PERFORMAgene™

Troubleshooting guide for PG-100 sample collection and extraction

Question	Possible cause	Explanation/solution
My sample contains solid material.	Debris was collected along with the nasal sample.	Solid material in the sample may be feed, hair, dust or other material from the animal's environment. The majority of this material will be removed during the first purification steps, primarily the high-speed centrifugation that follows the addition of the purifier reagent PG-L2P. Generally, insoluble material will not affect downstream analysis.
My sample is viscous prior to DNA extraction.	Sample may not have been incubated at 50°C as per protocol.	Ensure that the sample incubation step at 50°C in the original PG-100 collection tube was performed. Post-incubation, viscosity can also be an indicator of very high DNA yield. This is more likely to occur from samples from calves under 1 year old.
My extracted DNA is coloured.	Soluble material co-purified with DNA.	Occasionally, soluble, coloured material may co-purify with the DNA. There is generally no correlation between colour of the sample and downstream performance. If the 260/280 ratio (corrected for turbidity by subtracting the A_{320}) is >1.6 the sample should perform well in downstream analysis. A procedure for removing this material via chloroform extraction is available in MK-AN-017, *From turbidity to clarity: Simple methods to improve the A_{260}/A_{280} ratio of Oragene-purified DNA samples*, which can be found on our website.
Sample volume is less than 1 mL.	Liquid is trapped in sponge.	Ensure that the sponge has been wrung dry along the sides of the tube to collect as much sample as possible.
	Preservative was spilled during sample collection.	To determine how much sample is present weigh the PG-100 tube. A kit containing 1 mL of liquid will weigh approximately 9.28 g. If a kit weighs substantially less it is possible that the chemistry was dumped during sample collection. In this case, a new sample should be collected.
The yield/ concentration of Genomic DNA is low.	Incomplete lysis.	Ensure that the sample was incubated at 50°C in the original PG-100 collection tube as outlined in the extraction protocol. Failure to incubate the sample prior to extraction will result in reduced DNA yield.
	Improper precipitation.	Ensure that an equal volume of alcohol was added to the supernatant.
	Incomplete re-suspension of DNA.	PG-100 extracted DNA is very high molecular weight and takes time to dissolve. Incomplete hydration of the DNA is a cause of inaccuracy in estimating DNA concentration and failure of downstream applications. Samples should be vortexed after addition of TE buffer to ensure the pellet is dissolved. Incubate the sample overnight at room temperature to ensure that DNA is completely dissolved. Alternatively, incubate the DNA sample at 50°C for 1 hour with periodic vortexing.
	Incomplete recovery of precipitated DNA.	After precipitation and centrifugation, some of the DNA may be stuck to the side of the tube. This DNA can be recovered by vortexing the sample for 5 minutes after the addition of TE. This will ensure that all DNA is recovered from the tube.
	Natural biological variation.	DNA yields vary between animals and animals under 1 year may yield greater DNA concentrations. If a higher concentration of DNA is desired, less TE should be used to dissolve the DNA pellet.

