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TruSeq® Custom Amplicon Low Input Workflow on the MiniSeq™ System

TruSeq Custom Amplicon Low Input

Design Content	Prepare Library	Sequence	Analyze Data
			
< 1 day	6.5 hours	~ 24 hours	< 2 hours
For Custom Panels Only	TruSeq Amplicon Library Prep	MiniSeq System	• Local Run Manager Amplicon • BaseSpace® TruSeq Amplicon App • VariantStudio

This checklist supports the following workflow choices.

Sequencing Instrument:	MiniSeq System
Setup Option	Local Run Manager
Library Preparation Kit:	TruSeq Custom Amplicon Low Input Library Prep
Indexing:	Dual Indexing
Reagent Kits:	MiniSeq Kit
Analysis Workflow:	Amplicon
Analysis Software:	Local Run Manager

MiniSeq sequencing using TruSeq Custom Amplicon Low Input libraries

Set Run Parameters

- ☐1 Log in to Local Run Manager.
- ☐2 Click **Create Run**, and select **Amplicon**.
- ☐3 Enter a run name that identifies the run.
- ☐4 [Optional] Enter a run description.
- ☐5 From the Library Kit drop-down list, select TruSeq Amplicon.
- ☐6 Specify the number of cycles for the run.
- ☐7 Select a variant calling method.
- ☐8 Click **Show advanced module settings** and specify the Read Stitching and Variant Quality Filter settings.
- ☐9 Click **Import Manifests**.
- ☐10 Navigate to the manifest file.
- ☐11 Enter a unique sample ID.
- ☐12 [Optional] Enter a sample description.
- ☐13 Select an Index 1 adapter.
- ☐14 Select an Index 2 adapter.
- ☐15 Select a manifest file.
- ☐16 Select a reference genome.
- ☐17 Click **Save Run**.

Quantify and Dilute DNA

- ☐1 Quantify DNA using a fluorometric method.
- ☐2 Dilute DNA to 10–25 ng/µl in RS1.
- ☐3 Requantify the diluted DNA.
- ☐4 Dilute the desired input DNA amount in RS1 to a final volume of 4 µl, and then add 1 µl SS1.

Hybridize Oligo Pool

- ☐1 Dilute 2.5 µl CAT with 2.5 µl RS1 per sample well. Pulse vortex to mix, and then centrifuge briefly.
- ☐2 Dilute 2.5 µl ACP3 with 2.5 µl RS1. Pulse vortex to mix, and then centrifuge briefly.
- ☐3 Dilute 2 µl 2800M with 2 µl RS1 and 1 µl SS1. Pulse vortex to mix, and then centrifuge briefly.
- ☐4 Add 5 µl diluted 2800M to 1 well.
- ☐5 Add 5 µl diluted ACP3 to the well that contains diluted 2800M.
- ☐6 Add 5 µl RS1 to 1 well.
- ☐7 Add 5 µl diluted DNA to the remaining wells.
- ☐8 Add 5 µl diluted CAT to all wells except the well containing 2800M.
- ☐9 Add 15 µl OHS2 to each well. Pipette slowly to mix.
- ☐10 If bubbles form, centrifuge the plate at 100 × g for 20 seconds.
- ☐11 Place on the preprogrammed thermal cycler and run the HYB program.
- ☐12 Combine ELE and ELB as follows.

Samples	Instructions
16	Transfer 18 µl ELE to the ELB tube. Flick and invert to mix. Do not vortex.
96	Transfer 137 µl ELE to the ELB tube. Flick and invert to mix. Do not vortex.

- ☐13 Place the ELB/ELE mixture on ice.

Remove Unbound Oligos

- ☐1 Add 25 µl SPB. Pipette slowly to mix.
- ☐2 Incubate at room temperature for 5 minutes.
- ☐3 Place on the magnetic stand until liquid is clear.
- ☐4 Remove and discard all supernatant.
- ☐5 Wash 3 times with 80 µl SW1.
- ☐6 Using a 20 µl pipette, remove residual SW1.
- ☐7 Add 80 µl of 60% EtOH.
- ☐8 Incubate at room temperature for 30 seconds.
- ☐9 Remove and discard all supernatant.
- ☐10 Using a 20 µl pipette, remove residual EtOH.
- ☐11 Air-dry for a maximum of 5 minutes.

Extend and Ligate Bound Oligos

- ☐1 Add 22 µl ELB/ELE mixture to each well. Pipette to mix.
- ☐2 If bubbles form, centrifuge at 100 × g for 20 seconds.
- ☐3 Place on the thermal cycler and run the EXT_LIG program.
- ☐4 Combine EDP and EMM as indicated. Pipette to mix, and then centrifuge briefly.

Samples	EDP	EMM
1	1.1 µl	21 µl
16	17.6 µl	334 µl
96	106 µl	2006 µl

- ☐5 Place the EDP/EMM mixture on ice for the next step.

