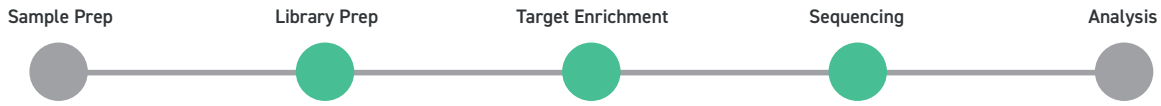
TWIST FOR ELEMENT

Trinity Freestyle™ Fast Hyb Workflow for Element AVITI™ Sequencing

For use with the Twist NGS Workflow

This Trinity Freestyle Fast Hybridization protocol details the steps for performing target enrichment and sequencing on Twist indexed libraries generated with the Twist UDI Primers for Trinity Freestyle. The Twist for Element, Trinity Freestyle Fast Hybridization Kit enables rapid enrichment of libraries generated from Twist's most commonly used library preparation kits, such as Enzymatic Fragmentation 2.0 and FlexPrep UHT kits. The resulting enriched DNA library can be sequenced on Element next-generation sequencing (NGS) systems using the Trinity Freestyle sequencing workflow. This protocol should only be performed with the reagents specified or their equivalents.



Twist NGS Workflow. *The complete NGS workflow takes you from sample preparation to NGS sequencing and data analysis.*

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

PROTOCOL COMPONENTS

Read the product packaging and storage recommendations carefully for each component, and store components as recommended below immediately upon arrival.

CATALOG #	NAME	DESCRIPTION	STORAGE
TWIST FOR ELEMENT TRINITY FREESTYLE REAGENTS AND KITS			
109298	Twist Fast Hybridization Reagents, Element Trinity Compatible, 12 Reactions	· Fast Hybridization Mix · Hybridization Enhancer · Blocker Solution	–20°C
	Trinity Freestyle Fast Hyb Binding Reagent	Trinity Freestyle Binding Reagent	–20°C
TWIST PROBE PANELS (ORDERED SEPARATELY)			
126855	Twist Exome 2.0 + Comp. Spike-in, Trinity FS, 96 Samples (Optional)	Twist Exome 2.0 + Comp. Spike-in, Trinity FS, 96 Samples	–20°C
Choice of panel type and reaction size	Twist Fixed Panel	Fixed content enrichment panel for hybridization reactions	–20°C
	Twist Custom Panel	Custom enrichment panel for hybridization reactions	–20°C
ELEMENT TRINITY REAGENTS AND KITS*			
860-00046	Trinity Freestyle 2x150 Sequencing Kit	Trinity Flow Cell	2-8°C
		AVITI Buffer Bottle	Room temperature
		· Trinity 2x150 Cartridge · Trinity Fast Hyb Loading Buffer · Trinity Freestyle Sequencing Reagent	–20°C
860-00045	Trinity Freestyle 2x75 Sequencing Kit	Trinity Flow Cell	2-8°C
		AVITI Buffer Bottle	Room temperature
		· Trinity 2x75 Cartridge · Trinity Fast Hyb Loading Buffer · Trinity Freestyle Sequencing Reagent	–20°C
830-00058	Trinity Freestyle PhiX Control (Optional)	Trinity Control	–20°C

**These reagents must be purchased from Element Biosciences (https://www.elementbiosciences.com).*

LEGAL

This document may contain references to other third-party resources such as sources of information, hardware or software, products, or services and/or web sites owned or licensed by third parties. Twist Bioscience does not control or take responsibility for any third-party resources, including, without limitation, the accuracy, reliability, copyright compliance, compatibility, performance, legality, or any other aspect of third-party resources. The inclusion of such resources in this document does not imply endorsement by Twist Bioscience of any third-party resources.

Certain processes described in this document may be subject to patent rights or licenses in local jurisdictions, including those owned or licensed by parties other than Twist Bioscience. Purchase of this product does not include a license to perform any such processes. Users of this product may, therefore, be required to obtain a patent license depending upon the particular application and country in which the product is used before performing such processes.

Twist Bioscience, the various Twist logos, and the Twist NGS logo used herein are trademarks of Twist Bioscience Corporation. All other trademarks are the property of their respective owners.

