



## Troubleshooting Guide for PERFORMA<sup>®</sup> DTR V3 96-Well Short Plates

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<b>Signal Intensity low</b>	Less than 10 $\mu$ l of sequencing reaction loaded	Adjust the sequencing reactions to 10 $\mu$ l before loading on the plate
	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>• Recalculate rpm to achieve 850 x g for your centrifuge</li> <li>• Change pre-spin time to 3 min at 750 x g or decrease pre-spin time to 2-2.5 min at 850 x g</li> <li>• Centrifuge samples for 5 min at 850 x g</li> <li>• If adjustable, use fast acceleration centrifugation mode</li> </ul>
	Sequencing reaction conditions were not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations
	Sample volume too low for sequencer	Add deionized water or sample buffer to purified reaction before loading on the sequencing instrument
<b>Dye blobs</b>	Too large a volume was loaded	<ul style="list-style-type: none"> <li>• Load no more than 15 <math>\mu</math>l</li> <li>• If using &gt; 3 <math>\mu</math>l BigDye<sup>®</sup> v 3.1 per 15 <math>\mu</math>l reaction, consider loading only 10 <math>\mu</math>l on the Performa<sup>®</sup> DTR V3 96-Well Short Plate</li> <li>• Consider using Performa<sup>®</sup> DTR Standard Plate for sample volumes &gt; 15 <math>\mu</math>l, or sequencing reactions containing more than 3 <math>\mu</math>l BigDye<sup>®</sup></li> </ul>
	The concentration of BigDye <sup>®</sup> v. 3.1 was too high ( <i>i.e.</i> More than 3 $\mu$ l BigDye <sup>®</sup> v 3.1 per 15 $\mu$ l reaction was loaded)	If the sequencing reaction conditions cannot be altered, consider loading only a portion of the reaction for cleanup and sequencing.



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<b>Dye blobs cont...</b>	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>• Be certain that the pre-spin is for 3 min at 850 x g</li> <li>• Recalculate rpm to achieve 850 x g for your centrifuge conditions</li> <li>• Decrease sample spin to 3 minutes at 850 x g</li> </ul>
	Samples were not loaded properly	Load the samples slowly, drop-wise to the center of the gel matrix
	Signal intensity too high	Apply only a portion of sequencing reaction to the plate; high signal strengths exaggerate the effect of small quantities of dye terminator in the purified reaction
	Sequencing reaction conditions were not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations
<b>Truncation of 5' sequence</b>	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>• Recalculate rpm to achieve 850 x g for your centrifuge</li> <li>• Change pre-spin time to 3 min at 750 x g or decrease pre-spin time to 2-2.5 min at 850 x g</li> <li>• Centrifuge samples for 5 min at 850 x g</li> </ul>
	Dye blobs present	See Dye blobs above
	Less than 10 $\mu$ l of sequencing reaction loaded	Adjust the sequencing reaction to 10 $\mu$ l before loading on the plate
	Sequencing reaction conditions not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations



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<b>Recovery volume higher than expected</b>	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>• Be certain that the pre-spin is performed for 3 min at 850 x g</li> <li>• Recalculate rpm for your centrifuge conditions</li> </ul>
	More than 15 $\mu$ l of sequencing reaction loaded	Decrease volume of sequencing reaction loaded to 10-15 $\mu$ l
	Plate was stored incorrectly. If you observe a high recovery volume after the first spin, it is likely the plate has been frozen	<ul style="list-style-type: none"> <li>• Store sealed plates at 4°C</li> <li>• Do not allow plates to freeze</li> </ul>
<b>Recovery volume lower than expected</b>	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>• Recalculate rpm for your centrifuge conditions</li> <li>• Spin samples for at least 5 min at 850 x g</li> </ul>
	Less than 10 $\mu$ l of sequencing reaction loaded	Increase volume of sequencing reaction to 10 -15 $\mu$ l
	Plates have not been stored correctly. If you observe a low recovery volume after the first spin, it is likely the plate has been dehydrated	<ul style="list-style-type: none"> <li>• Store plates at 4°C</li> <li>• Plates sealed in original bag can be left at room temperature for several days but should be stored at 4°C</li> </ul>

**For additional troubleshooting assistance, please contact Edge BioSystems at:**

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