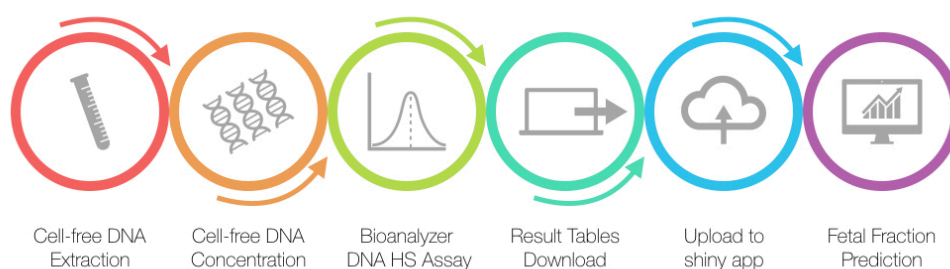# Fetal Fraction Percentage Predictor

This app uses our described prediction model based on Peak Height and Fetal Fragments Ratio to evaluate the cell-free fetal DNA percentage from a Bioanalyzer electrophoresis analysis (Agilent Technologies, Santa Clara, CA, USA).

Sample Requirements

- Extracted maternal cell-free DNA
- Target concentration: 1.5 ng/µL (cell-free DNA may require a concentration step)
- Due to the high sensitivity of the assay, the sample maximum salt concentration is 10 mM Tris and 1 mM EDTA

Bioanalyzer Assay

- Perform the micro-chip based electrophoresis using the Bioanalyzer High Sensitivity DNA Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions.

Agilent 2100 expert software set up

- Software version used B.02.08.SI648 (Fig 1):
  1. Select the experiment on the "Data" context
  2. Click on the "Assay properties" tab
  3. Set "Global" and "Advanced" in the Setpoints window
  4. Select "Sample Setpoints" menu
  5. Perform Baseline Correction
  6. Click "Regions" from the "Smear Analysis" section
  7. Insert the regions using the pop-up window
     - Region 1 from 78 bp to 143 bp