INTENDED USE

This product is for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease or condition. Twist Bioscience assumes no liability regarding use of the product for applications for which it is not intended.

TABLE OF CONTENTS

MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate libraries using the Twist for Element Workflow.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Ethanol (200 proof)	—
Molecular biology grade water	—
10 mM Tris-HCl pH 8 (optional)	—
Buffer EB (optional)	Qiagen
1.5-ml microcentrifuge tubes	VWR
5-ml Eppendorf tubes	Eppendorf
Thin-walled PCR 0.2-ml strip-tubes	Eppendorf
96-well thermal cycling plates	VWR
Qubit dsDNA Broad Range Quantification Assay	Thermo Fisher Scientific
Agilent DNA 7500 Kit	Agilent Technologies
EQUIPMENT	
Pipettes and tips	—
Vortex mixer	—
96-well compatible magnetic plate	Alpaqua, Permagen Labware
1.5-ml compatible magnetic stand	Beckman Coulter
Benchtop mini centrifuge for 0.2-ml tubes	—
Thermomixer (preferred) or heat block	Eppendorf
Thermal cycler (96-well) with heated lid	—
Fluorometer (Qubit 3.0)	Thermo Fisher Scientific
2100 Bioanalyzer	Agilent Technologies
Vacuum concentrator	—

GENERAL NOTES AND PRECAUTIONS

This protocol provides guidance on how to perform target enrichment of NGS libraries that incorporate P5 and P7 adapter sequences. General library pooling and hybridization strategies are provided for Trinity Freestyle sequencing on an Element Biosciences AVITI or AVITI24 instrument.

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol and follow the instructions provided. Twist cannot guarantee the performance of the Twist for Element, Trinity Freestyle Fast Hybridization Kit if modifications are made to the protocol.

Library preparation methods may yield more material than needed for target enrichment. Excess product can be stored at –20°C for later use.

Do NOT mix or combine the same reagents from different lots.

Test the compatibility of your thermal cycler and PCR tubes by incubating them at 95°C for up to 5 minutes to ensure the PCR tubes do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

This protocol details different methods for mixing reagents (gentle pipetting, flicking or tapping, vortexing), depending on the volume, vessel, and reagents involved.

The Fast Hybridization Mix is a viscous reagent. Pipette slowly to ensure accuracy.

FOR TECHNICAL SUPPORT, CONTACT CUSTOMERSUPPORT@TWISTBIOSCIENCE.COM.

PROTOCOL OVERVIEW

This protocol begins with amplified libraries indexed with Twist UDI Primers for Trinity Freestyle and generates target-enriched DNA libraries for subsequent sequencing on Element NGS systems.

	FAST HYBRIDIZATION AND TRINITY FREESTYLE SEQUENCING WORKFLOW (AMPLIFIED, INDEXED LIBRARIES)	TIME
STEP 1	**Prepare Libraries for Hybridization** Indexed library pool	**1 hour**
STEP 2	**Perform Hybridization and Trinity Sequencing Run Setup** Hybridize targets in solution and generate libraries ready for Trinity sequencing on an Element instrument	**1.5 hours**

STEP 1 PREPARE LIBRARIES FOR HYBRIDIZATION

This step involves aliquoting the appropriate amount of amplified, indexed libraries (generated previously in library preparation). Only libraries generated with Twist UDI Primers for Trinity Freestyle should be used in this workflow. When multiplexing, follow the pooling guidelines included in Appendix A: UDI Adapter Sequences and Pooling Guidelines.

