

# Olink® Explore 384/3072 Sequencing using NovaSeq<sup>™</sup> X Plus

User Manual

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### Part 1: Overview

### 1. Introduction

#### 1.1 Intended use

Olink® Explore 384, Olink® Explore 4x384, and Olink® Explore 3072, from now on called Olink® Explore 384/3072, is a multiplex immunoassay platform for human protein biomarker discovery. The product is intended for Research Use Only, and not for use in diagnostic procedures. The laboratory work shall only be run by trained laboratory staff. Data processing shall only be performed by trained staff. The results are meant to be used by researchers in conjunction with other clinical or laboratory findings.

#### 1.2 About this manual

This manual provides the instructions needed to sequence Olink® Explore 384/3072 libraries on Illumina® NovaSeq™ X and Illumina® NovaSeq<sup>™</sup> X Plus using the NovaSeq<sup>™</sup> X Series 1.5B Reagent Kit (100 Cycle) or the NovaSeq<sup>™</sup> X Series 10B Reagent Kit (100 Cycle). For full instructions on how to perform a sequencing run, refer to the manufacturer's instructions.

The instructions described in this manual must be strictly and explicitly followed. Any deviations throughout the laboratory steps may result in impaired data.

For instructions on how to run the Olink® Explore 384/3072 Reagent Kits, refer to the applicable Olink® Explore User Manual listed in 2. Associated documentation.

For data processing and analysis of the Olink® Explore sequence results, refer to applicable software User Manual listed in 2. Associated documentation.

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#### Definition of alert levels

The following alert levels are used in the Olink user manuals:



 $rac{I}{I}$  WARNING: Indicates a potentially hazardous situation which, if not avoided, could result in injury.



**IMPORTANT:** Indicates an important action that may impair the results if not performed correctly.



**NOTE:** Contains information that can make it easier to understand or perform a certain task.



> SAFE STOPPING POINT: Indicates a step where the protocol can be safely paused and restarted at a later time.



TIME SENSITIVE STEP: Indicates a step that must be performed within a limited time period. Results may be impaired if not performed correctly.

### 2. Associated documentation

### 2.1 Olink documentation

#### **Kit User Manuals**

- Olink® Explore Overview User Manual
- Olink® Explore 384 User Manual
- Olink® Explore 4 x 384 User Manual
- Olink® Explore 3072 User Manual

#### **Software User Manuals**

- Olink® NPX Explore 3072 User Manual
- NPX<sup>™</sup> Explore HT & 3072 User Manual
- Olink® Explore CLI Technical Information
- NPX™ Explore CLI HT & 3072 Technical Information

### 2.2 Other documentation

For information on the Illumina Novaseq X and the Illumina Novaseq X Plus, read the following technical information that can be found on

#### www.illumina.com:

Technical information for using the NovaSeq X or NovaSeq X Plus Sequencing System, Doc no 200027529

## 3. Technical support

For technical support, contact Olink Proteomics at <a href="mailto:support@olink.com"><u>support@olink.com</u></a>.

### Part 2: Laboratory instructions

# 4. Plan the sequencing run

### 4.1 Olink® Explore 384/3072 (96 samples)

Two Olink® Explore 384 (96 samples) libraries can be sequenced per NovaSeq X 1.5B flow cell. After pooling and dilution/denaturation, each library is loaded on a separate lane of the flow cell.

Eight Olink® Explore 384/3072 (96 samples) libraries can be sequenced per NovaSeq X 10B flow cell. After pooling and dilution/denaturation, each library is loaded on a separate lane of the flow cell.

#### 4.1.1 Install Olink® custom recipe

Install the applicable custom recipe on the NovaSeq X according to manufacturer's instruction and make sure to use the correct custom recipe for your planned sequencing run.

Library	Flow cell/ Reagent kit	Nr of lanes per library	Custom recipe	NovaSeq X control software	Cycle numbers
Olink Explore 384/3072 (96 samples)	1.5B (100 cycles)	1	Olink_NovaSeqX_1.5B_2Lib_V1	v1.2	24-0-0-0
Olink Explore 384/3072 (96 samples)	10B (100 cycles)	1	Olink_NovaSeqX_10B_8Lib_V2.x	v1.2	24-0-0-0

### 4.2 Olink® Explore 384/3072 (384 samples)

Four Olink® Explore 384/3072 (384 samples) libraries can be sequenced per NovaSeq X 10B flow cell. After pooling and dilution/denaturation, each library is loaded on 2 consecutive lanes on the flow cell (lane 1+2, lane 3+4, lane 5+6, or lane 7+8).

### 4.2.1 Install Olink® custom recipe

Install the applicable custom recipe on the NovaSeq X according to manufacturer's instruction and make sure to use the correct custom recipe for your planned sequencing run.

Library	Flow cell/ Reagent kit	Nr of lanes per library	Custom recipe	NovaSeq X control software	Cycle numbers
Olink Explore 384/3072 (384 samples)	10B (100 cycles)	2	Olink_NovaSeqX_10B_4Lib_V2.x	v1.2	24-0-0-0

### 5. Dilute Olink® Libraries

Olink recommends using a set library dilution of 1:160. The instructions below replace the instructions for library dilution in the manufacturer's instructions. Perform the instructions once per library to be sequenced.

#### **Prepare bench**

- PL/Lib Tube, prepared according to the applicable Olink Explore User Manual
- Illumina Resuspension Buffer (RSB) or nuclease free water
- 1x Microcentrifuge tubes (1.5 mL)
- Manual pipettes (0.5–10 μL, 100–1000 μL)
- Filter pipette tips

#### Before you start

- Thaw the PL/Lib tube if frozen.
- Mark the new microcentrifuge tube as "Dil".

#### Instructions

- 1. Add 795 µL of RSB into the Dil Tube.
- 2. Vortex the PL/Lib Tube and spin down briefly.
- 3. Transfer 5 µL from the PL/Lib Tube to the Dil Tube.
- 4. Vortex the Dil Tube and spin down briefly.
- 5. Use 34 uL of the diluted library for each denaturing reaction.



**NOTE:** One denaturing reaction is needed per lane to be loaded. When loading the same library on multiple lanes, the denaturing reaction can be scaled up accordingly.



**NOTE:** Store the PL/Lib Tube(s) at -20 °C in case of potential rerun(s).

Proceed according to manufacturer's instructions for denaturing the library, loading reagents, and performing the sequencing run. For more information, refer to 2.2 Other documentation.

# 6. Revision history

Version	Date	Description
1.0	2024-04-16	New

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