# Simplified $DV_{200}$ Evaluation with the Agilent 2100 Bioanalyzer System

## Technical Overview

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### Introduction

Quality assessment of RNA samples is crucial for the success of library preparation for RNA sequencing workflows. The Agilent 2100 Bioanalyzer system enables the highly reproducible evaluation of RNA sample quantity and integrity, with the Total RNA Nano and Pico assays. In general, the RNA integrity number (RIN) is a suitable parameter to assess sample integrity for RNA sequencing. The establishment of RNA sequencing approaches for samples originating from formalin-fixed paraffin-embedded (FFPE) tissue created the need for further differentiation of degraded RNA samples by fragment size distribution. The $DV_{200}$ quality metric represents the percentage of RNA fragments above 200 nucleotides and shows a high correlation to the precapture library yield of FFPE samples[1].

This Technical Overview describes the simplified evaluation of $DV_{200}$ using the DV200 RNA Nano and DV200 RNA Pico assays with the Agilent 2100 Bioanalyzer system.

Primary modifications of the $DV_{200}$ RNA Nano and Pico assays are:

- Predefined $DV_{200}$ region

- $DV_{200}$ presented in region table by default

- Result flagging according to the $DV_{200}$ range

- $DV_{200}$ range displayed in chip summary

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## Technical Details

The Agilent 2100 Bioanalyzer system with Agilent 2100 Expert Software version B.02.08, the Agilent RNA 6000 Nano Kit (p/n 5067-1511) and Agilent RNA 6000 Pico Kit (p/n 5067-1513) were used. Unless stated, the manufacturer's protocols and guidelines were followed. FFPE RNA samples were provided by the tissue bank of the National Center for Tumor Diseases (NCT, Heidelberg, Germany) in accordance with the regulations of the tissue bank and the approval of the ethics committee of Heidelberg University.

## Data Analysis

The DV200 RNA assay automatically defines a region from 200 to 8,000 nucleotides, as shown in Figure 1. A corresponding $DV_{200}$ is provided in the Region Table in the **% of Total** column. The RIN algorithm remains unaffected for all samples. If Results tables are selected, $DV_{200}$ can be included in exported and printed data. In addition, the $DV_{200}$ ranges can be copied from the Chip Summary table.

## Result Flagging

With the $DV_{200}$ metric, degraded RNA samples can be classified according to their size distribution. Some RNA sequence library protocols recommend adapting the input amount of RNA depending on the $DV_{200}$ range of the sample[1]. The DV200 RNA assay flags samples with different colors with RIN <4 for easy assessment of the $DV_{200}$ range (Figure 2).
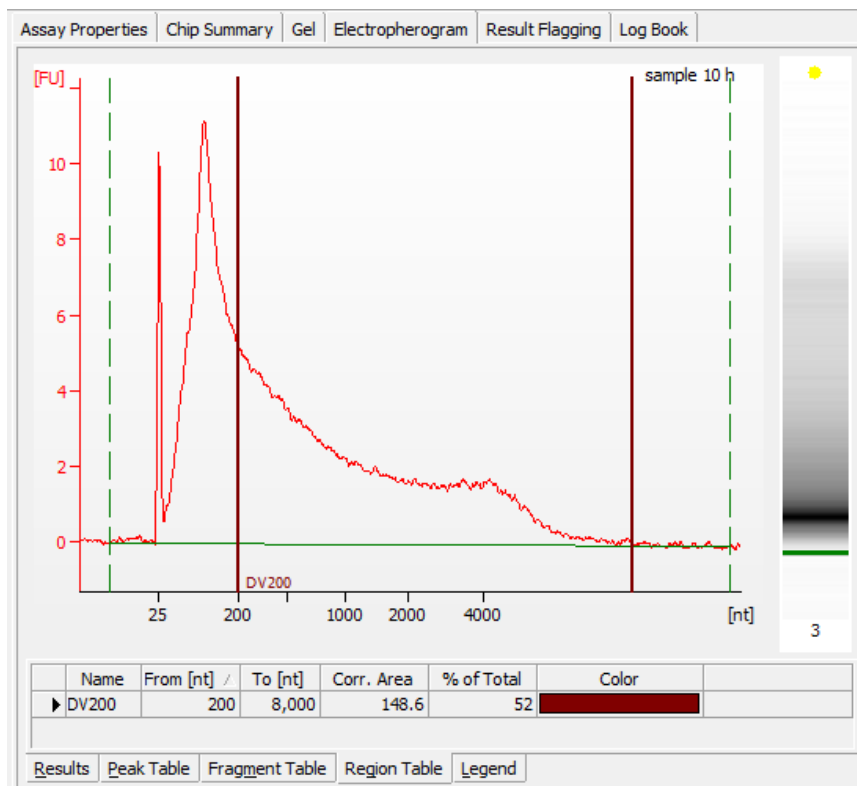


