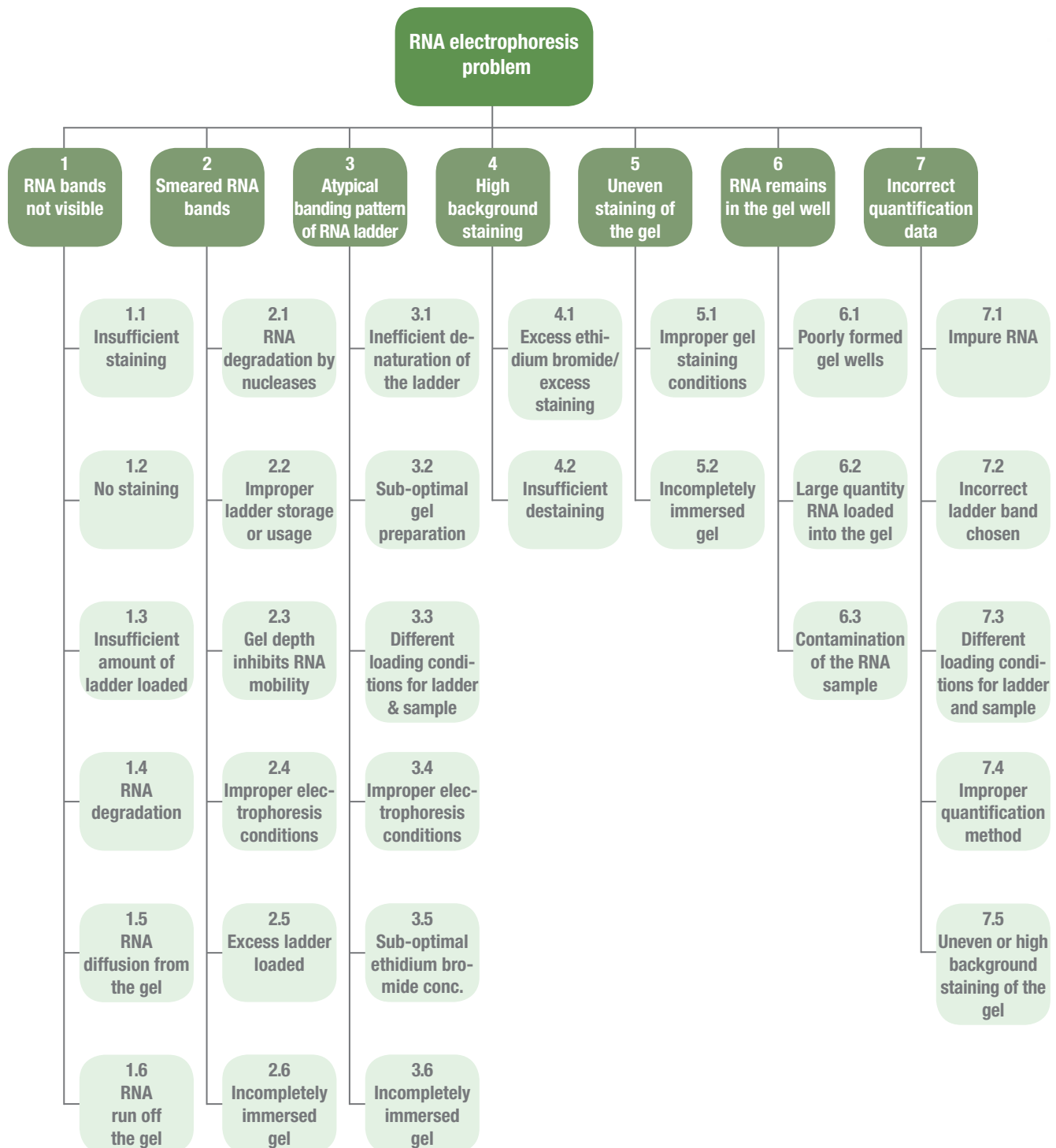




## Troubleshooting Guide for RNA Electrophoresis



**Table 10.1.** Troubleshooting Guide for RNA Electrophoresis.

Problem	Possible cause and recommended solution
<p><b>1. RNA bands are not visible</b></p>	<p><b>1.1. Insufficient staining.</b> Use the 2X RNA Loading Dye for both conventional RiboRuler™ RNA ladder and RNA sample preparation prior to electrophoresis. This solution includes ethidium bromide at a concentration sufficient to stain RNA on denaturing formaldehyde agarose gels. Ready-to-use RiboRuler™ RNA ladders are premixed with 2X RNA Loading Dye. If RNA fragments are separated on native agarose gels, additional staining with ethidium bromide (final concentration 0.5 µg/ml) is recommended. If RNA is separated on a denaturing glyoxal/DMSO agarose gel, stain the gel in ethidium bromide solution (final concentration 0.5 µg/ml) in 0.5 M ammonium acetate for 15-30 min after electrophoresis. Wash the gel in a fresh 0.5 M ammonium acetate solution for 15-30 min. If RNA is separated on a denaturing polyacrylamide gel with urea, soak the gel for about 15 min in 1X TBE to remove the urea prior to staining. Stain the gel in 0.5 µg/ml ethidium bromide in 1XT BE solution for 15 min.</p> <p><b>1.2. No staining.</b> If you are using loading dye which does not contain ethidium bromide, add ethidium bromide to both the agarose gel and electrophoresis buffer at a final concentration of 0.5 µg/ml. Alternatively, stain the gel after electrophoresis with ethidium bromide (0.5 µg/ml ethidium bromide) for 20 min, or SYBR® Green II (follow supplier recommendations).</p> <p><b>1.3. Insufficient amount of ladder was loaded.</b> Follow the recommendations for loading described in the certificate of analysis of the RiboRuler™ RNA ladders (0.25 µl per mm gel lane for conventional ladders; 0.5 µl per mm gel lane for ready-to-use ladders).</p> <p><b>1.4. RNA degradation.</b> Minimize exposure to UV light as this may cause RNA degradation/fading. RNA, including the RiboRuler™ RNA ladders, is extremely sensitive to degradation by ribonucleases. The use of fresh electrophoresis buffers, freshly poured gels, DEPC-treated solutions and protective gloves is recommended.