Troubleshooting Guide

Problem	Possible Reason	Solution
Poor bacterial growth	**Inoculated bacterial sample from a plate or a culture stock stored over a long time period**	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s).
	Inadequate shaking during incubation	Grow cells with vigorous shaking (e.g. 250 rpm). Adjust a suitable shaking speed according to the angular magnitude of an orbital shaker platform.
Poor cell lysis Low yield of plasmid DNA	**Use of excessive amount of bacterial cells harvested from a large or over-grown culture**	Up to 5 ml culture for high-copy plasmid. Up to 10 ml culture for low-copy plasmid. When the culture volume is larger than 5 ml, increase the amount of MX1, MX2 and MX3 Buffer.
	Insufficient amount of bacterial cells	Ensure that bacteria have been grown well (OD_{600}>1) after overnight incubation at vigorous shaking.
Low yield of plasmid DNA	**Overgrowth of bacteria**	Incubate bacterial culture with LB medium and do not incubate for more than 16 hours.
	Plasmid does not propagate	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s)
	Inefficient or incomplete DNA elution	Make sure that elution solution is at pH 7-8.5, in full contact with and completely absorbed by the membrane.

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Low yield of plasmid DNA	**Poor cell lysis**	Refer to Solution section of problem – "Poor cell lysis".
	Plasmid is larger than 10-kbp	Use elution solution preheated to 70°C.
Plasmid appears smeared or degraded	**Host strain is *end*A$^+$**	Use *end*A$^-$ strain if possible. Wash with additional 250 µl of MX3 Buffer before proceeding to the WN washing step.
	Overgrowth of bacteria	Incubate bacterial culture with LB medium, and do not incubate for more than 5 minutes.
Genomic DNA contamination in eluate	**Lysate improperly prepared**	After addition of MX2 Buffer, mix gently to prevent genomic DNA from shearing. Do not incubate for more than 5 minutes.
RNA contamination	**Insufficient RNase A activity in MX1 Buffer**	Ensure that entire RNase A is added into MX1 Buffer and stored at 4°C. After long-term storage (> 6 months), add RNase A into MX1 Buffer to a conc. of 70µg/ml and store at 4°C.
Plasmid of poor quality	**Ethanol in WS buffer is not completely removed**	Following WS washing step, discard the flow-through and centrifuge the column at full speed >/= 3 minutes.
	Use of excessive amount of bacterial cells harvested from a large or over-grown culture	Reduce the amount of sample used. Incubate bacterial culture with LB medium, and do not incubate for more than 16 hours.