Guidelines for sample preparation and shipping

DNA / RNA and Amplicon samples

Eurofins Genomics

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1. Service workflow and general guidelines for preparation and shipping

1.1. Sample assignment

- The sample assignment must be done in your online account.
- The sample submission form can be downloaded during the acceptance process of your online quote or order.
- Assign your samples to the corresponding barcodes available in your online account.

1.2. Sample shipment

- Please note, that the DropBoxes / Collection Points **cannot** be used for the shipment of NGS samples.
- You can buy UPS Labels for shipment within the European Union (no dry ice shipment, no shipment of S2 organisms).
- Assure that all necessary sample information and documents are available at project start including the quote acceptance / PO. Add the order confirmation and sample submission form into the package.
- **Tubes:**
  - **1.5 ml Eppendorf Safe-Lock Tubes** are accepted.
  - Add barcodes horizontally on the outside of the tube, sort samples by barcode.
  - Frozen barcodes tend to fall apart. Please ship in cryoboxes or other material that keep barcodes close to the sample.
  - If you use dry ice please store samples in a separate bag or box.
  - No usage of parafilm or tesafilm.
- **Plates:**
  - For most of our services we also accept samples in plates when the sample number exceeds 48 samples.
  - Accepted plate type: **Eppendorf twin.tec PCR Plate 96**, full-skirted / barcoded with the corresponding plate barcode. Please leave position G12 & H12 empty.
  - Arrange samples column-wise on plate in same order as on sample submission sheet (no empty wells).

For sample shipment in any other container a handling fee will be added and the delivery time might be increased.

- We recommend shipping the samples in a padded envelope, box, or other protective shipping package designed for mailing fragile items.
- Packages should be shipped overnight Monday to Thursday, especially if samples must be cooled. Recommended shipping temperature is found in the sections below.
- Include a "proforma invoice" with your shipment for smooth customs processing outside the EU. Declare the sample value and item descriptions, setting the amount to 1 Euro to minimize tax implications.
- For shipment of raw material for extraction, please refer to our "Sample preparation and shipping guide for Extraction"
- Shipping address can be found here: NGS Sample Submission

1.3. Contact

In case of any questions, please do not hesitate to contact Customer Care by email (ngs-support@genomics.eurofinseu.com) or by phone. All phone numbers are available at: www.eurofinsgenomics.eu/phone ; Operating hours: Mon-Fri from 8 am to 6 pm CET.

2. Starting material

I. RNA

Transcriptome Sequencing (RNA-Seq)

INVIEW Transcriptome Discover | INVIEW Virus

- Sample Type: total RNA (DNA-free)
- Purity (OD260/280): 1.8-2.2
- Recommended RIN: ≥8.0
- Resuspension Buffer: Nuclease-free water or Tris-HCL buffer (pH 7.5 to pH 8.0)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: Dry ice. Contact us about our recommendation for RNA Stabilization Tubes to ship RNA samples at ambient temperature.

RNA selection Method	target RNA	Minimum Amount	Volume	Concentration
none	Total RNA	20 ng	10 – 20 µl	2-20 ng/µl
Poly(A) selection	mRNA (eukaryotic)	150 ng	25 – 50 µl	6 - 200 ng/µl
rRNA depletion	mRNA+ lncRNA	375 ng	10 – 20 µl	37.5 - 200 ng/µl
globin + rRNA depletion	mRNA+ lncRNA (blood)	500 ng	10 – 20 µl	50 - 400 ng/µl

RNA-Seq - Bacteria

- Sample Type: total RNA (DNA-free)
- Purity (OD260/280): 1.8-2.2
- Recommended RIN: ≥5.0
- Resuspension Buffer: Nuclease-free water or Tris-HCL buffer (pH 7.5 to pH 8.0)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: Dry ice. Contact us about our recommendation for RNA Stabilization Tubes to ship RNA samples at ambient temperature.

