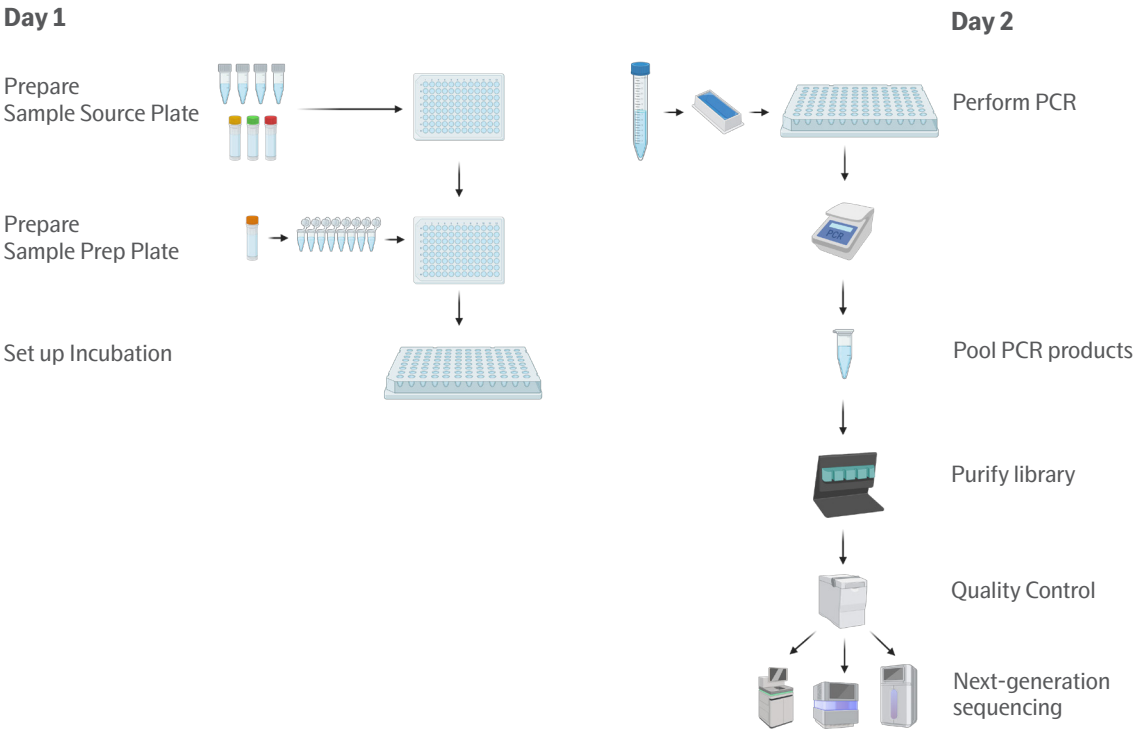
Olink®

Olink® Reveal

Laboratory instructions

For Research Use Only. Not for use in diagnostic procedures.
Before performing the protocol for the first time, please read the Introduction to the Olink Reveal Protocol.

Overview



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Important information

Unless stated otherwise, perform all steps at room temperature and DO NOT use a microplate vortexer like a MixMate.

Technical support and associated documentation

Associated documentation can be downloaded from *olink.com/downloads*.

For questions or guidance, please contact *support@olink.com*.

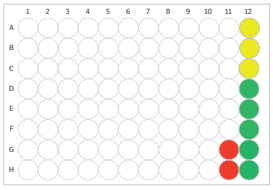
Day 1

Prepare bench	Storage	Fill volume	
Olink® Explore/Reveal Negative Control	-20 °C	160 µL	Thaw at room temperature
Olink® Explore/Reveal Plate Control	-80 °C	160 µL	Thaw at room temperature
Olink® Explore/Reveal Sample Control	-80 °C	95 µL	Thaw at room temperature
Olink® Reveal Sample Buffer	-20 °C	1375 µL	Thaw at room temperature
Olink® Reveal Incubation Plate	4 °C		Store at room temperature 15–20 minutes
Samples			Thaw at room temperature if frozen
2x 96-well PCR Plates			Mark one plate "Sample Source Plate"
			Mark one plate "Sample Prep Plate"
1x 8-well strip			
Single-channel pipettes			
Multi-channel pipettes			
Filter pipette tips			
Adhesive films			
Vortex mixer			
Mini centrifuge			
Plate centrifuge			

Prepare Sample Source Plate

1. Vortex then spin down the samples, Negative Control, Plate Control, and Sample Control.
2. Pipette 10 µL of the samples and controls into the Sample Source Plate according to the plate layout in ①.
3. Seal the Sample Source Plate with adhesive film and centrifuge at 400–1,000 x g for 1 minute.
4. Store the Sample Source Plate at 4 °C if used the same day, otherwise at -80 °C.

