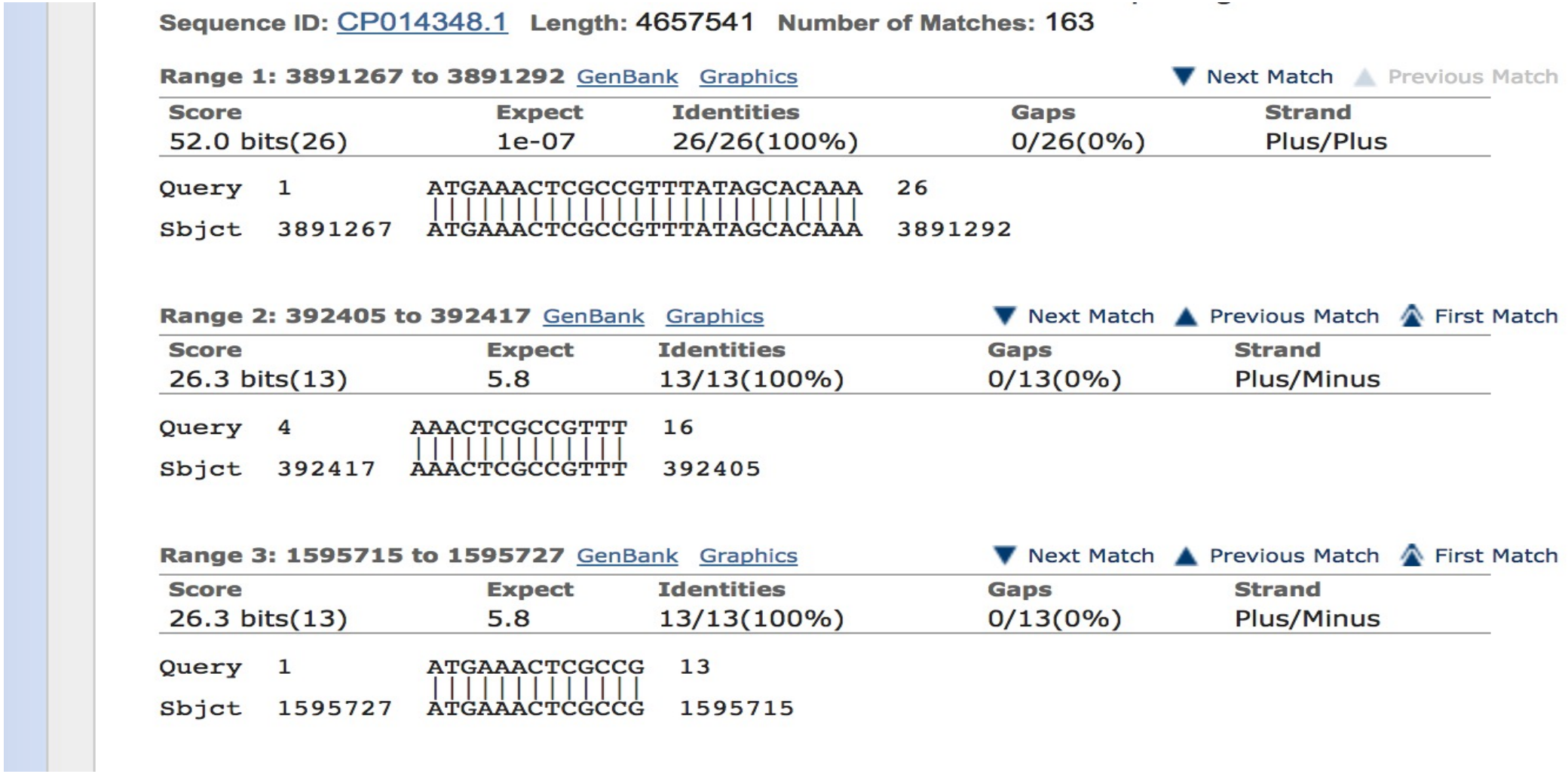
Consider how far upstream from start site to move to target promoter or operator

NT in this case = nucleotide

Use KEGG database genome map to identify location of your target gene relevant to other genes



