



## RELEASE NOTES

# AVENIO Edge KAPA User-Filled Kits (KUFK) EvoPrep and EvoPlus V2

### VERSION 1.0

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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# Preface

## Regulatory Disclaimer

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

The AVENIO Edge instrument can be used for Research Use Only (RUO) user-defined workflows. User-defined protocols created using AVENIO Designer Software are for research use only workflows, not for use in diagnostic procedures.

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### Manufacturer and Distribution





Manufacturer Roche Sequencing Solutions, Inc.  
2881 Scott Blvd  
Santa Clara, CA 95050 USA

Distribution Roche Diagnostics GmbH  
Sandhofer Strasse 116  
68305 Mannheim Germany

Distribution in USA Roche Diagnostics  
9115 Hague Road  
Indianapolis, Indiana 46256 USA

# Conventions Used in This Manual

## Symbols used in the publication

Symbol	Description
	<b>Important Note:</b> Highlights information that is critical for optimal performance of the system. May also indicate that loss of data or invalid data could occur if the precautions or instructions are not observed.
	<b>Information Note:</b> Identifies items of general interest and additional information about the topic or procedure being described.

## Text

Conventions	Description
Numbered listing	Indicates steps in a procedure that must be performed in the order listed.
<i>Italic type, blue</i>	Identifies a resource in a different area of this manual or on a web site.
<i>Italic type</i>	Identifies the names of dialog boxes, windows, tabs, panels, views, or message boxes in the software.
<b>Bold type</b>	Identifies names of menus and controls (buttons, checkboxes, etc.) in the software.

# 01

Preface	2
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Restrictions and Liabilities	2
Conditions of Use	2
Trademarks	2
Use Restrictions	2
Regulatory Disclaimer	3
Contact Information	3
Conventions Used in This Manual	4
Table of Contents	5

# 02

Chapter 1. Before You Begin	6
-----------------------------	---

---

Workflow Overview	7
Reagents and KAPA User-Filled Kits Available From Roche	9

# 03

Chapter 2. Library Preparation Protocols	11
------------------------------------------	----

---

Executing the EvoPrep Library Preparation Protocol	12
Executing the EvoPlus V2 Library Preparation Protocol	15

# 04

Chapter 3. Target Enrichment Protocols	18
----------------------------------------	----

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Executing a Target Enrichment UDW Protocol	19
--------------------------------------------	----

# Chapter 1.

## Before You Begin

## Workflow Overview

The AVENIO Edge EvoPrep and the AVENIO Edge EvoPlus V2 workflows utilize library preparation to create DNA sample libraries for subsequent target enrichment and sequencing. The AVENIO Edge EvoPrep and AVENIO Edge EvoPlus V2 library preparation workflows can be run with up to 48 samples.

The EvoPrep\_LP\_DEML library preparation steps include End Repair and A-tailing, followed by Ligation, bead cleanup, PCR amplification, and a final bead cleanup as shown in [Figure 1](#). The workflow utilizes the KAPA EvoPrep Kits, AVENIO Edge KAPA User-Filled Kits (KUFK), and other AVENIO Edge reagent kits. The output of this protocol are DNA libraries that can proceed directly into a target enrichment User-Defined Workflow (UDW) that is generated using the AVENIO Designer Software.

