

Use this as a guide to understand and interpret the batch summary reports that are generated for the following Sanger sequencing services:

- Capillary Separation (CS)
- Purified DNA (PD)
- Unpurified PCR Product (PD+)

## CONTENTS

<b>1</b>	<b>About Batch Summary Reports.....</b>	<b>1</b>
<b>2</b>	<b>Interpreting Batch Summary Reports .....</b>	<b>2</b>
2.1	Kb Q20 scores:.....	2
2.2	Signal intensity: .....	2
<b>3</b>	<b>Troubleshooting with the Batch Summary Report: .....</b>	<b>2</b>
3.1	The signal intensity is too high:.....	2
3.2	The signal intensity is too low: .....	2
3.3	The signal seems to be good, but the Q20 score is shorter than expected: .....	2

## 1 About Batch Summary Reports

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AGRF provides a Quality Control (QC) report for each batch of samples submitted for service sequencing. This allows you to view the quality scores of your sequencing results in a single file, and evaluate the overall performance of your submission easily.

Example of a Batch Summary Report table:

### AGRF Sanger Service: Batch Summary

Service:	AGRF Sanger Service
Batch:	TeamService323
Report Date:	16-Oct-2015

Sample Name	Q20 Bases	Intensity
KW1_G10	592	80
E65_G11	225	71

**Batch:** the unique identifier for the submission

**Report Date:** the date that the sequencing data was analysed

**Sample name:** the name of the sample specified by the submitter

**Q20 Bases:** the number of bases in the sample sequence that give a Q20 score (>99% confidence) for each base call.

**Intensity:** The average raw signal intensity of the sample (rounded to the nearest integer)

## 2 Interpreting Batch Summary Reports

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### 2.1 Kb Q20 scores:

The ideal Q20 score is dependent on the DNA sample that is being sequenced. For plasmids, or large PCR amplicons (>1000bp), a higher Q20 score is desirable. For PCR amplicons, a Q20 score larger than 85% of the total fragment length is good (a 100% Q20 score is not possible, due to the primer extension happening ~20-30bp into the amplicon).

### 2.2 Signal intensity:

This is the average signal of all four bases (A, G, C & T) across the length of the read. The ideal range for sequencing is between 2000-3000rfu (relative fluorescence unit), although valid signal intensities can be anywhere between 700-6000rfu. Sequences with signal intensities above 6000rfu may be too bright for the CCD sensors, resulting in “overloads” and high background (signal pull-ups). A signal intensity below 700rfu may be difficult to read, and can result in background signals interfering with the base calls. Data from sequences that have low signal intensities may not be as reliable as those with average signals above 1000rfu.

## 3 Troubleshooting with the Batch Summary Report:

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### 3.1 The signal intensity is too high:

Reduce the amount of DNA template that you are using for your submission (initially, try half the amount). Keep the primer concentration the same (0.8pmol/μL).

### 3.2 The signal intensity is too low:

The sequencing reaction is not performing optimally. Confirm that the T<sub>m</sub> of the primer and the concentration of the template/primer mix match the AGRF user guide. Also, try submitting more DNA template.

### 3.3 The signal seems to be good, but the Q20 score is shorter than expected:

This suggests that there are problems with the base calling. This is usually seen when there are multiple templates in the reaction (i.e. two different plasmids, or multiple PCR products). For PCR products, it may indicate the presence of a deletion mutation (indel) in the sample, or allelic variation. For DNA with regions of mononucleotide repeat, it may be the result of polymerase slippage during 5'-3' extension.

For further assistance with troubleshooting, refer to our user guide or contact the Sanger sequencing team at [sequencing@agrif.org.au](mailto:sequencing@agrif.org.au)