Use BLAST (Basic local alignment search tool) to assess off-target binding



Sequence ID: CP014348.1 **Length:** 4657541 **Number of Matches:** 163

Range 1: 3891267 to 3891292 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
52.0 bits(26)	1e-07	26/26(100%)	0/26(0%)	Plus/Plus

```
Query  1        ATGAAACTCGCCGTTTATAGCACAAA  26
               ||||||||||||||||||||||||||
Sbjct  3891267  ATGAAACTCGCCGTTTATAGCACAAA  3891292
```

Range 2: 392405 to 392417 GenBank Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Gaps	Strand
26.3 bits(13)	5.8	13/13(100%)	0/13(0%)	Plus/Minus

```
Query  4       AAACTCGCCGTTT  16
              |||||||||||||
Sbjct  392417  AAACTCGCCGTTT  392405
```

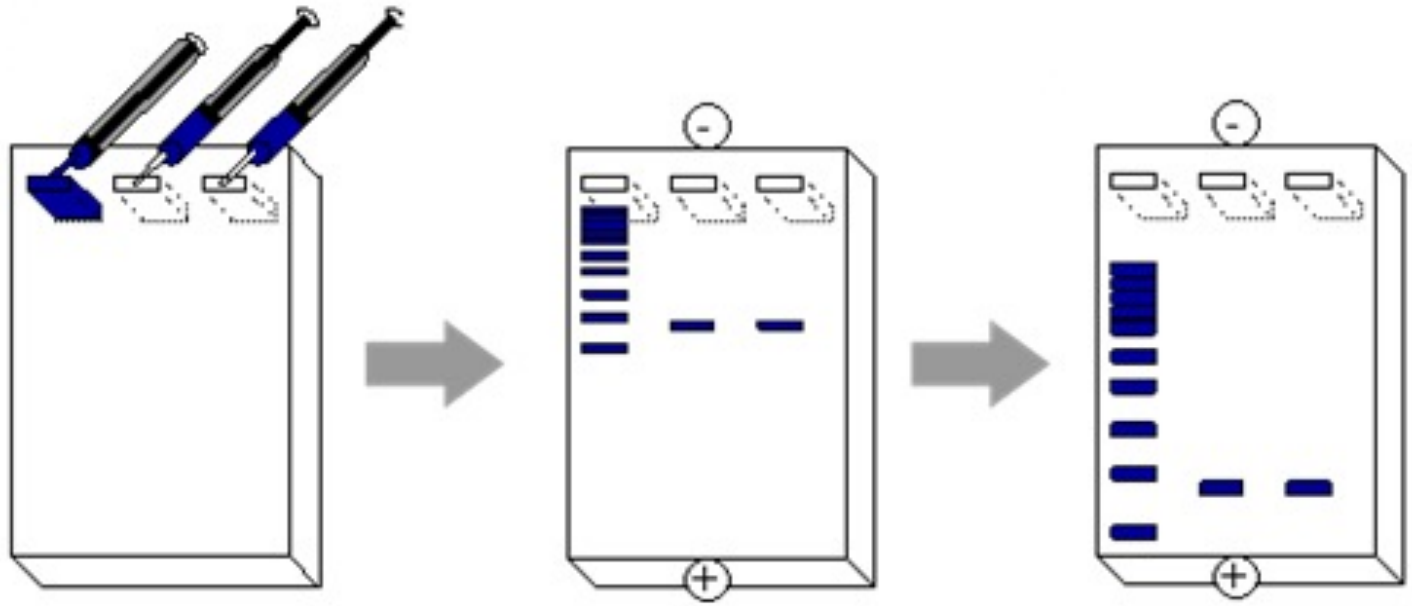
Range 3: 1595715 to 1595727 GenBank Graphics ▼ Next Match ▲ Previous Match First Match

Score	Expect	Identities	Gaps	Strand
26.3 bits(13)	5.8	13/13(100%)	0/13(0%)	Plus/Minus

```
Query  1        ATGAAACTCGCCG  13
               |||||||||||||
Sbjct  1595727  ATGAAACTCGCCG  1595715
```

