# Checks for MiSeq SAV

Criteria used to manually assign MiSeq runs as good or bad. Collated using information obtained during discussions with sequencing operators, the MiSeq SOP Illumina provided documentation and Illumina-run webinars(1–3). This will be used to form the basis of a new SOP, incorporating information on the immediate QC checks which should accompany each sequencing run.

* Inspect graphs under the Analysis tab
  + Look at Intensity over cycle plot (in Data By Cycle)
    - Intensities should be relatively high and not drop too much below 200 intensity
  + Look at box plots for cluster density and cluster density passing filter (in Data By Lane)
    - These should be close together and passing filter (green) should be lower than cluster density (blue)
  + Look at Q-score (in QScore Distribution)
    - >=Q30 should be over 85% (80% at the lowest- this indicates caution)
    - Under Q30 should be as flat as possible on the plot
  + Look at Q-score heatmap (in QScore Heatmap)
    - There should be a clear green line at Q30 (or above). Drops in the middle are not of concern and should be ignored
    - There should not be too much (visible) smearing under Q30
* Go to Summary tab
  + Cluster density should be within a sensible range (v2 should be ~1000, v3 should be ~1500)
  + Phasing/prephasing should be no greater than 0.5
* Go to Indexing tab (if available)
  + NTC should be close to 0
  + Samples should be roughly of equal proportions and not close to 0 (which indicates a lack of data from that sample)

1. All Wales Medical Genetics Service. MiSeq SOP. 2014.

2. Illumina Technical Assistance. Sequencing Analysis Viewer v1.10 Software Guide. (October 2015).

3. Illumina Inc. Sequencing Analysis Viewer (SAV) [Internet]. 2016 [cited 2016 Feb 17]. Available from: https://support.illumina.com/content/illumina-support/us/en/sequencing/sequencing\_software/sequencing\_analysis\_viewer\_sav.html