PERFORMAgene™

Laboratory protocol for manual purification of DNA from 0.5 mL of Performagene™ sample

The following step-by-step protocol describes how to purify DNA from a 0.5 mL aliquot of a sample that has been collected and preserved in Performagene chemistry with the PG-100 collection kit. Reagents required for manual purification are available with PG-AC1 reagent package or PG-AC4 reagent package.

When a DNA sample is collected and mixed with the Performagene solution, the DNA is immediately stabilized. Performagene samples are stable at room temperature for 1 year from the time of collection. If it is your laboratory practice to bank DNA samples, Performagene samples can be stored indefinitely at -15°C to -20°C, and can undergo multiple freeze-thaw cycles without deterioration of the DNA.

Equipment and reagents

- Microcentrifuge capable of running at 15,000 × *g*
- Air or water incubator at 50°C (Note: The false bottom tube will float in a water incubator, therefore an air incubator may be preferred.)
- Ethanol (95% to 100%) at room temperature
- DNA buffer: TE (10 mM Tris-HCl, 1mM EDTA, pH 8.0) or similar solution
- (Optional) Glycogen (20 mg/mL) (e.g., Invitrogen Cat. No. 10814-010)
- Ethanol (70%) at room temperature
- 5M NaCl solution

Procedure

Purification steps	Notes
1. Mix the sample by shaking vigorously for 5 seconds.	• This is to ensure that viscous samples are properly mixed with the Performagene solution.
2. Incubate the sample in a 50°C air incubator for a minimum of 2 hours, or in a 50°C water incubator for a minimum of 1 hour.	• DNA in Performagene is stable at room temperature even without the incubation step. • This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated. • This incubation step may be performed at any time after sample is collected from the animal and before it is purified. • Incubation of the entire sample is recommended. • The sample may be incubated at 50°C overnight if it is more convenient. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.
3. (Optional) Removal of collection sponge: Remove the cap and press the collection sponge against the inside of the tube to extract as much of the sample as possible. Discard sponge and cap.	• Sponge removal is dictated by preference of workflow. • Replacement caps (RC-1) are available for purchase.

For Animal Use Only

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Purification steps	Notes
4. Transfer 500 µL of the mixed Performagene sample to a 1.5 mL microcentrifuge tube.	• The remainder of the Performagene sample can be stored at room temperature or frozen (-15°C to -20°C). Do not store in refrigerator (4°C).
5. Add 20 µL (1/25th volume) of PG-L2P purifier to the microcentrifuge tube and mix by vortexing for a few seconds.	• The sample will become turbid as impurities and inhibitors are precipitated.
6. Incubate sample on ice for 10 minutes.	• Room temperature incubation can be substituted but will be slightly less effective in removing impurities.
7. Centrifuge at room temperature for 5 minutes at 15,000 × *g*.	• A longer period of centrifugation (up to 15 minutes) may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
8. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard pellet.	• The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged.
9. To 500 µL of supernatant, add 25 µL (1/20th volume) of 5 M NaCl, mix.	• Addition of NaCl is necessary to ensure efficient recovery of DNA.
10. To 500 µL of supernatant , add 600 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.	• During mixing with ethanol, the DNA will be precipitated. This may appear as a clot of DNA fibers or as a fine precipitate, depending upon the amount of DNA in the sample. • Even if no clot is seen, DNA will be recovered by carefully following the next steps.
11. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
12. Place the tube in the centrifuge in a known orientation (DNA pellet may not be visible after centrifugation). Centrifuge at room temperature for 2 minutes at >15,000 × *g*.	• For example, place each tube in the microcentrifuge with the hinge portion of the cap pointing away from the centre of the rotor. After centrifugation, the position of the pellet can be located (even if too small to be easily visible); it will be at the tip of the tube below the hinge.
13. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.	• This pellet contains DNA. Loss of the pellet will result in loss of the DNA. • Rotating the tube such that the pellet is on the upper wall will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant. • The supernatant may contain impurities and should be removed as completely as possible. • Excessive drying of the pellet can make the DNA more difficult to dissolve.

