

Gel Purification

Place the gel under UV light at an intensity just high enough to visualize the bands. Cut out the bands containing the insert and the vector with a razor and purify the DNA.

4.1 Place the gel under UV light at an intensity just high enough to visualize the bands. Cut out the bands containing the insert and the vector to purify the DNA.

4.2 Place the gel slice in a 1.5-mL microfuge tube and weigh it. Add two volumes of Buffer NT to one volume of gel (100 mg = 200 μ L). For gels >2% agarose, double the volume of Buffer NT.

4.3 Incubate the gel at 50° C for 5 – 10 min until the gel slice is completely dissolved. Vortex the tube every 2 – 3 min to speed up the dissolving process.

4.4 Place a spin column in one of the provided 2-mL collection tubes.

4.5 Place a NucleoSpin® column into a collection tube. Pipette the DNA solution onto the column.

Centrifuge the DNA solution at 13,000 g for 1 min. The maximum volume the column can hold is 800 μ L, so repeat this step using the same column if the volume is larger than that.

4.6 Discard flow-through from the previous step and place the column back in the collection tube.

4.7 Wash the DNA in the column by applying 600 μ L of buffer NT3. Centrifuge the column for 1 min at 13,000 g.

4.8 Discard the flow-through and spin the column for 2 additional minutes to dry the column.

4.9 Place the spin column in a clean 1.5-mL microfuge tube.

4.10 To elute the DNA, add 10 – 30 μ L of buffer EB to the center of the white matrix. Allow the column to sit for 1 min and then centrifuge it at 13,000 g for 1 min.

4.11 Quantify the DNA, which is eluted in the flow-through.