DNA genotek

"Super-pasteurization" of Oragene®/saliva samples†

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Some Oragene® users have inquired about the possible presence of infectious agents in saliva samples shipped under routine conditions. To address this question and to further prove the robustness and reliability of Oragene/saliva samples, experiments were conducted to demonstrate that Oragene/saliva samples can be "super-pasteurized" (treated for up to 3 hours at 72°C) with no effect on the quality and quantity of DNA recovered.

Introduction

The Oragene self-collection kit is a non-invasive method for collecting large amounts of DNA. The Oragene kits' ability to release and stabilize DNA from saliva for long periods of time at ambient temperature makes it an ideal collection method. The Oragene kit is increasingly being used to collect DNA samples around the world which has led to questions regarding potential pathogens in oral samples. Pasteurization, an industrial process typically associated with the dairy industry, has been documented to efficiently kill pathogens[1, 2, 3]. Pathogens can be inactivated by heat, given sufficient temperature and time of exposure[4].

The Oragene kit contains agents that are bactericidal for many microorganisms even at ambient temperature; this bactericidal effect will be even more effective at elevated temperatures. In this report, we describe a series of experiments conducted to further demonstrate the safety and integrity of transport and handling of saliva samples collected with the Oragene kit. In these experiments, Oragene/saliva samples were subjected to "super-pasteurization" conditions that are likely to be strongly bactericidal.

Materials and methods

Saliva collection

Two milliliters of saliva was collected from 2 donors using the Oragene kit. The Oragene kit was capped and the entire sample was mixed by inversion, allowing the DNA to be released and stabilized.

Sample treatment

As described in the prepIT™•L2P (DNA Genotek) purification protocol[5], samples were incubated in a water bath for 1 hour at 50°C. A 250 µL aliquot was immediately removed from each donor's sample. The remainder was placed in an air incubator and incubated at 72°C. From each donor's sample at 72°C, a 250 µL aliquot was taken at 30 minutes, 1 hour, 2 hours, and 3 hours.

Monitoring of sample temperature

A control Oragene/saliva sample was used to monitor the temperature within the collection tube. To monitor temperature, a remote temperature sensor was inserted through a modified cap.

DNA purification

The prepIT•L2P purification protocol[5] for extracting DNA from the Oragene/saliva samples was followed. In brief, 10 µL (1/25th volume) of prepIT•L2P reagent was added to each 250 µL sample. Each tube was mixed well and incubated on ice for 10 minutes. The tubes were centrifuged at 15,000 × *g* for 5 minutes. The supernatant was removed and transferred to a fresh tube; an equal volume of 95% EtOH at room temperature was added. The sample was mixed by inversion and allowed to stand 10 minutes at room temperature. The sample was centrifuged at 15,000 × *g* for 2 minutes and the supernatant was discarded. The DNA pellet was dissolved in 50 µL of TE solution.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

DNA analysis

DNA from the purified samples was quantified using a fluorescent dye, SYBR® Green I dye (Molecular Probes, Inc.). The A_{260}/A_{280} ratio was corrected by subtracting the A_{320} value. The molecular weight of the DNA was determined by electrophoresis on a 0.8% agarose gel.

Results and discussion

The saliva from 2 donors was used for these experiments. The average amount of DNA present in 2 mL of saliva from Donor A was 40.8 µg and from Donor B was 116.0 µg. The initial and subsequent A_{260}/A_{280} ratios were between 1.8 and 1.9 for all samples. Representative results of agarose gel electrophoresis analysis are shown in Figure 1.

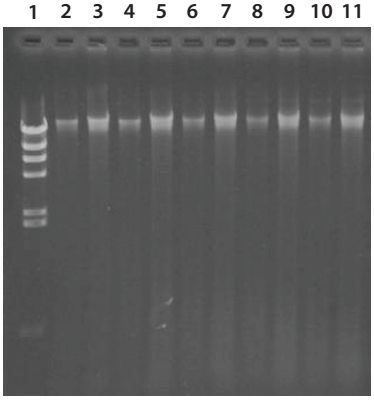

***Figure 1**: Samples were incubated at 72°C for 0 hours (lanes 2, 3); 0.5 hour (lanes 4, 5); 1 hour (lanes 6, 7); 2 hours (lanes 8, 9); 3 hours (lanes 10, 11). Donor A (lanes, 2, 4, 6, 8, 10); Donor B (lanes 3, 5, 7, 9, 11). Lane 1 – Lambda-Hind III marker.*

Pasteurization is a process for removing pathogens by the heating of a solution to a specific temperature for a defined time without allowing recontamination during the process. The accepted minimum conditions required for standard pasteurization are heating at 72°C for 16 seconds. These conditions are accepted by the dairy industry as being sufficient to destroy most pathogens in milk[2, 3]. Essentially all known pathogens are inactivated by heat, given sufficient temperature and exposure[4]. Because of recent concerns about avian influenza virus, several reports have studied thermal inactivation of the virus. The authors report that the virus is heat-sensitive and can be destroyed by standard pasteurization conditions[6, 7]. It is important to emphasize that the "super-pasteurization" conditions used in our experiments involved heating for up to 3 hours, that is, about 600 times longer than standard pasteurization and was carried out in the presence of strong detergents and alcohol.

Conclusions

Heating Oragene/saliva samples at 72°C for as long as 3 hours ("super-pasteurization") does not degrade DNA. When required, heat treatment could be used to further reduce risk from pathogens potentially present in an Oragene/saliva sample.

References

1 http://www.foodsci.uoguelph.ca/dairyedu/pasteurization.html
2 http://www.milk.org
3 http://www.cfis.agr.ca/english/regcode/hrt/juprodae.shtml
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6 Swayne, D.E. and Beck J.R. (2004). Heat inactivation of avian influenza and Newcastle disease viruses in egg products. *Avian Pathol.* 33(5):512-8.
7 Swayne, D.E. (2006). Microassay for measuring thermal inactivation of H5N1 high pathogenicity avian influenza virus in naturally infected chicken meat. *Int J Food Microbiol.* 108(2):268-71.

Oragene®•DNA is not available for sale in the United States.
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