

Miniprep of Plasmid DNA

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	Inoculate bacteria in 3mL of LB+antibiotic to grow for 16 hours at 37 degrees in a shaking incubator.	Biological exposure, breakage of glassware	Handling of bacteria outside of a Class I Biohazard Safety Cabinet risks generating aerosol particles which may be inhaled or ingested	Internal training is compulsory. Conduct routine bacteria work in a Class I Biohazard Safety Cabinet. Wear suitable PPE (e.g. lab coat, covered shoes, gloves); decontaminate equipment and/or small spills with 70% ethanol or DeCon90.	1	1	1	
2	Pellet Bacteria in 1.5mL tubes in a microcentrifuge at maximum speed for 5 minutes. Pour away the LB media into a waste container containing 10% bleach.	Biological exposure	Spillage of bacteria culture.	wear proper PPE (gloves, lab coat, covered shoes). Clean the bench with 70% ethanol after work for the day.	1	1	1	
3	Resuspend the bacterial cells in Resuspension solution, vortexing lightly to ensure pellet is fully resuspended in solution. Add Lysis buffer and incubate till solution is clear and there is no cloudy bacteria for 2 minutes. Add Neutralisation solution and invert 4-6 times to mix immediately.	Biological exposure	Spillage of bacteria culture, and buffers	wear proper PPE (gloves, lab coat, covered shoes). Clean the bench with 70% ethanol after work for the day.	1	1	1	
4	Pellet the lysed bacteria by centrifugation for 5 minutes. Run the flow through in DNA binding columns.	Spillage, Biological exposure, injury due to improper usage of centrifuge and fingers	Injury due to imbalanced centrifuge and trapping of limbs or fingers	wear proper PPE (gloves, lab coat, covered shoes). Clean the bench with 70% ethanol after work for the day. Ensure that the centrifuge is balanced.	1	1	1	

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5	Add wash buffer to wash through the columns and precipitate DNA. Centrifuge for 1 min, then pour out the remaining wash buffer in the tube. Centrifuge for a further 2 minutes to dry the column.	Spillage, Biological exposure , injury due to improper usage of centrifuge and fingers	Injury due to imbalanced centrifuge and trapping of limbs or fingers	wear proper PPE (gloves, lab coat, covered shoes). Clean the bench with 70% ethanol after work for the day. Ensure that the centrifuge is balanced.	1	1	1	
6	Add 30-50ul of nuclease free water to the column. Spin down the DNA and quantify concentration using the Thermo Scientific nanodrop.	Spillage, Biological exposure , injury due to improper usage of centrifuge and fingers	Injury due to imbalanced centrifuge and trapping of limbs or fingers	wear proper PPE (gloves, lab coat, covered shoes). Clean the bench with 70% ethanol after work for the day. Ensure that the centrifuge is balanced.	1	1	1	