RNA selection Method	Target RNA	Minimum Amount	Volume	Concentration
rRNA depletion	mRNA	375 ng	10 – 20 µl	37.5 - 200 ng/µl

II. DNA

Human/Mammal Whole Genome Sequencing (Short-Read)

- Sample Type: Genomic DNA (RNA-free)
- Minimum Amount: 900 ng
- Volume: 20 to 200 µL
- Concentration: >5 ng/µl
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: room temperature

Whole Genome Sequencing (Short-Read)

INVIEW Resequencing | INVIEW Metagenome | NGSelect - DNA | INVIEW Virus

- Sample Type: Genomic DNA (RNA-free)
- Minimum Amount: 600 ng
- Volume: 20 to 200 µL
- Concentration: >3 ng/µl
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: room temperature

Exome Sequencing (Short-Read)

INVIEW Exome | Clinical Research Exome - CRE V4 | INVIEW Oncoprofiling – 728

- Sample Type: Genomic DNA (RNA-free)
- Minimum Amount: 100 ng (gDNA), 200 ng (FFPE DNA)
- Volume: 20 to 100 µL
- Concentration: >1 ng/µl (gDNA), >2 ng/µl (FFPE DNA)
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: room temperature

Oncology solutions (Short-Read)

INVIEW Liquid Biopsy Oncoprofiling – 728 | INVIEW Liquid Biopsy Oncoexome

- Sample Type: DNA (RNA-free)
- Minimum Amount: 100 ng (gDNA), 200 ng (FFPE DNA), 30 ng (cfDNA)
- Volume: 20 to 100 µL (gDNA + FFPE DNA), 20 to 30 µL (cfDNA)
- Concentration: >1 ng/µl (gDNA, cfDNA), >2 ng/µl (FFPE DNA)
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: room temperature

Hybrid Assembly Bacteria & Yeast (Short-Read + Long-Read)

INVIEW Resequencing Polishing

- Sample Type: purified high-molecular weight DNA, ideally > 20kb fragment length, with recommended **>50% of the DNA above 15 kb in length***
- DNA to be provided in **two** separate tubes
- Minimum Amount: 2 x 600 ng,
- Volume: each tube 20 to 100 µL
- Concentration: 30 ng/µl using a fluorescence-based method
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes
- Shipment Method: Room temperature

*Please refer to our detailed instructions on how to prepare high molecular weight (HMW) in chapter 3.

Whole Genome Sequencing (Long-Read)

- Sample Type: purified high-molecular weight DNA, ideally > 20kb fragment length, with recommended **>50% of the DNA above 15 kb in length***
- Minimum Amount: 2 µg, recommended 4 µg
- Volume: > 50 µL
- Concentration: 40 ng/µl using a fluorescence-based method
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes
- Shipment Method: room temperature

*Please refer to our detailed instructions on how to prepare high molecular weight (HMW) in chapter 3.

Whole Genome Sequencing - Bacteria (Long-Read)

- Sample Type: purified high-molecular weight DNA, ideally > 20kb fragment length, with recommended **>50% of the DNA above 15 kb in length***
- Minimum Amount: 2 µg, recommended 4 µg
- Volume: > 50 µL
- Concentration: 40 ng/µl using a fluorescence-based method
- Purity (OD260/280): 1.8-2.0
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes
- Shipment Method: room temperature

*Please refer to our detailed instructions on how to prepare high molecular weight (HMW) in chapter 3.

INVIEW Microbiome profiling (Short | Long-Read) | MetaBarcoding

INVIEW Microbiome Profiling , **16S Full-Length Microbiome**, **Metabarcoding**

- Sample Type: Purified DNA
- Purity (OD260/280): 1.8-2.0
- Volume: 1 target: 20µL for each additional target plus 10 µL (e.g. 50 µL for 4 targets)
- Concentration: 10-50 ng/µL *
- Minimum Amount: 200 ng
- Resuspension Buffer Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, semi-skirted, leave position G12 & H12 empty
- Shipment Method: Room temperature/ice

*Defining an exact starting concentration is challenging, as the precise quantity of, for example, bacterial DNA within a background of host or environmental DNA is difficult to predict. Typically, we recommend an initial concentration of 10 ng/µL, ensuring the sample contains at least 10^4 16S/ITS/18S copies per µL.

