

Laboratory Protocol for Manual Purification of Bovine DNA from 0.5 mL of Performagene™.LIVESTOCK/ nasal sample

The following step-by-step protocol describes how to purify DNA from a 0.5 mL aliquot of a cattle nasal sample that has been collected and preserved in Performagene•LIVESTOCK 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 cattle nasal sample is collected and mixed with the Performagene•LIVESTOCK solution, the DNA is immediately stabilized. Performagene•LIVESTOCK /nasal samples are stable as is at room temperature for 1 year from the time of collection. If it is your laboratory practice to bank DNA samples, Performagene•LIVESTOCK /nasal samples can be stored indefinitely at -15 to -20°C, and can undergo multiple freeze-thaw cycles without deterioration of the DNA.

Equipment and reagents to be supplied by user

- Microcentrifuge capable of running at 13,000 rpm (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 cattle sample by shaking vigorously for 5 seconds.	<ul style="list-style-type: none"> • This is to ensure that viscous nasal samples are properly mixed with the Performagene•LIVESTOCK 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.	<ul style="list-style-type: none"> • DNA in Performagene•LIVESTOCK 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 nasal 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.	<ul style="list-style-type: none"> • Sponge removal is dictated by preference of workflow. • Replacement caps (RC-1) are available for purchase.

Quantification of DNA:

Assays that use fluorescent dyes are more specific than absorbance at 260nm for quantifying the amount of double-stranded DNA (dsDNA) in a nasal DNA sample. We recommend using fluorescent dyes such as Picogreen or Sybrgreen to quantify dsDNA since there is less interference by contaminating RNA. An inexpensive protocol using Sybrgreen can be found in the support section of our website. Alternatively, commercially available kits such as Invitrogen's Quant-iT™ Pico Green® 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 buffer and that 5 µL be used in the quantification assay.

Suggestions for quantifying by absorbance:

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. A detailed protocol "RNA removal by double RNase Digestion" can be found in the support section of our website. Please note that DNA from nasal samples 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 $A_{260} \times 10$ (dilution factor) $\times 50$ (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
$$\begin{aligned} & (A_{260} - A_{320}) \times 10 \text{ [dilution factor]} \times 50 \text{ [conversion factor]} \\ &= (0.295 - 0.025) \times 10 \times 50 \\ &= 0.270 \times 10 \times 50 \\ &= 135 \text{ ng/}\mu\text{L or } 135 \text{ }\mu\text{g/mL} \end{aligned}$$
3. The corrected A_{260}/A_{280} ratio will be
$$\begin{aligned} & (A_{260} - A_{320}) \div (A_{280} - A_{320}) \\ &= (0.295 - 0.025) \div (0.175 - 0.025) \\ &= 0.270 \div 0.150 \\ &= 1.80 \end{aligned}$$