

**USER GUIDE**

# Ovation® Target Capture Module

PART NO. 0332

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# I. Introduction

## A. Background

The Ovation Target Capture Module provides a library adaptor-specific blocking reagent mix and a PCR Primer mix to enable target capture workflows with NuGEN® library solutions for the Illumina® NGS platforms. Target, or Sequence Capture, methods allow researchers to “capture” genomic regions of interest. The resulting enriched products are utilized for sequencing, resulting in cost reduction, simplified data analysis and increased sample throughput as compared to whole genome sequencing. The Ovation Target Capture Module has been optimized for the SureSelect Target Enrichment System from Agilent Technologies.

Key benefits of the Ovation Target Capture Module include:

- Low DNA input — In conjunction with the Ovation Ultralow Library Systems, perform target capture starting with as little as 1 ng DNA
- Library pooling before capture — Combine barcoded libraries prior to target capture reaction for significant cost reduction and increased throughput
- Multiplexing options — Several barcoding options are available with NuGEN’s library solutions to optimize workflow needs

The Ovation Target Capture Module contains sufficient blocking reagent mix to perform 16 capture reactions using individual or pooled libraries. These reagents may be used with any of NuGEN’s library solutions, including multiplex systems utilizing either inline (IL) or dedicated read (DR) barcode designs. The objective of this protocol is to enable target enrichment of libraries generated using NuGEN kits that have adaptors specific for sequencing on Illumina platforms. The reagents provided in the module are compatible with the following NuGEN library kits for Illumina sequencing:

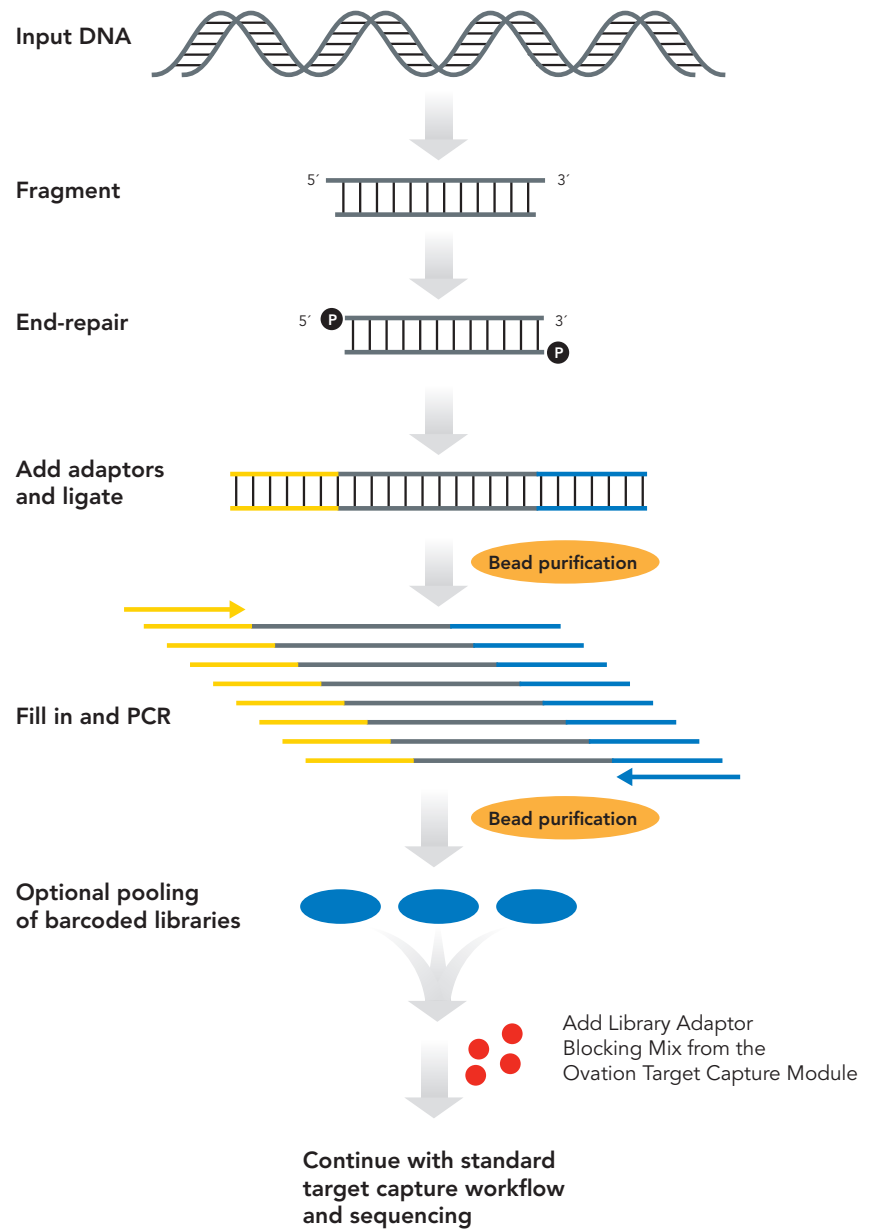
- Ovation Ultralow Library Systems (Part Nos. 0303, 0304, 0305, 0329, 0330, 0331)
- Encore 384 Library System (Part Nos. 0315A, 0315B, 0315C, 0315D, 0315E)
- Ovation SP Ultralow Library System and Ovation SP Ultralow DR Multiplex Systems (Part Nos. 8030, 8033, 8034)

