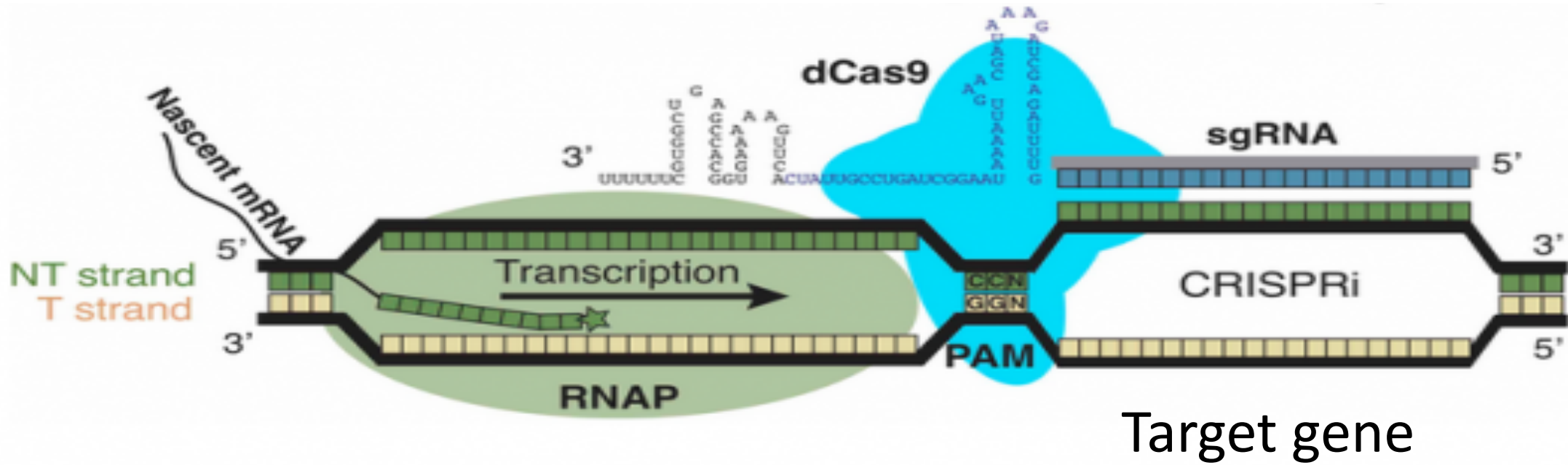


M3D2: Align gRNA sequences with genomic targets

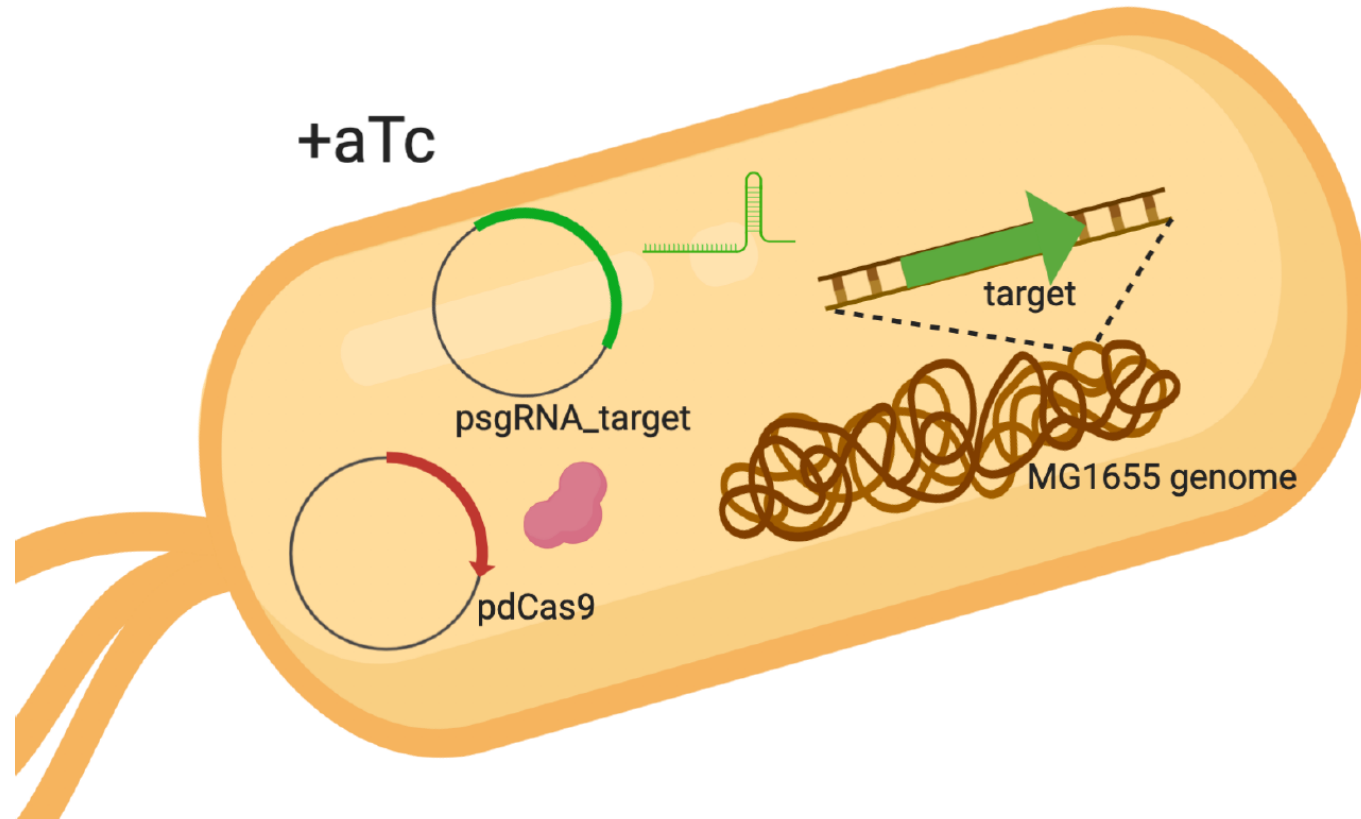
- Prelab discussion
- Assess design of previously utilized sgRNA
- **Comm Lab at 4pm!**



CRISPRi system can block the RNA polymerase



CRISPRi system overview



ON / OFF?

- aTc

+ aTc

pdCas9

pgRNA_target

Target gene

Workflow of sgRNA design for CRISPRi system

Design the gRNA DNA sequence



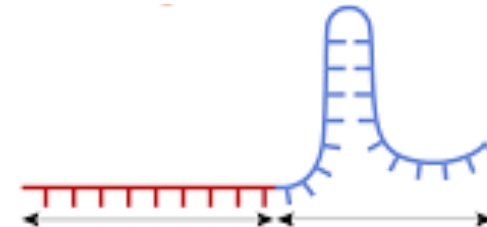
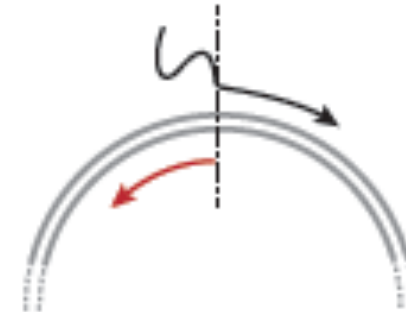
Create a plasmid (pgRNA)