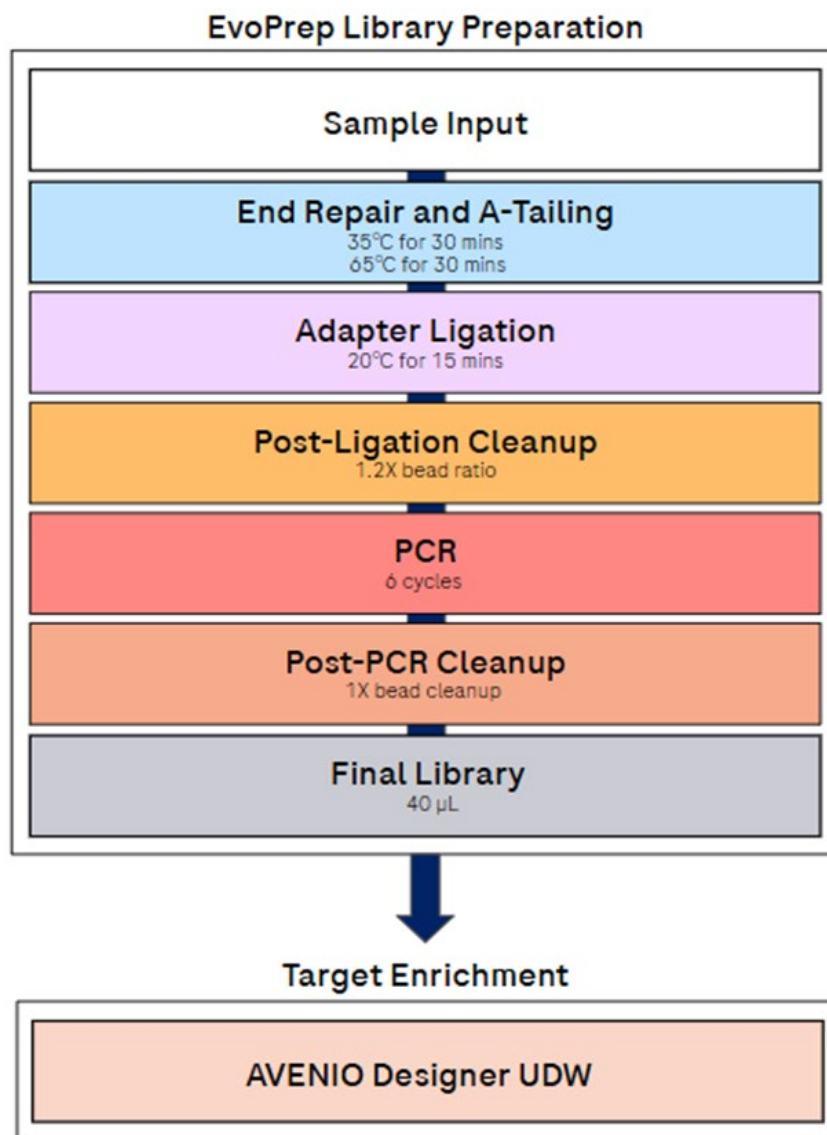


Figure 1: Overview of the AVENIO Edge EvoPrep workflow with subsequent target enrichment

The EvoPlusV2\_LP\_DEML library preparation steps include Fragmentation and A-tailing, followed by Ligation, bead cleanup, PCR amplification, and a final bead cleanup as shown in [Figure 2](#). The workflow utilizes the KAPA EvoPlus V2 Kits, AVENIO Edge KUFK plastics, and other AVENIO Edge reagent kits. The output of this protocol are DNA libraries that can proceed directly into a target enrichment UDW that is generated using the AVENIO Designer Software.