## B. Target Capture Process

The Ovation Target Capture Module protocol starts with 500 ng of sequencing library prepared with one of the NuGEN library preparation systems listed. This sample may be from a single, non-multiplexed sample or may represent a pool of barcoded libraries intended to be multiplex sequenced on a single lane of the flow cell. The Library Adaptor Blocking Mix is used in the appropriate target capture workflow. This blocking mix consists of a cocktail of reagents designed to block the specific adaptor sequences used in our sequencing libraries so these sequences do not impede efficient sequence capture.

# I. Introduction

Figure 1. Workflow diagram.



# I. Introduction

## C. Quality Control

Each Ovation Target Capture Module lot is tested to meet minimum performance specifications.

## D. Storage and Stability

The Ovation Target Capture Module is shipped on dry ice and should be unpacked immediately upon receipt. All components should be stored at  $-20^{\circ}\text{C}$  on internal shelves of a freezer without a defrost cycle.

This product has been tested to perform to specifications after as many as six freeze/thaw cycles. Kits handled and stored according to the above guidelines will perform to specifications for at least six months from date of receipt in your lab.

## E. Material Safety Data Sheet (MSDS)

An MSDS for this product is available on the NuGEN website at <http://www.nugen.com/nugen/index.cfm/support/user-guides/>.

## II. Components

### A. Reagents Provided

Library Adaptor Blocking Mix — a proprietary mixture of adaptor blocking reagents designed to prevent cross-hybridization of target and non-target library molecules that would otherwise significantly impair the efficacy of the enrichment procedure.

PCR primers — for post-capture amplification, these amplification primers are compatible with all NuGEN-generated Illumina libraries.

**Table 1. Reagents provided**

COMPONENT	0332 PART NUMBER	VIAL CAP	VIAL NUMBER
Library Adaptor Blocking Mix (300 $\mu$ M)	S01661	Yellow	—
Amplification Primer Mix (20 $\mu$ M each)	S01664	Red	P2 VER 5

### B. Additional Equipment, Reagents and Labware

#### Required Materials

- **Equipment**
  - Tube rotator
  - Microcentrifuge for individual 1.5 mL and 0.5 mL tubes
  - Microcentrifuge for 0.2 mL individual and 8 X 0.2 mL strip PCR tubes
  - 0.5–10  $\mu$ L pipette, 2–20  $\mu$ L pipette, 20–200  $\mu$ L pipette, 200–1000  $\mu$ L pipette
  - Vortexer
  - Thermal cycler with 0.2 mL tube heat block, heated lid, and 100  $\mu$ L reaction capacity
  - Appropriate spectrophotometer and cuvettes, or Nanodrop®
  - UV-Vis Spectrophotometer
- **Reagents**
  - Third-party Target Enrichment Kit and protocol, for example:
    - Agilent SureSelect
  - Dynal MyOne Streptavidin T1 beads (Invitrogen).
  - Agencourt AMPure XP beads
  - Nuclease-free water