Amplify Libraries

- ☐1 Arrange the Index 1 adapters in columns 1–12.
- ☐2 Arrange the Index 2 adapters in rows A–H.
- ☐3 Place the HYP plate on a TruSeq Index Plate Fixture.
- ☐4 Add 4 µl of each Index 1 adapter down each column.
- ☐5 Add 4 µl of each Index 2 adapter across each row.
- ☐6 Place the plate on ice or iceless cooler.
- ☐7 Add 20 µl EDP/EMM mixture. Pipette to mix.
- ☐8 Centrifuge at 280 × g for 1 minute.
- ☐9 Place the plate on ice or iceless cooler.
- ☐10 Immediately transfer to post-PCR area.
- ☐11 Place on the preprogrammed thermal cycler and run the PCR program for the appropriate number of cycles.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

- ☐1 Centrifuge the HYP plate at 280 × g for 1 minute.
- ☐2 Transfer 45 µl supernatant from the HYP plate to the CLP plate.
- ☐3 Add 36 µl SPB to the CLP plate.
- ☐4 Shake the plate at 1800 rpm for 2 minutes.
- ☐5 Incubate at room temperature for 5 minutes.
- ☐6 Centrifuge at 280 × g for 1 minute.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Wash 2 times with 200 µl 80% EtOH.
- ☐10 Using a 20 µl pipette, remove residual EtOH.
- ☐11 Remove from the magnetic stand and air-dry.
- ☐12 Add 25 µl RSB.
- ☐13 Shake the plate at 1800 rpm for 2 minutes.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Centrifuge at 280 × g for 1 minute.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Transfer 20 µl purified library from the CLP plate to the LNP plate.
- ☐18 From the liquid in the CLP plate, run an aliquot of the samples and control to confirm the PCR product sizes.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 3 months.

Normalize Libraries

- ☐1 Add 44 µl LNA1 per library to a new 15 ml conical tube.
- ☐2 Use a P1000 pipette to resuspend LNB1.
- ☐3 Transfer 8 µl LNB1 per library to the tube of LNA1.
- ☐4 Add 45 µl LNA1/LNB1 to the LNP plate.
- ☐5 Shake at 1800 rpm for 30 minutes.
- ☐6 Place on a magnetic stand until liquid is clear.
- ☐7 Remove and discard all supernatant.
- ☐8 Remove from the magnetic stand.
- ☐9 Wash 2 times with 45 µl LNW1.
- ☐10 Use a 20 µl pipette to remove residual LNW1.
- ☐11 Remove from the magnetic stand.
- ☐12 Add 30 µl fresh 0.1 N NaOH.
- ☐13 Shake at 1800 rpm for 5 minutes.
- ☐14 Place the LNP plate on a magnetic stand until liquid is clear.
- ☐15 Add 30 µl LNS2 to the SGP plate.
- ☐16 Transfer 30 µl supernatant from the LNP plate to the SGP plate.
- ☐17 Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 30 days.

Pool Libraries

- ☐1 Centrifuge at 1000 × g for 1 minute.
- ☐2 Transfer 5 µl of each library to an 8-tube strip.
- ☐3 Transfer the contents of the 8-tube strip to the PAL tube. Pipette to mix.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C for up to 7 days.

Prepare Consumables

- ☐1 Remove the reagent cartridge from -25°C to -15°C storage.
- ☐2 Thaw reagents in a room temperature water bath for 90 minutes.
- ☐3 Invert the cartridge 5 times to mix reagents.
- ☐4 Gently tap on the bench to reduce air bubbles.
- ☐5 Remove a new flow cell package from 2°C to 8°C storage.
- ☐6 Set the unopened flow cell package aside at room temperature for 30 minutes.
- ☐7 Remove the flow cell from the foil package and flow cell container.
- ☐8 Clean the glass surface of the flow cell with a lint-free alcohol wipe.
- ☐9 Dry with a lint-free lens cleaning tissue.

Denature, Dilute, and Load Libraries

- ☐1 Thaw the Hybridization Buffer at room temperature.
- ☐2 Vortex briefly before use.
- ☐3 Preheat the incubator to 98°C.
- ☐4 Combine the 5 µl pooled libraries and 995 µl prechilled Hybridization Buffer in a microcentrifuge tube.
- ☐5 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐6 Transfer 250 µl diluted library to a new microcentrifuge tube.
- ☐7 Add 250 µl prechilled Hybridization Buffer.
- ☐8 Vortex briefly and then centrifuge at 280 × g for 1 minute.
- ☐9 Place the tube on the preheated incubator for 2 minutes.
- ☐10 Immediately cool on ice.
- ☐11 Leave on ice for 5 minutes.
- ☐12 [Optional] Denature and dilute a PhiX control to 1.8 pM and a 1% spike-in to the final library.
- ☐13 Clean the foil seal covering reservoir **#16** using a low-lint tissue.
- ☐14 Pierce the seal with a clean 1 ml pipette tip.
- ☐15 Add 500 µl prepared libraries into reservoir **#16**.

Perform a Sequencing Run

- ☐1 From the Home screen, select **Sequence**.
- ☐2 Enter your user name and password.
- ☐3 Select **Next**.
- ☐4 Select a run name from the list of available runs.
- ☐5 Select **Next**.
- ☐6 Open the flow cell compartment door.
- ☐7 Press the release button to the right of the flow cell latch.
- ☐8 Place the flow cell on the flow cell stage over the alignment pins.
- ☐9 Close the flow cell latch to secure the flow cell.
- ☐10 Close the flow cell compartment door.
- ☐11 Open the reagent compartment door.
- ☐12 Slide the reagent cartridge into the reagent compartment until the cartridge stops.
- ☐13 Remove the spent reagents bottle from the compartment.
- ☐14 Discard the contents and slide the empty spent reagents bottle into the compartment.
- ☐15 Close the compartment door and select **Next**.
- ☐16 Confirm run parameters.
- ☐17 Select **Next**.
- ☐18 When the automated check is complete, select **Start**.
- ☐19 Monitor run progress, intensities, and quality scores as metrics appear on the screen.

View Analysis Results

- ☐1 From the Local Run Manager dashboard, click the run name.
- ☐2 From the Run Overview tab, review the sequencing run metrics.
- ☐3 [Optional] Click the **Copy to Clipboard** icon for access to the output run folder.
- ☐4 Click the Sequencing Information tab to review run parameters and consumables information.
- ☐5 Click the Samples and Results tab to view the analysis report.
- ☐6 [Optional] Click the **Copy to Clipboard** icon for access to the Analysis folder.