PRODUCT NOTE

Choosing the right molecular-weight size marker matters: Migration comparison of ssDNA, dsDNA and RNA on different electrophoresis platforms.

Authors

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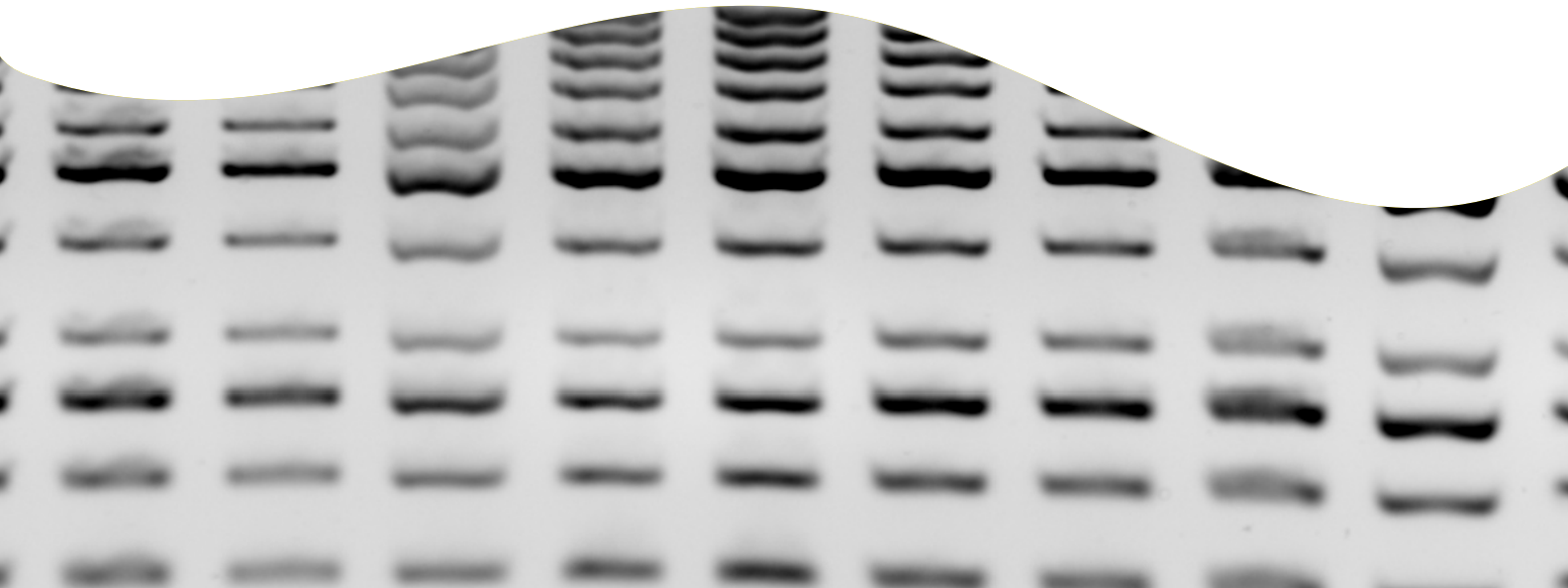
Introduction

A molecular-weight size marker, also referred to as a protein ladder, DNA ladder, or RNA ladder, is a set of size standards, which are a prerequisite for estimating the quality, size, and quantity of a molecule in electrophoresis systems. We recently developed a novel, large-size single-stranded DNA (ssDNA) 7K Ladder ranging from 1.1 kb to 7.2 kb. This ssDNA 7K Ladder is a great reference material for evaluating critical quality attributes (CQAs) of bio therapeutical products such as CRISPR Homology-Directed Repair (HDR) mediated long ssDNA genetic templates. In this report, we investigated its migration behavior and size resolutions in different electrophoresis systems. By comparing migrations of ssDNA to double-stranded DNA (dsDNA) and RNA fragments, we raised concerns of using dsDNA ladder or RNA ladder as reference for ssDNA analysis. The ssDNA/dsDNA/RNA size migration comparison study can be used as a resource and reference for researchers who are looking for reference standard and electrophoresis platform for ssDNA size analysis.



Key instruments and reagents

- Revvity ssDNA 7K Assay (Part# CLS158169), or ssDNA 7K Ladder (Part# CLS157950) with RNA Pico reagent (Part# CLS960012)
- Revvity LabChip® GXII Touch™ System (Part# CLS138160) and DNA 5K/RNA/CZE HT LabChip (Part# 760435)
- Agilent 2100 Bioanalyzer (Part# G2939BA) and RNA Pico Reagent (Part# 5067-1513)
- Agilent TapeStation 4200 (Part# G2991BA), High Sensitivity RNA ScreenTape (Part#5067-5579).