## II. Components

- Reagents for Post-Capture Amplification (these reagents can be used for post-capture amplification, or you may choose to use the reagents recommended in the third-party capture protocol you are following with the NuGEN provided PCR primers)
  - Taq DNA Polymerase (New England Biolabs Cat. #M0273)
  - 10x Standard Taq Buffer (included with New England Biolabs Cat. #M0273)
  - dNTP mixture (10mM each, New England Biolabs Cat. #N0447 or equivalent)
  - MgCl<sub>2</sub>
  - DMSO
- **Supplies and Labware**
  - Nuclease-free pipette tips
  - 1.5 mL and 0.5 mL RNase-free microcentrifuge tubes
  - 0.2 mL individual thin wall PCR tubes or 8 X 0.2 mL strip PCR tubes
  - Appropriate spectrophotometer cuvettes
  - Disposable gloves
  - Kimwipes
  - Ice bucket
- **Optional Materials**
  - Agilent 2100 bioanalyzer or other equipment for electrophoretic analysis of RNA
  - Real-time PCR system

### To Order:

- Agilent, [www.agilent.com](http://www.agilent.com)
- Beckman Coulter Genomics, [www.beckmangenomics.com](http://www.beckmangenomics.com)
- Invitrogen, [www.invitrogen.com](http://www.invitrogen.com)
- New England Biolabs, [www.neb.com](http://www.neb.com)



## III. Planning the Experiment

### A. Planning the Target Capture Experiment

The Ovation Target Capture Module allows the use of sequencing libraries prepared using a NuGEN sequencing library reagent system in commercially available target capture systems such as the Agilent SureSelect sequence capture kits. A list of the NuGEN library systems that are compatible with this module is given on pg. 1. A list of third-party protocols that can be used for the target enrichment can be found in Table 2.

The Ovation Target Capture Module contains a Blocking Reagent Mixture designed to block the adaptor sequences used in NuGEN library reagent systems, enabling high efficiency capture of targeted genomic sequences in the library. The presence of multiplex barcodes (either DR or IL types, or both) will not interfere with efficient blocking; therefore, it is possible to generate multiplexed sequencing libraries prior to the capture process, pool them and run a single target capture for each flow cell lane. The provided Amplification Primer Mix can then be used to amplify the captured material prior to loading on the flow cell. By enabling multiplexed libraries to be pooled prior to target capture, this module will provide for simplified workflows and significant cost savings.

Please note that this module is intended for use only with NuGEN sequencing libraries designed for the Illumina sequencing platforms, and it is not appropriate for use with sequencing libraries produced using other manufacturers' reagent systems.

### B. Using Nuclease-free Techniques

Nuclease contamination from equipment and work environment will lead to experimental failure. Follow these guidelines to minimize contamination:

- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could introduce DNases.
- Use only the reagents provided and recommended.
- Prior to initiating protocol, clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new DNase-free pipette tips and microcentrifuge tubes.

### C. Sequencing Library Storage

The sequencing libraries to be used as input for the target capture protocol may be stored at  $-20^{\circ}\text{C}$  for up to six months prior to labeling.

## III. Planning the Experiment

### D. Post-capture Library Storage

The post-capture sequencing library mixture should be handled and stored in accordance with the guidelines published by the target capture platform manufacturer.

## IV. Protocol

### A. Preparing the Sequencing Libraries

Sequencing libraries may be prepared using any of the following NuGEN library preparation methods.

- Ovation Ultralow Library Systems (Part Nos. 0303, 0304, 0305, 0329, 0330, 0331)
- Ovation SP Ultralow Library System and Ovation SP Ultralow DR Multiplex Systems (Part Nos. 8030, 8033, 8034)

Libraries may be prepared using either the Mondrian™ SP or SP+ digital microfluidics system or manual protocols.

Sequencing libraries should be fragmented to the appropriate size, then prepared in accordance with the protocols given in their respective user guides. If multiplex sequencing will be used, the multiplexing barcodes will be added during this step. Quantify the library yield using a NanoDrop and check size distribution on a Bioanalyzer as described in the appropriate user guide.

If multiplexing, determine the molar concentration of each library using an assay such as the KAPA Biosystems Library Quant kit or other suitable method for accurately determining sequencing library molarity. Use these data to create an equimolar mixture of libraries to be multiplexed in each lane. Ensure the mass of the mixture is sufficient for input into the hybridization step of the appropriate sequence capture protocol.

