

**Restriction Digests
(Guidelines)**

	<u>For 15µl Rxn Vol</u>	<u>Final Conc</u>
DNA (plasmid)	10-15µl	0.5µg
10X Buffer	1.5µl (1/10 vol)	1X
BSA (100X or 10mg/ml; enzyme dependent)	0.15µl (1/100 vol)	100µg/ml
RNase A (*10mg/ml; need for mini prep DNA)	0.15µl	100µg/ml
Restriction Enzyme	~0.1µl	1U/µg DNA
Incubate at appropriate temp	~1h	
Add Loading dye buffer	3µl	1/5vol
Load on gel or store away at -20°C		

Loading Dye:

<u>Stock</u>	<u>For 10 ml</u>	<u>Final Concentration</u>
80% Glycerol	9.4ml	75%
1M Tris(7.5)	0.1ml	10mM
5M NaCl	0.02ml	10mM
0.5M EDTA	0.2ml	10mM
10% SDS	0.1ml	0.1%
Bromophenol Blue	25mg	
Xylene Cyanol	25mg	

See Sambrook appendix B17 for how to prepare RNase A that is free of DNase