ONT Lite Bacterial Genome Sequencing (Long-Read)

Please find sample requirements here>>.

ONT Lite Whole Plasmid Sequencing (Long-Read)

Please find sample requirements here>>.

III. Amplicons

Amplicons on NovaSeq (150 - 270 bp)

NGSelect Amplicons | INVIEW CRISPR Check*

- Amplicon size: 150 - 270 bp
- Sample Type: Purified PCR products
- Purity (OD260/280): 1.8-2.0
- Volume: 25µL
- Concentration: >4 ng/µl
- Minimum Amount: 100 ng
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes
- Shipment Method: Room temperature/ice/dry ice

*Please refer to our Amplicon guideline for INVIEW CRISPR Check in chapter 5.

Amplicons on MiSeq (280 - 570 bp)

NGSelect Amplicons | INVIEW CRISPR Check*

- Amplicon size (not including the universal adapters): 280 - 570 bp
- Sample Type: Purified PCR products
- Purity (OD260/280): 1.8-2.0
- Volume: 25µL
- Concentration: >1 ng/µl
- Minimum Amount: 25 ng
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes; or for > 48 samples in Eppendorf twin.tec PCR Plate 96, full-skirted, leave position G12 & H12 empty
- Shipment Method: Room temperature/ice/dry ice

*Please refer to our Amplicon guideline for INVIEW CRISPR Check in chapter 5.

Custom Amplicon Sequencing (Long-Read)

- Amplicon size: 500 bp - 25 kb
- Sample Type: Purified PCR products
- Purity (OD260/280): 1.8-2.0
- Volume: 20µL
- Concentration: 30 ng/µl
- Minimum Amount: 600 ng (up to 5 kb amplicons), 2000 ng (up to 25 kb amplicons)
- Resuspension Buffer: Water, EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf Safe-Lock tubes
- Shipment Method: Room temperature

ONT Lite Clonal / linear amplicon sequencing (Long-Read)

Please find sample requirements here>>.

IV. “Ready to load” (compatible with Illumina sequencing and index primer)

NGSelect Ready-to-Load

- Sample Type: Purified library pool, compatible with Illumina sequencing and index primer *
- Volume: minimal 40 µL**
- Concentration: > 2.5 nM
- Total library size should be fitting to the required read mode (insert size + sequencing adapters):
    - 250 bp to 450 bp for Novaseq + PE150 read mode
    - 250 bp to 600 bp for MiSeq + PE300 read mode
    - minimum average size: 150 bp
    - maximum average size: 800 bp (MiSeq), 650bp (NovaSeq)
    - For custom options please contact us
- Buffer: RNase-, DNase- and protease-free Tris-HCl buffer (ph 7.5 - 8.0), EB, or low TE (<0.1 mM EDTA)
- Format: barcode labelled 1.5 ml Eppendorf safe-lock tubes
- Shipment Method: recommended dry ice

*Ready-to-load library pool must be purified using columns or beads to remove low molecular weight impurities, such as primers, nucleotides and reaction buffer.

**One tube with minimal 40µL per lane is mandatory. E.g. for a complete 10/25B flow cell, a total of 8 tubes are required. Samples to be sequenced on one lane, need to be pre-pooled.

3. General DNA guidelines

I. Requirements

- Double-stranded high molecular weight DNA with an OD 260/280 ≥ 1.8-2.0 and an OD 260/230 ≥ 1.8-2.2
- Preferably dissolved in RNAse-, DNAse- and protease-free Tris-HCl buffer (pH 8.0 – 8.5)
- The solution must not contain any impurities e.g., biological macromolecules (e.g., protein, polysaccharides, lipids), chelating agents (e.g., EDTA), divalent metal cations (e.g., Mg2+), denaturants (e.g., guanidinium salts, phenol), or detergents (e.g., SDS, Triton-X100).

