

### Instructions:

Each column that has a red triangle in the upper-right corner has a co

Some column headers have hyperlinks to NCBI webpages.

The YELLOW columns have drop-down menus that allow you to sele

**You must save spreadsheet under second tab (SRA\_data) as a T**

If you created samples previously, provide accessions in the form of **SAMN#** in the column *sample\_accession*. Otherwise provide the sample name used in the BioSample attributes spreadsheet.

Each row in the template represents a sequencing library with a unique combination of *sample + library + sequencing strategy + layout + instrument model*. Each row should have a **unique library\_id** that is short and meaningful (like an ID you might use in lab).

When libraries are indeed identical (same combination of *sample + library + strategy + layout + instrument model*), all files should be placed in the same row. To do this simply enter the file names consecutively in the same row by adding more columns with headers filename2, filename3, etc.... PAIRED files must always be listed in the same row.

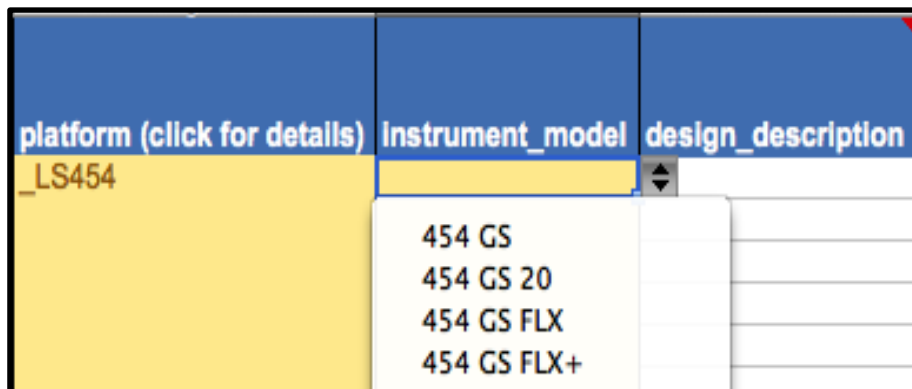
Provide **exact file names** (including extensions) in the *filename* columns.

File names must be **unique**.

red triangles indicate pop-up comments for that field

Many of the columns also have data checks - if you received a warnin

**NOTE:** There are data checks and autocomplete features in this sprea manually consult the platform and instrument information on the last



|  |                     |  |
|--|---------------------|--|
|  | 454 GS FLX Titanium |  |
|  | 454 GS Junior       |  |
|  | unspecified         |  |
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Example Drop Down Menu

Helpful Hyperlinks:

SRA submission overview:

SRA submission in Submission Portal:

You must save spreadsheet under second tab (SRA\_data) as a 1

comment that can be displayed if you hover over the header.

ect from a controlled vocabulary. Once specified for one row, these values can be copied-and-p

**TSV (tab-delimited file) to upload the TSV file for the SRA metadata tab.**

|                             |                           |                      |
|-----------------------------|---------------------------|----------------------|
| required for ALL data types | required for aligned data | paired-end data only |
|-----------------------------|---------------------------|----------------------|

g, please verify that you have entered a value from the drop-down menu. Example Below.

dsheet that are not compatible with Libre- and Open-Office. If you use one of these suites, please  
page.

<http://www.ncbi.nlm.nih.gov/sra/docs/submit/>  
<http://www.ncbi.nlm.nih.gov/sra/docs/submitportal/>

**TSV (tab-delimited file) to upload the TSV file for the SRA metadata tab.**

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ise



| sample_name  | library_ID      | title                  | library_strategy     |
|--------------|-----------------|------------------------|----------------------|
| biosample_id | nwss_sample_idX | Targeted Amplification | on sequencing of was |

| <u>library_source</u> | <u>library_selection</u> | <u>library_layout</u> |
|-----------------------|--------------------------|-----------------------|
| stewater sample       |                          |                       |

| platform | instrument_model  | design_description                    | filetype |
|----------|-------------------|---------------------------------------|----------|
| ILLUMINA | Illumina iSeq 100 | methods included in custom attributes |          |

filename

filename2 filename3 filename4

|                     |                |                            |
|---------------------|----------------|----------------------------|
| assembly fasta_file | enrichment_kit | amplicon_PCR_primer_scheme |
|---------------------|----------------|----------------------------|

|                         |               |                        |
|-------------------------|---------------|------------------------|
| library_preparation_kit | amplicon_size | quality_control_method |
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| quality_control_method_version | quality_control_determination | quality_control_issues |
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| quality_control_details | dehosting_method | sequence_submitter_contact_email |
|-------------------------|------------------|----------------------------------|

