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 I† or PicoGreen® (Molecular Probes). The reason is that RNA is co-purified with DNA and absorbed at 260 nm. 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/saliva 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, 1 mM EDTA, pH 8.0) or other standard buffer
- Microcentrifuge capable of running at 13,000 × g

Method

1. Purify a 500 μL aliquot of the Oragene® saliva sample† according to the prepIT™-L2P Laboratory protocol for manual purification of DNA from 0.5 mL of sample².
2. Resuspend the purified DNA pellet in 500 μL of 1× 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 minutes.
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 1× TE.

† Saliva samples were collected with Oragene®DNA or Oragene®DISCOVER
References

1. DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced with DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.

2. Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

Technical support is available Monday to Friday (9h00 to 17h00 EST):

- Toll-free (North America): 1.866.813.6354, option 6
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