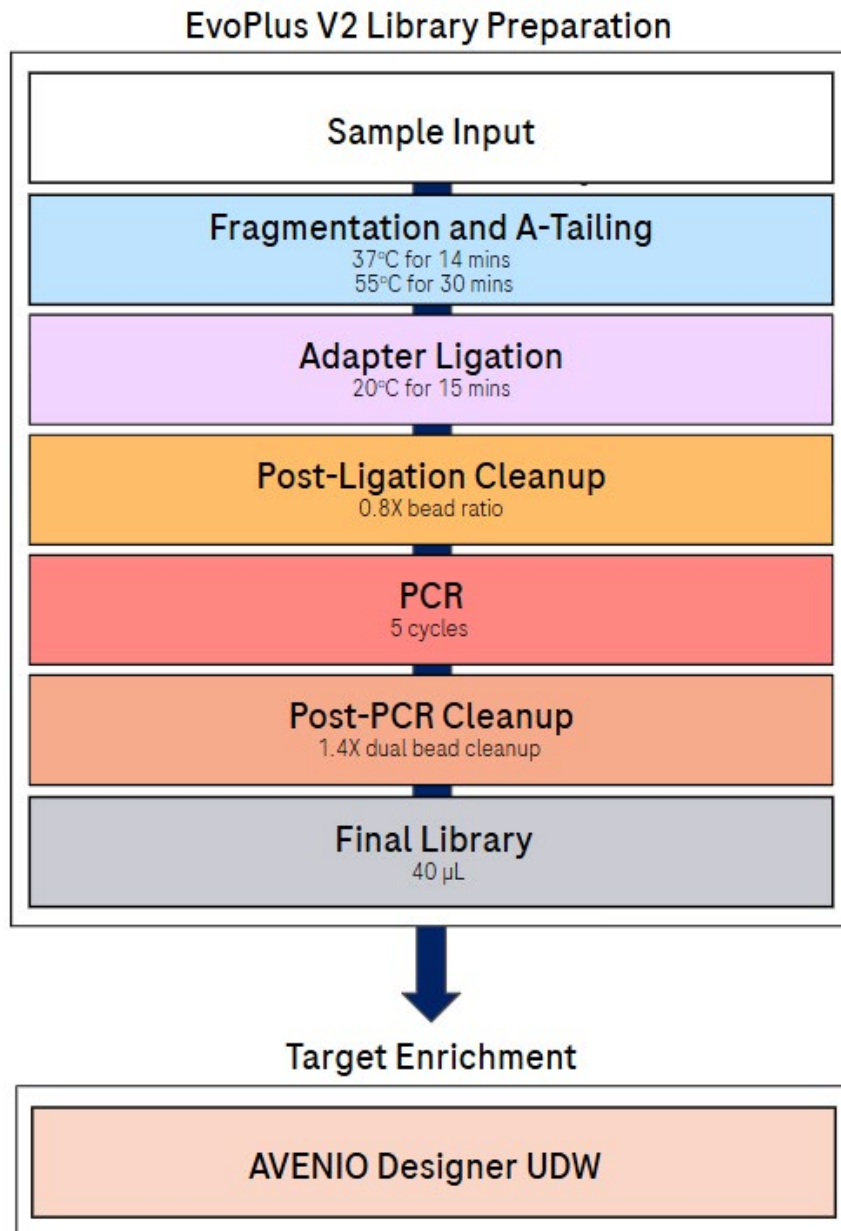


Figure 2: Overview of the AVENIO Edge EvoPlus V2 workflow with subsequent target enrichment

## Reagents and KAPA User-Filled Kits Available From Roche

### AVENIO Edge EvoPrep Library Preparation

Component	Package Size/Contents	Material Number
KAPA EvoPrep Kit (96 rxn)	Each kit contains: KAPA ERAT ReadyMix KAPA Ligation ReadyMix KAPA HiFi HotStart ReadyMix (2x)	10096039001
AVENIO Edge HyperPure Beads	Each kit contains: Cleanup Beads Empty 80% Ethanol Trough	09200967001
AVENIO Edge HyperPlex UMI Adapters (24 rxn)	Each kit contains: HyperPlex UMI Adapters (24 rxn)	09200983001
AVENIO Edge UDI Primer Mixes, 1-96	Dried down 96 primer pairs in a 96 well processing plate	09200959001
AVENIO Edge Empty Troughs Box A	Each box contains empty troughs: Ethanol Trough, b01 Trough 1, b91 Trough 2, b92* Trough 3, b93 Trough 4, b94 Trough 5, b95	10303042001
AVENIO Edge Empty 2.0 mL Tubes Box A	Each box contains empty 2 mL tubes: Tube 1, d91 Tube 2, d92* Tube 3, d93 Tube 4, d94* Tube 6, d96 Tube 7, d97 Tube 8, d98 Tube 9, d99 Tube 10, d9A	10303310001
AVENIO Edge Empty Mini Racks Box A	Each box contains empty mini racks: Mini Rack 1, a91* Mini Rack 2, a92 Mini Rack 3, a93 Mini Rack 4, a94 Mini Rack 5, a95	10302992001
AVENIO Edge Empty Mini Rack Tubes	Each box contains 240 empty mini rack tubes	10303581001

