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/yeastGFPOligoSequence.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
    - -H_2O 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