MethylEasy – TroubleShooting Guide

PROBLEMS	POSSIBLE SOLUTIONS
No PCR product was found for any sample	PCR has failed – make sure all the components were added to the PCR reaction tube and that the PCR cycle was correct.
	Confirm that the polymerase is within its storage date and that it retains its activity.
No PCR product was found for any sample except for control sample # 2	Modification has failed – check that the 3M NaOH solution was fresh and that combined Reagent #1 and Reagent #2 were no older than 4 weeks. Ensure that all the steps in the modification and clean up protocols were followed.
	DNA has been lost - ensure that the DNA pellets were not lost during the washing steps. Add carrier DNA to the sample before adding the isopropanol.
	DNA was degraded during modification - check that all reagents and tubes used during the procedure were of molecular biology quality (i.e. DNase free).
	Modification was incomplete. Return the samples to 95°C or 72°C (Method A or Method B, respectively) for a further 15 minutes incubation.

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	The starting DNA was not sufficiently pure. Re-purify the starting DNA using Qiagen mini-kit (Cat.# 51304). If this is not possible yet it is suspected that the DNA may contain impurities, it is recommended that the DNA is treated with phenol/chloroform before use.
PCR products were present only in the control reactions	Sample DNA was degraded before modification - check that the DNA has been stored/handled correctly.
	Check that the DNA concentration is not too dilute.
PCR products were present in the control reactions only when the control primers were used	PCR primers were not designed correctly - check Section 5 below for details on the primer design.
PCR products were present in all the lanes including the 'no-template' control	Check that the PCR-grade water and not the template was added to the negative control.
	Make sure that the PCR is being set up in a separate area with dedicated reagents and equipment to prevent cross contamination.
After incubation with 100% isopropanol a visible precipitate was observed.	Warm sample to room temperature for 5-10 mins, mixing by inversion every 2 mins until precipitate no longer visible. If precipitate is still visible, heat to 37^{o}C for 5 mins, and mix by inversion until precipitate is no longer visible. Incubate at 4^{o}C for a further 10 mins and proceed with step 9.