Ligation Protocol with T4 DNA Ligase

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 Thermocycler to 16 degrees hold.	Electric shock	Possibility of electric shock	wear proper PPE (gloves, lab coat, covered shoes)/	1	1	1	
2	Add aliquots of cut vector and insert to water and T4 ligase buffer in a 0.2mL pcr tube.	Biological exposure	Spillage of buffer and DNA	wear proper PPE (gloves, lab coat, covered shoes)/	1	1	1	
3	Lightly centrifuge the pcr tube in a microcentrifuge. Add an appropriate volume of ligase 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	2	2	
4	Place pcr tube into thermocycler and ligate overnight at 16 degrees.	Biological exposure	Spillage of buffer and DNA	wear proper PPE (gloves, lab coat, covered shoes)/	1	2	2	