XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Incomplete cell lysis.	Too many cells were taken for DNA purification.	The bacterial culture should be at a density of $A_{600} \leq 5,0$. For recommended sample volumes, see section IX. Sample preparation.
	Incomplete suspension of the bacterial pellet in the PMd1 Buffer.	The cell pellet should be mixed thoroughly in the PMd1 Buffer by intensive vortexing or pipetting until complete suspension.
	Salt precipitation in PMd2 Buffer occurred.	When PMd2 Buffer is stored below +20°C, a salt precipitation may occur. Re-dissolve any precipitate by warming the solution at 37°C, then mix well and cool down to the room temperature before use.
	The lysate is not clear.	Incubate the lysate at room temperature for 3 min. Do not incubate for longer than 5 min to avoid denaturation of supercoiled plasmid DNA.
Low yield of purified DNA.	Starting material contained low amount of bacterial cells.	Increase the amount of the starting material. For instructions, see section IX. Sample preparation.
	Old bacterial culture was taken for DNA isolation.	Culture cells in a broth medium containing antibiotic for no longer than 16 h.
	The bacterial cells do not contain plasmids.	Ensure that the appropriate antibiotics were added to every culture medium used.
	The culture medium was not removed completely from the cell pellet.	Carefully and accurately remove any residues of the culture medium from above the cell pellet.
	Incomplete cell lysis.	See the problem: „Incomplete cell lysis".
	Incomplete transfer of the lysate into a purification column.	Transfer the lysate into the pDNA MIDI Purification Column by a pipette.
	The purification column was not equilibrated.	Ensure that the pDNA MIDI Purification Column was equilibrated with the PMdQ Buffer.

Problem	Possible cause	Solution
Plasmid DNA has denatured.	Prolonged incubation with the PMd2 Buffer.	Do not incubate the sample for longer than 5 min before adding the PMd3 Buffer.
Isolated DNA is of poor purity.	Old bacterial culture has been processed.	Culture cells in broth medium containing antibiotic for no longer than 16 h.
	The culture medium was not removed completely from the cell pellet.	Some medium components may affect DNA purity. The LB medium is recommended for direct culture lysis. If another medium is used, the pellet should be suspended in water or TE buffer prior to lysis. Ensure complete removal of the culture medium from over the pellet.
Genomic DNA contamination present.	Old bacterial culture has been processed.	Culture cells in broth medium containing antibiotic for no longer than 16 h.
	Fragmentation of genomic DNA during cell lysis.	Do not vortex sample when the PMd2 Buffer has been added. It may cause the genomic DNA fragmentation and contamination of purified plasmid DNA sample.
RNA contamination present.	Improper preparation of PMd1 Buffer.	Add RNase A to the PMd1 Buffer.
	Improper storage of PMd1 Buffer.	The PMd1 Buffer with RNase A must be stored at +4°C.
Inhibition of downstream enzymatic reactions.	The plasmid DNA has denatured.	See the problem: „Plasmid DNA has denatured".