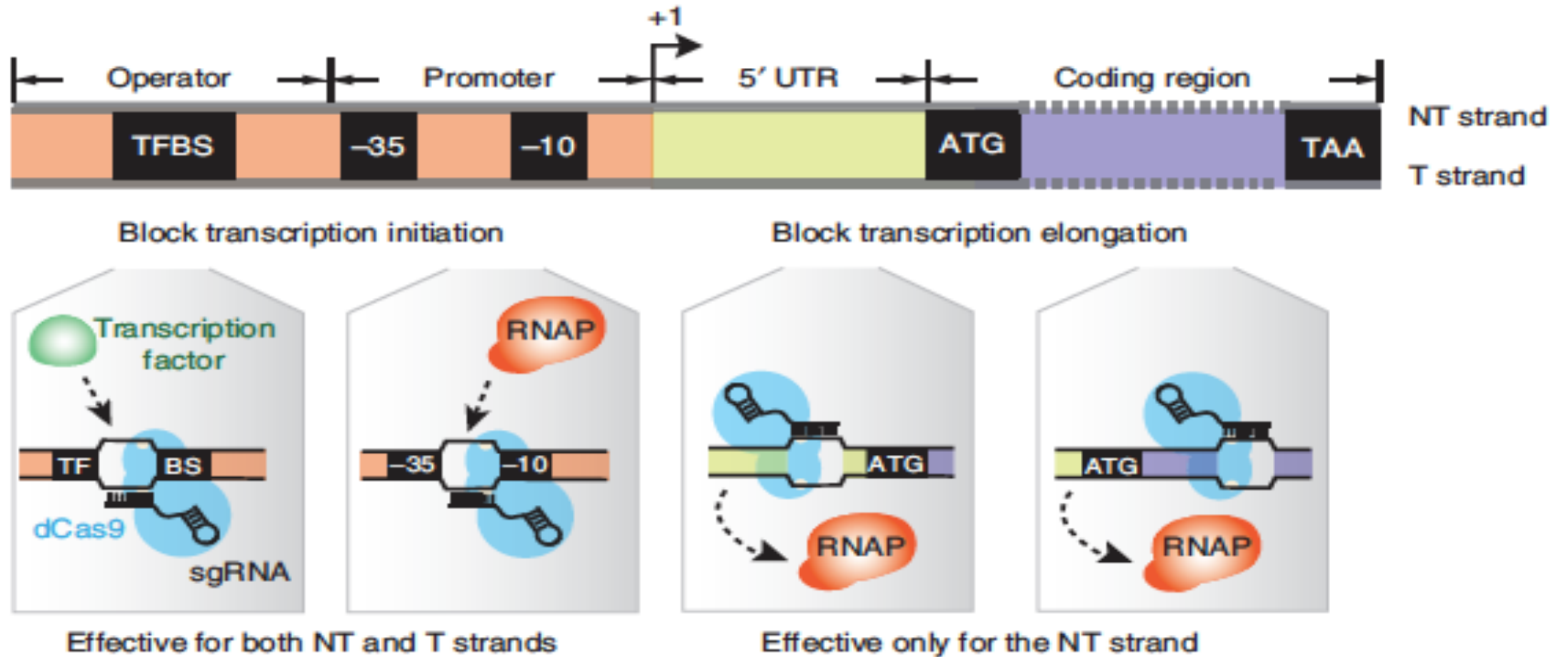
Generate the desired gRNA when transcribed



Target gene of interest



Which region of the gene will you target?



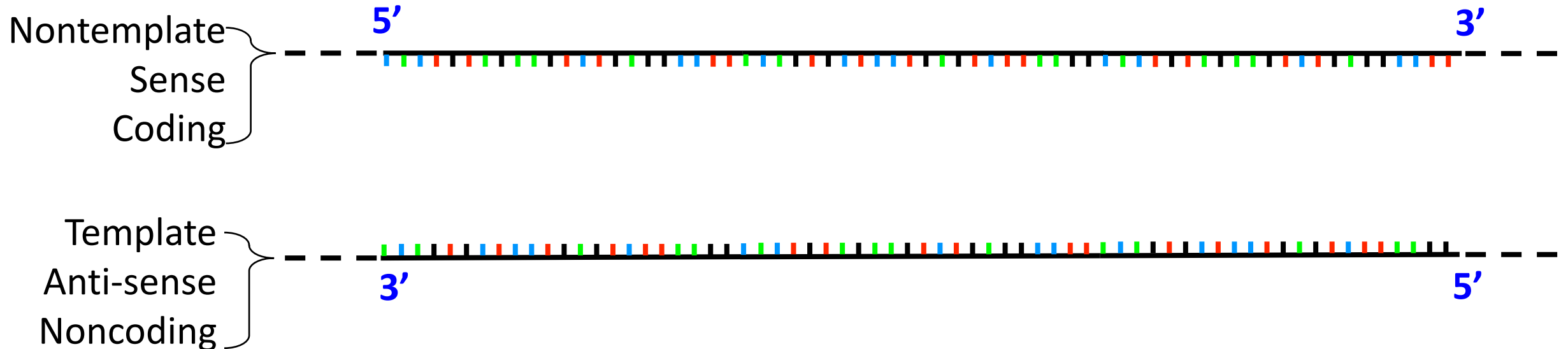
Design of gRNA for CRISPRi system

(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.

