

Using savR

R. Brent Calder

May 27, 2021

```
> library(savR)

> fc <- savR(system.file("extdata", "MiSeq", package="savR"))

> fc
savProject instance with 1 lanes, 82 total cycles, and 2 sequence reads ( 1 sequencing and 1 index)
Default naming convention.
With InterOp data for: savCorrectedIntensityFormat (correctedIntensities)
savQualityFormat (qualityMetrics)
savTileFormat (tileMetrics)
savExtractionFormat (extractionMetrics)

> pfBoxplot(fc)
```

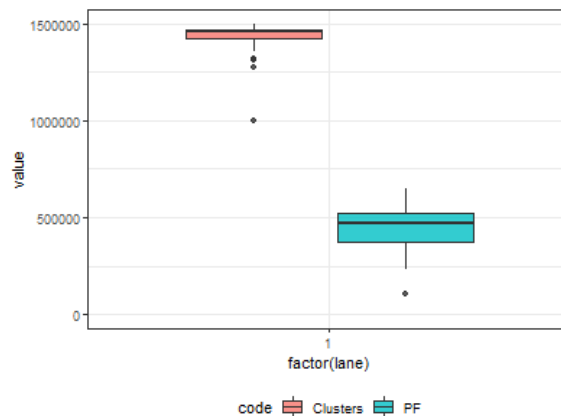


Figure 1: Boxplot of total vs. PF clusters

Introduction

The Illumina Sequence Analysis Viewer (SAV) is a Windows application provided by Illumina that presents graphs made in real time from data collected over the course of basecalling. This data was previously also made available in HTML format for inspection after the run; however, it is now preserved in binary format and not simply parsed by users who wish to perform automated quality assessment. Here is presented *savR*, an R package to parse the binary output, generate QC assessment plots and make the data available to users of Illumina sequencing instruments. For more information about Illumina SAV, please consult the Illumina iCom website and the Sequencing Analysis Viewer User's Guide, available *online*.

Description

The `savR` function is passed a path to an Illumina HiSeq or MiSeq run, and returns a `savProject` object, containing the parsed data. Accessor methods are available for information in the `RunInfo.xml` file as well as the parsed SAV Metrics files. These include corrected intensities, quality metrics, tile metrics, and extraction metrics. The *savR* package comes with an example MiSeq data set which can be loaded thusly:

```
> fc <- savR(system.file("extdata", "MiSeq", package="savR"))
```

RunInfo.xml

The `RunInfo.xml` file is parsed and stored in the slots of the `savProject` object. There are accessor methods for the project's `location`, `reads`, number of “ends” or `directions`, the run ID, the number of `cycles`, and a description of the `flowcellLayout`.

```
> directions(fc)
```

```
[1] 1
```

```
> reads(fc)
```

```
[[1]]
```

```
An object of class "illuminaRead"
```

```
Slot "number":
```

```
[1] 1
```

```
Slot "cycles":
```

```
[1] 76
```

```
Slot "index":
```

```
[1] FALSE
```

```
[[2]]
```

```
An object of class "illuminaRead"
```

```
Slot "number":
```

```
[1] 2
```

```
Slot "cycles":
```

```
[1] 6
```

```
Slot "index":
```

```
[1] TRUE
```

```
> cycles(fc)
```

```
[1] 82
```

```
> flowcellLayout(fc)
```

```
An object of class "illuminaFlowCellLayout"
```

```
Slot "lanecount":
```

```
[1] 1
```

```
Slot "surfacecount":
```

```
[1] 2
```

```
Slot "swathcount":
```

```
[1] 1
```

```
Slot "tilecount":
```

```
[1] 19
```

```
Slot "sectionperlane":
```

```
[1] NA
```

```
Slot "lanepersection":
```

```
[1] NA
```

```
Slot "tilenamingconvention":
```

```
[1] ""
```

Corrected intensities

Corrected intensity metrics (obtained from `CorrectedIntMetricsOut.bin`) can be inspected by the `correctedIntensities` accessor method:

```
> head(correctedIntensities(fc), n=1)
```

	lane	tile	cycle	avg_intensity	avg_cor_A	avg_cor_C	avg_cor_G	avg_cor_T
2	1	1101	1	80	72	17	116	101
				avg_cor_called_A	avg_cor_called_C	avg_cor_called_G	avg_cor_called_T	num_none
2			212	266	283	277	339	
	num_A	num_C	num_G	num_T	sig_noise			
2	97572	17051	136607	127150	6.704378			