①



Prepare Sample Prep Plate

Prepare the Sample Prep Plate no more than 1 hour before starting the incubation. Store at 4 °C until use.

1. Vortex the Sample Source Plate for 5–10 seconds then centrifuge it at 400–1,000 x g for 1 minute.
2. Vortex the Sample Buffer then spin it down.
3. Pipette 160 µL of Sample Buffer to each well of an 8-well strip tube and spin it down.
4. Reverse-pipette 12 µL of Sample Buffer from the 8-well strip to every well of the Sample Prep Plate. Use a multi-channel pipette without changing tips.
5. Forward-pipette 4 µL of the samples and controls from the Sample Source Plate to the corresponding well of the Sample Prep Plate. Use a multi-channel pipette and change pipette tips between each column.
6. Seal the Sample Prep Plate with adhesive film and vortex for 5–10 seconds. Make sure that all wells are vortexed thoroughly. Centrifuge at 400–1,000 x g for 1 minute.

Note that if a different plate map is used, such as to randomize the locations of the Negative Controls, Plate Controls, and Sample Controls, please record the locations and provide this to the NPX™ Map software later.

Set up incubation

1. Remove the Incubation Plate from the packaging.
2. Centrifuge the Incubation Plate at 400–1,000 x g for 1 minute before removing the seal.
3. Forward-pipette 4 µL from each well of the Sample Prep Plate to the corresponding well of the Incubation Plate.
   - Use a multi-channel pipette.
   - Dispense samples towards the wall of the wells as shown in ②.
   - DO NOT touch the dried reagents in the well with the pipette tip.
   - Change tips between each column.
4. Seal the Incubation Plate with adhesive film.
   DO NOT vortex the Incubation Plate.
5. Centrifuge at 400–1,000 x g for 1 minute.
6. Store the Incubation Plate at room temperature in the dark for 16–24 hours.

② 

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Note that when setting up for incubation, the room temperature range should be 18–27 °C. The best results are achieved when the temperature is consistently 25 °C. If using a thermal cycler for incubation, set the heated lid at 30 °C.

Prepare for day 2

7. Thaw the ● PCR Additive at room temperature overnight.
8. Thaw the ● PCR Solution at 4 °C overnight.
9. Store 20 mL Milli-Q water in the fridge at 4 °C.

Day 2

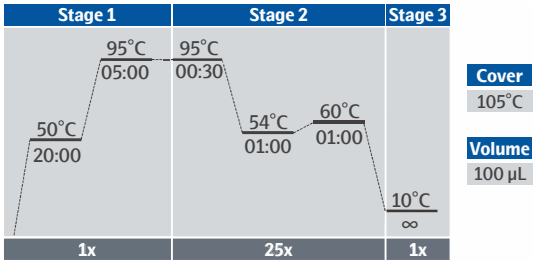
Prepare bench	Storage	Fill volume	
Olink® Reveal PCR Additive		1520 µL	Thawed from Day 1
Olink® Reveal PCR Solution	4 °C	1520 µL	Thawed from Day 1
Olink® Reveal Enzyme A	-20 °C	320 µL	Keep on freezing block at -20 °C
Olink® Reveal Enzyme B	-20 °C	50 µL	Keep on freezing block at -20 °C
Olink® Reveal Incubation Plate			Prepared from Day 1
1x 15 mL tube			Mark the tube "PCR Mix"
4x 2 mL tubes			Mark the tubes: "PCR Pool", "70% EtOH", "BP" and "Lib"
8-well strip with caps			
Reagent reservoir (25 mL)			
Multi-channel pipettes			
Single-channel pipettes			
Filter pipette tips			
Adhesive films			
AMPure XP beads			
96% ethanol			
Milli-Q water			
Magnetic stand			
Timer			
Thermal cycler with a 96-well block			
Vortex mixer			
Mini centrifuge			
Plate centrifuge			

Before you start

1. Keep the PCR Enzyme A and PCR Enzyme B on a freezing block at -20 °C.
2. Ensure that the PCR Additive and the PCR Solution are thawed and have reached room temperature.
3. Switch on the thermal cycler for it to pre-heat.

Perform PCR

1. Vortex the PCR Solution and PCR Additive then spin them down.
2. Set the PCR protocol as follows and start it. Pause the PCR protocol when then PCR block reaches 50 °C.



3. Take out the Incubation Plate from the storage and centrifuge at 400–1,000 x g for 1 minute.
4. Spin down PCR Enzyme A and PCR Enzyme B briefly. DO NOT vortex them. Keep them on a freezing block at -20 °C.

