The tools give us statistics or graphs:

	Good	**Bad**
Depth	≥ 30×	< 10×
Genome covered	≥ 90%	< 80%
GC bias	Flat graph	Roller coaster graph
Contamination	< 5%	> 10%
MAPQ	mostly ≥ 30	< 20
% mapped reads	> 90%	< 70%

These values are just general guidelines, not fixed rules. Different research projects may use different cut-offs based on what they are trying to study. For example, clinical studies (hospital/medical use) need very strict quality, while research or student projects can allow a bit lower quality. In the end, the acceptable quality depends on what the experiment is meant to do.

Parameters

1. Basic statistics - never raises warning
2. Per Base Sequence Quality-

- for good sequence
    - lower quartile for any base is > 10
    - median for any base is > 25.
- For bad sequence
    - the lower quartile for any base < 5
    - median for any base < 20.

3. Per Sequence Quality Scores
    - For good sequences most frequently observed mean quality > 27
    - For bad one it < 20
4. Per Base Sequence Content
    - Issues warning if the difference between A and T, or G and C is greater than 10% in any position.
    - fail if the difference between A and T, or G and C is greater than 20% in any position.

5.Per Base GC Content

- For good sequences GC content of any base should be < 5% from the mean GC content.
- Fail if the GC content of any base strays more than 10% from the mean GC content

6.Per Sequence GC Content

- warning is raised if the sum of the deviations from the normal distribution represents more than 15% of the reads.
- failure if the sum of the deviations from the normal distribution represents more than 30% of the reads.

7.Per Base N Content

- warning on N content of >5%
- Fail on N content of >20%

8.Sequence Length Distribution

- warning if all sequences are not the same length.
- error if any of the sequences have zero length.

9.Duplicate Sequences

- Non-unique (duplicate) sequences < 20% of total= good
- Non-unique sequences > 50%=bad

10.Overrepresented Sequences

- No individual sequence > 0.1% of total reads=good
- Any sequence represents > 1% of total reads=bad

11.Overrepresented Kmers

- All k-mers show ≤ 3-fold enrichment overall for good sequences
- Any k-mer enriched > 10-fold at any single base position for bad sequences