Protocol for mini-prep with Tiangen Mini-prep Kit

Pick a single colony from a selective plate and inoculate a culture of 4.5ml LB medium containing the appropriate antibiotic. Incubate for 10-12 h at 37℃ with vigorous shaking;

Harvest the bacteria cells by centrifuge at 12000 rpm in 1.5 ml microcentrifuge for 1min at room temperature and remove all traces of supernatant by inverting the open centrifuge tube;

Resuspend pelleted bacterial cells in 250ul buffer P1;

Add 250ul buffer P2 and mix thoroughly by inverting the tubes 4-6 times;

Add 350ul buffer P3 and mix gently by inverting the tube 6-8 times;

Centrifuge at 12000rpm for 10min;

Apply the supernatant from the last step to the column by decanting;

Centrifuge for 60s and then discard the flow-through;

Wash the column by adding 600ul buffer PW and centrifugeing for 60s. Discard the flow-through;

Repeat the last step;

Centrifuge for 2 min to remove the residual wash buffer;

Place the column in a clean 1.5ml microcentrifuge tube and add 40ul buffer EB to the center of the column; Let stand for 2min and centrifuge for 2min;

Note: add 500ul buffer BL to column, centrifuge at 12000rpm for 1 min and discard flow-through before use to maximally activate the column;

Reffernece: QIAprep® Miniprep Handbook