Table 1 Quality Metrics (Adopted from Roy et al)

Category Use Quality metric Performance Criteria \* Used for

Sample REQ DNA concentration Min, Max All sample types

Sample REQ DNA fragment size Min, Max All sample types

Sample REQ Library DNA quantification Min All sample types

Run metrics REQ Cluster density Min, Max All sample types on Illumina platforms that include this metric by default

Run metrics REQ % of bases higher than the

minimum Phred score of all bases called

Run metrics REQ Demultiplexing success (ie, all

molecular identifiers present and no unexpected molecular identifiers detected)

Run metrics REQ % of reads passing a minimum Phred

score criterion (eg, 99% of bases at Q30 or higher)

Min All sample types

Pass/fail All sample types when multiplexing is used

Min All sample types

Read ﬁlters REQ Mapping quality Min All sample types

Mapping*y* REQ Mean on-target coverage of reads Min All sample types

Mapping*y* REQ % of targeted bases with coverage

greater than a specified minimum

Mapping*y* REQ % of bases exceeding the minimum

Phred score mapped on target

Mapping*y* OHR % of aligned bases exceeding the

minimum Phred score that disagree with reference

Min All sample types

Min All sample types

Max Samples for germline analysis only

Mapping*y* OHR AT/GC bias Max All sample types

Mapping*y* REQ Mean insert size (bp) Min, Max All sample types for hybrid capture methods only

Mapping*y* REQ % PCR duplicates Max All sample types using none amplicon-based sequencing

Per variant REQ Depth of coverage at variant’s

position

Min All sample types

Per variant REQ Quality score Min All sample types

Per variant Opt Number of germline SNVs Min, Max (may have to

have separate criteria for different ethnicities)

All sample types

Per variant REQ Allele fraction Min All sample types

Per variant REQ Strand bias Max All sample types

Per variant Opt Haplotype bias Max All sample types

Per variant REQ Number of distinct vertical variants

at the same position

Per variant REQ Number of distinct horizontal

variants within a prescribed cluster window size (bp)

QC*y* OHR Estimate of % contamination from another sample

*S*2 All sample types

*S*1 All sample types

Max Samples for germline analysis only (optional for tumor samples)

QC*y* Opt Fingerprint genotypes match NGS results

Yes (no requires investigation/ explanation)

All sample types

QC*y* REQ Observed sex matches reported sex Yes (no requires investigation/

explanation)

All sample types if X/Y chromosomes are included in assay

QC*y* Opt % of bases called that are variants Min, Max Samples for germline analysis only (optional for tumor samples)

QC*y* Opt SNP/indel ratio Min, Max All sample types

QC*y* Opt Ti/Tv ratio Min, Max All sample types

QC*y* Opt Ratio of heterozygous/homozygous variants Min, Max Samples for germline testing only

QC*y* Opt Coverage profile compared with controls Goodness-of-fit test Critical for copy number analysis but also useful for assay QC

\*Each quality metric should have performance criteria (thresholds) established to determine the validity and accuracy of the assay. The criteria indicated in this column are required to be established and used when the metric itself is required for the sample type indicated.

*y*Evaluation of the quality metric per sample is needed.

Indel, insertion/deletion; Max, maximum threshold (value above which the sample or metric is considered unacceptable or failed); Min, minimum threshold (value below which the sample or metric is considered unacceptable or failed); NGS, next-generation sequencing; OHR, optional but highly recommended; Opt, optional; Q30, Phred (Quality) Score Z 30; QC, quality control; REQ, required for the sample types indicated; SNV, single-nucleotide variant; Ti/Tv, number of transitions/number of transversions.