> ➢ Region 2 from 163 bp to 168 bp

8. Click "Apply to all"

9. Check the electropherogram quality and the selected regions

10. From the export options select "Result Tables", "Exclude Markers" and "Include Ladders", then export the file.

Prediction App

- Go to: http://tools.cbm.fvg.it/Shiny_cfDNApredictor/

- Upload the "Result tables.csv" from 2100 Expert software

Upload OutputResults.csv	Manual Data Entry

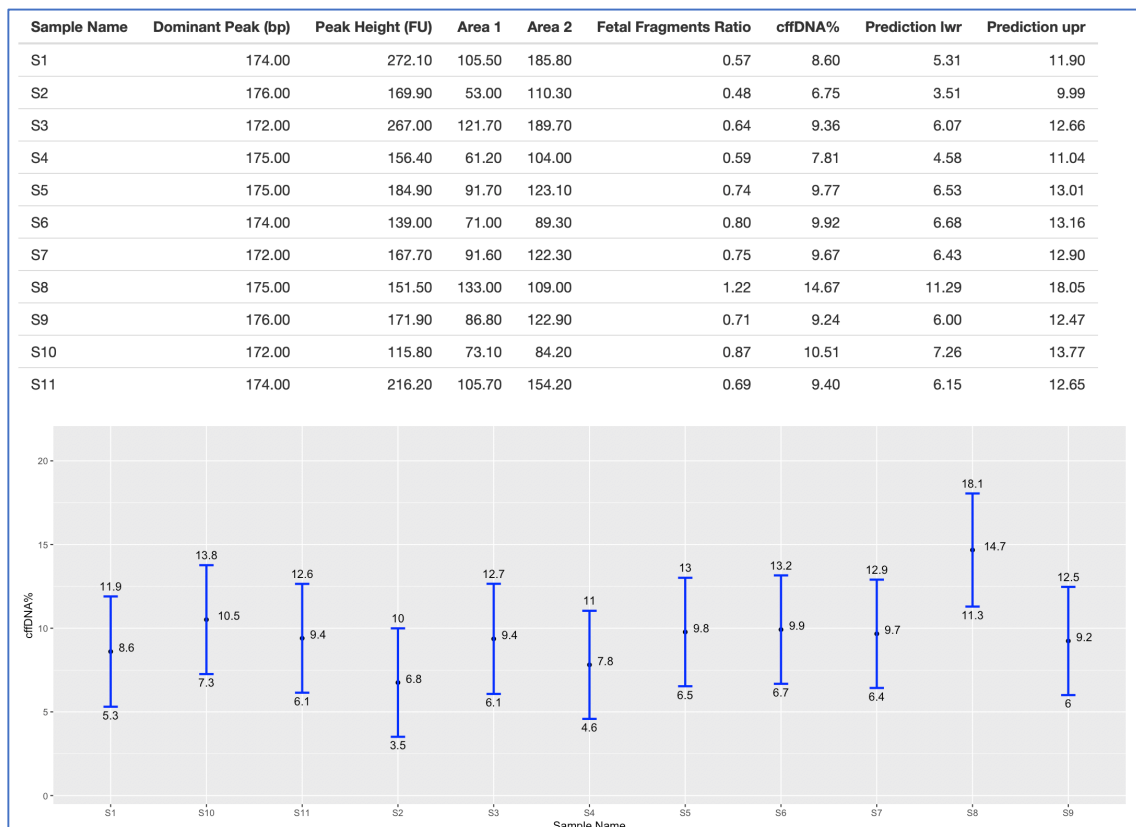# Fetal Fraction Percentage Predictor

Upload an OutputResults.csv from 2100 Expert Software

Choose a file

Browse...	No file selected

- The app returns a table that shows the electropherogram information for each sample, the predicted cffDNA% and the bounds of 95% prediction interval.

