



## Troubleshooting Guide for Performa® DTR Cartridges

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
<b>Signal intensity low</b>	Less than 10 $\mu$ l of sequencing reaction loaded	Adjust the sequencing reaction to 10-20 $\mu$ l before loading on the cartridge
	Centrifuge conditions were not optimal	Recalculate rpm to achieve selected speed for your dye conditions
	Sequencing reaction conditions were not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations
<b>Dye blobs</b>	Too large a volume was loaded	Decrease volume of reaction loaded
	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>Recalculate rpm to achieve selected speed for your dye conditions</li> <li>Be certain that the pre-spin is for 3 min at 850 x g for BigDye® v. 3.1</li> <li>Be certain that the pre-spin is 2 minutes at 750 x g for BigDye® v. 3.0 and other dye chemistries</li> </ul>
	Sample was not loaded properly	Load the sample slowly, drop-wise to the center of the gel column, taking care to avoid the crack
	Signal intensity too high	Apply only a portion of sequencing reaction to the cartridge; high signal strengths exaggerate the effect of small quantities of dye terminator in the purified reaction
	BigDye® v. 3.1 was used in sequencing reaction	Make sure you follow BigDye® v. 3.1 protocol: pre-spin at 850 x g for 3 minutes, load sample, spin sample at 850 x g for 3 minutes
<b>Truncation of 5' sequence</b>	Centrifuge conditions were not optimal	Recalculate rpm to achieve selected speed for your dye conditions
	Dye blobs present	See Dye blobs above
	Less than 10 $\mu$ l of sequencing reaction loaded	Increase the volume of reaction to 10-20 $\mu$ l before loading on the cartridge



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PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
	Sequencing reaction conditions were not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations
<b>Recovery volume higher than expected</b>	Centrifuge conditions were not optimal	<ul style="list-style-type: none"> <li>Recalculate rpm to achieve selected speed for your dye conditions</li> <li>Be certain pre-spin is 2 minutes at 750 x g for BigDye® v. 3.0</li> <li>Be certain pre-spin is 3 minutes at 850 x g for BigDye® v. 3.1</li> </ul>
	More than 20 µl of sequencing reaction loaded	Decrease volume of sequencing reaction loaded to 10-20 µl
	Cartridges have been stored incorrectly	<ul style="list-style-type: none"> <li>Store cartridges at 4°C</li> <li>Do not allow cartridges to freeze</li> <li>Unopened cartridges in a sealed bag can be left at room temperature for short periods of time</li> </ul>
<b>Recovery volume lower than expected</b>	Centrifuge conditions were not optimal	Recalculate rpm to achieve selected speed for your dye conditions
	Less than 10 µl of sequencing reaction loaded	Increase volume of sequencing reaction to 10-20 µl
	Cartridges were not stored correctly	<ul style="list-style-type: none"> <li>Store cartridges at 4°C</li> <li>Do not allow cartridges to freeze</li> <li>Do not allow cartridges to dehydrate; store unopened cartridges in a sealed bag</li> </ul>

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

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