### B. Target Capture

The sequencing libraries prepared in A above are used as input into the library hybridization step of the appropriate sequence capture protocol, using the Library Adaptor Blocking Mix from the Ovation Target Capture Module. It is critical to use the Library Adaptor Blocking Mix, as only the NuGEN blocking reagents are designed to work with libraries generated from NuGEN library systems.

The following table contains a list of third-party target enrichment protocols that are compatible with the Ovation Target Capture Module. The table contains information on how the Library Adaptor Blocking Mix should be used instead of, or in addition to, the blocking mix included with the third-party target enrichment kit.

## IV. Protocol

**Table 2. Third-party enrichment protocols that are compatible with the Ovation Target Capture Module.**

THIRD-PARTY CAPTURE REAGENT PROVIDER	THIRD-PARTY KIT	USER MANUAL	USE 0.6 µL LIBRARY ADAPTOR BLOCKING MIX...
Agilent	SureSelect <sup>XT</sup> Target Enrichment System for Illumina Paired-End Sequencing Library	G7530-90000	...instead of SureSelect Indexing Block #3 (brown cap) 0.6 µL (For details, see Appendix A.)
Agilent	Agilent SureSelect Automated Library Prep and Capture System	G7550-90000	...instead of SureSelect Indexing Block #3 (brown cap) 0.6 µL
Agilent	SureSelect <sup>XT2</sup> Target Enrichment System for Illumina Multiplexed Sequencing	G9630-90000	...in addition to SureSelect <sup>XT2</sup> Blocking Mix tube with blue cap (9 µL)

**Important:** The hybridization reaction is followed by a magnetic bead purification to isolate targeted sequences of interest from non-targeted sequences. During each wash step, we recommend pipetting forcefully (without creating bubbles) five to ten times. This will minimize clumping that can non-specifically trap DNA, which will result in improved enrichment of targeted sequences.

Continue with the sequence capture platform protocol until reaching the PCR step.

### C. Minimizing Evaporation During Incubation

It is critical to take steps to minimize evaporation during the hybridization process. Recommended equipment and consumables can be found in Agilent SureSelect User Manuals, such as in the table “Alternative Capture Equipment Combinations.” Additionally, if PCR plates are used for the hybridization, we recommend that you partially fill surrounding, non-reaction wells with nuclease-free water. This will help to minimize evaporation of your samples.

## IV. Protocol

### D. Post-capture Amplification

The following amplification protocol has been used successfully with the combination of NuGEN library kits, this Ovation Target Capture Module, and the Agilent SureSelect Target Enrichment System. You may also use the amplification recommendations from Agilent SureSelect, but be sure to use the amplification primer mix that is supplied with this kit, **not** the primers supplied with third-party capture kits.

Use the Amplification Primer Mix supplied in the Ovation Target Capture Module to perform the post-capture PCR amplification step. It is important to use our Amplification Primer Mix, as other post-amplification PCR primers may not work with NuGEN sequencing libraries. In addition, many systems use the post-amplification step to add multiplexing barcodes. When using the NuGEN sequencing libraries, the multiplex barcodes have already been added during the library preparation step during adaptor ligation.

**Table 3. Thermal Cycler Programming**

AMPLIFICATION	
<b>Program 1</b> Amplification	72°C – 2 min, 18 cycles* (94°C – 30 sec, 60°C – 30 sec, 72°C – 1 min), hold at 10°C

**Table 4. Amplification Mixture**

REAGENT	VOLUME
Purified, selected library	30 µL
10X Standard Taq Reaction Buffer (included with New England Biolabs Cat. #M0273)	8 µL
25 mM MgCl <sub>2</sub>	8 µL
5% DMSO	4 µL
Taq DNA polymerase (New England Biolabs Cat. #M0273)	1 µL
10 mM dNTP mix (each, New England Biolabs Cat. #N0447)	4 µL
Amplification Primer Mix (red: P2 VER 5)	4 µL
Nuclease-free Water	21 µL
<b>Total volume:</b>	<b>80 µL</b>

## IV. Protocol

1. Prepare a PCR enrichment mix by combining the reagents in a PCR tube according to the volumes shown in Table 4.
2. Place the tubes in a pre-warmed thermal cycler programmed to run Program 1 (Library Amplification; see Table 3).
3. Purify amplified library sample using Agencourt AMPure XP beads, following the Library Purification protocol from the appropriate NuGEN library system user guide.
4. Validate the library as described in the Illumina user guides for DNA library construction. Refer to the appropriate NuGEN library system user guide for information regarding analysis of multiplexed libraries.

