



## Troubleshooting Guide

Problem	Possible Cause	Recommendation
<b>No PCR product</b>	Missing component	- Check reaction set-up
	Defective component	- Check the aspect and the concentrations of all components as well as the storage conditions. If necessary test each component individually in controlled reactions - Redesign primers
	Cycling conditions not optimal	- Run a temperature gradient to determine the optimal annealing temperature - Increase the extension time, especially if amplifying a long target - Increase the number of cycles
	Difficult template	- Increase the initial denaturation time up to 3 min
<b>Smearing or Non-Specific products</b>	Excessive cycling	- Decrease the number of cycles
	DNA polymerase concentration too high	- Decrease the amount of DNA polymerase per reaction
	Extension time too long	- Decrease the extension time
	Annealing temperature too low	- Increase the annealing temperature
	Primer concentration too high	- Decrease primer concentration
	Suboptimal primer design	- Check that the primers are working in a control reaction - Check primer design
	Contamination	- Replace each component in order to find the possible source of contamination - Set-up the PCR reaction and analyze the PCR product in separated areas
<b>Low Yield</b>	Insufficient cycling	- Increase the number of cycles
	Extension time too short	- Increase the extension time up to 45 s/kb
	Not enough template	- increase template concentration
	DNA polymerase concentration too low	- Increase the amount of DNA polymerase per reaction

### Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact your local distributor or our Technical Support with details of reaction setup, cycling conditions and relevant data.

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### Associated Products

Product Name	Pack Size	Cat No
Agarose	500 g	BIO-41025
Agarose tablets	300 g	BIO-41027
HyperLadder™ 1kb	200 Lanes	BIO-33025
SureClean Plus	1 x 5 mL	BIO-37047

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