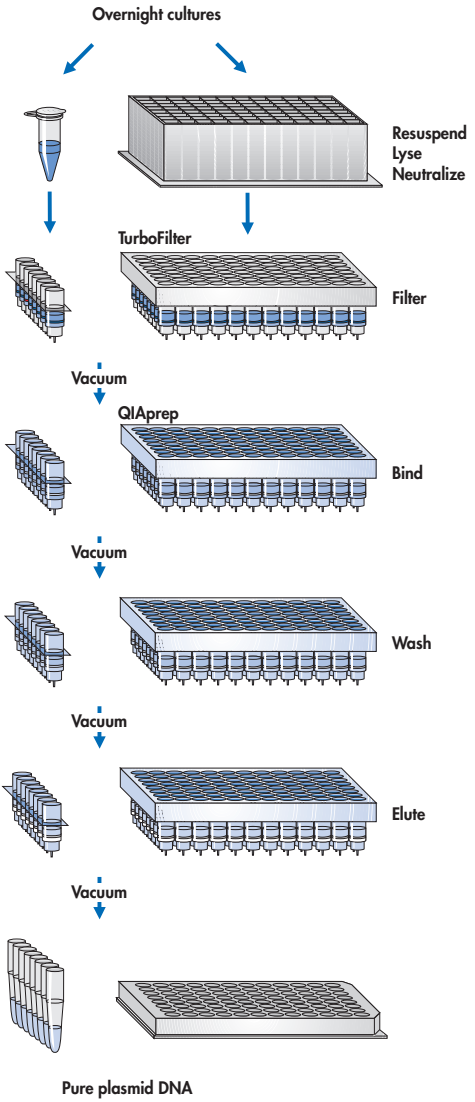
QIAprep 8 and 96 Turbo Procedure



QIAprep 8 Turbo Miniprep Kit Protocol

This protocol is designed for medium-throughput plasmid DNA minipreps using TurboFilter 8 and QIAprep 8 strips on QIAvac 6S. The kit accommodates 8–48 parallel preparations of up to 20 µg of high-copy plasmid DNA from 1–5-ml overnight cultures of *E. coli* grown in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 31.

DNA purification with the QIAprep 8 Turbo Miniprep Kit can be automated with the QIAGEN BioRobot™ 9600. Please call QIAGEN for more details.

! **Please read Important Notes for QIAprep Procedures on pages 14–15 before starting.**

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl of Buffer P1.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl of Buffer P2 to each sample, gently invert the tubes 4–6 times to mix, and incubate at room temperature for 5 min.**

   It is important to mix gently by inverting the tubes. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tubes until the solution becomes viscous and slightly clear.

   During incubation prepare QIAvac 6S:

   (**Note**: The following procedure applies to the manifold with a hinged lid and spring lock. See Appendix A on page 32).

   - Open QIAvac 6S lid and place TurboFilter 8 strips in the slots of the QIAvac top plate. Make sure the strips are seated tightly. Seal any unused slots with blanks (provided with QIAvac 6S), and close QIAvac 6S lid.
   - Place the strip holder inside the QIAvac base. Place QIAprep 8 strips into the strip holder such that a QIAprep strip is placed under each TurboFilter strip.
   - Place the top plate squarely over the base. Seal any unused wells of the TurboFilter strips with the strip caps provided. Attach the QIAvac to vacuum source.

3. **Add 350 µl of Buffer N3 to each sample and invert the tubes immediately but gently 4–6 times.**

   To avoid localized precipitation, mix the samples gently but thoroughly, immediately after addition of Buffer N3. The solutions should become cloudy.

4. **Pipet the lysates from step 3 (850 µl per well) into the wells of the TurboFilter strips. Unused wells of TurboFilter strips should be sealed with the strip caps provided. Apply vacuum until all samples have passed through TurboFilter.**

   Optimal flow rate is 1–2 drops/sec, which can be regulated by using a 3-way valve or vacuum regulator (see page 41) between QIAvac and the vacuum source.

5. **Switch off vacuum, and ventilate QIAvac 6S slowly. Discard the TurboFilter strips. Transfer the QIAprep strips containing the cleared lysates into the slots of the QIAvac top plate. Close QIAvac 6S lid. Replace strip holder in the base with the waste tray. Place the top plate squarely over the base. Seal any unused wells of the QIAprep strips with strip caps provided. Apply vacuum.**

   The flow-through is collected in the waste tray.

6. **(Optional): Switch off vacuum, and wash QIAprep strips by adding 1 ml of Buffer PB to each well and applying vacuum.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional step.

7. **Switch off vacuum. Wash QIAprep strips by adding 1 ml of Buffer PE to each well and applying vacuum.**

8. **Repeat step 7.**

9. **After Buffer PE in all wells has been drawn through, apply maximum vacuum for an additional 5 min to dry the membrane.**

   ! **IMPORTANT**: This step removes residual Buffer PE from the membrane. The removal is only effective when maximum vacuum is used (i.e. turn off vacuum regulator or leakage valves if they are used), allowing maximum airflow to go through the wells.

10. **Switch off vacuum, and ventilate QIAvac 6S slowly. Lift the top plate from the base (not the QIAprep strips from the top plate), vigorously rap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the QIAprep strips with clean absorbent paper. Proceed either to step 11a or 11b, as desired.**

    This step removes residual Buffer PE, which may be present around the outlet nozzles and collars of QIAprep strips. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

11a. For elution into provided collection microtubes:

Replace waste tray with the blue collection microtube rack (provided with QIAvac 6S) containing 1.2-ml collection microtubes. Place the top plate back on the base.

Rows of collection microtubes should be lined up with the QIAprep 8 strips.

11b. For elution into a 96-well microtiter plate:

Replace waste tray with empty blue collection microtube rack (provided with QIAvac 6S) and place a 96-well microtiter plate directly on the rack. Place the top plate back on the base.

12. **To elute DNA, add 100 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of each well of the QIAprep strips, let stand 1 min, and apply maximum vacuum for 5 min. Switch off vacuum and ventilate QIAvac 6S slowly.**

    For increased DNA concentration, an elution volume of 75 µl can be used.