Troubleshooting Guide for PurElute™ Bacterial Genomic Kit

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Low or no yield	Inadequate number of cells in the starting sample	Insufficient cells may result in a lower DNA concentration during the DNA precipitation step resulting in low DNA yield. On the other hand, too many cells may result in incomplete cell lysis also resulting in low DNA yield. Count cells prior to beginning cell lysis step ensuring approx. 2 x 10^9 cells/ml or an O.D. of 1.0.
	Ineffective cell lysis due to decreased activity of buffers.	The buffers are stable for up to 6 months if stored properly. Be sure to store all Buffers at 4ºC before and after use. Except the Spheroplast Buffer which should be stored at -20ºC. Keep all buffers on ice during use.
	DNA not completely dissolved	Ensure that the DNA is dissolved completely by gentle shaking at room temperature. If the DNA is still not fully dissolved, vortex at low speed. NOTE: Vigorous vortexing may shear the genomic DNA
	Insufficient resuspension of Harvested Cells	Ensure complete resuspension of pelleted cells by vortexing before addition of Spheroplast buffer.
	No DNA precipitate visible	Ensure that the sample is completely mixed after addition of isopropanol. Invert the tube at least 5 times after addition of isopropanol before centrifugation. Sometimes it is difficult to visually see the precipitated pellet because isopropanol precipitates are usually glassy.
	DNA was over dried	Avoid over drying DNA pellet after removal of 70% ethanol, over dried genomic DNA is difficult to redissolve

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Low or no yield *cont*…	Reason unknown	If fractions of DNA have been saved during the procedure, analysis by agarose-gel electrophoresis is the best way to determine at what stage the problem occurred. Prior to performing agarose gel electrophoresis, precipitate the DNA in the fractions by standard isopropanol precipitation.
No DNA in the cleared lysate	Harvested bacteria before early log phase of growth	Check that the conditions for optimal growth were met.
	Incorrect storage conditions or expiration of buffers	The buffers must be stored at 4°C except the Spheroplast buffer which should be stored at -20°C.
	Lysate prepared incorrectly	Follow the protocol carefully
	Lysis 1 precipitated	Redissolve by warming in 37°C until every precipitate goes into solution right before use. Then place back on ice.
	Incomplete cell resuspension	Pelleted bacterial cells should be completely resuspended in Spheroplast buffer. Do not add Lysis 1 and 2 solutions until an even suspension is obtained.
DNA does not perform well in downstream applications	Ethanol carryover	Make sure that all ethanol has evaporated before eluting by leaving the tube inverted on a clean piece of absorbent paper for a few minutes so the ethanol does not backflow from the rim and sides of the tube onto the pellet.

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DNA does not perform well in downstream applications *cont…*	Incorrect mass of DNA used	Amplification reactions are sometimes inhibited by excess DNA. Reduce the mass of DNA used to less than 50 ng as this is usually adequate for PCR reactions.
DNA sheared	Lysis Solutions added incorrectly	The lysate must be handled gently after addition of Lysis Solutions in order to prevent shearing. Reducing the culture volume may help if the lysate is too viscous for gentle mixing.
	Lysis time too long	Lysis must not exceed 5 minutes
	Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12–16 hours. Harvest bacteria during early log phase of growth.
	Prolonged vortexing after adding Extraction buffer	After the addition of the Extraction buffer make sure vortexing or shaking is not prolonged. Perform gentle vortexing for 10 seconds at low speed or shake for approximately 2–3 minutes.
	Vortexing during elution	Due to the large size of genomic DNA, it shears easily. If elution is difficult the sample can be eluted by gentle shaking (avoid vortexing) until it dissolves.
DNA concentration too low	Low DNA yield	Precipitate the DNA solution and elute in a smaller volume. Elution with volumes greater than 100 µl decreases the final DNA concentration in the eluate.

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Little or no DNA in the eluate	Bacterial pellet was frozen and thawed more than once	Use fresh samples or samples that have been thawed only once.
	Bacterial pellets were kept at room temperature for too long	DNA in the samples may degrade during prolonged storage at room temperature. Use fresh samples, or samples stored at 2–8°C
	Insufficient sample lysis	This can happen when the Lysis 1 and 2 solutions are stored at high temperatures for a prolonged period of time. Repeat the procedure using new samples and fresh Lysis 1 and 2 solutions. Always keep the lysis buffers at 4˚ when not in use and on ice during use. Warm Lysis 1 solution to dissolve precipitate right before use, then place back on ice.
	Sample/Lysis Solution mixture was mixed insufficiently	Mix Lysis solutions with resuspended samples by gently inverting the tube at least 5 times or until a uniform mixture is achieved.
	Low-percentage isopropanol was used	Repeat the purification procedure with new samples ensuring 0.7 volume of 100% isopropanol is used.
	Lysis 1 & 2 solutions were stored incorrectly	Make sure that the Lysis solutions are stored at 4°C. Repeat the lysis procedure with new samples, if available. Warm Lysis 1 solution at 37°C just before use, then place on ice or store at 4°C.

