Engineered Cells QC Report User Guide

Thank you for choosing EditCo Engineered Cells for your CRISPR experiment!

This QC Report User Guide can be used for iPSC, Immortalized and Primary engineered cell products, including cell pools, clones, knockout, and knock-in orders. In this document, we include information on how to interpret the information included in your QC document.

All QC documents include the following information:

- Product type, Order number, Cell line, Gene, Modification and Document Date
- Table containing the deliverables (**see further explanation below**). **iPSC only**: Pluripotency and Karyotyping are only added to the deliverables table when these additional services are requested. For karyotyping, in addition to the information on the table, a pdf with the karyotyping results is provided. For pluripotency, no additional data to the one included in the deliverables table is provided.
- Table containing the edit Information: Gene name, Transcript ID, Guide RNA sequences used, Guide RNA Cut location, Exon targeted (knockouts only), NGS primers (no adapters), Donor Sequence (knock-ins only)
- Table containing NGS sequencing information (**see further explanation below**)
- Figure showing the Indel percentage distribution
- Figure showing the sequence alignment (**see further explanation below**) except for large knock-ins, large fragment deletions, and non-human/mouse cell types. In this case an alignment between the Sanger sequencing data of the edited clone or a pool and the reference will be provided separately.

Engineered Cell Pools QC Reports

Notes:

- You will receive 2 vials of wild type controls per cell line in the order, regardless of the number of edits per cell type. If your order contains multiple edits and ships at different times, the wild type control information will be included in the QC report for the edited line that it has been shipped with.
- Edited cells correspond to parental cells electroporated with SpCas9 and target-specific sgRNA. All wild type cells (pools or clones) correspond to parental cells electroporated with SpCas9 only and confirmed to be unedited at target locus.
- If the knockout or knock-in editing causes the cell growth dynamics to shift, the cell population may change over time.
- For knock-ins, an additional silent/blocking mutation might be introduced to prevent gRNA-nuclease complex from re-editing the target site after successful knock-in of the desired inserts. Please refer to this help center article for more information.

Knockout Cell Pool QC Report

Deliverables Table

Below is the deliverables **sample** analysis table explaining each assay (by row number). Note that the assay results for your knockout cell pool are in the QC report provided with your order and not depicted in the table below.

	Sample	Wild Type Pool	Cell Pool
1	**Quantity of Vials**	2	2
2	**Quantity of Viable Cells**	1 x10^6	1 x10^6
3	**Pre-Cryopreservation Cell Viability (≥80%)**	Pass	Pass
4	**Genotype**	Mock transfected cell pool	SNRNP200 Knockout
5	**Passage**	4	5
6	**Mycoplasma Test**	Negative	Negative
7	**Pluripotency**	n/a	Pass

1. Total number of vials.
2. Approximate total number of viable cells in each vial. The viable cell number is estimated at the start of cryopreservation. An aliquot of cells in cryopreservation medium is taken for a total and dead cell count. The remaining cells are divided equally between the cryovials. Total cell number may vary between pools depending on each pool's expansion rate.
3. Approximate percentage of cells that are viable at the start of cryopreservation. At the time of cryopreservation, the vial/tube contains a minimum of 80% viable cells.
4. The desired edit. Mock transfected samples are wild type cells which underwent electroporation with SpCas9 only and in the absence of synthetic single-guide RNA.
5. The total number of passages that the wild type and edited cell line have undergone at EditCo.
6. The results of mycoplasma testing prior to shipment.
7. **iPSC only**: Pluripotency result. **Note**: This is an additional service and not a standard deliverable

Sequencing Information Table

Below is the sequencing information **sample** analysis table explaining each sequencing metric (by row number). Note that the sequencing results for your knockout cell pool are in the QC report provided with your order and not depicted in the table below.

