



firefly[®] + technical note

QIAseq[®] Targeted DNA Panel

Overview

- Based on the QIAseq[®] Targeted DNA Panel Handbook (06/2025), the workflow is split into two fully automated parts requiring no hands-on intervention.
- The QIAseq[®] Targeted DNA Panel enables targeted next-generation sequencing of DNA. The kit may be used with an input of 10-80ng for fresh DNA or 100-250 ng for formalin- fixed paraffin-embedded (FFPE) DNA.
- This method has been optimised using Eppendorf twin.tec, Eppendorf deep well plates and the Alpaqua Magnum FLX magnet. The use of alternative labware may require further optimization.
- This protocol has been written to be compatible with v2.0.3 and tested for 16 samples using the on deck thermo cycler script for target enrichment with less than 1500 primers.
- Instrument configuration: firefly plus 6-head, genomics, ATL head, 96 ODT.
- Development software version: v2.0.3.
- Variables are setup for FFPE, standard or cfDNA samples.
- Deviation(s) from published method:
 - Ethanol Additions during bead clean-up have been slightly reduced to allow for transfer removal of ethanol with 125ul tips.
 - Reduced total volume for beads cleanup.

firefly protocols

Protocol number	Protocol name	firefly run time (minutes)	Thermocycler run time (minutes)
1 of 2	QIAseq [®] Targeted DNA Panel Part 1	2h 6min	62min*
2 of 2	QIAseq [®] Targeted DNA Panel Part 2	2h 30min + 3h Pauses***	120min + 90min**

Table 1. Overview of the protocol to process 96 samples using QIAseq[®] Targeted DNA Panel on SPT Labtech's firefly. Times may vary based on sample number. *Run time will vary depending on the fragmentation time required. **Run time will vary depending on PCR cycles. ***Pauses are setup to wait for the long ODT program to finish before resuspension of settled beads.

Input variables

QIAseq® Targeted DNA Panel Part 1			
Input Variable	Interval	Range	
		Minimum	Maximum
Number of columns (1-12)	1	1	12
Adapter plate starting column	1	1	12
Fill volume (default 40ul, 20ul for cfDNA)	-	20	40
1st selection Beads volume (default 90ul, for cfDNA 98ul)	-	90	98
2nd selection Beads volume (default 50ul, 70ul for cfDNA)	-	50	70
Fragmentation ODTSC Script	-	-	-
Ligation ODTSC Script	-	-	-

Table 2: General variables for using QIAseq® Targeted DNA Panel Part 1 on SPT Labtech firefly liquid handler.

QIAseq® Targeted DNA Panel Part 2			
Input Variable	Interval	Range	
		Minimum	Maximum
Number of columns (1-12)	1	1	12
Adapter plate starting column	1	1	12
Post-target enrichment Fill volume (default 70ul, cfDNA 60ul)	-	60	70
Post-target enrichment Beads (default 90, cfDNA 96)	-	90	96
Target Enrichment ODTSC Script	-	-	-
Universal PCR ODTSC Script	-	-	-

Table 3: General variables for using QIAseq® Targeted DNA Panel Part 2 on SPT Labtech firefly liquid handler.

Reagent Calculations

QIAseq® Targeted DNA Panel Part 1	
Reagent	Total Required Volume (µl)
QIAseq Beads	2670.00
Fragmentation Mix + Enzyme	207.00
Adapter Ligation Mix	430.20
NF Water	2006.00

Table 4: Reagent volumes for processing 16 samples with QIAseq® Targeted DNA Panel on SPT Labtech firefly liquid handler. For details regarding the volumes required for variable sample inputs please reach out directly to SPT Labtech (see contact details below).

QIAseq® Targeted DNA Panel Part 2	
Reagent	Total Required Volume (µl)
QIAseq Beads	3360
Target Enrichment Mix	244.6
Universal PCR Mix	155
NF Water	3904

Table 5: Reagent volumes for processing 16 samples with QIAseq® Targeted DNA Panel on SPT Labtech firefly liquid handler. For details regarding the volumes required for variable sample inputs please reach out directly to SPT Labtech (see contact details below).

Consumables and Accessories:

Pipette Head Consumable Type	Product Number(s)	Part 1	Part 2
50 µL Tips (Filtered)	050-96-FF-AL-FS	1	1
125 µL Tips (Filtered)	125-96-FF-AL-FS	2	2

Table 6: This table outlines the Pipette Head consumables required for processing 16 samples, including product numbers and quantities.

Dispense Head Consumables Type	Product Number(s)	Part 1	Part 2
Syringes (Ultra-low Retention)	4150-07208	3	4
Syringes (Standard)	4150-07200	3	2
Reservoirs (Standard)	4150-07103	-	4
Reservoirs (Low Dead Volume)	4150-07202	6	2
Reservoirs (High Volume)	4150-07300	-	

Table 7: This table outlines the Dispense Head consumables required for processing 16 samples, including product numbers and quantities (quantities refer to the individual item).

Plate and Lid Type	Product Number(s)	Part 1	Part 2
Eppendorf twin.tec (96)	30128648	5 (including 1 AUDI Plate)	5 (including 1 XUDI Plate)
Eppendorf deep well plate	003050120	2	2
Azenta Auto-Sealing PCR Plate Lid	9 4ti-0291	1	2

Table 8: Table detailing the types and quantities of plates required for the protocol.

Accessory Type	Product Number(s)	Part 1	Part 2
Alpaqua Magnum FLX	A000400	1	1
40mm Upper Deck Riser	3276-01838	1	1
Thermo Adapter Block	3276-01065	1	1

Table 9: Table detailing the accessories needed for the protocol.

Workflow overview



Figure 1. Overview of the steps performed by firefly to automate QIAseq® Targeted DNA Panel.