Reagents Required

- Amplified and indexed linear library
- From Twist Fast Hybridization Reagents, Element Trinity Compatible:
  - Blocker Solution
- Twist Exome 2.0 + Comp. Spike-in, Trinity FS, 96 Samples Panel (or Trinity Freestyle Compatible catalog or custom panel)
- Trinity Freestyle Fast Hyb Binding Reagent (Twist Catalog, #126776)

Before You Begin

- Thaw all required reagents on ice, then pulse-vortex for 2 seconds to mix and pulse-spin for 2 seconds.
- In preparation for Step 3, thaw the Trinity sequencing cartridge in a water bath set to 20°C:
  - Thaw a 2 x 75 cartridge for about 90 minutes.
  - Thaw a 2 x 150 cartridge for about 2.5 hours.
  - Alternatively, thaw the cartridge overnight in a refrigerator.
  - Ensure proper time for cartridge thawing such that <30 minutes elapse between completion of hybridization and run setup

ALIQUOT AND DRY DOWN THE LIBRARY

1.1

Use the concentration of each amplified, indexed library to calculate the volume (in µl) of each library needed for hybridization:

- Each hybrid capture should be performed with 4000 ng of DNA
- Divide the amount of each indexed library per pool by the concentrations measured in ng/µl from the library preparation QC.
- This protocol supports up to an 8-plex hybridization capture for standard libraries or up to 96-plex for Twist FlexPrep UHT libraries
- Each Trinity flow cell can support up to 24 exome samples on an AVITI run or up to 32 exomes on an AVITI24 run. It is recommended to perform multiple 8-plex captures in parallel to maximize the flow cell capacity.
- For guidance on hybrid capture setup and loading of custom panels, see Appendix B: Hybrid Capture and Loading Recommendations for Custom Panels.

NUMBER OF INDEXED SAMPLES PER POOL*	AMOUNT OF EACH INDEXED LIBRARY PER POOL
4	1000 ng
8	500 ng

**For FlexPrep UHT libraries, pool up to 8 library pools (96 samples) into one 4000 ng hybridization reaction*

1.2 Transfer the calculated volumes from each amplified, indexed library to a tube or plate well for each hybridization being performed. A clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate is recommended to avoid unnecessary transfers in downstream steps.

PREPARE THE PRE-HYBRIDIZATION SOLUTION

1.3 Add the following volumes of reagents to each indexed library pool. Mix by flicking the tubes.

REAGENT	VOLUME*
Blocker Solution**	5 µl
Trinity Freestyle Binding Reagent	5 µl
Twist Exome 2.0 + Comprehensive Spike-in, Trinity FS, 96 Samples (or other Trinity Freestyle-compatible panels)	4 µl
Total	**14 µl**

**Prepare a master mix for multiple reactions.*

***Blocker Solution is designed for blocking human DNA. For non-human samples, replace with a compatible non-human blocker solution.*

1.4 Pulse-spin the indexed library pool tube for 2 seconds and ensure minimal bubbles are present.

1.5 Dry the indexed pool and pre-hybridization solution with a SpeedVac using low or no heat.

STOPPING POINT: If not proceeding immediately to Step 2: Fast Hybridization and Run Preparation, store the dried indexed library pool at –20°C for up to 24 hours.

PROCEED TO STEP 2: FAST HYBRIDIZATION

STEP 2 FAST HYBRIDIZATION

Use the dried indexed library pool from Step 1 to perform the hybridization reaction. Then, pool the hybridization reactions and sequence on an Element AVITI or AVITI24 instrument.

⚠ IMPORTANT: Before proceeding with this step, test the compatibility of your thermal cycler and PCR tubes or plates by incubating them at 95°C for up to 5 minutes to ensure they do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

Reagents Required

- Dried hybridization reaction from Step 1
- Trinity Sequencing Kit
  - Trinity Freestyle Sequencing Reagent
  - Trinity Fast Hyb Loading Buffer
- Twist Fast Hybridization Reagents, Element Trinity Compatible
  - Fast Hybridization Mix
  - Hybridization Enhancer

Before You Begin

- Set a heat block to 65°C.
- Thaw the Trinity Freestyle Sequencing Reagent, Fast Hybridization Mix, and Hybridization Enhancer on ice.
- Place the Trinity Fast Hyb Loading Buffer on ice.

RESUSPEND THE PRE-HYBRIDIZATION SOLUTION

2.1 Program a thermal cycler with the following conditions and set the lid temperature to 85°C.

STEP	TEMPERATURE	TIME
1	95°C	HOLD
2	95°C	5 minutes
3	72°C	1 hour
4	72°C	HOLD

2.2 Heat the Fast Hybridization Mix at 65°C for 10 minutes or until all precipitate is dissolved. Vortex to mix and use immediately.