Figure 1. $DV_{200}$ region of an RNA sample extracted from FFPE tissue.



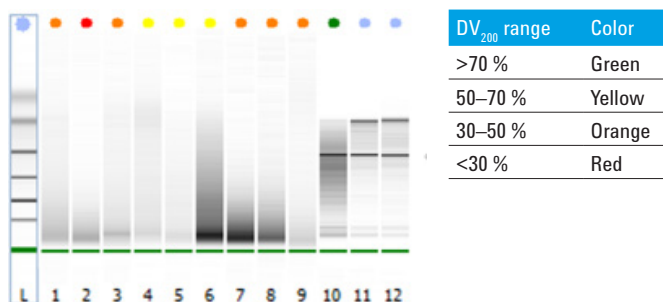| $DV_{200}$ range | Color |
|---|---|
| >70 % | Green |
| 50–70 % | Yellow |
| 30–50 % | Orange |
| <30 % | Red |

Figure 2. Result flagging with colors representing the $DV_{200}$ ranges of FFPE samples with a RIN <4. Samples 11 and 12 are eukaryotic total RNA samples with a RIN >4, and are unaffected by result flagging.

## Importing the Assay

The DV200 RNA Nano and Pico assays are compatible with the 2100 Expert Software version B.02.08 or higher. They can be used for reanalysis of existing data files as well as for new data acquisition.

To use the DV200 RNA Nano or Pico assay with the 2100 Expert Software, download it from the Agilent website: www.genomics.agilent.com. Store the DV200 RNA Nano.xsy or DV200 RNA Pico.xsy file in the RNA assay folder (C:\Program Files (x86)\Agilent\2100 bioanalyzer\2100expert\assays\RNA).

To apply the $DV_{200}$ on an existing data file:

1. Open the file with the 2100 Expert Software.

2. Navigate to the **Assay Properties** tab.

3. Click **Import Setpoints** and select the corresponding. xsy file (Figure 3).

The DV200 RNA Nano assay can only be used to reanalyze Eukaryote Total RNA Nano data files, and the DV200 RNA Pico assay is only for Eukaryote Total RNA Pico data files.

Assay setpoints and result flagging will be applied to the data file and can be saved afterwards.

To run samples with the DV200 RNA Nano and DV200 RNA Pico assay:

1. Prepare the samples and the RNA chip according to the RNA Nano or Pico Kit Guide.

2. When the chip is prepared and placed in the 2100 Bioanalyzer instrument, go to the instrument context, click **Assay Selection**, and choose the corresponding DV200 RNA assay. The assay will be part of the general assay selection when it has been stored in the RNA assay folder (Figure 4).