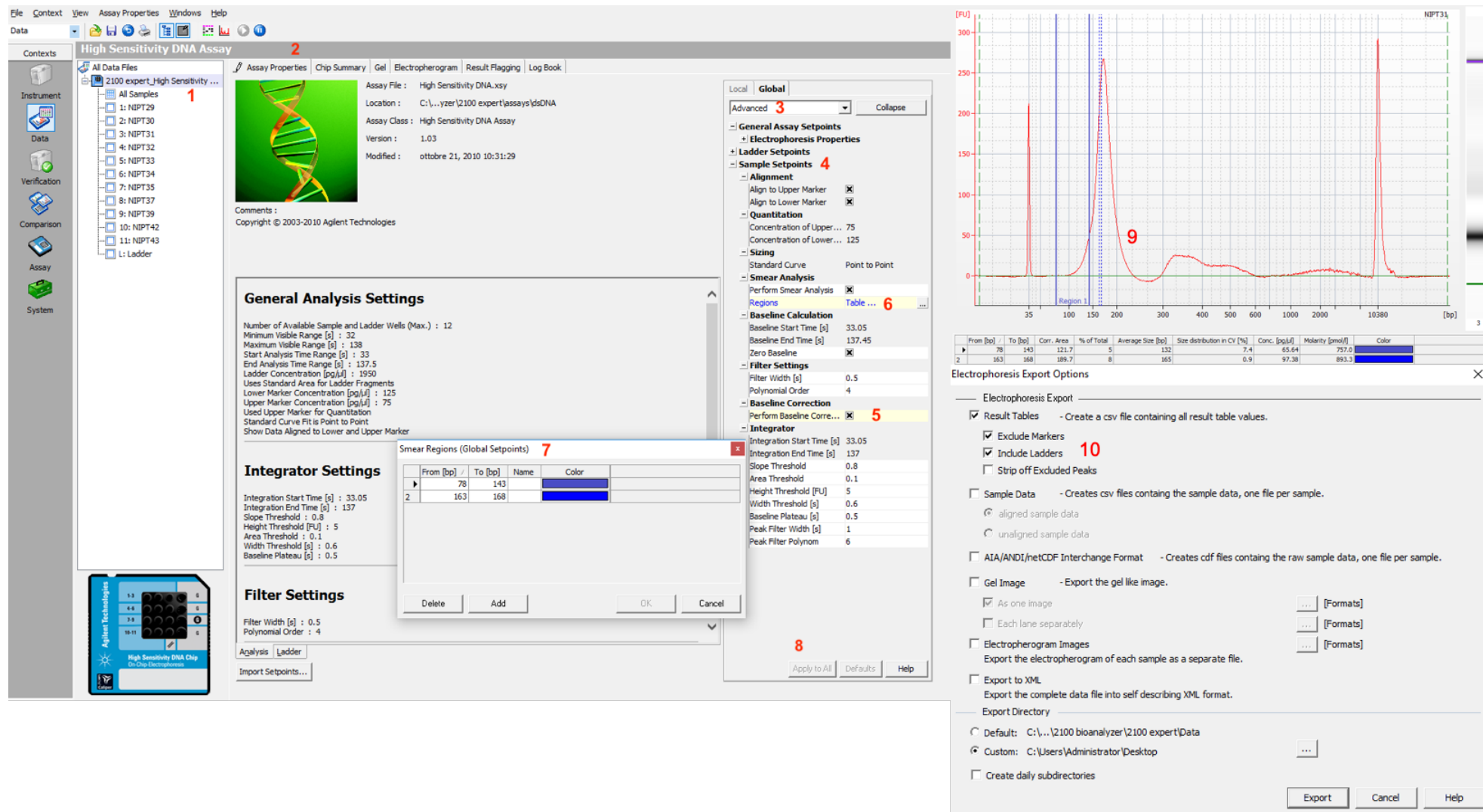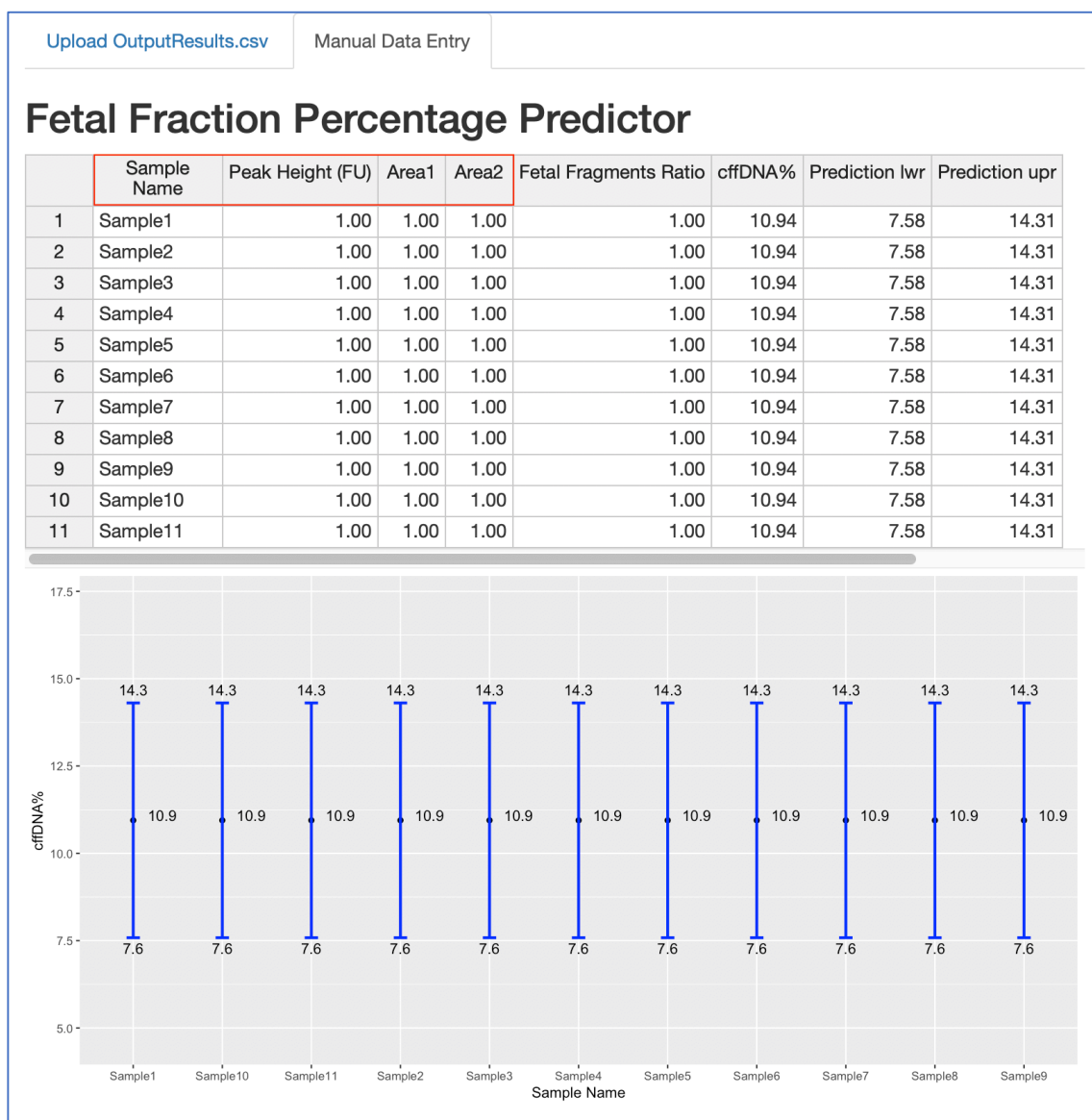| Sample Name	Dominant Peak (bp)	Peak Height (FU)	Area 1	Area 2	Fetal Fragments Ratio	cffDNA%	Prediction lwr	Prediction upr
S1	174.00	272.10	105.50	185.80	0.57	8.60	5.31	11.90
S2	176.00	169.90	53.00	110.30	0.48	6.75	3.51	9.99
S3	172.00	267.00	121.70	189.70	0.64	9.36	6.07	12.66
S4	175.00	156.40	61.20	104.00	0.59	7.81	4.58	11.04
S5	175.00	184.90	91.70	123.10	0.74	9.77	6.53	13.01
S6	174.00	139.00	71.00	89.30	0.80	9.92	6.68	13.16
S7	172.00	167.70	91.60	122.30	0.75	9.67	6.43	12.90
S8	175.00	151.50	133.00	109.00	1.22	14.67	11.29	18.05
S9	176.00	171.90	86.80	122.90	0.71	9.24	6.00	12.47
S10	172.00	115.80	73.10	84.20	0.87	10.51	7.26	13.77
S11	174.00	216.20	105.70	154.20	0.69	9.40	6.15	12.65

