As of 8/19/2014

* any extraction less than 5ng/µL we are putting on the back burner to continue processing at a later date
* anything that is more than 5ng/µL but makes less than 1µg of DNA when you multiply the quant result by 30µL (the digest addition amount) - calculate the amount of extract to make 1µg DNA and clean that amount, elute in 30µL and add to digest plate
* anything that makes more than 5µg of DNA when you multiply the quant result by 30µL - add 15µL of extract to plate and 15µL pH2O

Digestion recipe 50µL rxn:

30µL DNA (\*\*\*for bioanalyzer runs, combine enough dna to make 1ug with pH2O)

5µL 10x buffer

0.2µL PstI (want 20 U of 100,000U/mL conc)

2µL MluCI (want 20U of 10,000U/mL conc)

12.8µL pH2O

192 samples - round up to 210; master mix recipe in 15mL falcon tube:

1050µL 10x buffer

2688µL pH2O

42µL PstI

420µL MluCI

split 250µL into 16 wells - add 20µL to each sample (ran out during column 12 of last plate, make more than 200 worth)

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For a 50µL rxn, use:

1µg DNA - fill to volume w/ pH2O

5µL 10x buffer (50µL/10x=5)

Want a total of 10U in rxn

For single digest:

if enzyme is 10,000U/mL - 1µL = 10U

if enzyme is 20,000U/mL - 0.5µL = 10U

For double digest

if enzyme is 10,000U/mL - 0.5µL = 5U

if enzyme is 20,000U/mL - 0.25µL = 5U

Old Protocol:

50µL reaction:

44.5µL DNA

5.0 µL 10x buffer

0.25µL EcoRI

0.25µL SbfI

Incubate in thermocycler at 37˚ for 4 hours