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Little or no DNA in the eluate *cont…*	Elution buffer incorrect	DNA is eluted only in the presence of low-salt buffers (e.g., 10 mM Tris•HCl, pH 8.5) or water. DNA does not dissolve easily in acidic solutions, maximum efficiency is achieved between pH 7.0 and 8.5.
	Buffers not mixed well	Components of buffers may have separated out after being unused for a long period. Always mix buffers thoroughly before each purification procedure. Lysis 1 solution may have precipitated. Warm Lysis 1 solution at 37°C just before use until all precipitates are dissolved.
Cells are incompletely lysed	Too many cells were used	The amount of Cell Lysis Solution used was insufficient for the number of cells. If too many cells are used, cell lysis will be incomplete; the solution will become very viscous and cells will clump. To prevent incomplete cell lysis, count cells prior to beginning cell lysis step. We recommend using 2 X 10^9 cells/ml which corresponds to an O.D. of 1.0.
DNA Pellet loose or absent	Centrifuge speed too low	Centrifuge at highest speed.
DNA contaminated with RNA		In general, small amounts of residual RNA do not affect downstream applications. Residual RNA in the eluted DNA may be eliminated by further treatment with RNase.

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***A*260/*A*280 ratio for purified DNA is low**	Inefficient cell lysis	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Lysis Solutions immediately and thoroughly. Also be sure to store Lysis 1 and 2 Buffers at 4ºC immediately after use.
DNA Contamination	Carryover of particulate material	Ensure that no particulate material is transferred when supernatants are transferred to new micro-centrifuge tubes prior to isopropanol precipitation.
	Insufficient centrifugation during the pelleting steps	Increase the g-force if not spinning at highest speed and/or the correct centrifugation time.
Pellet difficult to redissolve	The DNA pellet was dried too long prior to elution	DNA pellets that are too dry will require a longer time to dissolve completely. High molecular weight DNA is very difficult to redissolve when over dried. The DNA solution can be given more time to redissolve or you may increase the volume of elution buffer.
	Jelly-like consistency of DNA pellet after Isopropanol precipitation and/or elution	Ensure that the sample was completely dissolved in step 6 by vortexing for 10 seconds or mixing for 3 minutes. If the pellet does not dissolve completely during elution, incubate with gentle shaking.
	Residual isopropanol in pellet	Ensure that the isopropanol is at room temperature during precipitation and pellets are washed with 70% ethanol to remove traces of isopropanol.

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Pellet difficult to redissolve ***cont…***	Buffer pH too low	DNA does not dissolve well in acidic solutions. Ensure that the buffer used for redissolving is 7.0 ≤ pH ≤ 8.5.
Bacterial sample does not become clear after lysis	**Low enzyme activity**	Spheroplast buffer was either stored incorrectly or expired. Make sure that the Spheroplast buffer solution is stored at 20°C.
Little or no DNA upon precipitation	DNA failed to precipitate	Ensure that the precipitate is centrifuged at 15,000rpm for at least 2 minutes. Recover DNA by centrifuging for a longer time at higher speeds.
	DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see, they may also be loosely attached to the side of the tube. Decant the supernatant carefully so as not to lose the pellet.
	DNA was poorly resuspended	Make sure the pellet is completely redissolved. Be sure to wash any DNA off the walls, particularly if using V-bottom tubes. It is recommended to use the standard 1.5 ml micro-centrifuge tubes since the pellets easily collect at the bottom of the tube.
Purified DNA showed multiple bands of different sizes or a smear	DNA is degraded	When samples are not collected or stored properly it can cause DNA to degrade. Eluting samples in water may cause unstable DNA and they may degrade over time. Redissolve DNA in TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.

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Purified DNA showed multiple bands of different sizes or a smear	**DNA is sheared**	Over-handling samples during Cell Lysis or DNA elution can cause the DNA to shear. Due to the large size of genomic DNA, it shears easily. If elution is difficult, the sample can be eluted by gentle shaking, avoid vortexing.
	Bacterial Cell pellet stored for a long time	Process the pellets promptly.
White precipitate in Lysis 1 Buffer		White precipitate may form at low temperature after prolonged storage. Any precipitate formed must be dissolved by incubating the buffer at 37°C for a few seconds or until it disappears right before use.

For additional troubleshooting assistance, please contact Edge BioSystems at:

Telephone: (800) 326-2685 or (301) 990-2685	Fax: (301) 990-0881
Email: info@edgebio.com	Web: http://www.edgebio.com