Troubleshooting Guide

Problem	Possible Reason	Solution
Low recovery of DNA fragment	**Incomplete dissolution of the gel slice**	Check dissolution mixture with a back light to see if there is any gel-like substance remained.
	Ineffective DNA elution	DNA elution is poor at acidic conditions. Make sure that ddH_2O or buffer is at a pH between 7.0 and 8.5
	Incomplete DNA elution	Complete DNA elution only takes place when elution solution is in full contact with the membrane. Make sure that no less than 15 µl of Elution Buffer is dispensed onto the center of the membrane and completely absorbed before centrifugation.
	TAE or TBE buffer is repeatedly used/has aged; incorrect pH	pH of repeatedly used TAE or TBE buffer usually increases. Use fresh TAE or TBE buffer each time.
	Column overloaded with too much agarose solution	Higher recovery is attained the smaller the agarose gel slice. Minimize the size of the gel slice by removing extra gel. When gel slice is more than 200 mg, use separate column to proceed with extraction.
	Size of DNA fragment is more than 5kb.	Preheat elution solution to 60°C prior to elution step.
Poor OD_{260}/OD_{280} ratio	**Use of acidic H_2O for dilution of eluted DNA.**	Make sure pH of H_2O is at a value of 7.0-8.5.

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Gel slice hard to dissolve	**High percentage agarose gel used**	When the agarose percentage is >2.0%, add GEX Buffer at 5 times the volume of the gel slice (100 mg= 0.5 ml). When the agarose percentage of the gel is > 2.5%, add GEX Buffer at 6 times the volume of the gel slice (100 mg = 0.6 ml). Mix every 1-2 minutes during the incubation until complete dissolution.
	Gel slice is too big (more than 200mg).	Use more than one column for gel slice bigger than 200 mg.
Poor performance in downstream applications	**Eluted DNA contains salt residues.**	Wash the column twice with 0.5 ml WS Buffer.
	Eluted DNA contains ethanol residue.	After washing with WS buffer, discard the wash and centrifuge the column for another 3 minutes. If necessary, stand the column at room temperature for a few minutes before eluting DNA. However, do not remove ethanol by heating the column in an oven or alike, as high temperature may affect the intactness of the column.
	DNA fragment is denatured and becomes single-stranded.	To re-anneal the single-stranded DNA, incubate the tube at 95°C for 2 minutes and let it slowly cool down to room temperature. Re-annealed DNA fragments are applicable for all downstream applications.