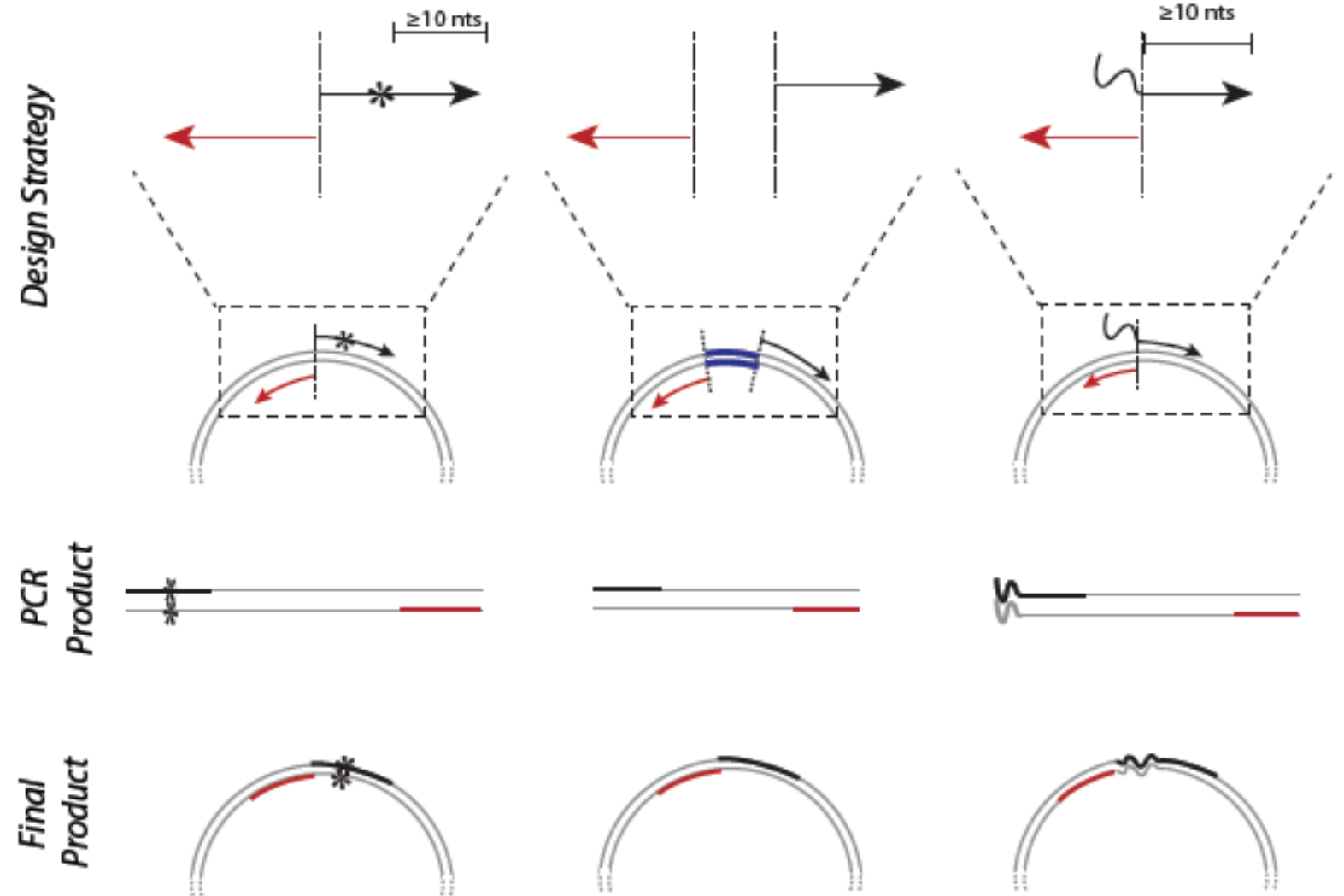


How can we insert the gRNA sequence into the sgRNA plasmid?