</p> <p><b>1.5. RNA diffusion from the gel.</b> Avoid prolonged electrophoresis or excessive staining and destaining procedures as this may cause diffusion of smaller RNA fragments from the gel. Avoid long term storage of the gel prior to photo documentation, as this may cause diffusion of RNA fragments and band fading.</p> <p><b>1.6. RNA has run off the gel.</b> Stop electrophoresis after the bromophenol blue passes two thirds down the length of the gel. In most denaturing agarose gel systems, bromophenol blue migrates slightly faster than 5S rRNA and xylene cyanol FF migrates slightly slower than 18S rRNA. Make sure that the electrophoresis tank is in a completely vertical position.</p>
<p><b>2. Smear RNA bands</b></p>	<p><b>2.1. RNA degradation by nucleases.</b> RNA, including the RiboRuler™ RNA ladders, is extremely sensitive to degradation by ribonucleases. The use of fresh electrophoresis buffers, freshly poured gels, DEPC-treated solutions and protective gloves is recommended.</p> <p><b>2.2. Improper storage or use of RNA ladders.</b> Store RiboRuler™ RNA ladders at -20°C for 6 months or at -70°C for 24 months. Thaw the ladders on ice.</p> <p><b>2.3. Excessive gel depth or sample volume.</b> Use thin (~0.5 cm) gels and avoid loading of large volumes in the gel lane.</p> <p><b>2.4. Improper electrophoresis conditions.</b> Ensure that there is enough electrophoresis buffer in the electrophoresis apparatus and that the gel is immersed completely. Do not use an excessively high voltage for electrophoresis. Run agarose gels at 5 V/cm (polyacrylamide/urea gels at 8 V/cm). To increase the band sharpness, use a lower voltage for several minutes at the beginning of electrophoresis. However, very low voltage during the entire run may result in band diffusion.</p> <p><b>2.5. Excessive RNA ladder loaded onto the gel.</b> Follow the recommendations for loading described in the certificate of analysis of the RiboRuler™ RNA ladders (0.25 µl per mm gel lane for conventional ladders; 0.5 µl per mm gel lane for ready-to-use ladders).</p> <p><b>2.6. Incompletely immersed gel.</b> Always ensure that there is enough electrophoresis buffer in the electrophoresis apparatus.</p>