Day 2 (cont.)

5. Prepare the PCR Mix tube using a 15 mL tube. DO NOT use a 50 mL tube. Add reagents according to the order listed, and invert and vortex the PCR Mix tube for 3–5 seconds where noted in the table.
   Pipette the PCR Additive and the PCR Solution carefully from the surface due to high volume in the tubes.

Order	Reagents	Volume	
1	Milli-Q water (4 °C)	10,000 µL	
2	Reveal PCR Additive	1,310 µL	Invert and vortex
3	Reveal PCR Solution	1,310 µL	
4	Reveal Enzyme A	263 µL	
5	Reveal Enzyme B	34 µL	Invert and vortex
	Total	12,917 µL	

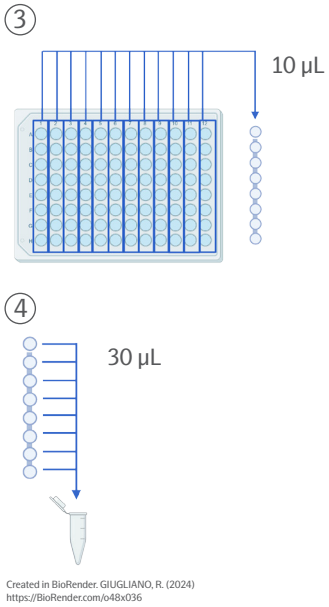
6. Pour the PCR Mix into the reagent reservoir.
7. Carefully remove the adhesive film from the Reveal Incubation Plate.
8. Set the timer for 10 minutes then start it. Transfer 96 µL of PCR Mix to the upper parts of each of the well walls of the Incubation Plate. Use reverse pipetting and DO NOT change tips.
   DO NOT let the tips come in contact with contents of the wells.
9. Seal the Incubation Plate with adhesive film and vortex for 3–5 seconds. Centrifuge it at 400–1,000 x g for 1 minute.
10. Wait till the timer ends then place the Incubation Plate in the thermal cycler. Set the PCR protocol to resume. The protocol takes 2 hours.
11. Continue to [Pool PCR products] or store the plate at 4 °C if used later on the same day.

Safe Stopping Point: The plates can be stored for -20 °C for up to 4 weeks.

Pool PCR products

1. Vortex the Incubation plate for 3–5 seconds then centrifuge it at 400–1,000 x g for 1 minute.
2. Pipette 10 µL from each column into one 8-well strip, one column at the time, according to ③. Use a multi-channel pipette and change tips after each column. Vortex and spin down the 8-well strip.
   Precipitate may form at this step. It is okay to include it for further steps.
3. Vortex and spin down the 8-well strip.
4. Pipette 30 µL from each well of the 8-well strip into the PCR Pool tube according to ④. Change tips after each well.
5. Vortex and spin down the PCR Pool tube.
6. Continue to [Purify library] or store the PCR Pool tube at 4 °C if used later on the same day.

Safe Stopping Point: The PCR Pool tube can be stored for -20 °C for up to 4 weeks.



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Day 2 (cont.)

Purify library

1. Let the refrigerated AMPure XP bottle reach room temperature.
2. Prepare fresh 70% ethanol in the 70% EtOH tube:

Milli-Q water	0.5 mL
96% ethanol	1.5 mL
Total	2 mL

3. Shake and vortex the bottle of AMPure XP vigorously to resuspend the magnetic beads.
4. Add PCR products from the PCR Pool tube and beads to the BP tube according to the table. Mix by pipetting the contents of the BP tube up and down 10 times.

PCR products	50 µL
Magnetic beads	80 µL
Total	130 µL

5. Incubate the BP tube for 5 minutes at room temperature.
6. Place the BP tube on the magnetic stand for 2 minutes to separate beads from the supernatant.
7. Open the caps carefully and discard 125 µL of the supernatant, leaving 5 µL behind. Use a single-channel pipette. DO NOT disturb the bead pellet and do not remove the tube from the magnetic stand.
8. Pipette 500 µL from the 70% EtOH tube to the BP tube. Pipette onto the opposite wall from the bead pellet. DO NOT disturb the bead pellet.
9. Incubate the BP tube for 30 seconds.
10. Aspirate and discard all the ethanol using a pipette. DO NOT disturb the beads pellet.
11. Repeat steps 8–10 one more time.
12. Use a smaller pipette to remove any residual ethanol.
13. Leave the BP tube with the lid open for 2 minutes on the magnetic stand to dry the bead pellet.
14. Remove the BP tube from the magnetic stand.
15. Add 50 µL of Milli-Q water and pipette mix towards the bead pellet 10 times to resuspend it.
16. Incubate the BP tube for 2 minutes at room temperature.
17. Place the BP tube back onto the magnetic stand and leave it for 1 minute to allow the bead pellet to separate from the eluted libraries.
18. Pipette 45 µL of eluate from the BP tube while it is on the magnetic stand to the Lib tube. DO NOT disturb or aspirate the bead pellet.
19. Discard the BP tube.