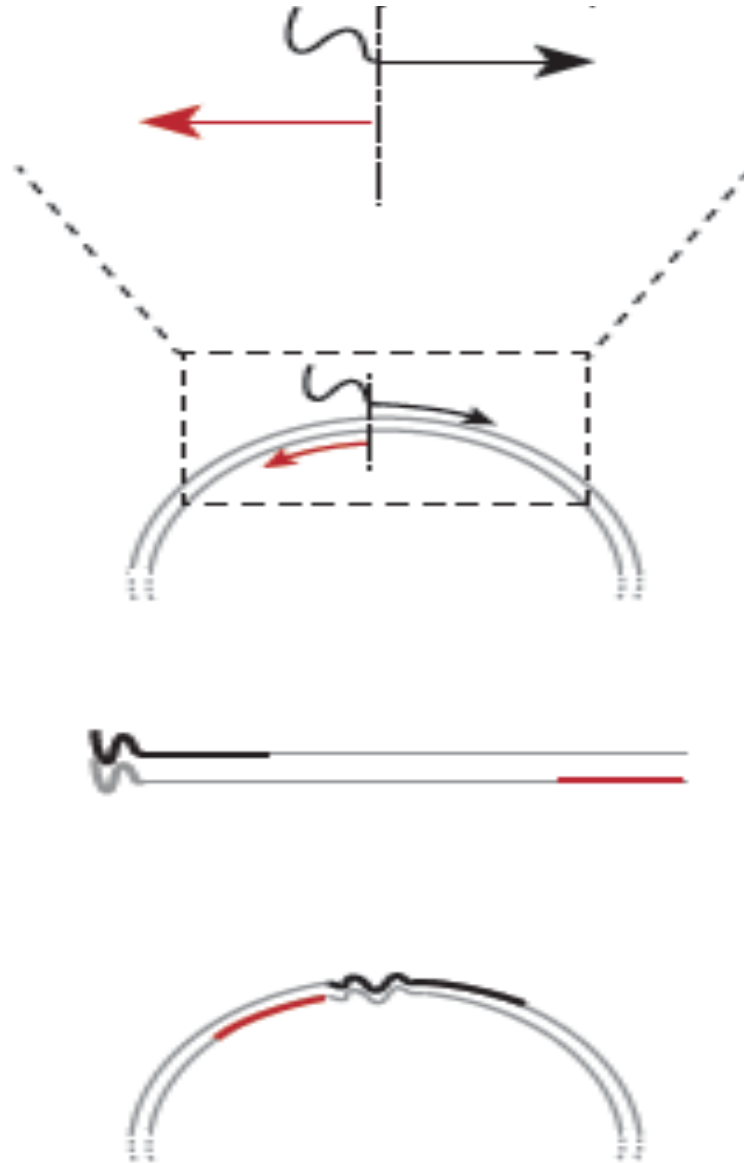
How did we do this in Mod2?

Use site-directed mutagenesis (SDM) to engineer plasmid DNA

- NEB Q5 SDM kit
- Create specific, targeted changes in double-stranded plasmid DNA
- Forward primer:
- PCR product:
- Final product:

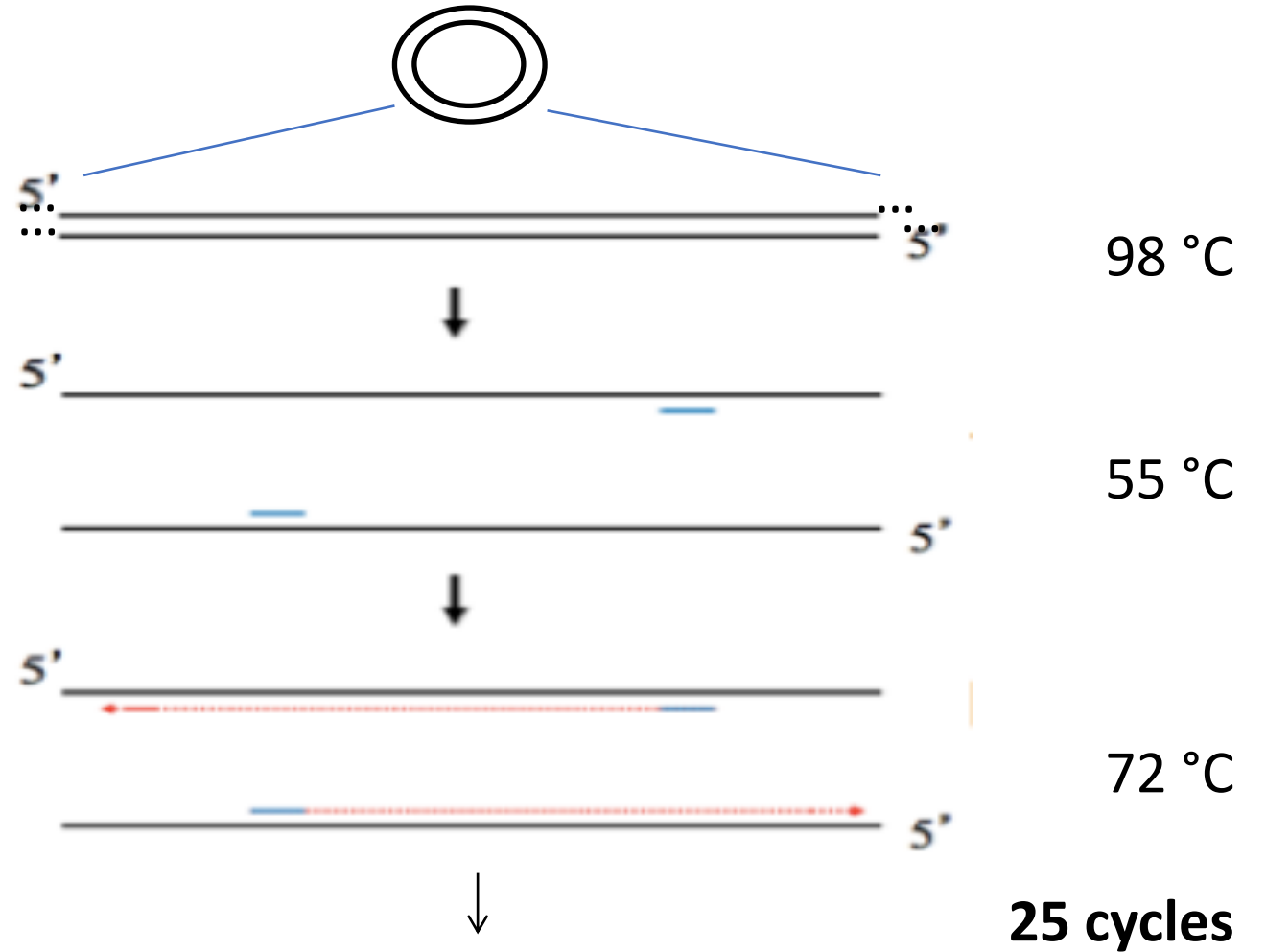


Insertion of DNA via SDM

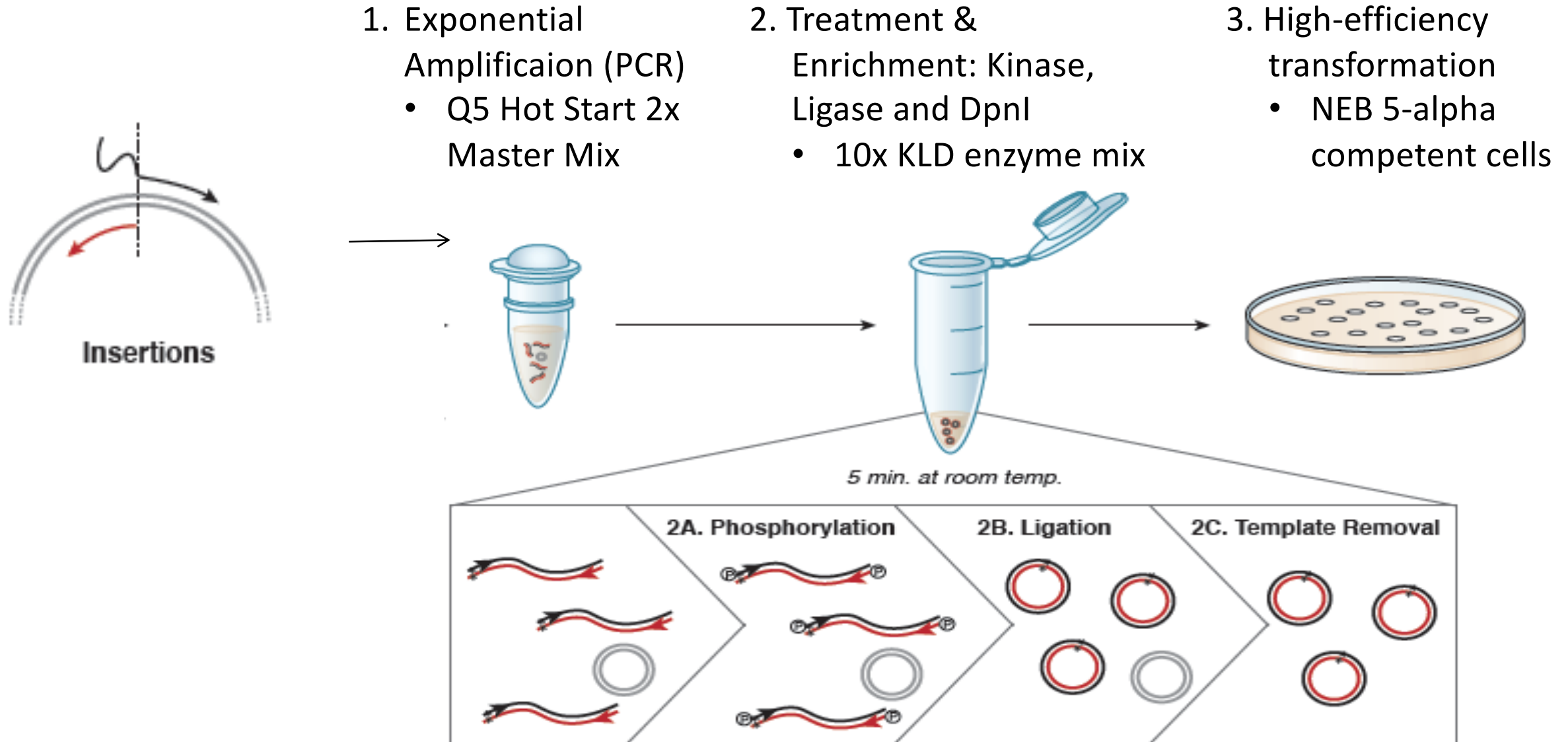


SDM Part 1: PCR amplification of DNA

Ingredients
dNTPs
Polymerase
Buffer (pH, cofactors like Mg^{2+})
H_2O