1	**Sample**	Cell Pool
2	**Gene Name**	SNRNP200
3	**Number of Reads used for Genotyping**	7000
4	**Q30**	85
5	**% Wild Type**	0
6	**% Editing**	100
7	**% KO Score**	100

1. Sample name
2. Gene name
3. The number of NGS reads which have aligned successfully to the internally sequenced wild type and subsequently have been used to determine the population's genotype
4. The percentage of bases with a quality score of 30 or higher. A quality score of 30 is equivalent to the probability of an incorrect base call 1 in 1000 times
5. Proportion of unmodified, unedited sequences in the sample
6. Includes all detected sequences that are different from wild type including all insertions/ deletions (indels) present in the sample. This provides a general indication of the editing efficiency in the sample
7. The proportion of sequences with either a frameshift or 21+ bp indel. This score is a useful measure for those who are interested in understanding how many of the contributing indels are likely to result in a functional Knockout (KO) of the targeted gene

Sequence Alignment Figure

Below is the sequence alignment **sample** figure. Note that the sequence alignment for your knockout cell pools are in the QC report provided with your order and not depicted in the table below.

The top sequence represents the wild type followed by the edited sample below. Please note that the black vertical bars represent the guide(s) cut site. Edits performed with multi-guide typically show large deletions as opposed to individual smaller indels observed with single guide edits. Sequences highlighted in red indicate differences from the wild-type amplicon sequence. EditCo's internal analysis pipeline removes genotype contributions below 5% abundance to ensure results accurately reflect the genotype of the cell population.

See below definitions:

1. Genotype: List the different genetic constitutions in the sample, it can include wild type, deletions, insertions, or substitutions

2. Indel: Insertions or deletions that constitute a specific genotype

3. Mismatches: Number of nucleotide discrepancies between the reference (wild type) and edited aligned sequences. Not counting deletions or insertions.

4. Contribution: Proportion of the specific genotype contributing to the overall genotype of the sample

Knock-in (Small) Cell Pool QC Report

Deliverables Table

Below is the deliverables **sample** analysis table explaining each assay (by row number). Note that the assay results for your knock-in (small) cell pool are in the QC report provided with your order and not depicted in the table below.

	Sample	Wild Type Pool	Cell Pool
1	**Quantity of Vials**	2	2
2	**Quantity of Viable Cells**	1 x10^6	1 x10^6
3	**Pre-Cryopreservation Cell Viability (≥80%)**	Pass	Pass
4	**Genotype**	Mock transfected cell pool	VAPB P56S (CCC>AGC)
5	**Passage**	4	5
6	**Mycoplasma Test**	Negative	Negative
7	**Pluripotency**	n/a	Pass

1. Total number of vials
2. Approximate total number of viable cells in each vial. The viable cell number is estimated at the start of cryopreservation. An aliquot of cells in cryopreservation medium is taken for a total and dead cell count. The remaining cells are divided equally between the cryovials. Total cell number may vary between pools depending on each pool's expansion rate.
3. Approximate percentage of cells that are viable at the start of cryopreservation. At the time of cryopreservation, the vial/tube contains a minimum of 80% viable cells
4. The desired edit. Mock transfected samples are wild type cells which underwent electroporation with SpCas9 only and in the absence of synthetic single-guide RNA
5. The total number of passages that the wild type and edited cell line have undergone at EditCo
6. The results of mycoplasma testing prior to shipment
7. **iPSC only**: Pluripotency result. **Note**: This is an additional service and not a standard deliverable

Sequencing Information Table

Below is the sequencing information **sample** analysis table explaining each sequencing metric (by row number). Note that the sequencing results for your knockout cell pool are in the QC report provided with your order and not depicted in the table below.

1	**Sample**	Cell Pool
2	**Gene Name**	VAPB
3	**Number of Reads used for Genotyping**	7000
4	**Q30**	85
5	**% Wild Type**	67.22
6	**% Editing**	32.78
7	**% KI Score**	32.78

1. Sample name
2. Gene name
3. The number of NGS reads which have aligned successfully to the internally sequenced wild type and subsequently have been used to determine the population's genotype
4. The percentage of bases with a quality score of 30 or higher. A quality score of 30 is equivalent to the probability of an incorrect base call 1 in 1000 times
5. Proportion of unmodified, unedited sequences in the sample
6. Includes all detected sequences that are different from wild type including all insertions/ deletions (indels) present in the sample. This provides a general indication of the editing efficiency in the sample
7. Proportion of sequences with the desired knock-in edit

Sequence Alignment Figure

Below is the sequence alignment **sample** figure. Note that the sequence alignment for your knockout cell pool are in the QC report provided with your order and not depicted in the table below.

