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# Survey for DNA sequencing SQ 1/25

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# Test instructions

Please note, that the **online entry of your results is mandatory for this survey.**

## Time schedule

**Test period**
Samples should be tested within four weeks.

**Submission deadline**
12.04.2025

## Sample material

You receive 5 labeled sample tubes (two samples and three primers) with this package.

**Color code:**

- sample 1 (blue) → PCR product 1
- sample 2 (red) → PCR product 2

Sequences of primer:

- Primer 1 forw. (yellow)
  5'- GCTAAGTGTCTTTGGAGTTC -3'
- Primer 1 rev. (green)
  5'- CCCCAACCCATAGACTGAG -3'`
- Primer 2 forw. (clear)
  5'- CCCTTTCAGCCTATGGAACA -3'`

The primers were designed using the UCSC Genome Browser (GRCh37/hg19) and Primer3.

Recommended storage temperature: +2 °C to +8 °C.

The homogeneity of the samples was checked.

The stability of the samples is guaranteed over the survey period.

If the samples do not reach you in flawless condition, please make a complaint immediately by telephone or e-mail, stating the participant number and the survey.

## Sample preparation

The samples are PCR-products and are provided as a dried pellet of approx. 250 ng.
Furthermore you receive 200 pmol per tube of sequencing primers as a dried pellet.
Please use exclusively the primer provided in concentrations according to your protocol.

**Please use primer 1 forw. and primer 1 rev. for sequencing sample 1**

**Please use primer 2 for. for sequencing sample 2.**

## Technical evaluation of sample 1:

- perform the **sequencing reaction** with the primers provided in sense and antisense direction,
- **edit** the raw data,
- provide the longest possible sequence of both sequencing runs following your editing steps **(i. e. without any mistakes or ambiguities "N")** (5 '→ 3'),
- identify the gene from the sequence string
- upload the raw data of the sense **and** antisense sequencing
- upload the edited sense **and** antisense sequence string
- report any sequence variations compared to the wild-type sequence according to HGVS recommendations

**Note:** Some parts of the surveys (packing, data transfer / sample preparation, manufacturing) can from time to time be subcontracted. When subcontracting occurs, it is placed with a competent subcontractor and the proficiency testing provider is responsible for this work.

## Technical evaluation of sample 2:

- perform the **sequencing reaction** with the primer provided (primer 2 forw.),
- **edit** the raw data,
- provide the longest possible sequence **without any mistakes or ambiguities (e. g. "N"),**
- identify the sequence string,
- upload the raw data of the antisense sequencing,
- upload the edited antisense sequence string.
- report any sequence variations compared to the wild-type sequence according to HGVS recommendations

## Documentation and further information

Please send your three electropherograms (ab1.files) to: Ulrike.pfeiffer@umm.de

All results (raw data and edited data) should be uploaded to the RfB web-page using your specific log-in. **We will evaluate the recent nomenclature of the sequence variations according to the recommendations of the Human Genome Variation Society (HGSV, see http://www.hgvs.org/mutnomen/).**

**Notes**

Please check your entries, as subsequent changes cannot be taken into account.

After entering your results, please send us the measured values by clicking on the corresponding button. To check your entries, you can request a protocol e-mail at any time during the test period.

## Evaluation

The final report of your test results will be available in the RfB online account approx. 8 weeks after submission deadline.