Results

We systematically compared ssDNA 7K Ladder migration behavior to dsDNA or RNA on four electrophoresis platforms: a) Revvity ssDNA 7K assay system; b) agarose electrophoresis; c) Agilent Bioanalyzer 2100 and d) Agilent TapeStation 4200. Details are described as below.

1. The Revvity ssDNA 7K assay system provides good resolution for ssDNA fragments where all six peaks were well separated. While dsDNA and RNA samples were also well resolved, ssDNA fragments ran slower than both dsDNA fragments and RNA fragments of similar length (Figure 1).

2. In the native agarose gel system interestingly, a reverse phenomenon was observed, where ssDNA fragments ran faster than dsDNA fragments of similar length (Figure 2).

3. In the Bioanalyzer 2100 platform, ssDNA fragments (1.1k to 7.2k) can also be separated, however, compared to the Revvity ssDNA 7K assay system, there is less resolution for large ssDNA fragments (>4k). Similar to the Revvity ssDNA 7K assay system is that ssDNA fragments run slower than both dsDNA and ssRNA fragments. However, the migration difference between ssDNA and dsDNA is more obvious in the Bioanalyzer than that in the Revvity ssDNA 7K assay system (Figure 3). For example, the 2.1kb ssDNA fragment migrated similarly to the 2.9kb dsDNA fragment in the Revvity ssDNA 7K assay system, while in the Bioanalyzer, it ran even slower than the 7.2kb dsDNA fragment. This observation tells us that different electrophoresis systems have different logarithmic scale of ssDNA sizing, any "estimation of ssDNA fragment using either dsDNA ladder or RNA ladder" assumption should be case by case. The assumption might be wrong or at least be skewed greatly in certain cases.

4. In the TapeStation 4200 system, ssDNA fragments run faster than RNA fragments and slower than dsDNA fragments when samples are in the same buffer (Figure 4A). When dsDNA samples were prepared in a buffer with a lower salt concentration, shorter ssDNA fragments (1.1kb, 2.1kb) ran slower than the dsDNA fragments at the same size while longer ssDNA fragments (3.1kb, 4kb, 5kb) fragments ran faster than the dsDNA fragments of the same size (Figure 4B). Interestingly, the longest fragment here (7.2kb ssDNA) ran slower than the 7.2kb dsDNA fragment. This data indicates that salt affects the migration of nucleic acid fragments substantially in the TapeStation 4200 system. In addition, the TapeStation 4200 system does not provide enough resolution for either dsDNA fragments or ssDNA fragments at sizes ranging from 3.2kb to 7.2kb under current electrophoresis setting. As shown in Figure 4, the four fragments 3.2kb, 4kb, 5.1kb and 7.2kb were not well separated.

Overall, the Revvity ssDNA 7K assay system produces the best resolution for ssDNA fragments at sizes from 1kb to 7.2kb. A complete summary of these four platform comparisons can be found in Table 1.

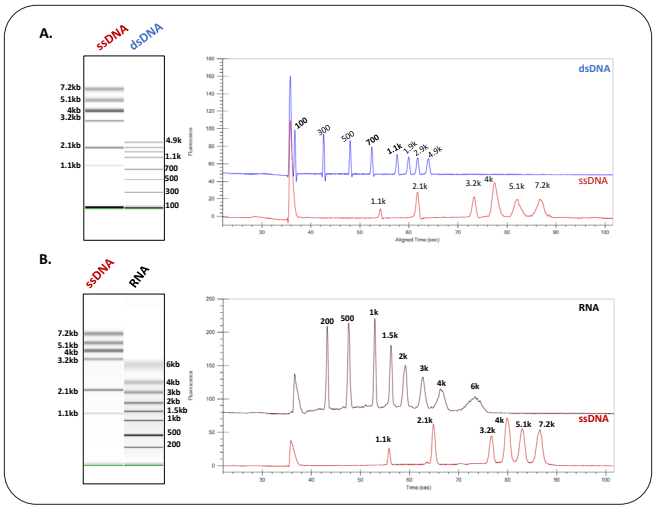


Figure 1. ssDNA migration pattern in Revvity ssDNA 7K assay system. (A) ssDNA 7K Ladder migration comparison to dsDNA. (B) ssDNA 7K Ladder migration comparison to RNA. The Revvity ssDNA 7K assay was run following the user guide[1]. ssDNA (in Red) represents ssDNA 7K Ladder. dsDNA (in Blue) represents dsDNA fragments (B). RNA (in Black) represents RNA ladder from RNA Pico assay kit. Fragment sizes are labeled beside the peaks/bands.