⚠ IMPORTANT: Do not allow the Fast Hybridization Mix to cool to room temperature.

2.3 Resuspend the dried indexed library and pre-hybridization solution from Step 1 in 20 µl Fast Hybridization Mix.

NOTES: Fast Hybridization Mix is viscous. Pipette slowly to ensure accurate pipetting. If the resuspended solution requires transfer to a secondary vessel for hybridization, vortex for 5 seconds and incubate at 65°C for an additional 5 minutes. Then, pulse-spin for 2 seconds, ensure no bubbles are present, and transfer to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate.

PERFORM THE HYBRIDIZATION REACTION

2.4 Add 30 µl Hybridization Enhancer to the top of the entire capture reaction.

2.5 Pulse-spin the tube or plate for 2 seconds to ensure all solution is at the bottom of the tube or wells.

NOTE: Hybridization Enhancer settles on top of the hybridization reaction after the pulse spin, which does not affect the final capture.

2.6 Place the tube or plate in the programmed thermal cycler and proceed to steps 2–4 of the thermal cycler program (95°C step of the thermal cycler program in Step 2.1 above).

STEP 3 SEQUENCING WITH THE TRINITY SEQUENCING WORKFLOW

Pool hybridization reactions and sequence on an AVITI or AVITI24 system.

For comprehensive instructions, see the user guide for your instrument on the Element website.

PREPARE CONSUMABLES

3.1 Gather the following consumables from the Trinity Sequencing Kit:

- Hybridization reaction (from Step 2.6)
- Trinity Freestyle Sequencing Reagent
- Trinity Fast Hyb Loading Buffer
- Trinity Sequencing Cartridge (thawed)
- Trinity Flow Cell
- AVITI Buffer Bottle

INITIATE A SEQUENCING RUN

3.2 On the Home screen, select New Run.

3.3 If prompted that the flow cell is missing, load a used flow cell.

3.4 Select Sequencing.

3.5 Select the side for sequencing: Side A, Both, or Side B.

3.6 For chemistry type, select Trinity.

3.7 For a Manual Run, proceed to Define Run Parameters. For a Planned Run, select the run and storage connection, and then select Next. Proceed to Inspect and Mix Reagents.

DEFINE RUN PARAMETERS

3.8 In the Run Name field, enter a unique name.

3.9 To import the run manifest, select Browse and navigate to the run manifest.

3.10 In the Storage drop-down menu, select a storage location.

3.11 In the Library Type drop-down menu, select Third Party.

3.12 In the Sequencing Kit drop-down menu, select the appropriate Trinity Sequencing Kit.

3.13 In the Panel drop-down menu, select Twist for Element, Trinity Exome Workflow or Other.

3.14 In the Cycles fields, enter the number of cycles for each read, and then select Next.

KIT SIZE	DEFAULT CYCLE VALUES FOR TRINITY FREESTYLE			
	INDEX 1	INDEX 2	READ 1	READ 2
2x75	10	10	76	76
2x150	10	10	151	151

3.15 For Twist FlexPrep libraries, select Advanced Run Settings, and type 8 in the PMG Shift field, to enable 8-cycle PMG shift.

INSPECT AND MIX REAGENTS

3.16 Gently invert the Trinity Sequencing Cartridge 10 times to mix reagents.

3.17 Tap the Trinity Sequencing Cartridge base on the benchtop to remove any large droplets from the tube tops.

3.18 Inspect the small tubes to make sure reagents are settled at the bottom.

3.19 Insert the Trinity Sequencing Cartridge into a clean cartridge basket and lock the clips.

PREPARE THE SEQUENCING SOLUTION

3.20 When the 1-hour incubation at 72°C is complete, remove the hybridization reaction(s) from the thermal cycler and immediately place them in a cold block on ice.

3.21 Pulse-spin the tube(s) containing the chilled hybridization reaction for 2 seconds to collect all liquid at the bottom of the tube. Add 150 µl chilled Trinity Fast Hyb Loading Buffer to each hybridization reaction. Keep on ice.