Safe Stopping Point: The Lib tube can be stored at -20 °C for up to 12 weeks.

Store the PCR Pool tube at -20 °C in case the purification needs to be repeated.

Quality control

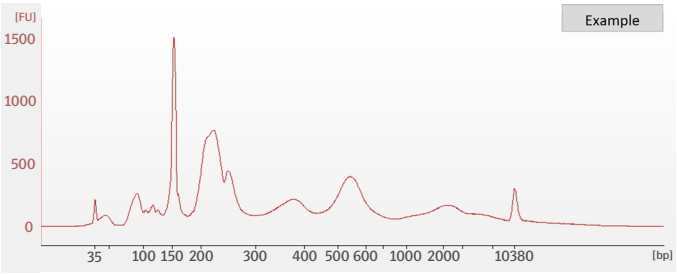
Prepare bench

- 2100 Bioanalyzer or 4200 TapeStation
- Bioanalyzer High Sensitivity DNA kit or TapeStation D1000 ScreenTape assay

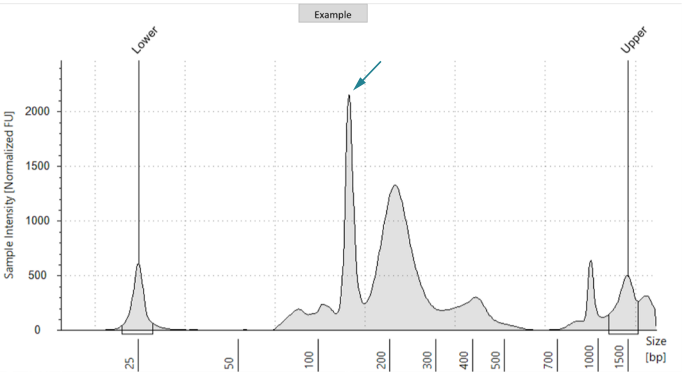
Quality control

1. Run the library on the 2100 Bioanalyzer or the 4200 TapeStation using the High Sensitivity DNA kit according to the manufacturer's instructions.
2. Check that the resulting electropherograms display a single or double peak at 150 bp. Peaks of larger sizes represent bubble products and do not impact sequencing results.

Electropherograms Bioanalyzer



Electropherograms TapeStation



Next-generation sequencing

The procedure for next-generation sequencing described below is intended for users who are already familiar with the method. Please refer to manufacturer's instruction for full details.

Before you start

1. Install the Olink custom recipe on the sequencer in advance. To do so, please contact *support@olink.com*.
2. Install ngs2counts, based on the document Olink® Map Preprocessing Technical Information.

Dilute libraries

3. Dilute the libraries based on the flowcell intended for use. Use the recommended dilution with either MilliQ water or resuspension buffer (RSB, provided by Illumina) given in the table. When diluting libraries, the volume of purified library used should be at least 4 µL.

Sequencer	Flowcell	Dilution	Dilution buffer	Sequencer control software	Custom recipe
Illumina NextSeq 2000	P4 XLEAP-SBS Reagent Kit (50 cycles)	1:100 and then 1:8	RSB	V1.7.x	Olink_NSQ2K_P4_V1
Illumina NovaSeq X or NovaSeq X Plus	1.5B Reagent Kit (100 cycles)	1:300	Milli-Q water	v1.2.x	Olink_NovaSeqX_ 1.5B_1Lib_V1
	10B Reagent Kit (100 cycles)	1:300	Milli-Q water	v1.2.x	Olink_NovaSeqX_ 10B_8Lib_V2.1
Illumina NovaSeq 6000	S1 Reagent Kit v1.5 (100 cycles)	1:350	Milli-Q water	v1.8	Olink_NovaSeq6K_ SP_S1_V1
	S4 Reagent Kit v1.5 (35 cycles)	1:350	Milli-Q water	v1.8	Olink_NovaSeq6K_ XP_SP_V2

4. Following the corresponding manufacturer's instructions on the next steps to load the libraries on to the flowcell. One library is added to each lane of the flowcells.

Note that usage of PhiX is not required.

Revision history

Version	Date	Description
1.0	2025-01-27	New

www.olink.com

1513, v1.0, 2025-01-27