Purification steps	Notes
14. Carefully wash the DNA by adding 250 µL of 70% ethanol. Let stand for 1 minute at room temperature. Remove the ethanol with a pipette tip without disturbing the pellet.	• Take care not to disturb the DNA pellet. • The DNA pellet may be small. Addition of a carrier such as glycogen at step 7 will increase the visibility of the pellet. • Should the pellet detach, centrifuge the sample for 5 minutes at 15,000 × *g*. • The 70% ethanol wash helps to remove residual inhibitors.
15. Centrifuge for 6 seconds to pool any remaining ethanol, remove with a pipette tip.	• Complete removal of ethanol is essential to prevent inhibition during downstream applications.
16. Add 100 µL of DNA buffer (e.g. TE buffer) to dissolve the DNA pellet. Vortex for at least 5 seconds. Let sit at room temperature overnight to ensure complete rehydration of the DNA. DNA can now be quantified and used in downstream applications.	• Note that large amounts of high molecular weight DNA can be slow to hydrate (dissolve) completely. • Incomplete hydration of the DNA is a cause of inaccuracy in estimating DNA concentration and of failure of downstream applications such as PCR.
17. (Optional) Additional steps to ensure complete hydration of the DNA. a) Additional vortexing, and/or b) Incubation at 50°C for 1 hour with occasional vortexing, and/or c) Incubation at room temperature for 1 to 2 days	• For applications that require very high molecular weight DNA, (c) is recommended.
18. Options for storage of the fully rehydrated DNA: a) **Recommended** in TE, in aliquots at -20°C for long-term storage, or b) In TE at 4°C for up to 2 months.	• Freezing of purified DNA in TE will cause DNA to precipitate. When thawing a sample of frozen purified DNA, pay careful attention to rehydration, as discussed in step 17.

Quantification of DNA

By fluorescence method

Assays that use fluorescent dyes are more specific than absorbance at 260 nm for quantifying the amount of double-stranded DNA (dsDNA) in a DNA sample. We recommend using fluorescent dyes such as PicoGreen® or SYBR® Green I to quantify dsDNA since there is less interference by contaminating RNA, see protocol[1]. Alternatively, commercially available kits such as Invitrogen's Quant-iT™ PicoGreen dsDNA Assay Kit (Cat. No. Q-33130) can be used. For either protocol, we recommend that the purified DNA be diluted 1:50 with TE solution and that 5 µL be used in the quantification assay.

By absorbance method

If you choose to quantify DNA by absorbance, we recommend that you first treat the purified sample with RNase to digest contaminating RNA and then remove the RNA fragments by ethanol precipitation of the DNA. See protocol[2] for details. Please note that DNA from a Performagene sample typically contains appreciably more RNA than found in blood samples. Ensure that alcohol-precipitated DNA is fully dissolved before reading the absorbance.

Conversion factor: An absorbance of 1.0 at 260 nm corresponds to a concentration of 50 ng/µL (50 µg/mL) for pure dsDNA.

- A spectrophotometer cuvette capable of reading a volume of 100 µL or less should be used to avoid using too large a volume of sample.
- Absorbance values at 260 nm should be between 0.1 and 1.5. Lower values may not be reliable. If the undiluted sample is used, care must be taken to ensure that the cuvette is very clean or that disposable cells are used to avoid cross-contamination of samples. Absorbance values >1.5 at 260 nm are not reliable; the sample should be diluted and re-read.

Method:

1. Dilute a 10 µL aliquot of purified RNase-treated DNA with 90 µL of TE (1/10 dilution). Mix by gently pipetting up and down. Wait for bubbles to clear.
2. Use TE in the reference (blank) cell.
3. Measure absorbance at 320 nm, 280 nm and 260 nm.
4. Calculate corrected A_{280} and A_{260} values by subtracting the absorbance at 320 nm (A_{320}) from the A_{280} and A_{260} values.
5. DNA concentration in ng/µL = corrected A_{260} × 10 (dilution factor) × 50 (conversion factor).
6. A_{260}/A_{280} ratio: Divide corrected A_{260} by corrected A_{280}.

Example

1. Assume the measured A_{320}= 0.025, A_{280}= 0.175 and A_{260}= 0.295
2. The DNA concentration of the undiluted sample will be:
   $(A_{260} - A_{320}) \times 10$ [dilution factor] × 50 [conversion factor]
   $= (0.295 - 0.025) \times 10 \times 50$
   $= 0.270 \times 10 \times 50$
   = 135 ng/µL or 135 µg/mL
3. The corrected A_{260}/A_{280} ratio will be:
   $(A_{260} - A_{320}) \div (A_{280} - A_{320})$
   $= (0.296 - 0.025) \div (0.175 - 0.025)$
   $= 0.270 \div 0.150$
   = 1.80

References

[1] DNA quantification of Oragene/saliva samples using SYBR Green I Dye and a micro-plate reader. DNA Genotek. PD-PR-075.
[2] RNA removal by double-RNase digestion. DNA Genotek. PD-PR-040.

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

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