**raw\_sequence\_data\_processing\_method**

| Column Name                      | Required?                   | Comments                                                                                                                                                                                 |
|----------------------------------|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| sample_name                      | required for ALL data types | Targeted Amplification sequence                                                                                                                                                          |
| library_ID                       | required for ALL data types |                                                                                                                                                                                          |
| title                            | required for ALL data types |                                                                                                                                                                                          |
| <u>library_strategy</u>          | required for ALL data types |                                                                                                                                                                                          |
| <u>library_source</u>            | required for ALL data types |                                                                                                                                                                                          |
| <u>library_selection</u>         | required for ALL data types |                                                                                                                                                                                          |
| library_layout                   | required for ALL data types |                                                                                                                                                                                          |
| <u>platform</u>                  | required for ALL data types |                                                                                                                                                                                          |
| instrument_model                 | required for ALL data types |                                                                                                                                                                                          |
| design_description               | required for ALL data types |                                                                                                                                                                                          |
| filetype                         | required for ALL data types |                                                                                                                                                                                          |
| filename                         | required for ALL data types |                                                                                                                                                                                          |
| filename2                        |                             |                                                                                                                                                                                          |
| filename3                        |                             |                                                                                                                                                                                          |
| filename4                        |                             |                                                                                                                                                                                          |
| assembly                         |                             |                                                                                                                                                                                          |
| fasta_file                       |                             |                                                                                                                                                                                          |
| enrichment_kit                   | required for ALL data types |                                                                                                                                                                                          |
| amplicon_PCR_primer_scheme       | required for ALL data types |                                                                                                                                                                                          |
| library_preparation_kit          | required for ALL data types |                                                                                                                                                                                          |
| amplicon_size                    |                             | <p>Would it work if both method a</p> <p>Is version required?</p> <p>Can be intentionally left blank</p> <p>Can be left blank if no issues po</p> <p>Any additional details one wish</p> |
| quality_control_method           |                             |                                                                                                                                                                                          |
| quality_control_method_version   |                             |                                                                                                                                                                                          |
| quality_control_determination    |                             |                                                                                                                                                                                          |
| quality_control_issues           |                             |                                                                                                                                                                                          |
| quality_control_details          |                             |                                                                                                                                                                                          |
| dehosting_method                 | required for ALL data types |                                                                                                                                                                                          |
| sequence_submitter_contact_email |                             |                                                                                                                                                                                          |
| raw_sequence_data_processing_met | required for ALL data types |                                                                                                                                                                                          |

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| <u>library_selection</u>            |  |
| library_layout                      |  |
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| instrument_model                    |  |
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| enrichment_kit                      |  |
| amplicon_PCR_primer_scheme          |  |
| library_preparation_kit             |  |
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| quality_control_method              |  |
| quality_control_method_version      |  |
| quality_control_determination       |  |
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## fasta\_file

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library\_preparation\_kit  
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filename4

assembly

fasta\_file

enrichment\_kit

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## fasta\_file

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sed for amplification  
or amplicon generation  
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nd version were in 1 cell?