This is a `data.frame` of intensity metrics; one line for each set of lane, tile and cycle measurements. Reported statistics include average intensity, corrected intensity (for cross-talk between bases and phasing/pre-phasing), called corrected intensities, number of called bases and signal to noise ratio. There are methods which act upon `savProject` objects to produce QC plots, for example `plotIntensity` to assess signal intensity for each channel as in figure 2.

```
> plotIntensity(fc)
```

Quality Metrics

The quality metrics (`QMetricsOut.bin`) file contains per-lane/tile/cycle metrics for the number of clusters with quality at each PHRED value from 1-50.

```
> head(qualityMetrics(fc), n=1)
```

	lane	tile	cycle	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17
1	1	1101	1	0	0	0	0	0	0	0	0	0	0	0	17087	0	0	0	0	0
				Q18	Q19	Q20	Q21	Q22	Q23	Q24	Q25	Q26	Q27	Q28	Q29	Q30	Q31	Q32	Q33	
1	0	0	0	6562	0	5323	5901	0	0	13362	0	0	250	22276	13477	12757				
				Q34	Q35	Q36	Q37	Q38	Q39	Q40	Q41	Q42	Q43	Q44	Q45	Q46	Q47	Q48	Q49	Q50
1	281724	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

```
> qualityHeatmap(fc,1,1)
```

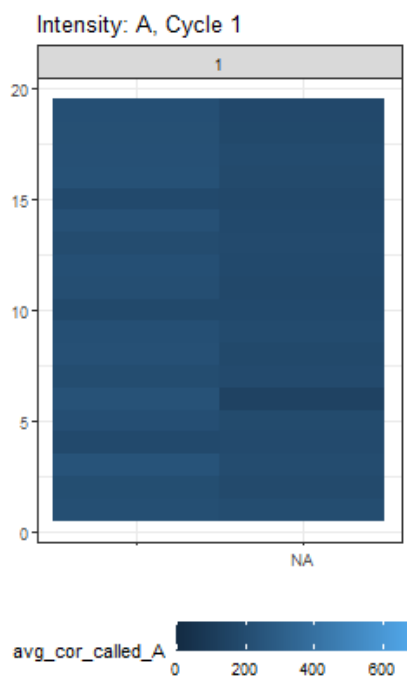


Figure 2: Corrected intensity plot: cycle 1, base “A”.

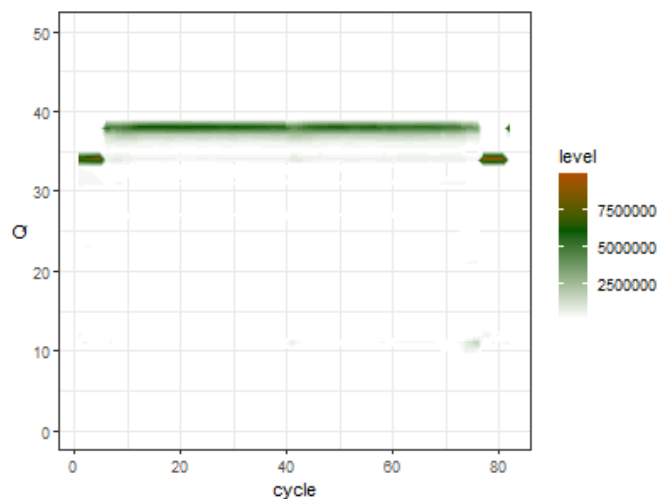


Figure 3: Quality heatmap: lane 1, read 1.

Tile Metrics

The tile metrics (`TileMetricsOut.bin`) file contains coded information about per-lane/cycle/tile cluster density, pass-filter clusters, phasing and pre-phasing data. Consult the `tileMetrics` help page for more information.

```
> head(tileMetrics(fc), n=4)
```

	lane	tile	code	value
39	1	1101	100	1271891.8
41	1	1102	100	1455525.0

```
43    1 1103  100 995404.6
45    1 1104  100 1463691.8
```

Extraction Metrics

The extraction metrics (`ExtractionMetricsOut.bin`) file contains per-lane/cycle/tile information about per-base FWHM (full width pixel size of clusters at half maximum) and 90th %-ile intensity of signal intensity.

```
> head(extractionMetrics(fc), n=1)
  lane tile cycle  FWHM_A  FWHM_C FWHM_G  FWHM_T int_A int_C int_G int_T
2    1 1101     1 2.235387 2.308783 1.86132 2.174398  180  326  357  400
```

Coda

There is a convenience function (`buildReports`), which partially reconstructs the Illumina reports folder that was previously generated by the Illumina instrument software and which was superseded by SAV and InterOp files.