\*KUFK plastics used in the EvoPrep\_LP\_DEML library preparation protocol

## AVENIO Edge EvoPlus V2 Library Preparation

Component	Package Size/Contents	Material Number
KAPA EvoPlus V2 Kit (96 rxn)	Each kit contains: KAPA FragTail ReadyMix KAPA Ligation ReadyMix KAPA HiFi HotStart ReadyMix (2X)	09420053001
AVENIO Edge HyperPure Beads	Each kit contains: Cleanup Beads Empty 80% Ethanol Trough	09200967001
AVENIO Edge HyperPlex UMI Adapters (24 rxn)	Each kit contains: HyperPlex UMI Adapters (24 rxn)	09200983001
AVENIO Edge UDI Primer Mixes, 1-96	Dried down 96 primer pairs in a 96 well processing plate	09200959001
AVENIO Edge Empty Troughs Box A	Each box contains empty troughs: Ethanol Trough, b01 Trough 1, b91 Trough 2, b92* Trough 3, b93 Trough 4, b94 Trough 5, b95	10303042001
AVENIO Edge Empty 2.0 mL Tubes Box A	Each box contains empty 2 mL tubes: Tube 1, d91 Tube 2, d92 Tube 3, d93 Tube 4, d94* Tube 5, d95 Tube 6, d96* Tube 7, d97 Tube 8, d98 Tube 9, d99 Tube 10, d9A	10303310001
AVENIO Edge Empty Mini Racks Box A	Each box contains empty mini racks: Mini Rack 1, a91* Mini Rack 2, a92 Mini Rack 3, a93 Mini Rack 4, a94 Mini Rack 5, a95	10302992001
AVENIO Edge Empty Mini Rack Tubes	Each box contains 240 empty mini rack tubes	10303581001

\*KUFK plastics used in the EvoPlusV2\_LP\_DEML library preparation workflow

# Chapter 2.

## Library Preparation Protocols

## Executing the EvoPrep Library Preparation Protocol

### AVENIO Edge EvoPrep Library Preparation

Reagent Type	KUFK Plastic	Reagent	Exact Fill Volume
Reagent Cool Tubes	Tube 2, d92	KAPA EvoPrep ERAT ReadyMix	825 µL
	Tube 4, d94	KAPA EvoPrep Ligation ReadyMix	430 µL
Reagent Trough	Trough 2, b92	10mM Tris-HCl pH 8.0	30 mL
Reagent Mini Rack	Mini Rack 1, a91	N/A	N/A
		KAPA HiFi HotStart ReadyMix (2X)	865 µL
		N/A	N/A
		N/A	N/A

Table 1: Overview of KUFK plastics and their respective reagents

### KUFK Reagent Cool Tubes and Reagent Troughs

For AVENIO Edge KUFK reagent cool tubes and reagent troughs, refer to the [AVENIO Edge KAPA User-Filled Kits \(KUFK\) EvoPrep Whole-Genome Sequencing \(WGS\) PCR-free Workflow](#) for detailed instructions. The reagent cool tubes and reagent troughs should be prepared as shown in [Table 1](#).

### KUFK Reagent Mini Racks

For AVENIO Edge KUFK reagent mini racks, refer to the instructions as described below.

1. Obtain the reagent, AVENIO Edge Empty Mini Rack, and AVENIO Edge Mini Rack Tube as shown in [Table 2](#).



**When collecting the reagent, check the name on the reagent bottle label to confirm that the KAPA HiFi HotStart ReadyMix (2X) is pulled to thaw.**

2. Thaw the KAPA HiFi HotStart ReadyMix (2X) on ice for approximately 90 minutes. Keep reagent on ice once thawed.



**Ensure that the reagent is fully thawed prior to proceeding.**

Reagent Mini Rack Supplies		
AVENIO Edge Mini Rack	Mini Rack Tube Quantity Needed Per Rack	KAPA Reagent
Mini Rack 1, a91	1	KAPA HiFi HotStart ReadyMix (2X)

Table 2: Reagent and KUFK plastics per each reagent mini rack

3. Place the mini rack tube into the reagent mini rack in the position indicated in [Table 3](#). Reagent mini rack orientation convention is indexed from Position 0 to Position 3 starting from the side with the printed label. Any tube position with contents listed as “N/A” is not used in the workflow and does not need a reagent mini rack tube loaded in that position. See [Figure 3](#) for an example of the reagent mini rack tube placement.



Figure 3: Reagent mini rack preparation. Tube is placed into reagent mini rack position 1. Positions 0, 2, and 3 are empty.

4. Firmly press down the mini rack tube into place; an audible clicking sound should be heard when the tube is fully seated. [Figure 4](#) shows the correct placement of the mini rack tubes.