The top sequence represents the wild type followed by the edited sample below. Please note that the black vertical bars represent the guide(s) cut site. Sequences highlighted in orange represent the inserted sequences that match the homology-directed repair template and in some instances the silent mutation. Sequences highlighted in red indicate differences from the wild-type amplicon sequence. EditCo's internal analysis pipeline removes genotype contributions below 5% abundance to ensure results accurately reflect the genotype of the cell population.

See below definitions:

1. Genotype: List the different genetic constitutions in the sample, it can include wild type, deletions, insertions, or substitutions.

2. Indel: Insertions or deletions that constitute a specific genotype.

3. Mismatches: Number of nucleotide discrepancies between the reference (wild type) and edited aligned sequences. Not counting deletions or insertions.

4. Contribution: Proportion of the specific genotype contributing to the overall genotype of the sample.

Knock-in (Large) Cell Pool QC Report

Please see the deliverables **sample** analysis table explaining each assay (by row number) in the knock-in (small) cell pool QC report section above (except for the % editing efficiency information in the genotype section). Note that the assay results for your large knock-in cell pool are in the QC report provided with your order and not depicted in the table above.

Note: Due to the size of this insertion, we cannot confirm the desired editing outcome using our NGS analysis for large knock-ins. However, we provide the Sanger sequencing data to confirm the presence of the insert. The alignment of the inserted sequence and the intended sequence is partially included in the QC report. The file containing an alignment between the inserted sequence and the Sanger sequencing data for the junction PCR will be provided separately.

Engineered Cell Clones QC Reports

Notes:

- You will receive 2 vials of wild-type controls per cell line in the order, regardless of the number of edits per cell type. If your order contains multiple edits and ships at different times, the wild type control information will be included in the QC report for the edited line that it has been shipped with.
- Edited cells correspond to parental cells electroporated with SpCas9 and target-specific sgRNA. Wild type cells correspond to parental cells electroporated with SpCas9 only and confirmed to be unedited at target locus. If a clone order contains a wild type clone, this clone is generated using the wild type cells (mock transfected cell pool).
- For clonal populations, the genotype of your edit is stable and will not change over time.
- Knockouts in clonal populations may be homozygous (alleles contain the same frame-shifting indel) or compound heterozygous (alleles contain different frame-shifting indels, depending on the gene's copy number in the cell line, the number of unique indels may vary in a compound heterozygous knockout clone). Despite the difference in indel composition, the knockout phenotype should be the same whether a clone is homozygous or compound heterozygous. The number of unique indels within the population may vary depending on the number of alleles present in a particular cell line (i.e. the ploidy of the cells).
- Knock-in clonal populations may be homozygous (alleles contain the same desired edit), compound heterozygous (alleles contain a combination of the desired edit and indels) or true heterozygous (one allele contains the desired edit and the other allele is wild type). The number of unique indels within the population may vary depending on the number of alleles present in a particular cell line (i.e. the ploidy of the cells).
- For knock-ins, an additional silent/blocking mutation might be introduced to prevent gRNA-nuclease complex from re-editing the target site after successful knock-in of the desired inserts. Please refer to this help center article for more information

Knockout Cell Clones QC Report

Deliverables Table

Below is the deliverables **sample** analysis table explaining each assay (by row number). Note that the assay results for your knockout cell clone are in the QC report provided with your order and not depicted in the table below.

	Sample	Wild Type Pool	Clone B3	Clone B4	Wild Type Clone C3
1	**Quantity of Vials**	2	2	2	2
2	**Quantity of Viable Cells**	0.5 x10^6	0.5 x10^6	0.5 x10^6	0.5 x10^6
3	**% Pre-Cyro Viability (≥80%)**	Pass	Pass	Pass	Pass
4	**Genotype**	Mock transfected cell pool	PKMYT1 Knockout, heterozygous	PKMYT1 Knockout, heterozygous	Clone from the mock transfected cell pool
5	**Passage**	29	33	33	33
6	**Mycoplasma Test**	Negative	Negative	Negative	Negative
7	**Pluripotency**	n/a	Pass	Pass	Pass
8	**Karyotyping**	n/a	Pass	Pass	Pass

* Compound heterozygous KO refers to a genotype where the alleles contain unique indels causing loss-of-function frameshift mutations. Depending on the gene's copy number in the cell line, the number of unique indels may vary in a compound heterozygous KO clone.

