

## Laboratory Protocol for ‘Quick to PCR’ Processing of Bovine DNA from 0.1 mL of Performagene™•LIVESTOCK/ nasal sample

The following step-by-step protocol describes how to quickly process DNA from a 0.1 mL aliquot of a cattle nasal sample that has been collected and preserved in Performagene•LIVESTOCK chemistry with the PG-100 collection kit.

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 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.)
- DNA buffer: TE (10 mM Tris-HCl, 1mM EDTA, pH 8.0) or similar solution

### Procedure

Purification Steps	Notes
1. Mix the cattle sample by shaking vigorously for 5 seconds.	<ul style="list-style-type: none"> <li>• This is to ensure that viscous nasal samples are properly mixed with the Performagene•LIVESTOCK solution.</li> </ul>
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"> <li>• DNA in Performagene•LIVESTOCK is stable at room temperature even without the incubation step.</li> <li>• This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated.</li> <li>• This incubation step may be performed at any time after nasal sample is collected from the animal and before it is purified.</li> <li>• Incubation of the entire sample is recommended.</li> <li>• The sample may be incubated at 50°C overnight if it is more convenient.</li> <li>• Longer incubation times may help in reducing sample viscosity and facilitating pipetting in subsequent steps.</li> </ul>
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"> <li>• Sponge removal is dictated by preference of workflow.</li> <li>• Replacement caps (RC-1) are available for purchase.</li> </ul>
4. Transfer a 100 µL aliquot of the mixed Performagene•LIVESTOCK/nasal sample to a 0.2 mL tube.	<ul style="list-style-type: none"> <li>• The remainder of the Performagene•LIVESTOCK/nasal sample can be stored at room temperature or frozen (-15°C to -20°C). Do not store in refrigerator (4°C).</li> </ul>
5. Add 4 µL (1/25th the volume) of Purifier (PG-L1) to the sample and mix by vortexing for two seconds.	<ul style="list-style-type: none"> <li>• The sample will become turbid as impurities and inhibitors are precipitated.</li> </ul>



Purification Steps	Notes
6. Incubate sample on ice for 10 minutes.	<ul style="list-style-type: none"><li>• Room temperature incubation can be substituted but will be slightly less effective in removing impurities.</li><li>• Incubation on ice is strongly recommended.</li></ul>
7. Centrifuge samples for 3 minutes at > 13,000 x g.	<ul style="list-style-type: none"><li>• A longer period of centrifugation (up to 15 min) may be beneficial in reducing the turbidity (high A<sub>320</sub>) of the final DNA solution.</li></ul>
8. Carefully transfer 50 µL of the supernatant to a new 0.2 mL PCR tube containing 50µL of Direct reagent (PG-L2).	<ul style="list-style-type: none"><li>• A new 0.2 mL PCR tube containing 50 µL of Direct reagent can be prepared during the incubation and/or centrifugation step.</li></ul>
9. Vortex the sample.	<ul style="list-style-type: none"><li>• At this point, samples can be quantified using a fluorescent dye method (2 – 5 µL of sample should be used).</li><li>• Refer to the “DNA Quantification using SYBR Green I Dye and Micro-Plate Reader” Protocol under literature in the support section of the website</li></ul>
10. Heat the diluted samples in a heating block, water bath or PCR machine at 90°C for 15 minutes. Cool to room temperature.	<ul style="list-style-type: none"><li>• The heating step is necessary to inactivate components of the Performagene•LIVESTOCK chemistry before use in downstream applications.</li></ul>

### Quantification of DNA:

Quantification by absorbance should not be performed on samples prepared using the method outlined above. Samples prepared according to this method are not fully purified and contain components that interfere with quantification by absorbance. However, the remaining components will not interfere with downstream applications.

As an alternative, quantification can be carried out using a method based on fluorescence as described in the protocol.

We recommend using fluorescent dyes such as Picogreen or SYBR Green to quantify dsDNA since there is less interference by contaminating RNA. An inexpensive protocol using SYBR Green 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.