**Tubes that are not fully locked into place can cause volume detection issues or hardware failures with the pipetting arm on the AVENIO Edge instrument.**

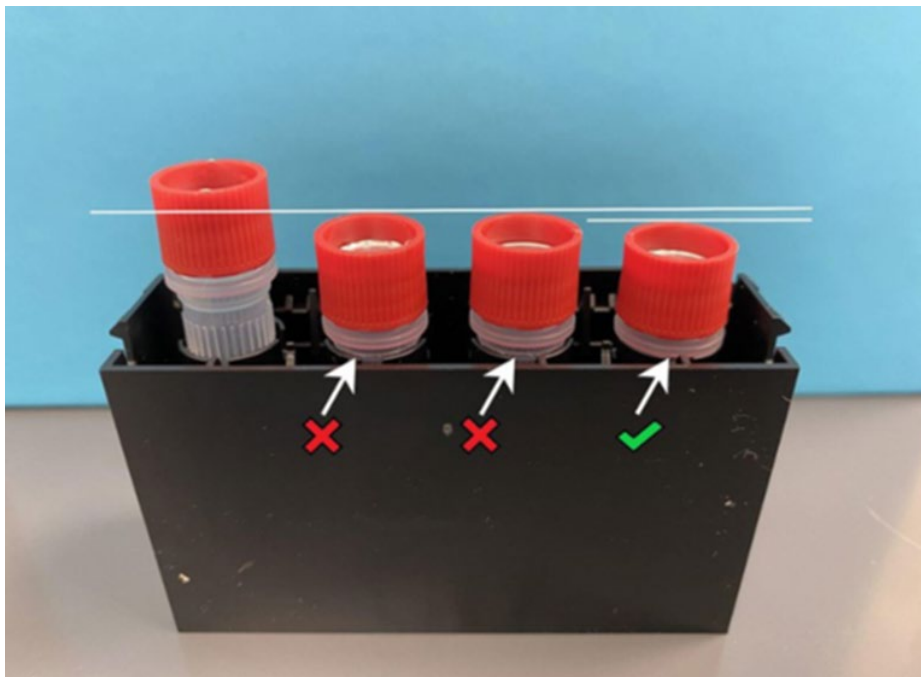


Figure 4: Reagent mini rack tubes at three different height placements in the reagent mini rack with arrows and reference lines highlighting the differences. Position 0 tube is loosely set into the reagent mini rack. Position 1 and 2 tubes are partially pressed down into place as indicated by the small gap between the tube lip and the reagent mini rack tube frame. Position 3 tube is fully pressed down into the reagent mini rack.



**Press the tube down before adding any reagents. Pressing down an uncapped tube that contains liquid into the reagent mini rack will result in reagent splashback.**

5. Visually check that the bottom of the mini rack tube is flush with the bottom of the reagent mini rack to confirm the tube is fully seated.
6. Fully remove the cap from the mini rack tube.
7. Fill the mini rack tube with the reagent and volume indicated in [Table 3](#). Note that Position 0 starts at the reagent mini rack tube position that is closest to the reagent mini rack printed label. This reagent requires accurate and precise filling. Use a low-retention pipette tip when transferring the reagents into the KUFK plastics.



**Do not over or underfill the reagent mini rack.**

Reagent Mini Rack Fill Volumes			
AVENIO Edge Mini Rack	Position	Contents	Exact Fill Volume (µL)
Mini Rack 1, a91	0	N/A	N/A
	1	KAPA HiFi HotStart ReadyMix (2X)	865 µL
	2	N/A	N/A
	3	N/A	N/A

Table 3: Reagent mini rack fill volumes

8. Cover the tube with a pierceable reagent mini rack cap and fully tighten the cap. Color-coding is not required and is up to the user.
9. Recheck that the reagent mini rack tube is firmly pressed down into the reagent mini rack.
10. Hold the reagent mini rack upright between your fingers. Swing the reagent mini rack outward by moving your arm away from your body with a quick downward flick of the wrist. Repeat this action 3 times in order to bring the liquid contents to the bottom of the reagent tube.
11. Tap the reagent mini rack gently on a firm flat surface at least 5 times to ensure the liquid has settled to the bottom of the tube.
12. Place prepared reagent mini rack over ice.
13. Refer to page 32 of the [AVENIO Edge User-Defined Workflow DNA Hybridization Workflow](#) for detailed instructions on loading the prepared reagent mini rack.