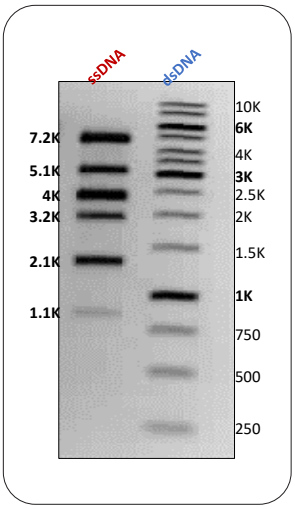


Figure 2. ssDNA migration on native agarose gel. 200ng DNA samples were mixed with 6x DNA loading dye and Gel Red (Final concentration: 1x), and then loaded onto a 1% TAE agarose gel and run at 10V/cm gel length for 45min in 1X TAE buffer. ssDNA represents ssDNA 7K Ladder; dsDNA represents GeneRuler 1KB dsDNA Ladder. Fragment sizes are labeled beside the peaks/bands.

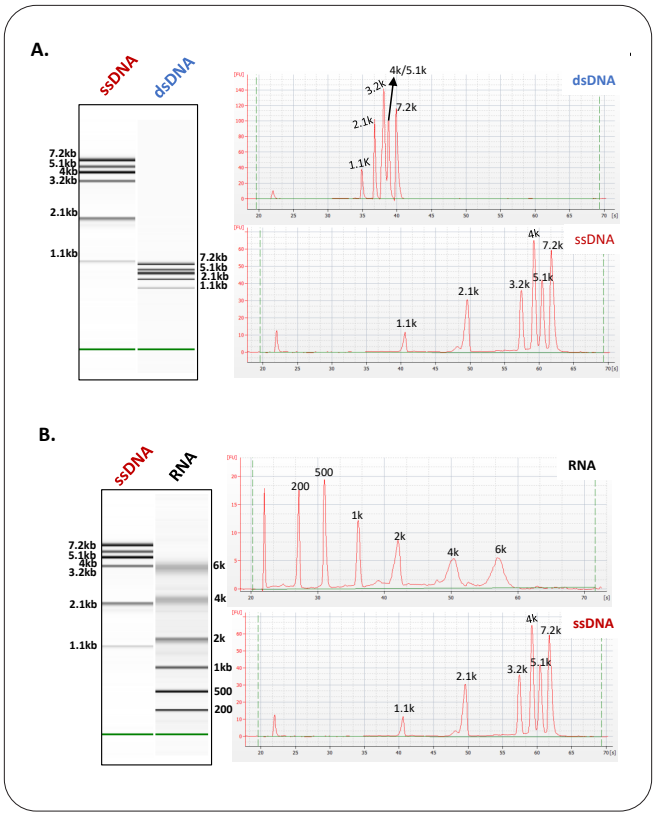


Figure 3. ssDNA migration pattern in 2100 Bioanalyzer. (A) ssDNA 7K Ladder migration comparison to dsDNA. (B) ssDNA 7K Ladder migration comparison to RNA. RNA Chip was prepared according to the Agilent user guide for RNA Pico 6000 assay. 2.5ng/µl samples were loaded onto the sample wells and RNA Pico 6000 ladder was loaded on to the ladder well. ssDNA (in Red) represents ssDNA 7K Ladder; dsDNA (in Blue) represents dsDNA fragments. RNA (in Black) represents RNA fragments. Fragment sizes are labeled beside the peaks/bands.