1. Total number of vials
2. Approximate total number of viable cells in each vial. The viable cell number is estimated at the start of cryopreservation. An aliquot of cells in cryopreservation medium is taken for a total and dead cell count. The remaining cells are divided equally between the cryovials. Total cell number may vary between pools depending on each pool's expansion rate.
3. Approximate percentage of cells that are viable at the start of cryopreservation. At the time of cryopreservation, the vial/tube contains a minimum of 80% viable cells
4. The desired edit. Mock transfected samples are wild type cells which underwent electroporation with SpCas9 only and in the absence of synthetic single-guide RNA
5. The total number of passages that the wild type and edited cell line have undergone at EditCo
6. The results of mycoplasma testing prior to shipment
7. **iPSC only**: Pluripotency result. **Note**: This is an additional service and not a standard deliverable
8. **iPSC only**: Karyotyping result. **Note**: This is an additional service and not a standard deliverable

Sequencing Information Table

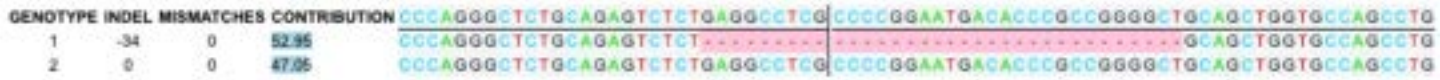
Below is the sequencing information **sample** analysis table explaining each sequencing metric (by row number). Note that the sequencing results for your knockout cell clone are in the QC report provided with your order and not depicted in the table below.

1	**Sample**	Clone B3
2	**Gene Name**	PKMYT1
3	**Number of Reads used for Genotyping**	7000
4	**Q30**	85
5	**% Wild Type**	47.1
6	**% Editing**	53.0
7	**% KO Score**	52.9

1. Sample name
2. Gene name
3. The number of NGS reads which have aligned successfully to the internally sequenced wild type and subsequently have been used to determine the population's genotype
4. The percentage of bases with a quality score of 30 or higher. A quality score of 30 is equivalent to the probability of an incorrect base call 1 in 1000 times
5. Proportion of unmodified, unedited sequences in the sample
6. Includes all detected sequences that are different from wild type including all insertions/ deletions (indels) present in the sample. This provides a general indication of the editing efficiency in the sample
7. The proportion of sequences with either a frameshift or 21+ bp indel. This score is a useful measure for those who are interested in understanding how many of the contributing indels are likely to result in a functional Knockout (KO) of the targeted gene

Sequence Alignment Figure

Below is the sequence alignment **sample** figure. Note that the sequence alignment for your knockout cell clone are in the QC report provided with your order and not depicted in the table below.



The top sequence represents the wild type followed by the edited sample below. Please note that the black vertical bars represent the guide(s) cut site. Edits performed with multi-guide typically show large deletions as opposed to individual smaller indels observed with single guide edits. Sequences highlighted in red indicate differences from the wild-type amplicon sequence. EditCo's internal analysis pipeline removes genotype contributions below 5% abundance to ensure results accurately reflect the genotype of the cell population.

See below definitions:

1. Genotype: List the different genetic constitutions in the sample, it can include wild type, deletions, insertions, or substitutions.

2. Indel: Insertions or deletions that constitute a specific genotype.

3. Mismatches: Number of nucleotide discrepancies between the reference (wild type) and edited aligned sequences. Not counting deletions or insertions.

4. Contribution: Proportion of the specific genotype contributing to the overall genotype of the sample.

Knock-in (Small) Cell Clone QC Report

Deliverables Table

Below is the deliverables **sample** analysis table explaining each assay (by row number). Note that the assay results for your knock-in (small) cell clone are in the QC report provided with your order and not depicted in the table below.