## AVENIO Edge Reagents

Refer to the [AVENIO Edge User-Defined Workflow DNA Hybridization Workflow](#) for detailed instructions on handling (pages 18-19) and loading (page 32) of the following:

- AVENIO Edge HyperPlex UMI Adapters (24 rxn)
- AVENIO Edge UDI Primer Mixes

## Executing the EvoPlus V2 Library Preparation Protocol

### AVENIO Edge EvoPlus V2 KUFK Overview

Reagent Type	KUFK	Reagent	Exact Fill Volume
Reagent Cool Tubes	Tube 6, d96	KAPA EvoPlus V2 FragTail ReadyMix	825 µL
	Tube 4, d94	KAPA EvoPlus V2 Ligation ReadyMix	430 µL
Reagent Trough	Trough 2, b92	10mM Tris-HCl pH 8.0	30 mL
Reagent Mini Rack	Mini Rack 1, a91	N/A	N/A
		KAPA HiFi HotStart ReadyMix (2X)	865 µL
		N/A	N/A
		N/A	N/A

Table 4: Overview of KUFK reagent cool tubes, KUFK reagent troughs, and reagents

### KUFK Reagent Cool Tubes and Reagent Troughs

For AVENIO Edge KUFK reagent cool tubes and reagent troughs, refer to the [AVENIO Edge KAPA User-Filled Kits \(KUFK\) EvoPlus V2 Whole-Genome Sequencing \(WGS\) PCR-free Workflow](#) for detailed instructions. The reagent cool tubes and reagent troughs should be prepared as shown in [Table 4](#).

### KUFK Reagent Mini Racks

For AVENIO Edge KUFK reagent mini racks, refer to the instructions as described below.

1. Obtain the reagent, AVENIO Edge Empty Mini Rack, and AVENIO Edge Mini Rack Tube as shown in [Table 5](#).



**When collecting the reagent, check the name on the reagent bottle label to confirm that the KAPA HiFi HotStart ReadyMix (2X) is pulled to thaw.**

2. Thaw the KAPA HiFi HotStart ReadyMix (2X) on ice for approximately 90 minutes. Keep reagent on ice once thawed.



**Ensure that the reagent is fully thawed prior to proceeding.**

Reagent Mini Rack Supplies		
AVENIO Edge Mini Rack	Mini Rack Tube Quantity Needed Per Rack	KAPA Reagent
Mini Rack 1, a91	1	KAPA HiFi HotStart ReadyMix (2X)

Table 5: Reagent and KUFK plastics per each reagent mini rack

3. Place the mini rack tube into the reagent mini rack in the position indicated in [Table 6](#). Reagent mini rack orientation convention is indexed from Position 0 to Position 3 starting from the side with the printed label. Any tube position with contents listed as “N/A” is not used in the workflow and does not need a reagent mini rack tube loaded in that position. See [Figure 5](#) for an example of the reagent mini rack tube placement.



Figure 5: Reagent mini rack preparation. Tube is placed into reagent mini rack position 1. Positions 0, 2, and 3 are empty.

4. Firmly press down the mini rack tube into place; an audible clicking sound should be heard when the tube is fully seated. [Figure 6](#) shows the correct placement of the mini rack tubes.



**Tubes that are not fully locked into place can cause volume detection issues or hardware failures with the pipetting arm on the AVENIO Edge instrument.**