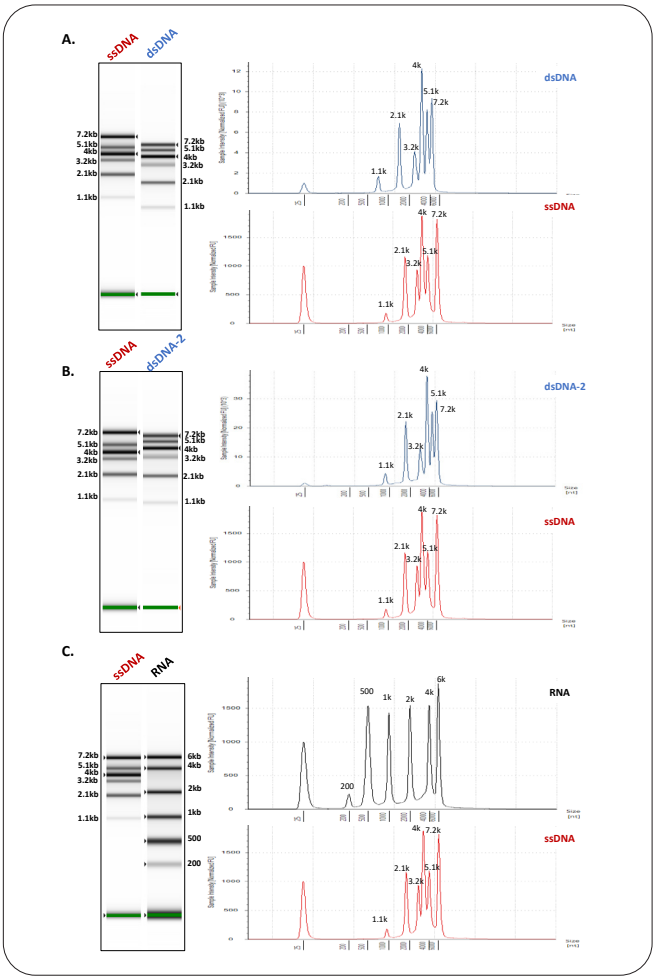


Figure 4. ssDNA migration pattern in TapeStation 4200. (A)(B) ssDNA 7K Ladder migration comparison to dsDNA. (C) ssDNA 7K Ladder migration comparison to RNA. 2 µL sample/Ladder was mixed with 1 µL High Sensitivity RNA ScreenTape Sample Buffer. After mixing, all samples were heated up at 70°C for 3 minutes and then left on ice immediately for 3 minutes. Then samples in 8-strip and High Sensitivity RNA ScreenTape were loaded on to TapeStation 4200. ssDNA (in Red) represents ssDNA 7K Ladder; dsDNA or dsDNA-2 (in Blue) represents dsDNA fragments. RNA (in Black) represents RNA fragments. Fragment sizes are labeled beside the peaks/bands. Note: ssDNA in (A)(B) and dsDNA in (A) contains 1.25 mM K^+, 0.5 mM Tris, 0.25mM Mg^{2+}, 0.5 mM EDTA in final, dsDNA-2 in (B) contains 0.25 mM Tris in in final sample concentration.

Table 1. Comparison of ssDNA migration speed with dsDNA and RNA on different electrophoresis platforms.

Electrophoresis platforms	Migration speed comparison for fragments at similar size	
	ssDNA vs. dsDNA	ssDNA vs. RNA
Revvity ssDNA 7K assay system	ssDNA < dsDNA	ssDNA < RNA
Native Agarose Gel	ssDNA > dsDNA	NA*
Agilent 2100 Bio analyzer	ssDNA < dsDNA	ssDNA < RNA
Agilent TapeStation 4200	complicated**	ssDNA > RNA

*NA: not available

**ssDNA fragments migrated slower than dsDNA fragments when samples were in the same buffer condition tested here. When samples are in different buffers, further investigation is required to determine the migration pattern of ssDNA and dsDNA.

Discussion

Several parameters are recommended to be considered when developing nucleic acid electrophoresis study: a) choosing a ladder which is the same type as the study samples; b) choosing a ladder that the study sample size is within the ladder's range; c) choosing the electrophoresis system (such as gels, buffers, charge, and time, etc.) that achieve high size resolution of the study samples. In this study, we have demonstrated the migration difference of ssDNA ladder comparing to dsDNA or RNA fragments across electrophoresis platforms, which depends on multiple properties such as electrophoresis system, dye concentration, the size and sequence of the fragments of interest. In addition, some salt change in samples can reach the opposite conclusion on relative migration behavior of ssDNA vs. dsDNA on certain electrophoresis platform (as shown in Figure 4B). Therefore, we raise the concerns of using dsDNA ladder or RNA ladder for ssDNA size analysis, and strongly recommend that using the right type of ladder (here is ssDNA ladder) will be beneficial for electrophoresis system optimization and proper size calling in ssDNA analysis. As an example, the Revvity ssDNA 7K assay system[1,2] has undergone internal optimization, presenting the best separation and resolution for large-size ssDNA fragment analysis.

References

1. Revvity, Inc. ssDNA 7K Assay Kit
2. Revvity, Inc. ssDNA 7K Ladder Reagent

Technical contact information

Please see instrument, reagent and software section listed above for ordering. Please send questions or requests for user guide and/or evaluation reagent to: dxsupportamericas@perkinelmer.com or Zhixiang.Lu@revvity.com by adding "ssDNA-" at the beginning of the subject line.

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