For Animal Use Only

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Fluorescence and absorbance quantification values do not match.	RNA is present in sample.	It is normal to obtain different values when measuring yields with absorbance vs fluorescence, as absorbance will measure both DNA and RNA content, while fluorescence will only measure DNA content. We suggest quantifying by fluorescence as it is a more accurate way of measuring DNA concentrations. If quantification by absorbance is necessary, we recommend RNase-treating the samples. A detailed protocol is described in PD-PR-040, *RNA removal by double-RNase digestion,* and can be found on our website.
Low OD ratios.	The PG-L2P purifier was not added.	The PG-L2P purifier must be added to precipitate impurities and inhibitors from the sample.
	Incorrect blank.	Ensure that you blank your spectrophotometer using the same buffer that was used to dissolve the DNA pellet (e.g., TE).
	Absorbance readings were not corrected for turbidity.	When measuring by absorbance it is essential to subtract the A_{320} reading from your A_{280} and A_{260} readings before calculating the 260/280 ratio. This eliminates the contribution of any turbid material present in your sample. Note that this turbid material is inert and should not affect your downstream applications. The formula for this subtraction can be found in the purification protocol. Some spectrophotometers may perform this correction automatically. Please see the documentation for your instrument.
	Residual ethanol not removed.	Residual ethanol should be collected at the bottom of the tube by brief centrifugation and removed using a pipette. If residual ethanol is evaporated, impurities left in the wash will concentrate and be left behind in the sample affecting the 260/280 ratio.
	Post PG-L2P purifier pellet was disturbed.	The pellet contains turbid impurities. Even a small carryover of this pellet can reduce ratios. If accidentally disturbed, the tube should be re-centrifuged before removing the supernatant. A longer period of centrifugation, (up to 15 minutes) after the addition of PG-L2P and 10 minutes incubation in ice may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
	Turbid material in extracted DNA.	There are several methods to help remove insoluble particulate from the DNA. These include high-speed centrifugation and chloroform treatment. More details are available in MK-AN-017, *From turbidity to clarity: Simple methods to improve the A_{260}/A_{280} ratio of Oragene-purified DNA samples*, which can be found on our website.
Storage of raw PG-100 samples and extracted DNA.	Short-term storage of raw sample.	Unpurified samples may be stored at room temperature in their original tube for up to 1 year.
	Long-term storage of raw sample.	For long-term storage, we recommend transferring the samples to a cryo-tube with an O-ring and storing at -20°C or -80°C. Samples may be split into aliquots and stored in microcentrifuge tubes. To ensure sample homogeneity, heat the entire sample for 2 hours at 50°C before aliquoting.
	Storage of purified DNA sample.	Store purified DNA in 1×TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0 or greater). Samples may be stored short-term (weeks) at 4°C or long term frozen at -20°C.

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DNA does not perform well on downstream applications.	Ensure 70% ethanol wash was performed.	The 70% ethanol wash step is critical to ensure that potential inhibitors are completely removed.
	Ethanol carryover.	Remove all of the wash using a small volume pipettor. Pulse-spin the tubes in order to collect the remaining liquid so it can be removed. Ethanol carryover is known to interfere with downstream applications. Residual ethanol should not be allowed to evaporate; it should be removed with a pipette.
	Overestimation of DNA concentration.	If quantifying DNA by absorbance, RNA will also be quantified resulting in an overestimation of DNA. We recommend quantifying by fluorescence.
	Insufficient rehydration time.	If the DNA is not rehydrated completely it is possible to underestimate the amount of DNA in the sample causing a risk of overloading the downstream application.
	Compatibility with 3rd-party purification methods.	In addition to PG-L2P, Performagene samples have been tested with column-based purification using DNA Genotek's PT-C2D kit and with magnetic bead-based purification using the MagaZorb DNA Miniprep Kit (Promega).
	DNA concentration may be inappropriate.	The amount of DNA recovered from a nasal sample varies from animal to animal based on a number of factors (age, breed, species, and environment). If concentrations are too low, the DNA can be concentrated by re-precipitating or by eluting in a smaller volume during the initial extraction. If concentrations are too high (as recommended by the assay manufacturer), samples should be diluted appropriately using TE or other suitable diluent.
DNA is not visible or appears degraded on agarose gel.	No high molecular weight DNA present.	Verify that sample was properly collected according to the instructions supplied with the kit. If sample was properly collected, extract another aliquot of the sample following the precautions discussed above.
	Smearing on agarose gel.	Overloading the gel will make smearing more apparent. We recommend loading no more than 100 ng of DNA on the gel. A small proportion of the DNA may shear during sample processing and appear as a smear on the gel. Generally, the vast majority of the DNA will remain above 23 kb, as evidenced by a bright band at the top of the gel.
	Band at bottom of gel.	RNA co-purifies with DNA and may be visible as a distinct band at the bottom of the gel. RNA does not normally affect the performance of samples on downstream applications. If you wish to remove RNA, a detailed protocol is described in PD-PR-040, *RNA removal by double-RNase digestion,* and can be found on our website.