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es to include about QC goes here  
nan-read scrubber. Note if other methods are used instead

ssing"

## Strategy

|                                         |                                                                                                                             |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| WGA                                     | Random sequencing of the whole genome following non-pcr amplification                                                       |
| WGS                                     | Random sequencing of the whole genome                                                                                       |
| WXS                                     | genome                                                                                                                      |
| RNA-Seq                                 | Random sequencing of whole transcriptome                                                                                    |
| miRNA-Seq                               | Random sequencing of small miRNAs                                                                                           |
| WCS                                     | Random sequencing of a whole chromosome or other replicon isolated from a genome                                            |
| CLONE                                   | Genomic clone based (hierarchical) sequencing                                                                               |
| POOLCLONE                               | Shotgun of pooled clones (usually BACs and Fosmids)                                                                         |
| AMPLICON                                | Sequencing of overlapping or distinct PCR or RT-PCR products                                                                |
| CLONEEND                                | Clone end (5', 3', or both) sequencing                                                                                      |
| FINISHING                               | Sequencing intended to finish (close) gaps in existing coverage                                                             |
| ChIP-Seq                                | Direct sequencing of chromatin immunoprecipitates                                                                           |
| MNase-Seq                               | Direct sequencing following MNase digestion                                                                                 |
| DNase-Hypersensitivity                  | Sequencing of hypersensitive sites, or segments of open chromatin that are more readily cleaved by DNaseI                   |
| Bisulfite-Seq                           | Sequencing following treatment of DNA with bisulfite to convert cytosine residues to uracil depending on methylation status |
| Tn-Seq                                  | Sequencing from transposon insertion sites                                                                                  |
| EST                                     | Single pass sequencing of cDNA templates                                                                                    |
| FL-cDNA                                 | Full-length sequencing of cDNA templates                                                                                    |
| CTS                                     | Concatenated Tag Sequencing                                                                                                 |
| MRE-Seq                                 | Methylation-Sensitive Restriction Enzyme Sequencing strategy                                                                |
| MeDIP-Seq                               | Methylated DNA Immunoprecipitation Sequencing strategy                                                                      |
| MBD-Seq                                 | Direct sequencing of methylated fractions sequencing strategy                                                               |
| Synthetic-Long-Read                     |                                                                                                                             |
| ATAC-seq                                | Assay for Transposase-Accessible Chromatin (ATAC) strategy is                                                               |
| ChIA-PET                                | Direct sequencing of proximity-ligated chromatin immunoprec                                                                 |
| FAIRE-seq                               | Formaldehyde Assisted Isolation of Regulatory Elements. reveal                                                              |
| Hi-C                                    | Chromosome Conformation Capture technique where a biotin-                                                                   |
| ncRNA-Seq                               | Capture of other non-coding RNA types, including post-translat                                                              |
| RAD-Seq                                 |                                                                                                                             |
| RIP-Seq                                 | Direct sequencing of RNA immunoprecipitates (includes CLIP-S                                                                |
| SELEX                                   | Systematic Evolution of Ligands by EXponential enrichment                                                                   |
| ssRNA-seq                               | strand-specific RNA sequencing                                                                                              |
| Targeted-Capture                        |                                                                                                                             |
| Tethered Chromatin Conformation Capture |                                                                                                                             |

|       |                                                                                          |
|-------|------------------------------------------------------------------------------------------|
| OTHER | Library strategy not listed (please include additional info in the "design description") |
|-------|------------------------------------------------------------------------------------------|

## Source

|                            |                                                                                                                     |
|----------------------------|---------------------------------------------------------------------------------------------------------------------|
| GENOMIC                    | Genomic DNA (includes PCR products from genomic DNA)                                                                |
| TRANSCRIPTOMIC             | Transcription products or non genomic DNA (EST, cDNA, RT-PCR, screened libraries)                                   |
| METAGENOMIC                | Mixed material from metagenome                                                                                      |
| METATranscriptomic         | Transcription products from community targets                                                                       |
| SYNTHETIC                  | Synthetic DNA                                                                                                       |
| VIRAL RNA                  | Viral RNA                                                                                                           |
| GENOMIC SINGLE CELL        |                                                                                                                     |
| TRANSCRIPTOMIC SINGLE CELL |                                                                                                                     |
| OTHER                      | Other, unspecified, or unknown library source material (please include additional info in the "design description") |