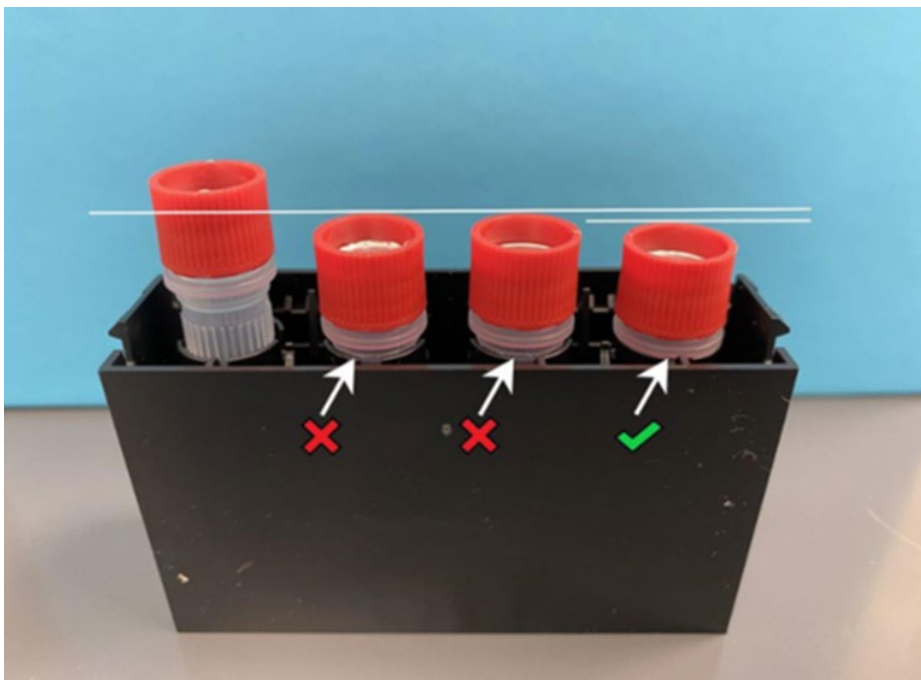


Figure 6: Reagent mini rack tubes at three different height placements in the reagent mini rack with arrows and reference lines highlighting the differences. Position 0 tube is loosely set into the reagent mini rack. Position 1 and 2 tubes are partially pressed down into place as indicated by the small gap between the tube lip and the reagent mini rack tube frame. Position 3 tube is fully pressed down into the reagent mini rack.



**Press the tube down before adding any reagents. Pressing down an uncapped tube that contains liquid into the reagent mini rack will result in reagent splashback.**

5. Visually check that the bottom of the mini rack tube is flush with the bottom of the reagent mini rack to confirm the tube is fully seated.
6. Fully remove the cap from the mini rack tube.
7. Fill the mini rack tube with the reagent and volume indicated in [Table 6](#). Note that Position 0 starts at the reagent mini rack tube position that is closest to the reagent mini rack printed label. This reagent requires accurate and precise filling. Use a low-retention pipette tip when transferring the reagents into the KUFK plastics.



**Do not over or underfill the reagent mini racks.**

Reagent Mini Rack Fill Volumes			
AVENIO Edge Mini Rack	Position	Contents	Exact Fill Volume (µL)
Mini Rack 1, a91	0	N/A	N/A
	1	KAPA HiFi HotStart ReadyMix (2X)	865 µL
	2	N/A	N/A
	3	N/A	N/A

Table 6: Reagent mini rack fill volumes

8. Cover the tube with a pierceable reagent mini rack cap and fully tighten the cap. Color-coding is not required and is up to the user.
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10. Hold the reagent mini rack upright between your fingers. Swing the reagent mini rack outward by moving your arm away from your body with a quick downward flick of the wrist. Repeat this action 3 times in order to bring the liquid contents to the bottom of the reagent tube.
11. Tap the reagent mini rack gently on a firm flat surface at least 5 times to ensure the liquid has settled to the bottom of the tube.
12. Place prepared reagent mini rack over ice.
13. Refer to page 32 of the [AVENIO Edge User-Defined Workflow DNA Hybridization Workflow](#) for detailed instructions on loading the prepared reagent mini rack.

## AVENIO Edge Reagents

Refer to the [AVENIO Edge User-Defined Workflow DNA Hybridization Workflow](#) for detailed instructions on handling (pages 18-19) and loading (page 32) of the following:

- AVENIO Edge HyperPlex UMI Adapters (24 rxn)
- AVENIO Edge UDI Primer Mixes

# Chapter 3.

## Target Enrichment Protocols



## Executing a Target Enrichment UDW Protocol

Refer to the [AVENIO Edge User-Defined Workflow DNA Hybridization Workflow](#) for detailed instructions on generating a target enrichment protocol using the AVENIO Designer Software, reagent handling and loading, and running a target enrichment protocol.



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4300 Hacienda Drive

Pleasanton, CA 94588 USA

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