

Sanger Sequencing Proficiency Testing Program

The Objectives of the Sanger Sequencing PT scheme are:

- To maintain a high level of quality in Sanger sequencing tests.
- To monitor flaws in the testing procedure using pre-characterized DNA samples.
- To provide feedback on the lab's sequencing procedure.
- To provide guidance and recommendations for improvements in test procedures.

The Sanger Sequencing PT Program

Twice a year, QualiGene sends two PCR products, potentially harboring specific mutations (detectable by Sanger sequencing), forward and reverse sequencing primers and instructions. The laboratory determines the sample's genotype and submits the results to QualiGene for evaluation. QualiGene verifies the accuracy of the analysis and reports its findings back to the participating laboratory.

Sanger sequencing PT Laboratory Practice

- The tested laboratory needs to treat the PT test samples as if these were routine Sanger sequencing tests.
- The Sanger sequencing procedure will be carried out with the reagents and protocols, normally used for this purpose in your laboratory.

Sanger sequencing PT material storage instructions

- The PCR products and primers should be stored at 4°C

The Sanger Sequencing PT Protocol

- QualiGene will inform all participating laboratories two weeks before sample shipment.
 - A submission date will be set for **90 days from delivery day**. (The delivery day will be announced and **late submission, without a prior approval for extension by QualiGene, will result in grade reduction**)
 - The results will be submitted by fax or e-mail, on a result form provided by QualiGene.
 - The raw data (forward and reverse sequences of both samples) will be attached to the result form .
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- The lab will receive QualiGene's evaluation and feedback within four weeks from the results submission deadline (assuming you have submitted your results on time).
 - Grades and PT results will not be reported back until all participants have submitted their results.
 - At the end of each year, the registered laboratories will receive a certificate attesting to their participation in QualiGene's PT program.
 - Following each PT round, QualiGene will publish a report containing, Sanger sequencing PT related statistics, general information and conclusions.

Appeals

- Each participating laboratory may appeal the grade, reported back by QualiGene, up to one week from grade receipt.
- The appeals should be submitted by e-mail, specifying the reason for the appeal and supporting arguments.
- QualiGene will re-evaluate the results submitted by the participating laboratory and will report its decision to it - up to 10 days from appeal receipt.

Confidentiality

- All participating laboratories' names are kept strictly confidential.
- QualiGene's evaluation and grading of your results is confidential and will be submitted back only to the laboratory manager.
- Statistics and grades published in QualiGene's report are always anonymous.
- Grades of Israeli laboratories are reported annually to the Israeli MOH.

Grading Criteria (percent of total grade)

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| • Correct identification of the tested mutations | 40% |
| • Sequence quality | 45% |
| • Nomenclature | 5% |
| • Timely submission of results | 10% |