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Problem	Possible cause and recommended solution
<b>3. Atypical banding pattern</b>	<p><b>3.1. Inefficient denaturation of the ladder.</b> All RiboRuler™ RNA ladders should be heated to 70°C for 10 min, chilled on ice for 3 min and briefly centrifuged before loading on the gel in order to completely denature the RNA. Sample RNA should be prepared the same way.</p>
	<p><b>3.2. Sub-optimal gel preparation.</b> Older formaldehyde has an acidic pH which may cause extra RNA bands on the gel. Use only fresh formaldehyde for optimal results.</p>
	<p><b>3.3. Different loading conditions for the sample and the ladder.</b> Both ladder and sample RNA should be prepared with the same loading dye solution and loaded under the same conditions. After electrophoresis of total RNA samples in the presence of ethidium bromide, the 28S and 18S human rRNA should be clearly visible under UV illumination. Fast-migrating bands composed of 5.8S RNA and 5S RNA may also be visible depending on the RNA purification procedure. The intensity of the 28S RNA should be approximately twice the intensity of the 18S RNA. The 28S human rRNA band migrates at approximately 5000 b and the 18S human rRNA band migrates at approximately 1900 b.</p>
	<p><b>3.4. Improper electrophoresis conditions.</b> Excessively long electrophoresis runs may result in migration of small RNA fragments off the gel. Very short electrophoresis runs may result in incompletely resolved bands. Run agarose gels at 5 V/cm (polyacrylamide/urea gels at 8 V/cm) until the bromophenol blue passes 2/3 of the gel length. TAE buffer is recommended for analysis of larger RNA, and TBE buffer is used to resolve RNA fragments smaller than 1500 b and for denaturing polyacrylamide gel electrophoresis. The correct gel percentage is important for optimal separation of the ladder RNA; take into account the following: RiboRuler™ High Range RNA Ladder (#SM1821/3) can be loaded on: – native 0.8-1.5% agarose gel with TAE buffer – denaturing formaldehyde 0.8-1.5% agarose gel with MOPS buffer – denaturing glyoxal/DMSO 0.8-1.5% agarose gel with sodium phosphate buffer RiboRuler™ Low Range RNA Ladder (#SM1831/3) can be loaded on: – native 1.7-2.5% agarose gel with TBE buffer – denaturing formaldehyde 1.7-2.5% agarose gel with MOPS buffer – denaturing glyoxal/DMSO 1.7-2.5% agarose gel with sodium phosphate buffer – denaturing 4-8% polyacrylamide gel with TBE buffer</p>
	<p><b>3.5. Sub-optimal ethidium bromide concentration in sample and ladder.</b> The 2X RNA Loading Dye allows for RNA visualization without additional staining of denaturing agarose gels. Addition of extra ethidium bromide to the ladder or sample is not recommended and may result in RNA migration in the direction of the cathode. If RNA fragments are separated on native agarose gels or on polyacrylamide/urea gels, additional staining with ethidium bromide after electrophoresis is recommended.</p>
	<p><b>3.6. Incompletely immersed gel.</b> Always ensure that there is enough electrophoresis buffer in the electrophoresis apparatus.</p>
<b>4. High background staining</b>	<p><b>4.1. Excessively high ethidium bromide concentration or prolonged staining.</b> Use ethidium bromide at a final concentration of 0.5 µg/ml. Avoid prolonged staining of the gels.</p>
	<p><b>4.2. Insufficient gel destaining.</b> If the gel is extensively stained with ethidium bromide, additional destaining in water is needed to remove background staining. Wash glyoxal/DMSO agarose gels after staining in a fresh 0.5 M ammonium acetate solution for 15-30 min.</p>

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Bulk quantities and custom formulations available upon request

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<b>5. Uneven staining of the gel</b>	<b>5.1. Improper gel staining conditions.</b> Ethidium bromide migrates in the opposite direction of the RNA during electrophoresis. Therefore, if ethidium bromide is only added to the agarose gel and not to the electrophoresis buffer, it may result in uneven RNA fragment staining. When 2X RNA Loading Dye is used for both conventional RiboRuler™ RNA ladders and RNA sample preparation prior to electrophoresis, additional staining is not required as the loading dye includes sufficient ethidium bromide to stain RNA on denaturing formaldehyde agarose gels. For native agarose gels, ethidium bromide (0.5 µg/ml) should be added to both the electrophoresis buffer and the agarose. This ensures an even distribution of ethidium bromide during electrophoresis so that the intensity of the bands upon exposure to UV light will be proportional to the quantity of RNA present.
	<b>5.2. Incompletely immersed gel.</b> Always ensure that there is enough electrophoresis buffer in the electrophoresis apparatus or enough of the staining solution during the staining so that the gel is always immersed completely.
<b>6. RNA remains in the gel well</b>	<b>6.1. Poorly formed gel wells.</b> Remove the gel comb only after complete polymerization of the gel. Pour the buffer onto the gel immediately. Rinse the wells with electrophoresis buffer to remove urea from denaturing polyacrylamide gels prior to loading the sample.
	<b>6.2. Large quantity of RNA loaded into the gel.</b> Follow the recommendations for loading described in the certificate of analysis of the RiboRuler™ RNA ladders (0.25 µl per mm gel lane for conventional ladders; 0.5 µl per mm gel lane for ready-to-use ladders).
	<b>6.3. Contamination of the RNA sample.</b> Make sure that your sample RNA solution does not contain any precipitate.
<b>7. Incorrect quantification data</b>	<b>7.1. Impure RNA.</b> Free NTPs and truncated transcripts remaining in the sample after <i>in vitro</i> transcription can interfere with spectrophotometrical measurements and lead to inaccurate quantification of sample RNA. RiboRuler™ RNA ladders are produced from chromatography-purified RNA transcripts and are free of any NTPs and truncated transcripts. Therefore the gel quantification data is compatible with the spectrophotometrical measurements of RiboRuler™ RNA ladders.
	<b>7.2. Incorrect RiboRuler™ band chosen for quantification of the sample.</b> Always compare the sample band with a similar size ladder band.
	<b>7.3. Different loading conditions for the ladder and samples.</b> Both sample and ladder RNA should be loaded under the same conditions. Use the supplied 2X RNA Loading Dye for the sample and ladder. Load equal volumes of sample RNA and ladder RNA. The required volume of sample RNA can be obtained by diluting with a mixture (1:1) of DEPC-treated Water (#R0603) and 2X RNA Loading Dye.
	<b>7.4. Improper quantification method used.</b> If possible, quantify by video-densitometry measurements while subtracting the gel background as this method is more precise than a visual comparison of the bands.
	<b>7.5. Uneven staining of the gel and high background staining</b> can also interfere with gel quantification results (see Problem 4 and 5 above).