II. DNA isolation

- There are numerous commercial kits and a wide variety of suitable protocols available for the isolation of DNA (e.g., QIAGEN DNeasy kit). Researchers should select a protocol that meets their particular needs. Organic extraction methods (such as phenol or trizol) should not be used for the purification of total DNA as they can inhibit enzymes used during the library preparation and therefore increase the risk of failure of library preparation. If the use of phenol- or trizol-based methods is unavoidable (e.g. to obtain high molecular DNA), the total removal of these compounds should be guaranteed (that means, an extra clean-up step after extraction should be necessary).
- Wash bacterial cell pellets before DNA extraction with PBS to remove potential inhibitors
    - Add 1ml of PBS and resuspend cells by pipetting
    - Pellet the cells by centrifugation and discard the supernatant
- For best results use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -80°C for the DNA isolation in order to minimize degradation through endogenous nucleases.
- DNA should have undergone a minimum of freeze-thaw cycles, no exposure to high temperatures, no exposure to pH extremes (< 6 or > 9) and no exposure to intercalating fluorescent dyes or ultraviolet radiation.
- DNA should be treated with RNase (e.g., from QIAGEN) to minimize contamination through RNA, which otherwise might be reflected in the sequencing results.

III. High molecular weight (HMW) DNA isolation

We recommend: New England Biolabs Monarch® Spin gDNA Extraction Kit, QIAGEN Genomic-tip-500/G, QIAGEN MagAttract HMW DNA Kit, QIAGEN Puregene Yeast/Bacteria Kit. In general, all extraction methods that allow the generation of HMW gDNA fulfilling the above requirements can be used:

- Wash bacterial cell pellets before DNA extraction with PBS to remove potential inhibitors
    - Add 1ml of PBS and resuspend cells by pipetting
    - Pellet the cells by centrifugation and discard the supernatant
- Avoid vortexing and fast or unnecessary pipetting; use wide-bore tips only
- Elute in nuclease-free elution buffer, not water
- Avoid over-drying of gDNA
- Do not expose DNA to high temperatures (>37°C) for >1 hour
- Use buffer with appropriate pH 7.5- 8.5

- Avoid intercalating fluorescent dyes, or UV radiation
- Avoid freeze-thaw cycles; store gDNA at 4°C for 1-2 months

Short DNA fragments are typically not ideal for long-read sequencing, as they can result in poor assembly quality. We recommend that at least 50% of the DNA is above 15 kb in length. To improve sequencing outcomes, we recommend removing shorter DNA fragments from your sample before submission. One effective protocol involves using 4X diluted SPRISelect beads (35% volume by volume) to eliminate fragments smaller than 3-4 kb.

Please note that the DNA quantity will decrease following this process, so it's essential to quantify the remaining DNA to ensure sufficient material is available for sequencing.

1. Transfer each sample into a clean 1.5 ml Eppendorf DNA LowBind tube.
2. Dilute SPRISelect beads with Elution Buffer to 35% (volume by volume).
3. Resuspend the diluted SPRISelect beads by vortexing.
4. Add 4x volume of the resuspended diluted SPRISelect Beads to each gDNA and mix by flicking the tube.
5. Incubate for 5 minutes at room temperature.
6. Prepare sufficient fresh 80% EtOH in nuclease-free water for all of your samples.
7. Spin down the samples and pellet the beads on a magnet until the eluate is clear and colourless. Keep the tubes on the magnet and pipette off the supernatant.
8. Keep the tube on the magnet and wash the beads with freshly prepared 80% EtOH without disturbing the pellet. The EtOH volume must be enough to cover the beads entirely. Remove the EtOH using a pipette and discard.
9. Repeat the previous step.
10. Briefly spin down and place the tubes back on the magnet for the beads to pellet. Pipette off any residual EtOH. Allow to dry for 30 seconds, but do not dry the pellets to the point of cracking.
11. Remove the tubes from the magnetic rack and resuspend the pellet in e.g. 50 µL nuclease-free 1xTE or EB. Spin down and incubate for 10 minutes at 37°C with gentle agitation (700rpm) on a shaker.
12. Pellet the beads on a magnet until the eluate is clear and colorless.
13. Remove and retain the eluate into a clean 1.5 ml Eppendorf Safe Lock Tubes™

