

RNA removal by double-RNase digestion

Introduction

DNA quantification by absorbance at 260 nm with a spectrophotometer is fast and easy, but may be less accurate than fluorescent quantification with dyes like SYBR[®] Green or PicoGreen[®] (Molecular Probes). The reason is that RNA is co-purified with DNA and absorbed at 260nm. This may lead to an over-estimation of the amount of DNA. This protocol describes the use of double-RNase digestion to remove the RNA in Oragene samples. After this RNase treatment, the DNA samples will give similar quantification results by absorbance or fluorescence.

Double-RNase digestion

This protocol uses two ribonucleases for double-digestion of RNA because treatment with Ribonuclease A alone is not sufficient to degrade RNA into alcohol-soluble fragments. This is because Ribonuclease A cleaves only at U- and C-nucleotides, leaving fragments large enough to be precipitated with alcohol. By including Ribonuclease T1 (which cleaves at G-nucleotides), RNA can be digested into very small fragments that are not precipitated by alcohol.

Equipment and Reagents

- Ribonuclease A (Stock concentration: 1 mg/mL) (e.g. Sigma-Aldrich, Cat. No. R4875)
- Ribonuclease T1 (Stock concentration: 6,000 Units/mL) (e.g. Sigma-Aldrich, Cat. No. R1003)
- Sodium chloride (NaCl) (Stock concentration: 5 M)
- Ethanol (95 to 100%) at room temperature
- TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0), or other standard buffer
- Microcentrifuge capable of running at 13,000×g
- Water-bath or air incubator, heated to 37°C

Method

1. Purify a 500 µL aliquot of the Oragene/saliva sample according to the standard Oragene DNAPurification Protocol (ref.1).
2. Resuspend the purified DNA pellet in 500 µL of 1x TE.
3. Add 5 µL of Ribonuclease A (final concentration of 10 µg/mL), and also add 2 µL of Ribonuclease T1 (final concentration of 25 Units/mL).
4. Incubate at 37°C for 30 min.
5. Add 10 µL of NaCl (final concentration of 0.1 M), and also add 1,000 µL of 95% ethanol (two volumes).
6. Mix well and incubate at room temperature for 10 minutes.
7. Collect the precipitated DNA by centrifugation at room temperature for 2 minutes at 13,000×g.
8. Discard the supernatant and redissolve the DNA pellet in 500 µL of 1x TE.

References

1. Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene[®]•DNA/Saliva. PD-PR-006, 2008