## Selection

|                                        |                                                                                                                                                                                                                 |
|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| RANDOM                                 | Random selection by shearing or other method                                                                                                                                                                    |
| PCR                                    | Source material was selected by designed primers                                                                                                                                                                |
| RANDOM PCR                             | Source material was selected by randomly generated primers                                                                                                                                                      |
| RT-PCR                                 | Source material was selected by reverse transcription PCR                                                                                                                                                       |
| HMPR                                   | Hypo-methylated partial restriction digest                                                                                                                                                                      |
| MF                                     | Methyl Filtrated                                                                                                                                                                                                |
| CF-S                                   | Cot-filtered single/low-copy genomic DNA                                                                                                                                                                        |
| CF-M                                   | Cot-filtered moderately repetitive genomic DNA                                                                                                                                                                  |
| CF-H                                   | Cot-filtered highly repetitive genomic DNA                                                                                                                                                                      |
| CF-T                                   | Cot-filtered theoretical single-copy genomic DNA                                                                                                                                                                |
| MDA                                    | Multiple displacement amplification                                                                                                                                                                             |
| MSLL                                   | Methylation Spanning Linking Library                                                                                                                                                                            |
| cDNA                                   | complementary DNA                                                                                                                                                                                               |
| ChIP                                   | Chromatin immunoprecipitation                                                                                                                                                                                   |
| MNase                                  | Micrococcal Nuclease (MNase) digestion                                                                                                                                                                          |
| DNase                                  | Deoxyribonuclease (MNase) digestion                                                                                                                                                                             |
| Hybrid Selection                       | Selection by hybridization in array or solution<br>Reproducible genomic subsets, often generated by restriction<br>fragment size selection, containing a manageable number of<br>loci to facilitate re-sampling |
| Reduced Representation                 |                                                                                                                                                                                                                 |
| Restriction Digest                     | DNA fractionation using restriction enzymes                                                                                                                                                                     |
| 5-methylcytidine antibody              | Selection of methylated DNA fragments using an antibody<br>raised against 5-methylcytosine or 5-methylcytidine (m5C)                                                                                            |
| MBD2 protein methyl-CpG binding domain | Enrichment by methyl-CpG binding domain                                                                                                                                                                         |
| CAGE                                   | Cap-analysis gene expression                                                                                                                                                                                    |
| RACE                                   | Rapid Amplification of cDNA Ends                                                                                                                                                                                |
| size fractionation                     | Physical selection of size appropriate targets                                                                                                                                                                  |
| Padlock probes capture method          | Circularized oligonucleotide probes                                                                                                                                                                             |

|                            |                                                                                                                                 |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------|
| other                      | Other library enrichment, screening, or selection process (please include additional info in the "design description")          |
| unspecified                | Library enrichment, screening, or selection is not specified (please include additional info in the "design description")       |
| cDNA_oligo_dT              |                                                                                                                                 |
| cDNA_randomPriming         |                                                                                                                                 |
| Inverse rRNA               | depletion of ribosomal RNA by oligo hybridization.                                                                              |
| Oligo-dT                   | enrichment of messenger RNA (mRNA) by hybridization to Olig                                                                     |
| PolyA repeat fractionation | PolyA selection or enrichment for messenger RNA (mRNA); sho<br>Selection for less repetitive (and more gene rich) sequence thrc |

### Platforms

|                   |                     |                              |
|-------------------|---------------------|------------------------------|
| _LS454            | <b>_LS454</b>       | <b>ILLUMINA</b>              |
| ABI_SOLID         | 454 GS              | HiSeq X Five                 |
| BGISEQ            | 454 GS 20           | HiSeq X Ten                  |
| CAPILLARY         | 454 GS FLX          | Illumina Genome Analyzer     |
| COMPLETE_GENOMICS | 454 GS FLX+         | Illumina Genome Analyzer II  |
| HELICOS           | 454 GS FLX Titanium | Illumina Genome Analyzer IIX |
| ILLUMINA          | 454 GS Junior       | Illumina HiScanSQ            |
| ION_TORRENT       |                     | Illumina HiSeq 1000          |
| OXFORD_NANOPORE   |                     | Illumina HiSeq 1500          |
| PACBIO_SMRT       |                     | Illumina HiSeq 2000          |
|                   |                     | Illumina HiSeq 2500          |
|                   |                     | Illumina HiSeq 3000          |
|                   |                     | Illumina HiSeq 4000          |
|                   |                     | Illumina iSeq 100            |
|                   |                     | Illumina NovaSeq 6000        |
|                   |                     | Illumina MiniSeq             |
|                   |                     | Illumina MiSeq               |
|                   |                     | NextSeq 500                  |
|                   |                     | NextSeq 550                  |
|                   |                     | NextSeq 1000                 |
|                   |                     | NextSeq 2000                 |
|                   |                     | <br>Illumina HiSeq X         |