Alternatively, we recommend the DNA clean-up and size selection for long-read sequencing from Jones et al. 2021 (Jones A, Torkel C, Stanley D, Nasim J, Borevitz J, et al. (2021) High-molecular weight DNA extraction, clean-up and size selection for long-read sequencing. PLOS ONE 16(7): e0253830. https://doi.org/10.1371/journal.pone.0253830; https://www.protocols.io/view/dna-clean-up-and-size-selection-for-long-read-sequ-261ge6ymol47/v4)

IV. Quantitative assessment

- Preferred measurement method: fluorescence-based methods like Qubit® (Invitrogen, Life Technologies), Quant-iT™ (Invitrogen) or Quantifluor (Promega).
- HMW gDNA often requires extra homogenization effort (longer incubation time, increased incubation temperature, very extensive gentle mixing, etc.) to obtain accurate quantification. If separate DNA quantifications from the top and bottom of the sample are within 15% of each other, this is usually a good indication of adequate homogeneity.

- If you have less than the requested amount of gDNA, we strongly recommend that you perform additional extractions to increase yield.
- When using UV-spectrometer-based methods, please be aware that they tend to overestimate the DNA concentration as they are not double-stranded DNA specific but also measure contaminants like e.g., RNA.

V. Qualitative assessment

- Preferred measurement method: capillary electrophoresis-based methods like Fragment Analyzer or Bioanalyzer. High molecular weight DNA is greater than 20 kb in size and shows minimal smearing. Contamination, damage and degradation are revealed through a low molecular weight smear and should be removed using alternative cleanup strategies.
- Alternatively, we recommend UV electrophoresis on an agarose gel (low-percentage for gDNA or high percentage for amplicons).

VI. Purity

- Phenol, chloroform and other related reagents will inhibit the library preparation and must be completely removed. Although spectrophotometric measurements may not detect all types of contamination, we highly recommend performing them to assess sample purity. Ideally, the 260/280 ratio should be above 1.8, and the 260/230 ratio should fall between 2.0 and 2.2. If the sample is contaminated and does not meet this metric, either re-extract the sample or clean up the sample to remove the contaminants using a Qiagen cleanup kit or AMPure XP beads. Your samples:
    - Must not contain RNA; we strongly recommend RNase treatment during extraction
    - Must not contain denaturants (guanidinium salts, phenol, etc.) or detergents (SDS, Triton-X100, etc.)
    - Must not contain residual contaminants from the organism/tissue (heme, humic acid, polyphenols, etc.)
    - Must not contain insoluble material or be colored or cloudy

VII. Shipping temperature

DNA can be sent at room temperature; we recommend shipping samples refrigerated (4°C).

4. General RNA guidelines

I. Requirements

- High quality RNA with an OD 260/280 ratio ~2.0 and an OD 260/230 ratio between 2.0-2.2.
- RNA Integrity Number (RIN) or RNA quality indicator (RQI; Bio-Rad's Experion) value ≥ 8.
- Preferably dissolved in RNase-, DNase- and protease-free molecular grade water (do not use DEPC-treated H2O).
- The solution must not contain any impurities e.g., biological macromolecules (e.g., protein, polysaccharides, lipids), chelating agents (e.g., EDTA), divalent metal cations (e.g., Mg2+), denaturants (e.g., guanidinium salts, phenol), or detergents (e.g., SDS, Triton-X100).