SDM Part 2: Recover circular plasmid product using Kinase-Ligase-Dpn1 (KLD) enzyme mix

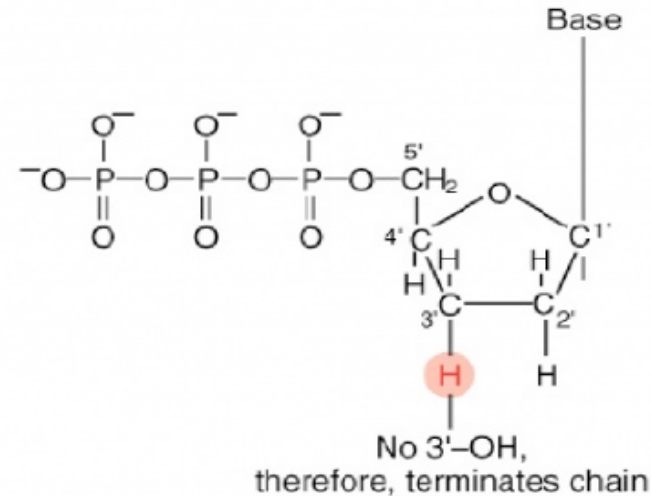
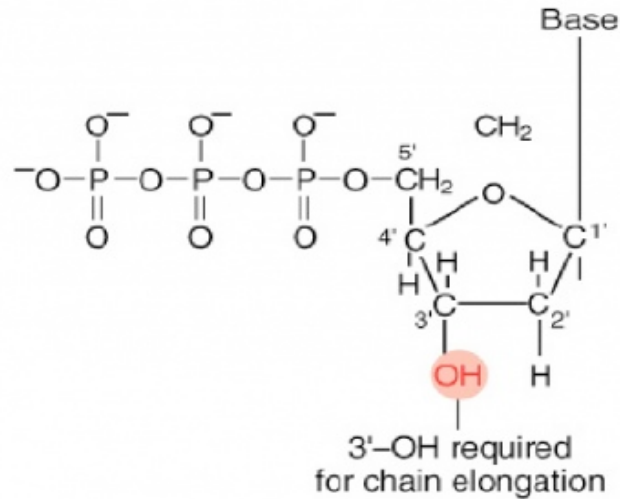


How can we determine that our Site Directed Mutagenesis was successful?

