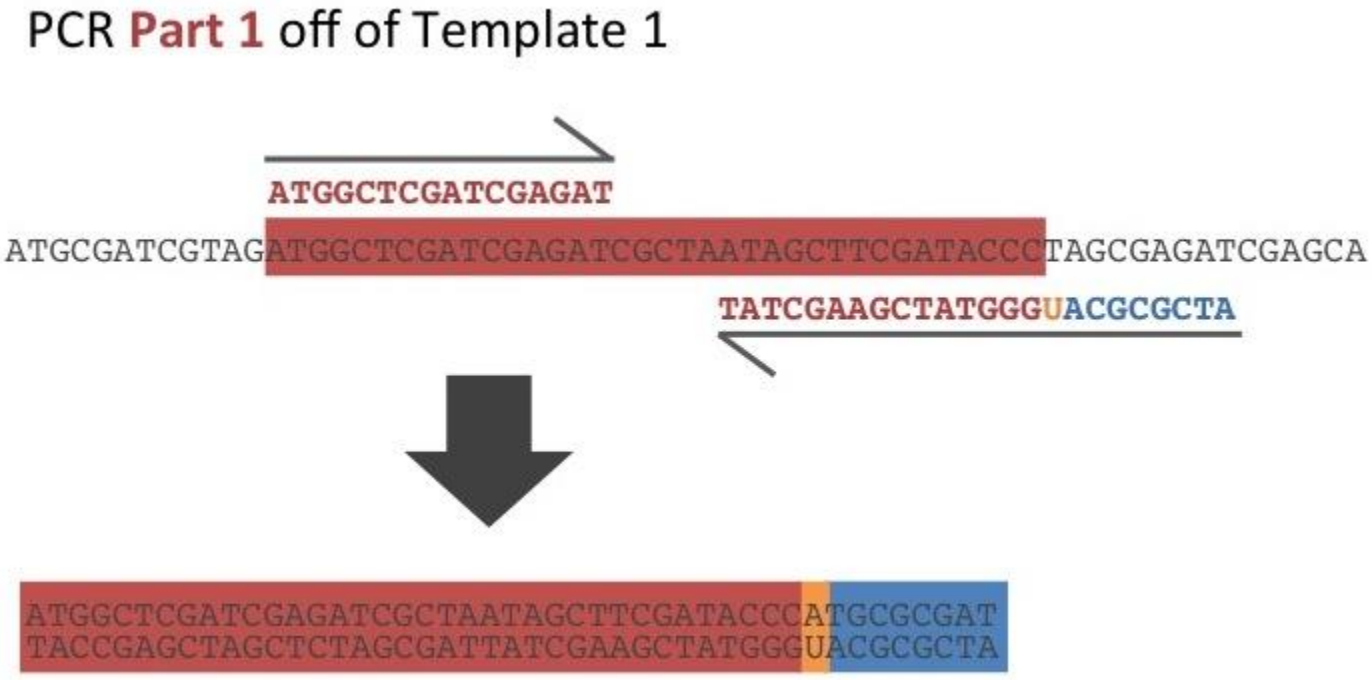
USER Cloning

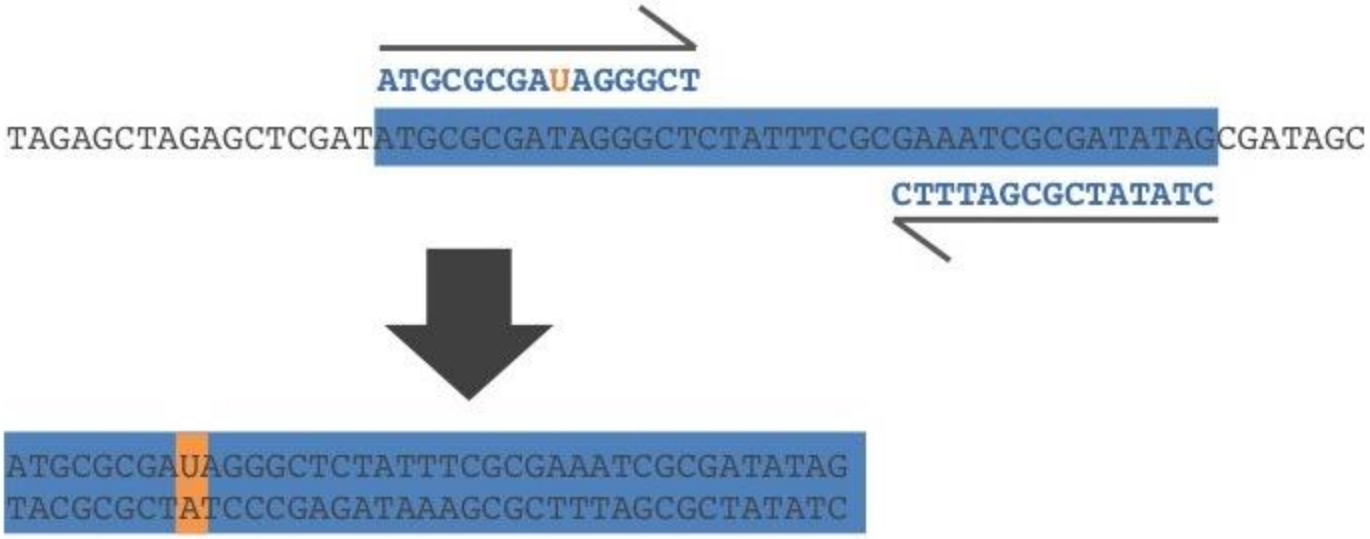
By Penn iGEM 2013 and Spencer Glantz

Goal: *To scarlessly combine two linear fragments of DNA, each of which contain uracil residues, into