Agilent OneSeq Target Enrichment

Product Overview Guide

For Research Use Only. Not for use in diagnostic procedures.

Agilent Technologies

What is OneSeq Target Enrichment?

OneSeq is a target-enrichment product for Next Generation Sequencing (NGS) that enables you to survey the entire genome for copy number variations (CNVs) and copy-neutral losses of heterozygosity (cnLOH intervals) while simultaneously searching for point mutations and indels in specific regions of interest.

Each OneSeq Capture Library tube includes two components:

- Backbone design (high-resolution or low-resolution) for identifying genome-wide CNVs
- Targeted design for identifying point mutations and indels in regions of interest

The high-resolution backbone design has a genome-wide resolution of 300 kb with a higher resolution of 25–50 kb in disease-associated ClinGen regions. For cnLOH intervals, the resolution is 5 Mb.

The low-resolution backbone design has a genome-wide resolution of 1 Mb and a resolution of 10 Mb for cnLOH intervals.

For the OneSeq Constitutional Research Panel, the backbone design is the high-resolution 300 kb CNV backbone and the targeted design is the Agilent SureSelect Focused Exome Panel, which enriches for regions with known disease associations, as identified by the HGMD, OMIM, and ClinVar databases. For the Custom OneSeq Capture Libraries, the targeted design is a user-selected SureSelect gene panel or custom SureSelect design. Use Agilent's SureDesign web application to create and order a custom OneSeq design.

In a OneSeq target enrichment protocol, you process your experimental DNA samples side-by-side with an Agilent DNA reference sample or with another DNA reference sample of your choosing. Male and female reference samples can be ordered separately from Agilent, but any sample can be used as a reference sample. Figure 1 is an overview of the OneSeq workflow.

Agilent's SureCall software (v3.5 and higher) has algorithms for analyzing OneSeq sequencing data. The results for the backbone design and targeted design are reported together in the Triage View window and in the Reports tab. SureCall uses the probes in the backbone design for CNV and cnLOH detection, and the probes in the targeted design for detection of point mutations and indels.

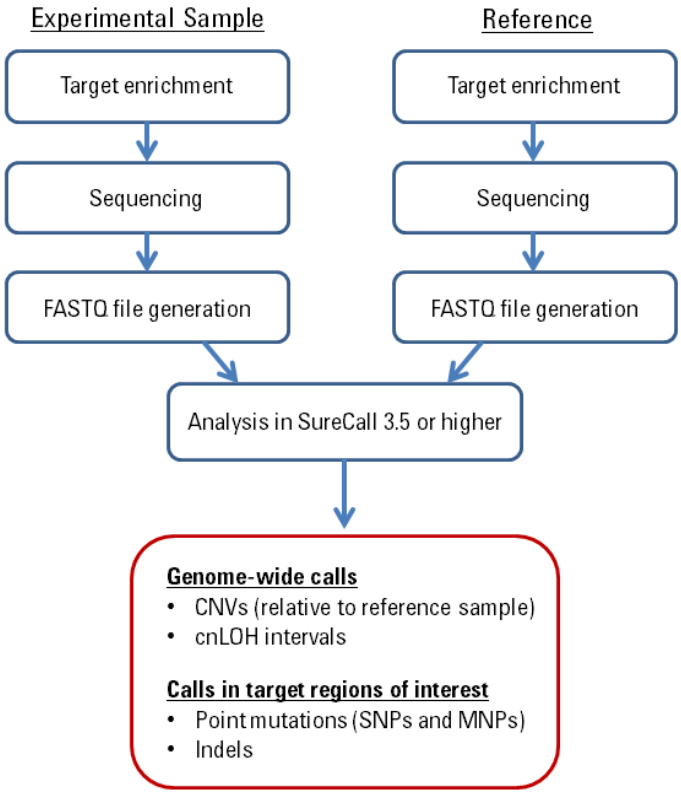

Figure 1 Overview of the OneSeq workflow

Kit Part Numbers

The part numbers for the available OneSeq Capture Library kits are listed in Table 1 and Table 2. The part numbers for the male and female reference DNA are listed in Table 3. Table 4 lists the part numbers for the SureSelect^{XT} reagent kits.