used to study genome-wide chromatin accessibility. alternative method to DNase-seq that uses formaldehyde crosslinking and immunoprecipitation.

Identifies regions of open chromatin

A labeled nucleotide is incorporated at the ligation junction, enabling selective purification of chromatin regions associated with specific RNA types such as snRNA (small nuclear RNA) or snoRNA (small nucleolar RNA), or

or other RNA types (e.g., HITS-CLIP and PAR-CLIP).

o-dT.

uld replace cDNA enumeration.

ough Cot filtration (CF) or other fractionation techniques based on DNA kinetics.

#### **HELICOS**

Helicos HeliScope

#### **ABI\_SOLID**

AB 5500 Genetic Analyzer

AB 5500xl Genetic Analyzer

AB 5500x-WI Genetic Analyzer

AB SOLiD 3 Plus System

AB SOLiD 4 System

AB SOLiD 4hq System

AB SOLiD PI System

AB SOLiD System

AB SOLiD System 2.0

AB SOLiD System 3.0

#### **COMPLETE\_GENOMICS**

Complete Genomics

#### **PACBIO\_SMRT**

PacBio RS

PacBio RS II

PacBio Sequel

PacBio Sequel II

s an engineered Tn5 transposase to cleave DNA and to integrate primer DNA sequences into the cle

meric DNA ligation junctions followed by deep sequencing

expression regulation types such as siRNA (small interfering RNA) or piRNA/piwi/RNA (piwi-interacti

**ION\_TORRENT**

Ion Torrent PGM  
Ion Torrent Proton  
Ion Torrent S5 XL  
Ion Torrent S5

**CAPILLARY**

AB 310 Genetic Analyzer  
AB 3130 Genetic Analyzer  
AB 3130xL Genetic Analyzer  
AB 3500 Genetic Analyzer  
AB 3500xL Genetic Analyzer  
AB 3730 Genetic Analyzer  
AB 3730xL Genetic Analyzer

**OXFORD\_NANOPORE**

GridION  
MinION  
PromethION

**BGISEQ**

BGISEQ-500  
DNBSEQ-G400  
DNBSEQ-T7  
DNBSEQ-G50  
MGISEQ-2000RS

aved genomic DNA

ng RNA).

**enrichment\_kit**

### **amplicon\_PCR\_primer\_scheme**

ARTIC v5.4.2

ARTIC v5.3.2

ARTIC v5.3

ARTIC v5.2.0

ARTIC v5.1.0

ARTIC v5.0.0

ARTIC v4.1

ARTIC v4

ARTIC v3

ARTIC v2

ARTIC v1

NEB VarSkip Short / VarSkip Short v1a

NEB VarSkip Short v2 / VarSkip v2a

NEB VarSkip Short v2b

NEB VarSkip / VarSkip Long / VarSkip Long 1a

Midnight

Midnight v2

Midnight v3

AmpliSeq SARS-CoV-2 Research Panel (Illumina)

ARTIC SARS-CoV-2 Amplicon Panel (IDT)

CleanPlex SARS-CoV-2 FLEX Kit (Paragon Genomics)

CleanPlex SARS-CoV-2 Kit (Paragon Genomics)

CleanPlex SARS-CoV-2 Emerging Variants Panel Add-on v2 (Paragon Genom

CleanPlex Respiratory Virus Research Panel v2 (Paragon Genomics)

Clear Dx FlexPro: Wastewater (SARS-CoV-2) (Clear Labs)

Clear Dx SARS-CoV-2 WGS v2.0 (Clear Labs)