NOTE: The diluted hybridization reaction can be stored at –20°C for up to a week.

3.22 If sequencing multiple hybrid captures, pool the entire contents of each diluted hybridization reaction from Step 3.21 (~200 µl each) together in a 1.5-ml tube on ice. Mix thoroughly by gentle pipetting.

3.23 To prepare the sequencing solution, add the following reagents to a 5-ml tube on ice. Ensure the pooled, diluted hybridization reaction(s) from Step 3.22 are well mixed immediately before dilution. Mix the sequencing solution thoroughly by gentle pipetting.

COMPONENT	VOLUME PER RUN*
Trinity Fast Hyb Loading Buffer	2114 µl
Trinity Freestyle Sequencing Reagent	36 µl
Pooled, diluted hybridization reaction(s)	50 µl
Total	**2200 µl**

**50 µl hybridization loading volume is recommended for the Exome 2.0 + Comp Spike-in, Trinity FS panel. For loading volumes of other panels, refer to Appendix B: Hybrid Capture and Loading Recommendations for Custom Panels.*

3.24 (Optional) Add 4-8 µl Trinity Freestyle PhiX Control to the sequencing solution. Pipette gently to mix.

ADD SEQUENCING SOLUTION TO THE CARTRIDGE

3.25 Using a new 1-ml pipette tip, pierce the Library well of the sequencing cartridge. Push the foil to the edges.

3.26 Transfer the 2200 µl of prepared sequencing solution to the Library well in the sequencing cartridge.

CONFIRM REAGENT PREPARATION

3.27 Select the Invert cartridge checkbox to confirm that the reagents are mixed.

3.28 Select the Insert into basket checkbox to confirm that the Trinity Sequencing Cartridge is in the cartridge basket.

3.29 Select the Load hybed reaction checkbox to confirm that the Trinity Sequencing Cartridge contains the hybridized reaction.

3.30 Select Next.

LOAD REAGENTS AND BUFFER

3.31 Open the reagent bay door and remove any used consumables.

3.32 Slide the basket containing the thawed Trinity Sequencing Cartridge into the reagent bay until it stops.

3.33 Slide the AVITI Buffer Bottle into the reagent bay until it stops.

3.34 Close the reagent bay door, and then select Next.

EMPTY WASTE AND PRIME REAGENTS

3.35 Open the waste bay door, remove the waste bottle, and close the transport cap.

3.36 Open the transport cap and the vent cap and empty the waste bottle.

3.37 Close the vent cap and reload the empty waste bottle.

3.38 Select Next to automatically start priming.

3.39 During priming, bring a new Trinity Flow Cell to room temperature for ≥ 5 minutes. Do not open the pouch.

3.40 When priming is complete, select Next. The nest door opens automatically.

LOAD THE FLOW CELL

3.41 Remove the used flow cell from the nest.

3.42 Unpackage the new Trinity Flow Cell and load it onto the nest.

3.43 Select Close Nest, and then select Next.

REVIEW AND START THE RUN

3.44 Review the run parameters to ensure proper setup.

3.45 Select Run to start sequencing.

END OF WORKFLOW

APPENDIX A: UDI ADAPTER SEQUENCES AND POOLING GUIDELINES

UDI SEQUENCES

For a complete guide to the Twist Adapter System - Trinity Freestyle-compatible UDIs, please refer to the Twist Adapter System - Trinity Freestyle Reference Spreadsheets and Sample Sheet Templates.

NOTE: Given that the Twist UDI Primers for Trinity Freestyle are compatible with commonly used adapter sequences, there is the potential to mix adapter plates. The Trinity Freestyle indices and adapter system are optimized for compatibility with Trinity Freestyle Chemistry. The indices contain 10-nucleotide barcodes that share sequences with the Twist Universal Adapter System - TruSeq Compatible. To avoid potential index clashes, do not sequence these UDI sets together within a sequencing run.

POOLING GUIDELINES

Twist UDI primers are base balanced for next-generation sequencing on a column basis. When pooling unique dual-indexed libraries for 8-plex hybridization, it is recommended that libraries be selected from a single column. Multiple columns may be selected in any desired combination across a single plate or multiple plates for sequencing.