	Sample	Wild Type Pool	Clone B3	Clone B4	Wild Type Clone C3
1	**Quantity of Vials**	2	2	2	2
2	**Quantity of Viable Cells**	0.5 x10^6	0.5 x10^6	0.5 x10^6	0.5 x10^6
3	**% Pre-Cyro Viability (≥80%)**	Pass	Pass	Pass	Pass
4	**Genotype**	Mock transfected cell pool	RING1 R95Q (CGA>-CAA) Homozygous	RING1 R95Q (CGA>-CAA) Homozygous	Clone from the mock transfected cell pool
5	**Passage**	4	16	16	12
6	**Mycoplasma Test**	Negative	Negative	Negative	Negative
7	**Pluripotency**	n/a	Pass	Pass	Pass
8	**Karyotyping**	n/a	Pass	Pass	Pass

1. Total number of vials
2. Approximate total number of viable cells in each vial. The viable cell number is estimated at the start of cryopreservation. An aliquot of cells in cryopreservation medium is taken for a total and dead cell count. The remaining cells are divided equally between the cryovials. Total cell number may vary between pools depending on each pool's expansion rate.
3. Approximate percentage of cells that are viable at the start of cryopreservation. At the time of cryopreservation, the vial/tube contains a minimum of 80% viable cells
4. The desired edit. Mock transfected samples are wild type cells which underwent electroporation with SpCas9 only and in the absence of synthetic single-guide RNA
5. The total number of passages that the wild type and edited cell line have undergone at EditCo
6. The results of mycoplasma testing prior to shipment
7. **iPSC only**: Pluripotency result. **Note**: This is an additional service and not a standard deliverable
8. **iPSC only**: Karyotyping result. **Note**: This is an additional service and not a standard deliverable

Sequencing Information Table

Below is the sequencing information **sample** analysis table explaining each sequencing metric (by row number). Note that the sequencing results for your knockout cell clone are in the QC report provided with your order and not depicted in the table below.

1	**Sample**	Clone B3
2	**Gene Name**	RING1
3	**Number of Reads used for Genotyping**	7000
4	**Q30**	85
5	**% Wild Type**	0
6	**% Editing**	100
7	**% KI Score**	100

1. Sample name
2. Gene name
3. The number of NGS reads which have aligned successfully to the internally sequenced wild type and subsequently have been used to determine the population's genotype
4. The percentage of bases with a quality score of 30 or higher. A quality score of 30 is equivalent to the probability of an incorrect base call 1 in 1000 times
5. Proportion of unmodified, unedited sequences in the sample
6. Includes all detected sequences that are different from wild type including all insertions/ deletions (indels) present in the sample. This provides a general indication of the editing efficiency in the sample
7. Proportion of sequences with the desired knock-in edit

Sequence Alignment Figure

Below is the sequence alignment **sample** figure. Note that the sequence alignment for your knockout cell clone is in the QC report provided with your order and not depicted in the table below.

The top sequence represents the wild type followed by the edited sample below. Please note that the black vertical bars represent the guide(s) cut site. Edits performed with multi-guide typically show large deletions as opposed to individual smaller indels observed with single guide edits. Sequences highlighted in red indicate differences from the wild-type amplicon sequence. EditCo's internal analysis pipeline removes genotype contributions below 5% abundance to ensure results accurately reflect the genotype of the cell population.

See below definitions:

1. Genotype: List the different genetic constitutions in the sample, it can include wild type, deletions, insertions, or substitutions.
2. Indel: Insertions or deletions that constitute a specific genotype.
3. Mismatches: Number of nucleotide discrepancies between the reference (wild type) and edited aligned sequences. Not counting deletions or insertions.
4. Contribution: Proportion of the specific genotype contributing to the overall genotype of the sample.

Knock-in (Large) Cell Clone QC Report

Please see the deliverables sample analysis table explaining each assay (by row number) in the knock-in (small) cell clone QC report section above. Note that the assay results for your large knock-in cell clone are in the QC report provided with your order and not depicted in the table above.

Note: Due to the size of this insertion, we cannot confirm the desired editing outcome using our NGS analysis for large knock-Ins. However, we provide the Sanger sequencing data to confirm the presence of the insert. The alignment of the inserted sequence and the intended sequence is partially included in the QC report. The file containing an alignment between the inserted sequence and the Sanger sequencing data for the junction PCR will be provided separately.

We confirm large homozygous knock-in by the absence of wt/indels in all the alleles. However, if it's a heterozygous clone, we won't be able to distinguish between true heterozygous (other allele is wt) or compound heterozygous (other allele contains indels) since our genotyping method does not allow the distinction between wt or indel in the non-knock-in allele.

Additional Information

For an up-to-date list of all protocols and other resources, please visit this link.

For technical assistance, contact our Scientific Support Team at technicalsupport@editco.bio.

For additional support and resources, please visit this link.