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PARTIAL DIGESTS OF PLASMID DNA

(Restriction digests that affect only a subset of the target restriction sites; for difficult clonings)

- Have a timer or watch handy
- Pour an agarose gel to separate your digestion products
- Label four 0.6ml microcentrifuge tubes as follows: 0, 1, 5 and 20
- Into each tube, pipette 3 μ l STOP mix
- Prepare a restriction enzyme digest as follows
 - ~4 μ g plasmid DNA
 - 4 μ l 10x restriction enzyme buffer
 - 4 μ l 1 mg/ml BSA
 - 0.5 μ l RNase (0.5 mg/ml, if necessary)
 - adjust volume to 40 μ l with dsH₂O
- Remove 10 μ l from the mixture and place it into the tube labeled "0"
- Add ~0.5 μ l restriction enzyme to the digestion mixture from step □; mix; note the time
- After 60s, remove 10 μ l from the reaction mix and transfer it to the tube labeled "1"; mix well
- After 4 more minutes, remove 10 μ l from the reaction mixture and transfer it to the tube labeled "5"; mix well
- After 15 more minutes, remove 10 μ l from the reaction mixture and transfer it to the tube labeled "20"; mix well
- Load samples onto agarose gel

STOP mix

100 μ l 0.5M EDTA, pH 8

200 μ l 6x loading dye