a larger linear or circular fragment.*

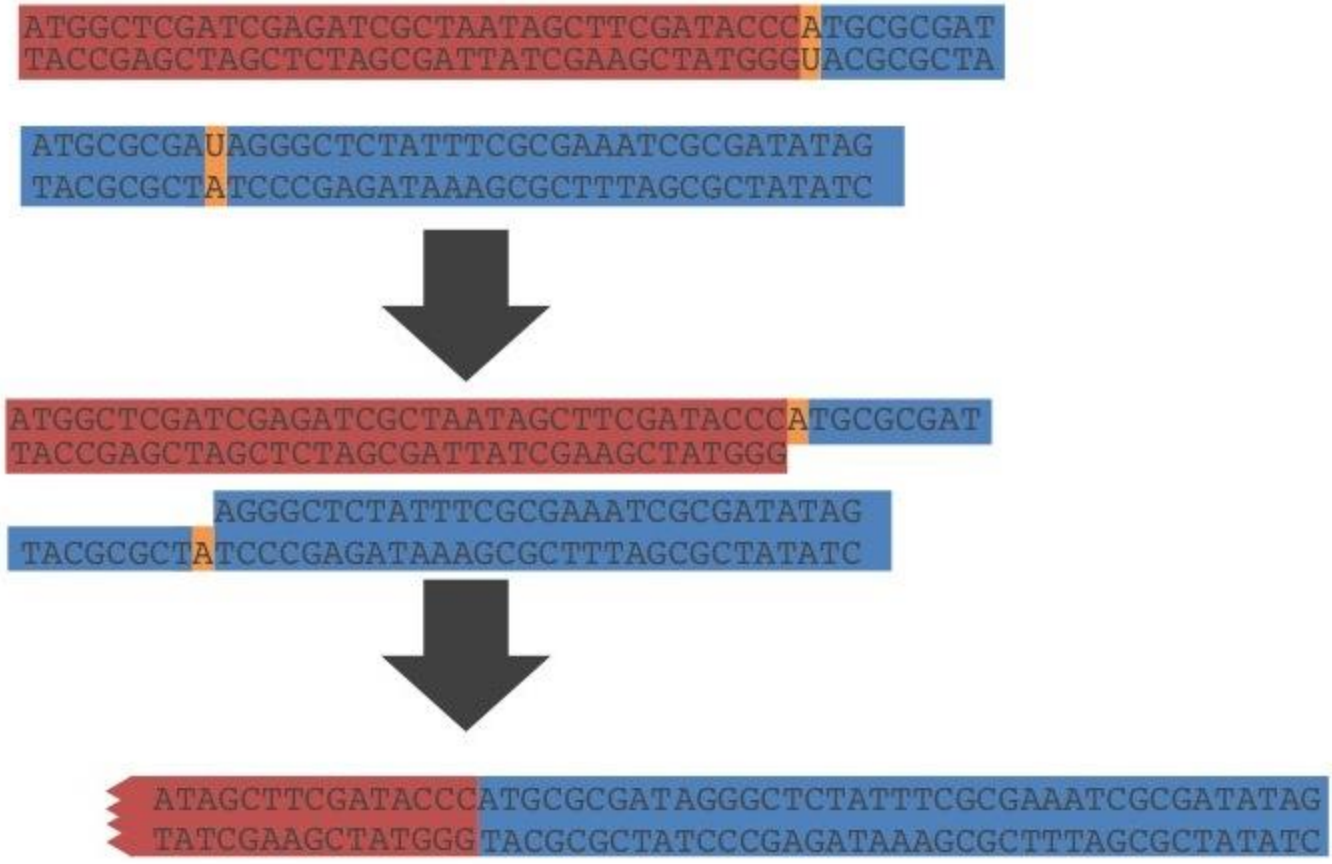
See figures below that explain the concept behind USER cloning (for a hypothetical situation where one wants to scarlessly ligate Part1 to Part2)