Twist Adapter System - Trinity Freestyle

96 Samples, Plate 1 to 4 (126792, 126793, 126795, 126796)

Plate 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Plate 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

Plate 3.

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

Plate 4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	297	305	313	321	329	337	345	353	361	369	377
B	290	298	306	314	322	330	338	346	354	362	370	378
C	291	299	307	315	323	331	339	347	355	363	371	379
D	292	300	308	316	324	332	340	348	356	364	372	380
E	293	301	309	317	325	333	341	349	357	365	373	381
F	294	302	310	318	326	334	342	350	358	366	374	382
G	295	303	311	319	327	335	343	351	359	367	375	383
H	296	304	312	320	328	336	344	352	360	368	376	384

APPENDIX B: HYBRID CAPTURE AND LOADING RECOMMENDATIONS FOR CUSTOM PANELS

The Trinity Freestyle Workflow performs hybrid capture without the need for washes and post-capture amplification, saving users time and additional library handling steps. In Trinity Freestyle, DNA that goes into hybrid capture is loaded directly onto the flow cell. This means optimal loading of the flow cell must be determined during hybrid capture setup. Optimal flow cell loading is based primarily on the fraction of library molecules within the genome that are targeted by the custom capture panel. As the fraction of the genome the panel covers decreases, the total mass of library needed to fill a Trinity flow cell increases. Twist has validated the mass needed to maximize the number of reads generated on a Trinity run for custom capture panels of various sizes.

CAPTURE CONDITION AND LOADING VOLUME FOR CUSTOM PANELS

PANEL TARGET SIZE	NUMBER OF CAPTURES/ SAMPLES PER CAPTURE/ MASS PER SAMPLE	TOTAL MASS TO LOAD ON SEQUENCER	VOLUME OF DILUTED HYBRIDIZATION REACTION TO LOAD
70 Mb	1 Capture/ 96-plex/ 500 ng (or per FlexPrep pool)	500 ng	25 µl
36 Mb	3 Captures/ 8-plex/ 500 ng	1200 ng	50 µl
7 Mb	2 Captures/ 96-plex/ 500 ng (or per FlexPrep pool)	6 µg	300 µl
2.5 Mb	2 Captures/ 12-plex/ 500 ng	12 µg	400 µl

For large panels, only a subset of the total hybridization will be loaded onto the flow cell to avoid overloading. For smaller panels, the full pooled, diluted hybridization reaction volume from Step 3.22 will need to be loaded to ensure sufficient polony generation. When preparing the sequencing solution in Step 3.23 ensure the total volume is 2200 µl. Increase or decrease the volume of Trinity Fast Hyb Loading Buffer used in the sequencing solution to account for the volume of the hybridization reaction listed in the table above.

The optimal number of hybridizations and level of multiplexing will depend on the sequencing depth necessary for a user's specific applications. Some examples of different Trinity applications are provided in the table below. These recommendations are a starting point to guide hybrid capture setup and loading, and should be tested for each application. Contact Twist customer support for more specific guidance.

EXAMPLE EXPERIMENTAL SETUP FOR A TRINITY FREESTYLE RUN ON AVITI AND AVITI24

PANEL	PANEL TARGET REGION*	DESIRED SEQUENCING DEPTH	SAMPLES PER RUN	RECOMMENDED HYBRIDIZATION CONFIGURATION
Genotyping - Human 600K	70 Mb	1 Gb	AVITI: 96	AVITI: 1 x 96-plex FlexPrep Pool
Exome 2.0 + Comp	37 Mb	6 Gb	AVITI: 24 AVITI24: 32	AVITI: 3 x 8-plex AVITI24: 4 x 8-plex
Custom SNP	7.8 Mb	0.5 Gb	AVITI: 192	AVITI: 2 x 96-plex FlexPrep Pool
Twist Oncology - DNA CGP Panel	2.4 Mb	5 Gb	AVITI: 24	AVITI: 2 x 12-plex