## V. Technical Support

For help with any of our products, please contact NuGEN Technical Support at 650.590.3674 (direct) or 888.654.6544, option 2 (toll-free, U.S. only). You may also send faxes to 888.296.6544 (toll-free) or email [techserv@nugen.com](mailto:techserv@nugen.com).

In Europe contact NuGEN at +31(0)135780215 (Phone) or +31(0)135780216 (Fax) or email [europe@nugen.com](mailto:europe@nugen.com).

In all other locations, contact your NuGEN distributor for technical support.

### A. Using the Ovation Target Capture Module with Agilent SureSelect Target Enrichment

The following instructions describe how to use NuGEN NGS libraries and the Ovation Target Capture Module (Part No. 0332) with Agilent SureSelect Target Enrichment (Agilent User Guide G7530-90000). We recommend reviewing section I.A. of this user guide for a list of compatible NuGEN NGS library kits.

1. When preparing libraries prior to target capture, follow the Library Preparation protocol for the appropriate NuGEN NGS library kit rather than following the protocol in Chapter 2 (Sample Preparation) of the Agilent SureSelect User Guide.
2. When performing target enrichment of the NuGEN libraries, follow Chapter 3 (Hybridization) of the SureSelect User Guide with the following modifications:
  - If you intend to perform a multiplex capture experiment, you may pool samples at this stage. However, you must ensure that the total input of pooled material for hybridization step is 500 ng in 3.4  $\mu$ L.
  - When making the "SureSelect Block Mix" (Step 1.6, Table 20, page 42), replace the SureSelect Indexing Block #3 (brown cap) with an equal volume (0.6  $\mu$ L per reaction) of Library Adaptor Blocking Mix (yellow cap) from NuGEN's Ovation Target Capture Module kit.
  - Complete the steps in Chapter 3, including the final bead purification.
3. During post-capture library amplification, use the NuGEN-provided Amplification Primer Mix (P2 VER 5, red cap) from the Ovation Target Capture Module instead of the amplification primers provided by Agilent. You may use either:
  - a. the amplification reagents and conditions recommended in the Ovation Target Capture Module User Guide or
  - b. the amplification conditions in Step 1, Chapter 4 (Addition of Index Tags by Post-Hybridization Amplification) of the SureSelect User Guide, using 2.5  $\mu$ L of the NuGEN Amplification Primer Mix for a 50  $\mu$ L reaction.

**Note:** For the first few times performing this protocol, and whenever changes are made, such as changing the input amount or hybridization time, we recommend monitoring PCR amplification both pre- and post-capture in order to avoid unnecessary amplification cycles and minimize PCR bias. This can be accomplished by measuring DNA concentration using an intercalating dye (such as Invitrogen Qubit or Picogreen) or by running an aliquot on an agarose gel between cycles.

4. After post-amplification purification, analysis and sequencing, follow the protocol in Chapter 4 of the SureSelect User Guide from step 2 onwards.



## VI. Appendix

### B. Frequently Asked Questions (FAQs)

**Q1. Can I use the PCR primers provided with the target capture system?**

No. The Amplification Primer Mix provided with the Ovation Target Capture Module is specifically designed for use with the workflow described in this user guide. Many systems use the post-capture PCR step to add multiplexing barcodes. This is not required with the NuGEN Ovation Target Capture Module workflow.

**Q2. Is the Ovation Target Capture Module compatible with sequencing libraries prepared by other methods?**

No. The Ovation Target Capture Module is only compatible with the NuGEN sequencing library preparation kits listed in this user guide.

**Q3. Can I use the Ovation Target Capture Module with Ovation Rapid Library Systems?**

The yield of the Ovation Rapid Library Systems is not sufficient for the Target Capture methods discussed in this user guide. If the end user has a Target Capture method for which Ovation Rapid Library Systems produce sufficient amounts of library, the Ovation Target Capture Module would be suitable for blocking and subsequent PCR enhancement.

### C. Update History

This document, the Ovation Target Capture Module user guide (M01291 v6) includes the following updates:

Description	Section	Page(s)
Changed product name from Encore Target Capture Module to Ovation Target Capture Module	Throughout	Throughout

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