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[1] 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 sample*[2].
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
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com

Oragene®•DNA is not available for sale in the United States.
Oragene®•DISCOVER is for research use only, not for use in diagnostic procedures.
®Oragene is a registered trademark and prepIT™ is a trademark of DNA Genotek Inc. All other brands and names contained herein are the property of their respective owners.
All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.

© 2011 DNA Genotek Inc., a subsidiary of OraSure Technologies, Inc., all rights reserved.