PCR PRIMERS:

* A primer’s job is to determine specificity of amplification
* Designed to obtain desired product at high yield, with minimum amplification of unwanted sequences
* You can just make them from scratch! They don’t need to be taken from any organism. Follow the considerations below to make yours today!

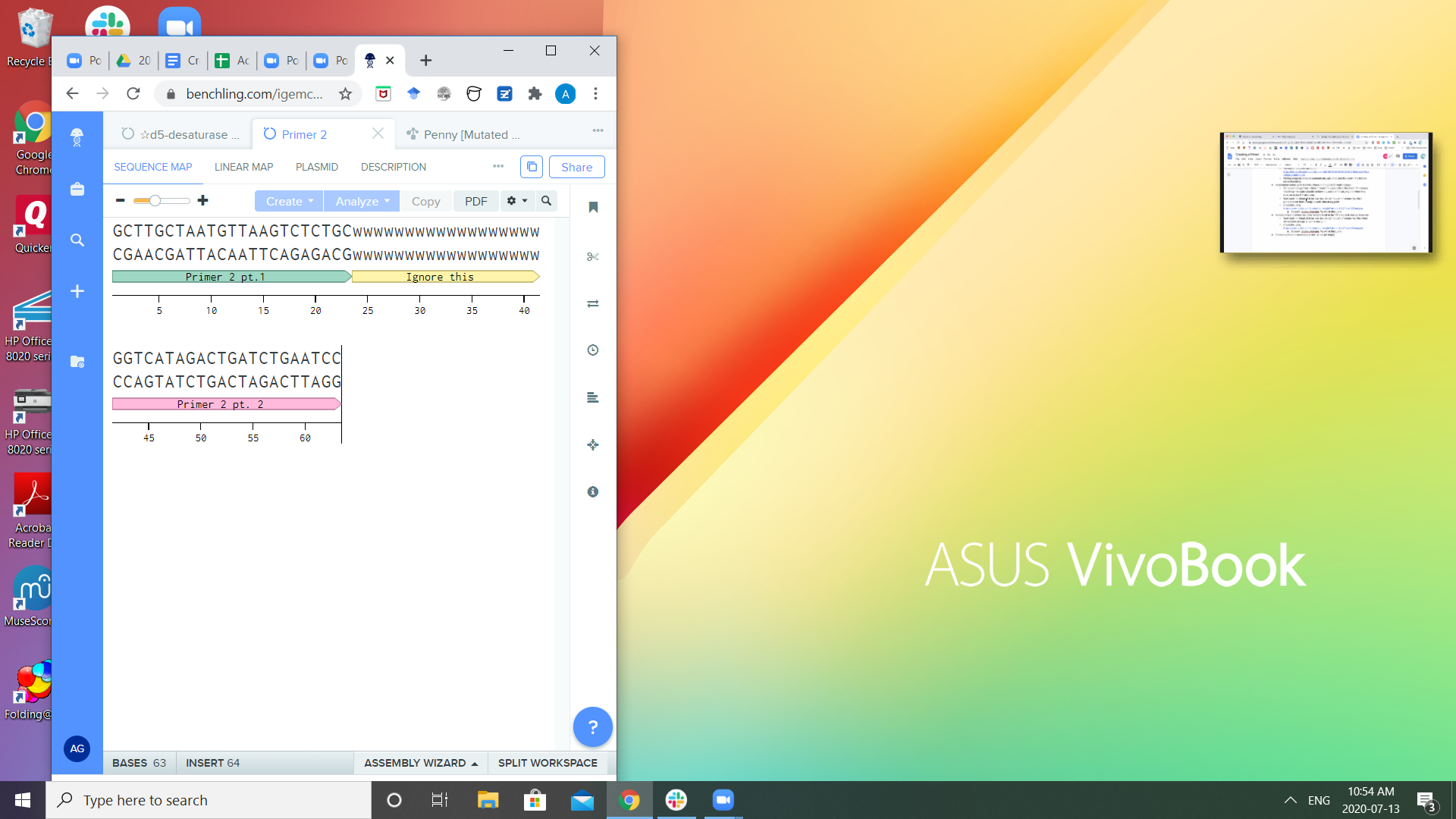
CONSIDERATIONS:

* Each primer is 18-24 nucleotides in length. Primer pairs should not differ from each other in length by >3 nucleotides. Generally, longer primers are for priming longer sequences
  + GC richness should be 40-60%, with no long runs of bases. Start and end with 1-2 G/C pairs.
  + GC richness is automatically calculated and displayed in the bottom bar on Benchling.
* Melting temperature (Tm) of 50-60°C
  + Calculated Tm values of primer pair shouldn’t differ by > 5 °C
  + Tm values of the amplified product should be low enough to ensure complete separation of strands during denaturation step of PCR.
  + Melting Temperature Calculator: <http://insilico.ehu.es/tm.php?primer=GCATGCTAATGATATCGTATCTGC&basic=1&cp=200&cs=50&cmg=0>
  + Melting temperature is also automatically calculated and displayed in the bottom bar on Benchling.
* Heterodimerization (when the two primers bind together) is bad
  + Do not want primer:primer interaction, especially at the 3’ end. This means that the primer pairs should not have any significant complementary regions when they both run in the 5’-3’ direction.
  + Want each individual heterodimer ΔG to be less than -5kcal/mole, which means that the 2 primers do not bind strongly to each other at any point
  + Check this using: <https://www.idtdna.com/site/account/login?returnurl=%2Fcalc%2Fanalyzer>
    - Account: sravyakakumanu, Password: bhsc\_pha!
* Homodimerization (when the primer binds to itself in the 3’-5’ direction), also bad
  + Want each individual homodimer ΔG to be less than -5kcal/mole, which means that the primer will not bind strongly to itself at any point
  + Check this using: <https://www.idtdna.com/site/account/login?returnurl=%2Fcalc%2Fanalyzer>
    - Account: sravyakakumanu, Password: bhsc\_pha!
* Hairpin loops (when a single strand of DNA folds in half to bind with itself)
  + Want each individual hairpin loop ΔG to be less than **-2**kcal/mole, which means that the primer does not very strongly want to form a hairpin loop
  + Check using same tool as the previous two, where it says ‘hairpin loops’

RECOMMENDATIONS FOR DESIGNING A PRIMER

* Use Benchling to create your primers.
* Create both primers on the same Benchling page (insert an obvious divider between the two primers) to easily make changes when correcting homodimerization and heterodimerization.
* You can also change the width of your browser window to align the two primers vertically on top of each other for easy visual comparison (as seen in Image 1 below).
* Go to the scissors on the right baron Benchling, then click **Cut Sites Visible on Maps,** then **None, except selected and compatible with them.** This can make it easier to compare them visually right next to each other because the restriction sites are no longer shown.

Image 1 :



HOW TO USE OLIGO ANALYZER (checking homo/heterodimerization)

Homodimerization:

* Copy and paste your primer into the sequence box and click “Self-dimer” on the right hand menu to run your analysis.
* A sample results list is shown below. The ΔG in each individual box should be less than -5kcal/mole.
* Repeat with your second primer.