Clean deletions

pMAD see Arnaud et al., Appl Environ Microbiol 2004, 70:6887
(http://www.ncbi.nlm.nih.gov/pubmed/15528558)

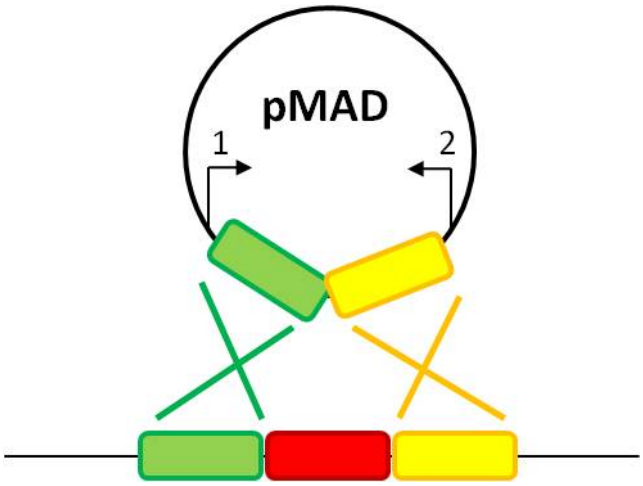
1. Cloning
    a. Amplify up and down fragments (~1 kb upstream and downstream from the fragment to be deleted)
    b. Join both fragments (see joining PCR in PCR methods)
    c. Clone joined fragments in pMAD (in *E. coli* DH5α)

2. Transform the sequenced plasmid into *E. coli* BMH7118 and isolate it again

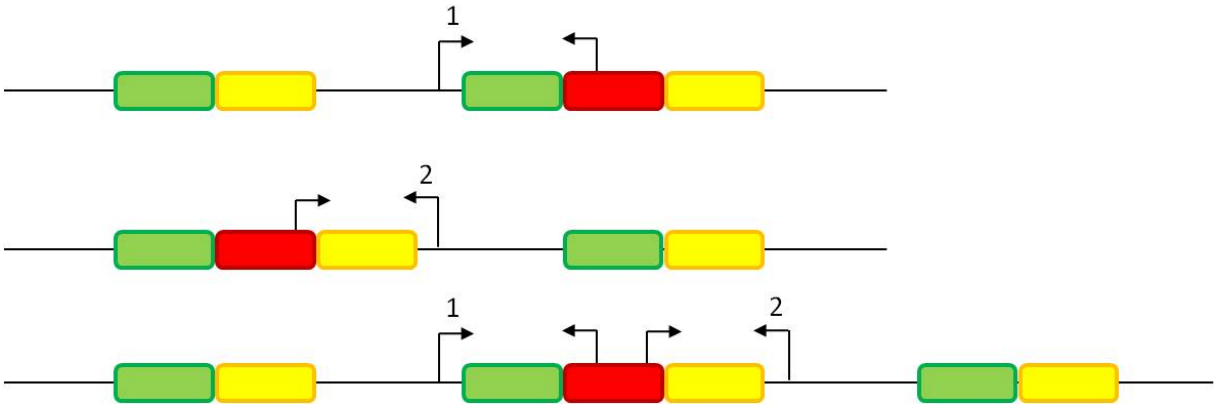
3. Transformation in *B. subtilis*
    a. Transform the plasmid isolated from *E.* coli BMH7118 (~1,5 – 2 µg) in *B. subtilis* W168
    b. Select the transformants at 30°C on plates with X-gal (100 µg/ml) and MLS selection
    c. Pick blue colonies

4. 1st temperature shift
    a. Prepare an overnight culture with MLS selection at 30°C
    b. Inoculate 10 ml LB with MLS at OD_{600} 0,1 and incubate at 30°C for 2 hours
    c. Shift the temperature to 42°C and incubate for 6 hours
    d. Plate out dilutions (10^{-2} to 10^{-5}) on plates with X-gal and MLS and incubate at 42°C

5. Colony PCR - check the integration of pMAD in the genome



Possibilities after recombination:



1&2 pMAD check fwd and rev primers (253 & 254)

6. 2nd temperature shift
    a. Inoculate the positive cones in LB without selection and incubate for 6 hours at 30°C
    b. Shift the temperature to 42°C and incubate for 3 hours
    c. Plate out dilutions (10^{-2} to 10^{-5}) on plates with X-gal (without selection) and incubate at 42°C
    d. Pick white colonies and check the deletion with colony PCR
    e. Check if the mutants are MLS sensitive!

Protocol generously provided by the lab
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