Table 1 OneSeq 1Mb CNV Backbone part numbers

Product	16 Reactions	96 Reactions	96 Reactions, Automated
OneSeq 1Mb CNV Backbone + Custom, 1 – 499 kb	5190-9462	5190-9463	5190-9464
OneSeq 1Mb CNV Backbone + Custom, 0.5 – 2.9 Mb	5190-9465	5190-9466	5190-9467
OneSeq 1Mb CNV Backbone + Custom, 3 – 5.9 Mb	5190-9468	5190-9469	5190-9470
OneSeq 1Mb CNV Backbone + Custom, 6 – 11.9 Mb	5190-9471	5190-9472	5190-9473
OneSeq 1Mb CNV Backbone + Custom, 12– 24Mb	5190-9474	5190-9475	5190-9476

Table 2 OneSeq 300 kb CNV Backbone part numbers

Product	16 Reactions	96 Reactions	96 Reactions, Automated
OneSeq Constitutional Research Panel	5190-8702	5190-8703	5190-8704
OneSeq Hi Res CNV Backbone + Custom, 1 – 499 kb	5190-8705	5190-8887	5190-8888
OneSeq Hi Res CNV Backbone + Custom, 0.5 – 2.9 Mb	5190-8889	5190-8890	5190-8891
OneSeq Hi Res CNV Backbone + Custom, 3 – 5.9 Mb	5190-8892	5190-8893	5190-8894
OneSeq Hi Res CNV Backbone + Custom, 6 – 11.9 Mb	5190-8895	5190-8896	5190-8897

Table 3 OneSeq Reference DNA part numbers

Product	Part Number
OneSeq Human Reference DNA, Female	5190-8850
OneSeq Human Reference DNA, Male	5190-8848

Table 4 SureSelect^{XT} Reagent Kit part numbers

Product	16 Reactions	96 Reactions
SureSelect^{XT} Reagent Kit, Illumina HiSeq platform	G9611A	G9611B
SureSelect^{XT} Automation Reagent Kit, Illumina HiSeq platform	—	G9641B
SureSelect^{XT} Reagent Kit, Illumina MiSeq platform	G9612A	G9612B
SureSelect^{XT} Automation Reagent Kit, Illumina MiSeq platform	—	G9642B

Using the OneSeq Capture Libraries

Performing the target enrichment protocol

To perform OneSeq target enrichment, use 200 ng DNA and refer to the appropriate SureSelect^{XT} target enrichment protocol.

- For manual (non-automated) target enrichment using a 16-Reaction or 96-Reaction OneSeq kit, refer to the Agilent protocol *SureSelect^{XT} Target Enrichment for Illumina Paired-End Sequencing Library* and follow the instructions for capture libraries ≥ 3.0 Mb. This publication is available on the Agilent website at: www.chem.agilent.com/Library/usermanuals/Public/G7530-90000.pdf
- For automated target enrichment using a 96-Reaction Automated OneSeq kit, refer to the Agilent protocol *SureSelect^{XT} Automated Target Enrichment for Illumina Paired-End Multiplexed Sequencing* and follow the instructions for target sizes > 3.0 Mb. This publication is available on the Agilent website at: www.chem.agilent.com/Library/usermanuals/Public/G7550-90000.pdf

IMPORTANT

Follow the instructions in the appropriate SureSelect protocol listed above, but with the following modification:

For the OneSeq Constitutional Research Panel and all OneSeq 300 kb CNV Backbone + Custom panels, in the Post-Capture PCR cycling program, run Segment 2 for 10 cycles, regardless of the Capture Library size.

Use of a reference sample

Include a sex-matched or mismatched reference sample each time you perform target enrichment on a set of experimental samples. You can order male and female reference DNA separately from Agilent. Use 200 ng of the reference DNA as the starting material in the protocol.

