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 3µl STOP mix
- Prepare a restriction enzyme digest as follows

  ~4µg plasmid DNA
  4µl 10x restriction enzyme buffer
  4µl 1 mg/ml BSA
  0.5µl RNase (0.5 mg/ml, if necessary)
  adjust volume to 40µl with dsH_20

- Remove 10µl from the mixture and place it into the tube labeled "0"
- Add ~0.5µl restriction enzyme to the digestion mixture from step □; mix; note the time
- After 60s, remove 10µl from the reaction mix and transfer it to the tube labeled "1"; mix well
- After 4 more minutes, remove 10µl from the reaction mixture and transfer it to the tube labeled "5"; mix well
- After 15 more minutes, remove 10µl from the reaction mixture and transfer it to the tube labeled "20"; mix well
- Load samples onto agarose gel

STOP mix

100µl 0.5M EDTA, pH 8

200µl 6x loading dye