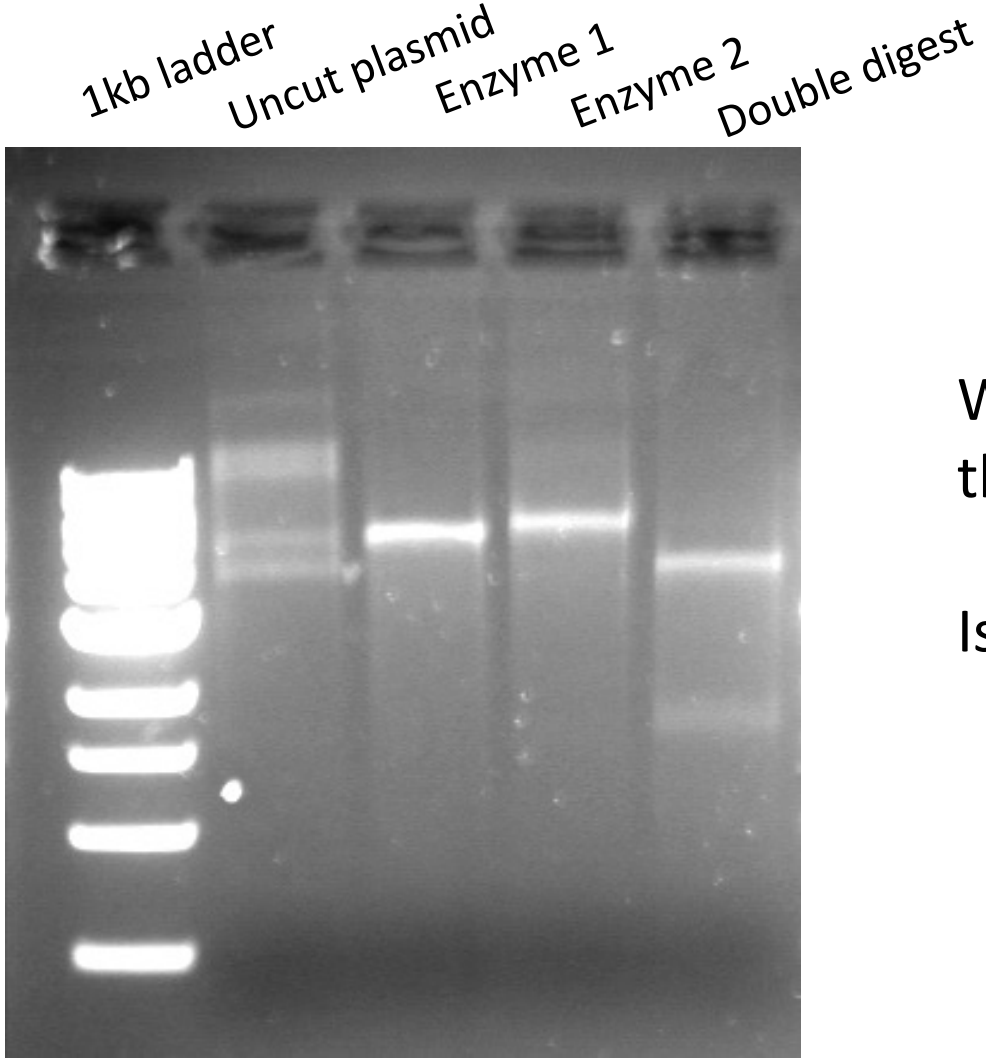
DNA gel electrophoresis

- Similar concept to SDS-PAGE
- (-) charged DNA migrates through 1% agarose gel toward positive anode
- DNA fragments are separated by size



bio.libretexts.org

Diagnostic Digest example



Why are there multiple bands in the uncut plasmid?

Is that OK?

For Today:

- Load and electrophorese DNA gel of diagnostic digest
- Design gRNA primer to improve ethanol or acetate production
  - MUST upload primer to Wiki before leaving class!

For M2D3:

- Read your journal club article and chose 4 figures which are most important to the paper's main conclusion
  - Answer questions on the wiki about those 4 figures
- Email Noreen about presentation day

- Today is a lot of design work on your computer!

- BE Faculty Conference Room is open for you
  - **16-339**

- Office hours begin at 5pm in 16-339
  - Don’t need to have specific questions
  - Space to work