Standard restriction analysis:

General procedure:

1) Extract plasmid DNA from clone(s). PCR products may also be used but then it is recommended to ensure that the concentration of the products is sufficiently high.

2) Mix the sample with the following components (total volume 10 µl):
   - Plasmid product 5 µl
   - Restriction buffer 1 µl (provided together with the restriction enzyme)
   - Restriction enzyme 0,25 µl (e.g. Eco RI)
   - Sterile MQ 3,25 µl
   - RNase 0,5 µl

2) Incubate for minimum 2 h or overnight at 37° C.

3) Pipette products on a 1 % agarose gel (remember to add DNA ladder and loading dye if no dye was provided in the PCR buffer) and develop the gel with an appropriate dye solution (e.g. standard EtBr).

4) Evaluate restriction patters – either manually or with special softwares.

T-RFLP

General procedure, similar to standard restriction analysis, with the following modifications:

1) PCR is performed with fluorescently labeled PCR primers (either the Forward or the Reverse primer on the 5´Positon, or both (differently labeled)). For example:
   a. 8mF, labeled with 6-FAM: 5´-AGAGTTTGATCMTGGCTCAG-3´
   b. 1541R, labeled with TET: 5´- AAGGAGGTGATCCAGCCGCA-3´

2) The PCR products are digested with minimum one or more restriction enzymes (e.g. Eco RI and H*Ha*i). For further selection of appropriate restriction enzymes see web links in review by Schütte et al., 2008, Advances in T-RFLP, Applied Microbiol Biotechnol, 80, 365.

3) The digested products are visualized via gel electrophoresis or fragment analysis on an ABI DNA sequencer (e.g. at GATC, www.gatc-biotech.com/de/index.html).

4) Restriction patterns are evaluated either manually or with special softwares. See review by Schütte et al., 2008, Advances in T-RFLP, Applied Microbiol Biotechnol, 80, 365.