Clear Dx SARS-CoV-2 WGS v3.0 (Clear Labs)

COVID Mini v14: 576 samples (ONT)

COVIDSeq Assay (96 samples) (Illumina)

COVIDSeq Test (RUO Version) (Illumina)

COVIDSeq v3 Primer Pool (Illumina)  
COVIDSeq v4 Primer Pool (Illumina)  
EasySeq SARS-CoV-2 WGS Library Prep Kit v4 (NimaGen)  
EasySeq SARS-CoV-2 WGS Library Prep Kit v5 (NimaGen)  
HIFIVIRAL SARS-COV-2 (PacBio)  
Ion AmpliSeq SARS-CoV-2 sequencing (Thermo Fisher)  
Midnight RT PCR Expansion (ONT)  
NEBNext ARTIC SARS-CoV-2 Companion Kit - ARTIC v3 (NEB)  
NEBNext ARTIC SARS-CoV-2 Companion Kit - VarSkip Short v2 (NEB)  
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit - ARTIC v3 (NEB)  
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit - VarSkip Short v2 (NEB)  
NEBNext ARTIC SARS-CoV-2 Library Prep Kit - ARTIC v3 (NEB)  
NEBNext ARTIC SARS-CoV-2 RT-PCR Module - ARTIC v3 (NEB)  
NEBNext ARTIC SARS-CoV-2 RT-PCR Module - VarSkip Short v2 (NEB)  
QIAseq DIRECT SARS-CoV-2 (Qiagen)  
QIAseq DIRECT SARS-CoV-2 Boosted (Qiagen)  
SNAP - Swift Normalase Amplicon SARS-COV-2 Additional Genome Coverage  
SNAP - Swift Normalase Amplicon SARS-COV-2 Panel (Swift Biosciences)  
SNAP - Swift Normalase Amplicon SARS-COV-2 S Gene Panel (Swift Biosciences)  
xGen SARS-CoV-2 Amplicon Panel (IDT)  
xGen SARS-CoV-2 Midnight Amplicon Panel (IDT)  
xGen SARS-CoV-2 S-gene Amplicon Panel (IDT)

**amplicon\_PCR\_primer\_scheme\_no\_longer\_included**

Cotten  
Eden  
Gonzalez-Reiche  
Itokawa  
Resende  
SARS-CoV-2 SNAP primer pool

UNZA Sanger  
Midnight 1200/Freed primers

**library\_preparation\_kit**

Covidmini kit  
Illumina DNA prep  
Illumina Nextera XT  
Illumina TrueSeq  
Illumina RNA Prep with Enrichment, (L) Tagmentation  
Illumina COVIDSeq Assay  
Ligation sequencing kit  
Midnight expansion kit  
NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)  
NEBNext Ultra II FS DNA Library Prep for Illumina  
QIAseq DIRECT Unique Dual Index prep  
Rapid barcoding kit

**dehosting\_method**

ncov-dehoster  
Nanostripper 0.1alpha  
SRA human read removal tool

ics)

Panel (Swift Biosciences)

es)

**quality\_control\_method\_name**

GalaxyTrakr SSQuAWK  
CFSAN Wastewater Analysis Pipeline (C-WAP)

**quality\_control\_method\_version**

**quality\_control\_determination**

no quality control issues identified  
minor quality control issues identified  
sequence flagged for potential quality control issues  
not performed

**quality\_control\_issues**

low quality sequence  
sequenced contaminated  
low average genome coverage  
low % genome captured  
read lengths shorter than expected  
sequence amplification artifacts  
low signal to noise ratio  
low coverage of characteristic mutatio

**quality\_control\_details**

ons

## **Update from v5.7 to v5.8**

**Date Updated:** 2025-04-21

1. The **"enrichment\_kit"** field is now a free-text field, allowing users to provide the name of the
2. The **"amplicon\_PCR\_primer\_scheme"** field has been updated: outdated primer scheme optio

PCR kit used for amplification instead of selecting from a predefined drop-down list.

ns were removed, and the list was expanded to include primers and kits used for amplicon gene

eration.