

DNA isolation

Using UltraClean Kit from QIAGEN for isolating DNA from swabs

Prep:

- 01 If precipitated, heat solution SL at 55 °C until precipitates dissolve.
- 02 **Vortex** the collection tube with the swab for several seconds, follow by **quick spin**.
- 03 Transfer **300 µL** of the supernatant from the collection tube to the PowerBead tube.
- 04 Add **50 µL** of Solution **SL** to the PowerBead tube. **Vortex** the mixture.
- 05 Incubate the PowerBead tube at **70 °C for 5 minutes**. Quick spin afterwards.
- 06 Secure PowerBead tubes horizontally on the plate vortex using a red box with tape.
Vortex at maximum speed for 10 minutes.
- 07 Centrifuge the tubes at **10 000 g for 1 minute**.
- 08 Transfer the supernatant into a new 2 mL tube.
- 09 Add **100 µL** of Solution **IRS** and **vortex for 5 s**. Incubate at **4 °C for 5 minutes**.
- 10 Centrifuge the tubes at **10 000 g for 1 minute**.
- 11 Transfer the supernatant into a new 2 mL tube. Avoid the white pellet.
- 12 Add **900 µL** of Solution **SB** and **vortex for 5 s**. Shake the Solution SB before use.
- 13 Transfer **700 µL** of the mixture from step 12 into an **MB Spin Column**.
Centrifuge at **10 000 g for 1 minute**. Mark both the column and the collection tube.
- 14 Discard the flow-through. Transfer another **700 µL** of the mixture from step 12 into an **MB Spin Column**. Centrifuge at **10 000 g for 1 minute**.
- 15 Discard the flow-through. Transfer the remaining mixture from step 12 into an MB Spin Column and centrifuge again at **10 000 g for 1 minute**. Discard the flow-through.
- 16 Add **300 µL** of Solution **CB** into the MB Spin Column. Shake the Solution SB before use.
Centrifuge at **10 000 g for 1 minute**.
- 17 Discard the flow-through. Place the MB Spin Column in a new 2 ml collection tube.
Centrifuge at **10 000 g for 1 minute**.
- 18 Discard the flow-through. Place the MB Spin Column in a new 2 ml collection tube.
Be careful not to splash any of the remaining liquid on the MB Spin Column.
- 19 Add **50 µL** of Solution **EB** to the **centre** of the white filter membrane.
- 20 **Incubate at RT for 5 minutes**.
- 21 Centrifuge at **10 000 g for 1 minute**.
- 22 Discard the MB Spin Column and **keep the flow-through**.