Troubleshooting Guide for MagDTR™ Technology

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Dye peaks present	Incomplete aspiration of ethanol after binding and/or wash steps	• Complete removal of ethanol is critical for successful cleanup. Residual ethanol can be removed by aspiration at a pipet setting of 150µl or higher, coupled with slow aspiration. There should be no need for a second aspiration. However, if a second aspiration is necessary, care should be taken to ensure that ethanol is not reintroduced into the wells from the pipet tip. • Increase the number of washes from 2 to 3 washes in order to ensure that all the salt and dye have been removed.
	Excessive dye present	• Increase the number of washes from 2 to 3 washes to ensure that all salt and dye have been removed. • More than 4µl BigDye® used per sequencing reaction. • Use lower dye concentration. Dilute the dye with the appropriate sequencing buffer. • Increase the number of cycles during thermal cycling. • Increase the concentration of the template in the sequencing reaction.
	Sequencing reaction conditions not optimal	• Optimize template and primer concentrations. Consult dye manufacturer's recommendations. • Start with high purity template. • Check thermal cycler for program entry error. • Check for potential machine failure

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Dye peaks present *cont…*	Wrong volume of ethanol used for precipitation	Use the correct volume of 100% ethanol for the reaction. Too high of a final concentration can lead to dye terminator retention.
	Wrong concentration of ethanol used for washes	• Check ethanol concentrations used. Be certain that freshly prepared 80% ethanol is used for the washes • Use at least 100µl of freshly prepared 80% ethanol per wash.
	Incompatible magnet	• Use the Edge BioSystems' MagWell Magnetic Separator. • Optimize the protocol for use with an alternative magnet.
	Incorrect storage of pre and post purified samples	• Avoid prolonged exposure to light. • Avoid extended storage at temperatures above 4°C. • Store post purified samples at -20°C.
Low recovery	Over dried samples	• Aspirate all ethanol. Reduce the drying time at room temperature to 3 minutes. Resuspend immediately • Aspirate all ethanol. Heat to 98°C for 30 seconds. Resuspend immediately. • At the elution step, add recommended volume of deionized water. Incubate for one minute at room temperature, then pipet mix to resuspend.

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Low recovery *cont…*	Incomplete elution at the resuspension step	• Use the recommended volume of deionized water. Incubate for one minute at room temperature, then pipet mix to resuspend. • Vortex sample at medium speed for at least 30 seconds to properly resuspend the samples. • In the absence of a vortex, pipet aggressively.
	Formamide used as an elution buffer	• Direct elution with formamide is not recommended • Use the recommended volume of deionized water. Incubate for one minute at room temperature, then pipet mix to resuspend. • If the sample must be in formamide, the sample can be eluted in water then dried down and resuspended in formamide.
	Incomplete drying of sample before resuspension	• Increase drying time at room temperature or incubate at 98°C for 30 seconds.
	Failure to precipitate sequencing products	• Check ethanol concentration used. Be certain that 100% ethanol is used for precipitation. • Be certain that the correct volume of 100% ethanol was used.
	Failure to retain sequencing products during washes	• Check ethanol concentration used. Be certain to that freshly prepared 80% ethanol was used during the washes. • Try to remain within the suggested number of washes unless your reaction was done with greater than 4µl of BigDye®, then up to 3 washes may be required.

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Low recovery *cont…*	Incomplete removal of reaction salts during the wash steps causing high background	• Check ethanol concentration used. Be certain that freshly prepared 80% ethanol is used for the washes. • Increases the number of washes from 2 to 3 washes to ensure that all salts and dye have been removed.
	Insufficient quantity of template used	Consult dye manufacturer's recommendations.
	Poor quality template used	• Start with high purity template • For optimal performance, use Edge BioSystems' QuickStep™2 for PCR purifications.
	Incompatible magnet used	• Used Edge BioSystems' MagWell Magnetic Separator. • Optimize protocol for use with an alternative method.
Noisy background on the sequence	Incomplete removal of reaction salts	• Check ethanol concentration used. Be certain that freshly prepared 80% is used for the washes. • Increase the number of washes from 2 to 3 washes to ensure that all salt and dye have been removed.
	Low signal strength or high signal strength	• Follow the steps recommended for troubleshooting low signal sequences. • If the signal is high, dilute the sample before loading onto the sequencer
	Incomplete drying of samples before resuspension	Increase drying time at room temperature or incubate at 98°C for 30 seconds.
	Contaminated template or primer	A clean template and a clean primer are required to obtain optimal results.

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Noisy background on the sequence *cont…*	Thermal cycler failure	• Check thermal cycler program for entry error. • Check thermal cycler for mechanical or electrical failure. • Troubleshoot your thermal cycler to ensure optimal conditions for preparing reactions.
Resin settles too fast	Nature of the resin	• Shake well or vortex the resin prior to use. • If using a robotic workstation, make sure to pipet mix the resin prior to each aspiration step. • If using reagent reservoir, rock back and forth 8-10 times to resuspend resin before pipetting.
Resin forms aggregates during storage	Resin is exposed to strong magnetic field	Shake well or vortex the resin, then sonicate for 5-10 minutes before using.
	Resin is stored for extended period of time	Shake well or vortex the resin, then sonicate for 5-10 minutes before using.

For additional troubleshooting assistance, please contact Edge BioSystems at:

Telephone: (800) 326-2685 or (301) 990-2685	Fax: (301) 990-0881
Email: **info@edgebio.com**	Web: **http://www.edgebio.com**