Endogenous tagging of yeast genes
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-This protocol can be used with any pFA6a-based tagging vector and the F2/R1 primer pairs used to construct the yeast GFP collection (http://yeastgfp.yeastgenome.org/yeastGFP OligoSequence.txt)

-We use NEB Q5 polymerase for amplification, but any high-fidelity polymerase can be used

-For small epitope tags (i.e. 3xFLAG, 3xHA), a 45" extension time works well. For large tags (i.e. FRB, 3xFLAG-MNase), a 1'15" extension time is needed

1. For each PCR, prepare the following reaction mix:
   -10 µL 5X Q5 reaction buffer
   -1 µL 10 mM dNTPs (2.5 mM each dNTP)
   -2.5 µL 10 µM F2 primer
   -2.5 µL 10 µM R1 primer
   -X µL DNA (1-5 ng)
   -0.5 µL Q5 polymerase
   -H2O to 50 µL

2. Cycle using the following parameters:
   -98°C, 30"
   -25 cycles of:
     -98°C, 10"
     -55°C, 30"
     -72°C, 45" or 1'15"
   -72°C, 2’
   -Hold at 8°C

3. Analyze 5 µL of the reaction on an agarose gel