Fig 1 Agilent 2100 expert software set up

- The app allows the "Manual Data Entry". Perform the *"Agilent 2100 expert software set up"* section and at the end of the analysis identify from the 2100 expert software (Fig 2):
  - ➢ Sample Name
  - ➢ Peak Height of the dominant peak (~ 170bp)
  - ➢ Area1 (of region 1 from 78 bp to 143 bp)
  - ➢ Area2 (of region 2 from 163 bp to 168 bp)

  Insert the abovementioned information in the table; based on the data provided, Fetal Fragments Ratio, cffDNA%, and the bounds of 95% prediction interval are automatically computed.

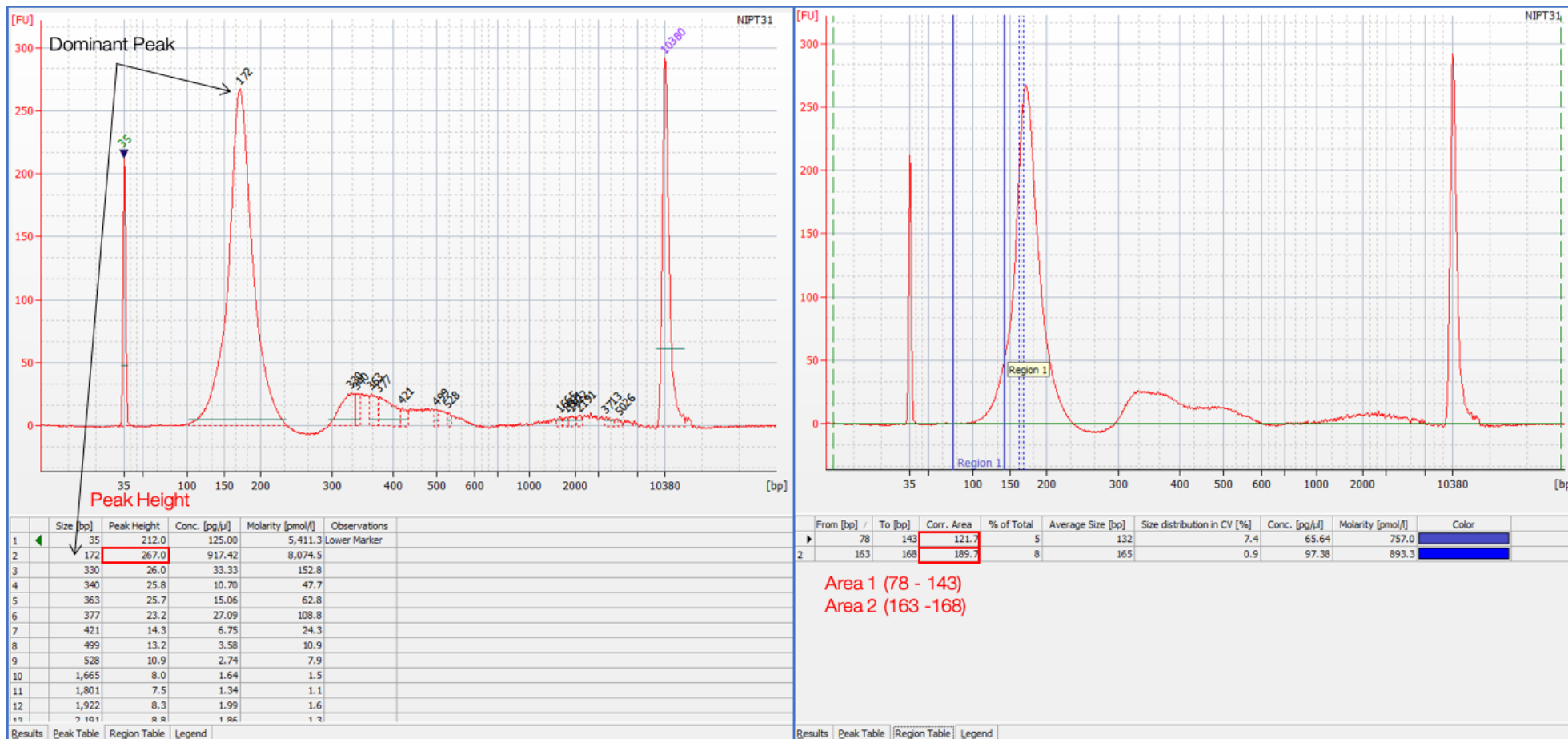|	Sample Name	Peak Height (FU)	Area1	Area2	Fetal Fragments Ratio	cffDNA%	Prediction lwr	Prediction upr
1	Sample1	1.00	1.00	1.00	1.00	10.94	7.58	14.31
2	Sample2	1.00	1.00	1.00	1.00	10.94	7.58	14.31
3	Sample3	1.00	1.00	1.00	1.00	10.94	7.58	14.31
4	Sample4	1.00	1.00	1.00	1.00	10.94	7.58	14.31
5	Sample5	1.00	1.00	1.00	1.00	10.94	7.58	14.31
6	Sample6	1.00	1.00	1.00	1.00	10.94	7.58	14.31
7	Sample7	1.00	1.00	1.00	1.00	10.94	7.58	14.31
8	Sample8	1.00	1.00	1.00	1.00	10.94	7.58	14.31
9	Sample9	1.00	1.00	1.00	1.00	10.94	7.58	14.31
10	Sample10	1.00	1.00	1.00	1.00	10.94	7.58	14.31
11	Sample11	1.00	1.00	1.00	1.00	10.94	7.58	14.31

Fig 2 Information from 2100 Expert Software