**Sequencing panels that target under 1 Mb of genome space are not recommended for the Trinity Freestyle Workflow.*

***It is not recommended to pool more than 2 captures on a Trinity Freestyle run using panels between 1-25 Mb of target capture space. For applications requiring more than 16 samples per Trinity Flow Cell, we recommend plexing up to 24 samples per hybridization. Such conditions should be empirically validated for performance.*

APPENDIX C: MODIFIED CAPTURE FOR TWIST GENOTYPING PANEL - HUMAN 600K

The Twist Genotyping Panel - Human 600k has been optimized to enrich over 600,000 genetic variants (covering commonly used variants in genotyping assays for population screening studies). The panel can be used in combination with standard library preparation protocols or with the Twist FlexPrep UHT library preparation workflow. The panel spans a ~70 Mb area of the human genome. This workflow has been optimized for maximum performance, accounting for the panel's size and the stoichiometry of Trinity Sequencing within the Trinity Freestyle workflow.

PREPARE THE PRE-HYBRIDIZATION SOLUTION

C1.3 Add the following volumes of reagents to each indexed library pool. Mix by flicking the tubes.

REAGENT	VOLUME PER REACTION
Blocker Solution	5 µl
Trinity Freestyle Binding Reagent	5 µl
Twist Genotyping Panel - Human 600K	2 µl
Total	**12 µl**

C1.4 Proceed to Step 1.4 and continue with the protocol through Step 1.5. Then, in place of Step 2.1, proceed to Step C2.1 below.

RESUSPEND THE PRE-HYBRIDIZATION SOLUTION

C2.1 Program a thermal cycler with the following conditions and set the lid temperature to 85°C.

STEP	TEMPERATURE	TIME
1	95°C	HOLD
2	95°C	5 minutes
3	60°C	1 hour
4	65°C	5 minutes
5	70°C	5 minutes
6	70°C	HOLD

C2.2 Proceed to Step 2.2 and continue with the protocol through Step 3.22. Then, in place of Step 3.23, proceed to Step C3.23 below.

PREPARE THE SEQUENCING SOLUTION

C3.23 Add the following reagents on ice and mix thoroughly by gentle pipetting.

COMPONENT	VOLUME PER RUN
Trinity Fast Hyb Loading Buffer	2139 µl
Trinity Sequencing Reagent	36 µl
Pooled, Diluted Hybridization Reaction(s)	25 µl
Total	**2200 µl**

C3.24 Proceed to Step 3.24 and continue with the protocol until completion.

APPENDIX D: KIT COMPONENTS

The following table details part numbers for each component provided in the kits required for this protocol.

BOX	COMPONENT	COMPONENT PART NUMBER
Twist Fast Hybridization Reagents, Element Trinity Compatible, 12 Reactions	Fast Hybridization Mix	100962
	Hybridization Enhancer	100937
	Blocker Solution	100864
Trinity Freestyle Fast Hyb Binding Reagent	Trinity Freestyle Binding Reagent, 12 Rxns	830-00059
Twist Universal Adapters - TruSeq Compatible, 96 Samples	Twist Universal Adapters - TruSeq Compatible, 96 Samples	101226
Twist UDI Primers for Trinity Freestyle, 96 Samples, Plate 1	Twist UDI Primers for Trinity Freestyle, 96 Samples - Plate 1	126792
Twist UDI Primers for Trinity Freestyle, 96 Samples, Plate 2	Twist UDI Primers for Trinity Freestyle, 96 Samples - Plate 2	126793
Twist UDI Primers for Trinity Freestyle, 96 Samples, Plate 3	Twist UDI Primers for Trinity Freestyle, 96 Samples - Plate 3	126795
Twist UDI Primers for Trinity Freestyle, 96 Samples, Plate 4	Twist UDI Primers for Trinity Freestyle, 96 Samples - Plate 4	126796
Trinity Freestyle Sequencing Kit	Trinity Cartridge	820-00030 (2x75) 820-00031 (2x150)
	Trinity Flow Cell	810-00015
	Trinity Freestyle Sequencing Reagent	830-00060
	AVITI Buffer Bottle (Universal Wash Buffer)	820-00002
	Trinity Fast Hyb Loading Buffer	830-00030
Trinity Freestyle PhiX Control	Trinity Freestyle PhiX Control	830-00058