

Restriction Digest with NEB Enzymes

No	Description/Details of Steps in Activity	Hazards	Possible Accident / Ill Health & Persons-at-Risk	Existing Risk Control (Mitigation)	Severity	Likelihood (Probability)	Risk Level	Additional Risk Control
1	Adjust Water bath to 37 degrees.	Electric shock, heat shock	Possibility of electric shock	wear proper PPE (gloves, lab coat, covered shoes)	1	1	1	
2	Add aliquots of DNA to water and Cut Smart buffer in a 1.5mL centrifuge tube.	Biological exposure	Spillage of buffer and DNA	wear proper PPE (gloves, lab coat, covered shoes)	1	1	1	
3	Lightly centrifuge the centrifuge tube in a microcentrifuge. Add an appropriate volume of NEB restriction enzymes and centrifuge again.	Spillage, Biological exposure , injury due to improper usage of centrifuge and fingers	Injury due to imbalanced centrifuge and trapping of limbs or fingers	Internal training is compulsory.wear proper PPE (gloves, lab coat, covered shoes); handle cells in the biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand. When using the centrifuge, ensure centrifuge is balanced and rotor is placed correctly, and that all tubes are capped tightly. Close centrifuge properly and ensure that there are no funny sounds when centrifuge is running.	1	1	1	
4	Place 1.5mL tube into waterbath and digest for 2 hours at 37 degrees. When Restriction digest is done, take the tube out and stop reaction by adding Purple loading dye and cooling on ice.	Electric shock, heat shock	Spillage of buffer and DNA	wear proper PPE (gloves, lab coat, covered shoes)	1	1	1	