II. RNA isolation

- There are numerous commercial kits and a wide variety of suitable protocols available for the isolation of RNA (e.g., mirVana™ miRNA Isolation Kit from Ambion or QIAGEN RNeasy kit). Researchers should select a protocol that meets their particular needs.
- Extract and stabilize RNA as quickly as possible after obtaining samples and wear gloves at all times to minimize degradation of crude RNA by limiting the activity of endogenous RNases.
- All reagents should be prepared from RNAse-free components.
- We strongly recommend performing a final clean-up of the RNA using a DNAse treatment (e.g., from QIAGEN).
- Organic extraction methods (such as phenol or trizol) should not be used for the purification of total RNA as they can inhibit enzymes used during the library preparation protocol and therefore increase the risk of failure of library construction. If the use of phenol- or trizol-based methods is unavoidable, the total removal of these compounds should be guaranteed (i.e. an extra clean-up step after extraction should be necessary, using e.g. RNeasy kit from QIAGEN).

III. Quantitative assessment

- Preferred measurement method: fluorescence-based methods like Qubit® (Invitrogen, Life Technologies), Quant-iT™ (Invitrogen) or Quantifluor (Promega).
- Please note that DNA contamination will result in an underestimation of the amount of RNA.

IV. Qualitative assessment

Preferred measurement method: capillary electrophoresis-based methods like Fragment Analyzer or Bioanalyzer to determine the RNA Integrity Number (RIN). Alternatively, we recommend running a 1% formaldehyde agarose gel to assess RNA integrity. The mRNA should appear as a smear ranging from 500 bp to 8 kb, with two distinct bands corresponding to the rRNA subunits. If the rRNA subunits appear intact and are not smeared, it indicates that the mRNA is also likely intact.

V. Shipping temperature

- RNA should ideally be shipped on dry ice. Contact us about our recommendation for RNA Stabilization Tubes to ship RNA samples at ambient temperature.
- If it is not possible to ship on dry ice, then you can precipitate your RNA in ethanol. Recommended precipitation protocol:
    - add 1/10 volume 3 M sodium acetate to 1 volume RNA (pH 5.2)

- add 1/50 volume glycogen (5 mg /ml), final concentration will be 100 µg/ml
- vortex to mix
- add 3 volumes ice cold 100 % ethanol
- vortex to mix thoroughly
- Please do not decant and discard the supernatant, but send the sample diluted in ethanol.

Please note: If you send your RNA samples in EtOH it is important that you inform us during ordering (sample questionnaire)

5. Amplicon guideline for INVIEW CRISPR Check

Amplicon Length Requirements

- Amplicon size needs to be adapted to the length of InDels you want to be able to analyse, the sequencing mode you chose must allow merging of read pairs. Please find below the wildtype amplicon size requirements for two experimental cases.

INVIEW CRISPR Check Product type	Detection of deletions up to 100 bp and insertions up to 30 bp	Detection of deletions up to 50 bp and insertions up to 30 bp
Amplicons on MiSeq (280 - 570 bp)	450 – 500 bp	400 – 500 bp
NGSelect Amplicons on NovaSeq (150 - 270 bp)	200 – 225 bp	150 – 225 bp

Table: Wildtype amplicon size requirement for two experimental cases.

- Wildtype amplicon size for INVIEW CRISPR Check MiSeq does not include the universal adaptors that are included in your lab
- Wildtype amplicon size for INVIEW CRISPR Check NovaSeq does not include the 10 nucleotide tags on forward primers that you optionally include in your lab
- Target regions can be mutated in your unmodified cells and might differ from the available reference sequence/expected amplicon sequence. To evaluate this, we strongly recommend including at least one wildtype control sample for your experiment.
- The INVIEW CRISPR Check Gene Editing Bioinformatics Analysis requires a minimum of 10 000 read pairs per sample and target site. In case you are using the INVIEW CRISPR Check Adaptor Ligation approach with sample tagging, please plan accordingly.