Instead of using the Agilent reference samples recommended for the OneSeq kits, you can use your own reference sample. The key requirement is that the sample is diploid and has no or minimal CNV aberrations. Additionally, if you use your own reference, you must first validate it by analyzing a well-characterized sample with known aberrations to make sure that SureCall is calling CNVs accurately.

Analyzing the results in SureCall

SureCall version 3.5 (and higher) has the necessary tools and algorithms for analyzing sequencing data from a OneSeq enrichment. SureCall can perform the analysis using unaligned FASTQ files or aligned BAM files. The steps for analyzing OneSeq data in SureCall are summarized in Figure 2.

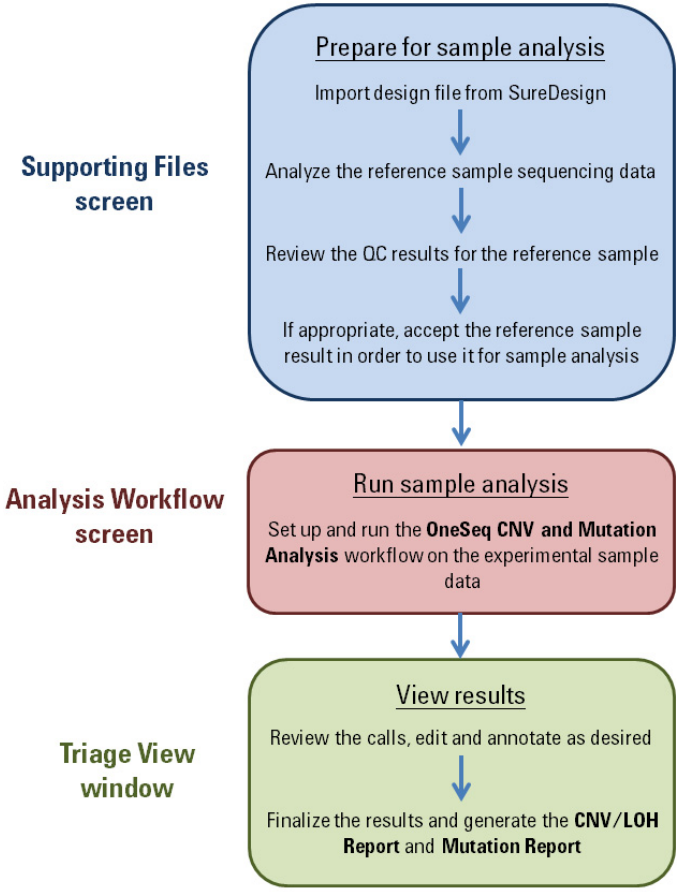

Figure 2 Steps for running a OneSeq analysis in SureCall

The SureCall help system has specific instructions on each of these steps. Press F1 from any screen in the SureCall software to access the help system.

NOTE

The SureCall analysis method *Default OneSeq Backbone CN* is suitable for all OneSeq analyses except for enrichments that use the OneSeq 1Mb CNV Backbone with custom content ≥ 24 Mb. In such cases, create a custom version of the Default OneSeq Backbone CN analysis method that has the following modifications.

- In the **CNV and SNP Caller** parameters, set the CNV parameter called **False Positive Control threshold cutoff** to 0.7.
- In the **CNV Filter** parameters, set the **Minimum absolute log ratio** to 0.3 and set the parameter called **Merge two aberrations if distance less than (kb)** to 100.

These modifications help reduce false positive calls. See the SureCall help system for instructions on creating a custom analysis method.

Agilent Technical Support

For technical product support, contact your local Agilent Support Services representative.

For US and Canada, call (800) 227-9770 (option 3,4,4). For other countries, find your support center telephone numbers at www.agilent.com/chem/contactus.

Or send an e-mail to: SureSelect.Support@agilent.com

www.agilent.com

In this book

This book provides an overview of Agilent's OneSeq products for NGS target enrichment.

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