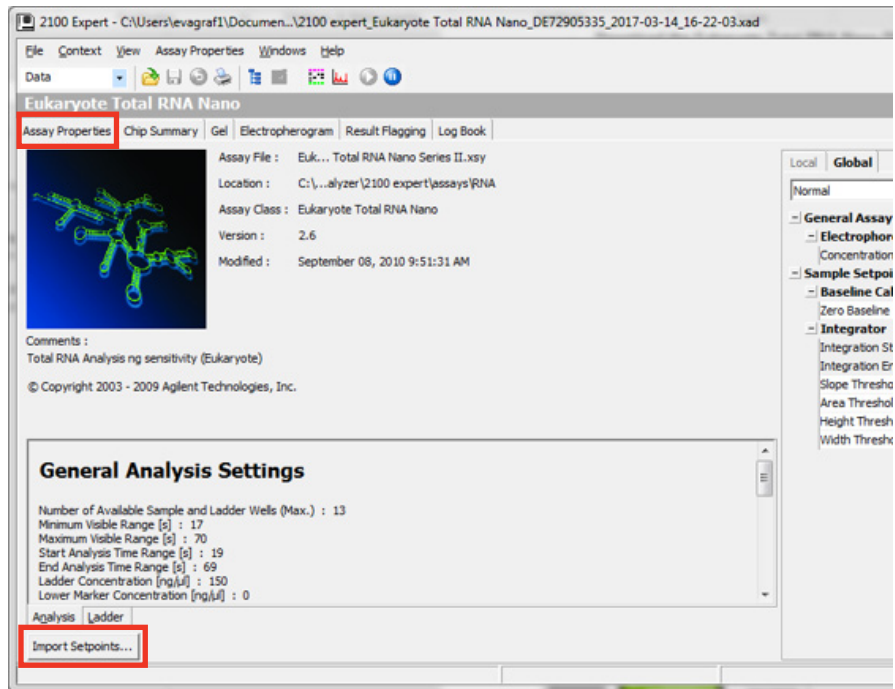3. To start the run, click **Start**.



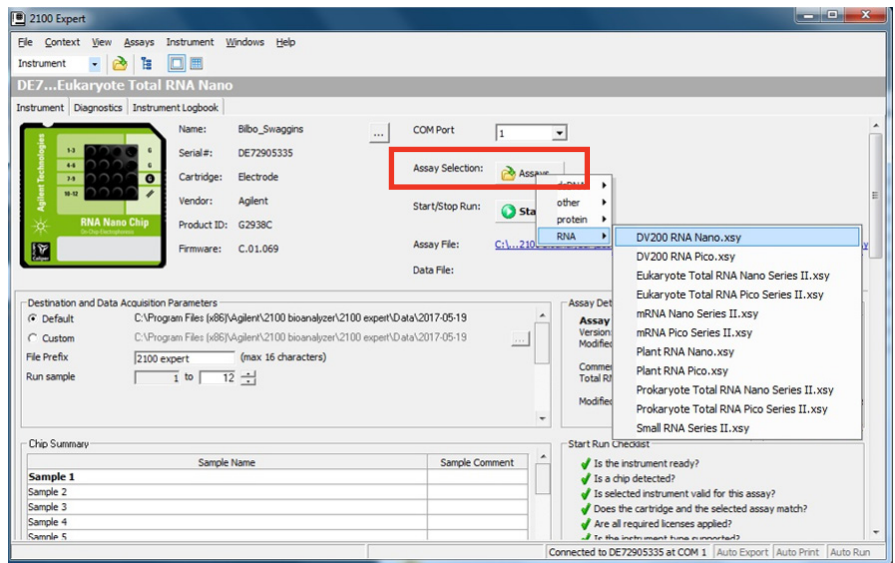Figure 3. Importing the DV200 RNA assay into existing data files.



Figure 4. Selecting the DV200 RNA assay to run an RNA chip.

## Conclusion

The DV200 RNA Nano and DV200 RNA Pico assays are useful tools to automatically define $DV_{200}$ regions for degraded RNA samples extracted from FFPE tissue. Once the assay is imported, it can be used to run the Agilent 2100 Bioanalyzer RNA chips, or to reanalyze existing data files. The $DV_{200}$ results can be saved, exported, and displayed in reports.

## References

1. Evaluating RNA Quality from FFPE Samples. Illumina, *Technical Note*, publication number 470-2014 001. https://www.illumina.com/documents/products/technotes/technote-truseq-rna-access.pdf

www.agilent.com/genomics/bioanalyzer

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