PCR **Part 2** off of Template 2



Digest PCR Products with DpnI and User Enzyme



Protocol:

(1) Amplify target sequences (Parts 1 and 2) with primers that contain an adenine at the 5' end and a uracil roughly 8-11 bp later (in place of a thymine). In between the 5' adenine and the uracil will be the USER cloning overhang sequence - which may be standardized or part of the gene you are trying to clone. When designing overgangs, I try to find and A and T roughly 8-11 bp away from each other with a lower GC content (higher specificity). Use Pfu Turbo Cx polymerase to minimize PCR errors - although I have only had success with this polymerase for amplicons ≤ 2 kb.

Pfu Turbo Cx Amplification Reaction Mix (20 uL)

1 uL Pfu Turbo Cx
1 uL 10 uM Fwd Primer
1 uL 10 uM Rev Primer
1 uL DNA template
1 uL 10 mM dNTPs
2 uL Pfu Turbo Cx Buffer (10x)
13 uL H2O

Thermocycler Settings:

95ºC for 3 minutes (or 5 minutes for colony/genomic DNA templates)
30 Cycles of:
    95ºC for 30 seconds
    55ºC for 30 seconds
    68ºC for extension time (1 kb/minute) + some (I'm generous here)
1 Cycle of:
    68ºC for 20 minutes
    Hold at 4ºC

(2) Perform USER cloning reaction:

Note, run reactions with just each part on its own as negative controls to see how much "background" you are getting from each one - replace missing parts with H2O. This is most important for parts that contain an origin of replication and an antibiotic resistance marker

2-way assembly Reaction Mixture (4.8 uL)
0.4 uL DpnI
0.4 uL USER enzyme
2 uL Part 1
2 uL Part 2

3-way assembly Reaction Mixture (4.8 uL)
0.4 uL DpnI
0.4 uL USER enzyme
1.33 uL Part 1
1.33 uL Part 2
1.33 uL Part 3

On the thermocycler:

37ºC for 1 hour.
22ºC (room temperature) for 15 minutes.
Cool to 4ºC.

Directly transform 1 uL of the product into chemically competent cells (follow plasmid transformation protocol) - Plate everything and should get 20 - 50 colonies.