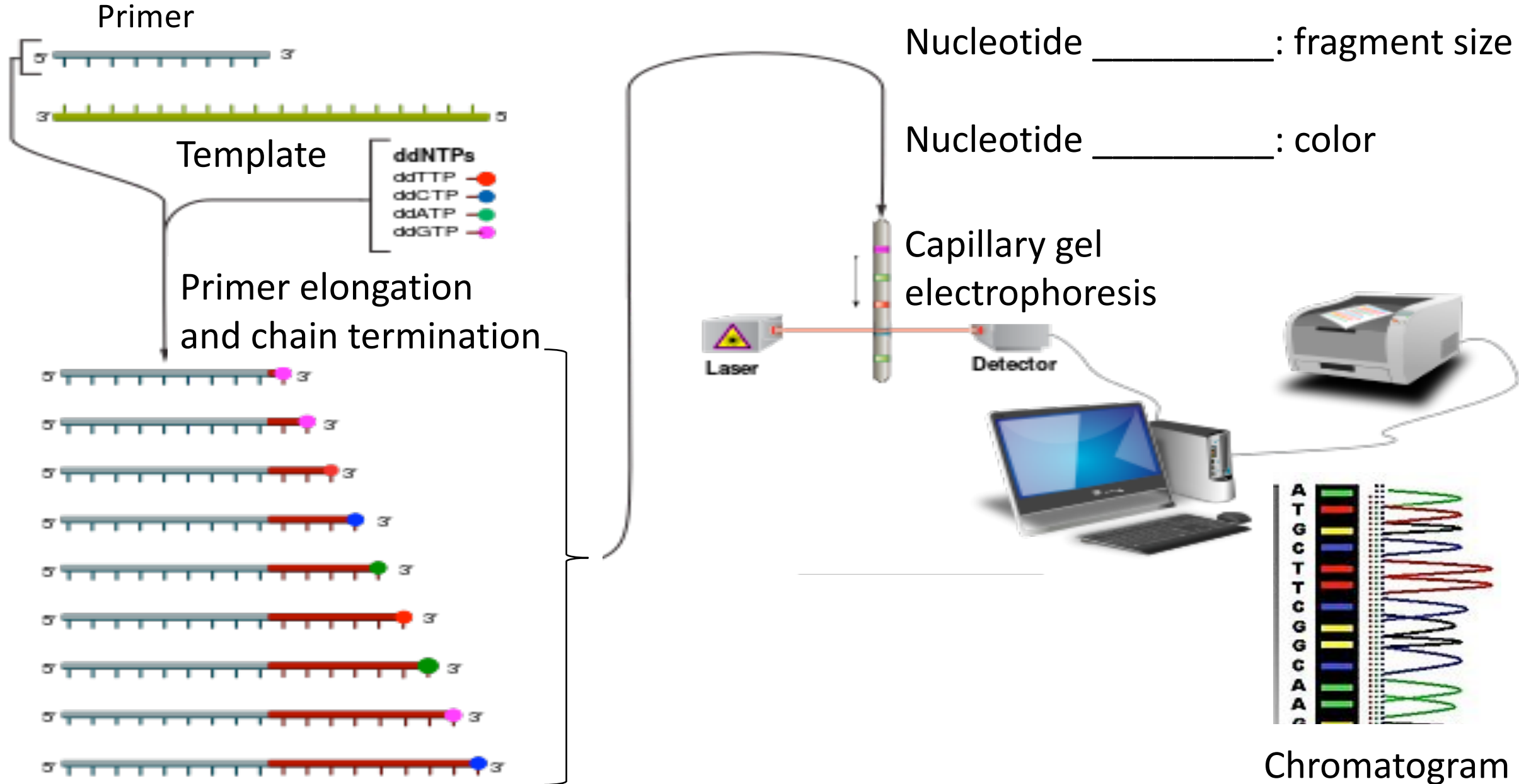
How did we determine successful cloning in Mod2?

Use sequencing to determine if we have the intended plasmid with gRNA_target

- Sanger DNA Sequencing, 1977
 - di-deoxynucleotides terminate elongation
 - good to have both forward and reverse coverage



Sanger sequencing by Genewiz



For Today

- Work through wiki to identify creation and alignment of sgRNA in the CRISPRi system
- Comm Lab at 4pm about preparing the Research Proposal

For M3D3...

- Work **with your lab partner** to write up a short description of your idea for the research proposal
 - Use the guidelines on the homework section of the wiki
- This does not have to be your final idea