

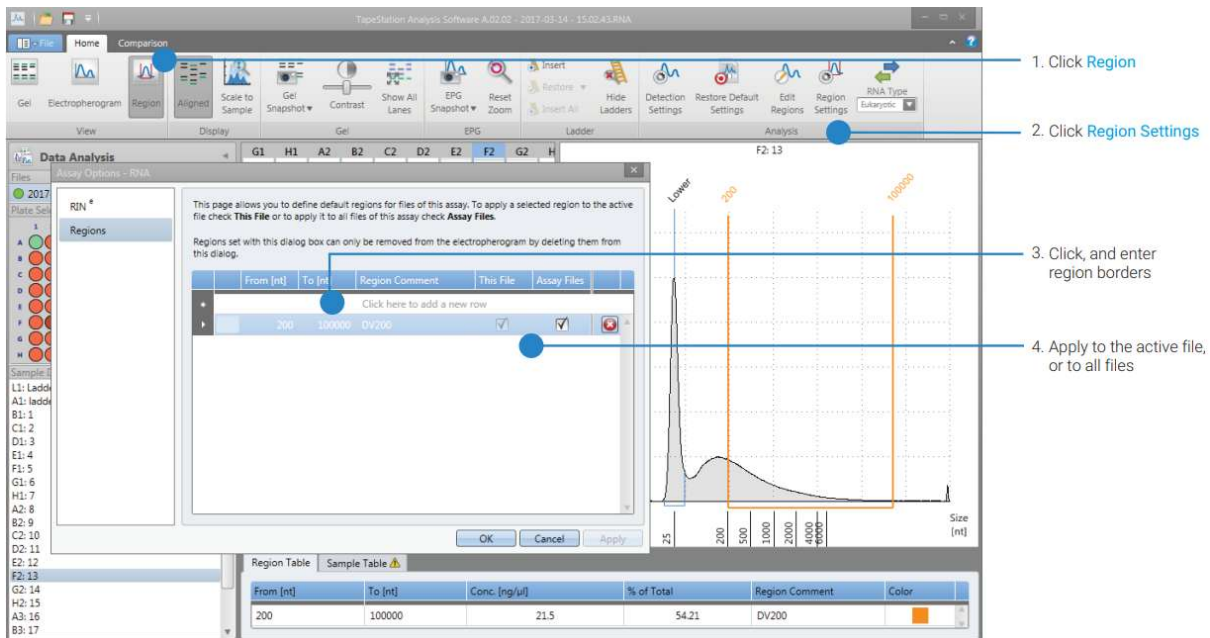
## DV200 CALCULATION FOR FFPE SAMPLES WITH THE TS4200 APPLIED TO nCOUNTER (Nanosttring)

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RNA quality is a critical factor in any molecular biology study involving gene expression. DV200 is a key indicator of RNA integrity, especially in degraded samples. Unlike RIN (RNA Integrity Number), which can be unreliable under these conditions, DV200 provides a more accurate measure of the amount of RNA useful for applications such as sequencing or transcriptomics.

The DV200 of a sample is defined as the percentage of fragments larger than 200 nucleotides (nt) and can be calculated using a capillary electrophoresis system such as the TapeStation (TS4200). This value is obtained from the analysis of the RNA fragmentation profile by following the following steps:

1. Install the program: Agilent TapeStation System Software. This program can be downloaded from the Agilent website or requested to be installed at SAU. <https://explore.agilent.com/Software-Download-TapeStation-Systems>
2. Open the file with the RNA quality analysis (HS RNA or RNA Screentape) provided by the platform with the **Agilent TapeStation System Software**
3. .Click on Region
4. Click on Region Settings
5. Write 200 in the from (nt) box and 100,000 in the To (nt) box
6. Write DV200 in the Region comment box
7. Click on This File to apply this calculation to all the samples in the open file
8. Click Apply
9. We obtain the DV200 value in the % of total column of the Region table of each sample



The screenshot shows the TapeStation Analysis Software interface. The 'Region' menu is selected, leading to the 'Region Settings' dialog box. The dialog box has a table with the following data:

From [nt]	To [nt]	Region Comment	This File	Assay Files
200	100000	DV200	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Below the dialog box, the 'Region Table' is visible, showing the calculated DV200 value:

From [nt]	To [nt]	Conc. [ng/ul]	% of Total	Region Comment	Color
200	100000	21.5	54.21	DV200	Orange

Four numbered callouts point to the following actions in the software:

1. Click Region
2. Click Region Settings
3. Click, and enter region borders
4. Apply to the active file, or to all files

To determine the real **adjusted by DVD200 (ng) Input** that we will need to use for the nCounter experiment, we need to apply this formula:

$$\text{Input Real * adjusted by DV200} = \frac{\text{theoretical input}}{\left( \begin{array}{c} \% \text{ sample} \\ >200 \end{array} \right) \times 100}$$

Example:

FÓRMULA:  
Input teòric / (DV200 \*100)

MOSTRA	DV200 (%)	INPUT TEÒRIC (100 ng)	Qubit ng/μl	INPUT REAL AJUSTAT PER DV200	μL mostra	QC
1	50	100	250	200,0	0,8	PASS
2	12	100	104	833,3	8,0	FAIL
3	48	100	103	208,3	2,0	PASS
4	60	100	110	166,7	1,5	PASS
5	23	100	67	434,8	6,5	FAIL
6	55	100	87,2	181,8	2,1	PASS
7	70	100	20	142,9	7,1	FAIL

Volum > 5μl (CodeSet) i a >7μl (TagSet)

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**DV200:** value obtained by the TS4200 (recommended > 50%).

**INPUT TEÒRIC:** fixed value 100 ng. Can be modified if the IP has more diluted samples (talk to Genomics).

**Qubit (ng/μl):** sample concentration measured with Qubit.

**INPUT REAL AJUSTAT PER DV200:** total ng that needs to be hybridized, value that results from applying the formula (\*).

**μl mostra:** sample volume in the nCounter hybridization reaction (maximum 5μl for Panels/CodeSets and 7μl for TagSets).

**QC:** Pass o Fail depending on the quantity and volume of the sample. If the sample volume exceeds the maximum hybridization volume, the researcher either places the maximum possible or discards the sample (IP decision).

**ATTENTION:** Once the sample volume (μl) has been calculated, add 2μl more of sample to the same tube, for internal platform quality controls.