Workflow details

Protocol 1 of 2 - QIAseq® Targeted DNA Panel Part 1

This protocol performs the following sections described in the QIAseq Targeted DNA Panel Handbook (06/2025):

- Fragmentation, End-Repair, and A-addition
- Adapter Ligation

Prior to executing this protocol:

- The QIAseq Targeted DNA Panel (96) Part 1 protocol requires a pre-filled sample input plate (Input DNA Plate). The Reaction mix for fragmentation, end-repair, and A-addition (Frag Mix + Enzyme) volume must be adjusted accordingly.
- This protocol also requires a pre-filled fresh 80% ethanol plate containing 800ul/well. Prepare the reaction mix for fragmentation, end-repair, and A-addition.
- Prepare the reaction mix for adapter ligation

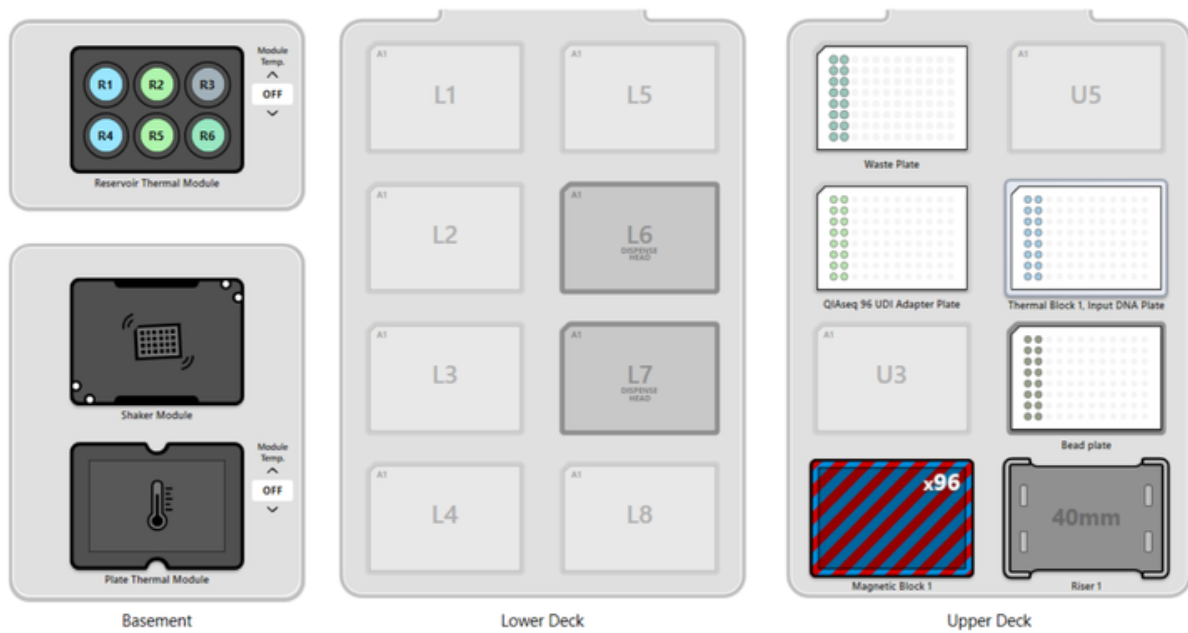


Figure 2: QIAseq® Targeted DNA Panel Part I initial deck layout.

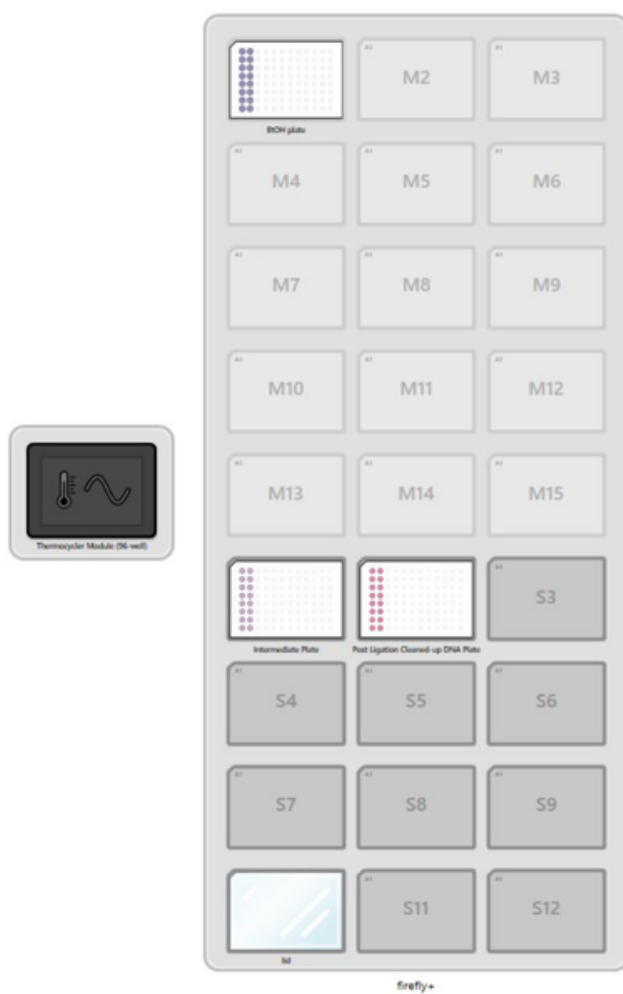


Figure 3: QIAseq® Targeted DNA Panel plus module initial layout.

Protocol 1 of 2 - QIAseq® Targeted DNA Panel Part 2

This protocol performs the following sections described in the QIAseq Targeted DNA Panel Handbook (06/2025):

- Target Enrichment
- Universal PCR

There is an user interaction to place UDIX on deck after ca. 3 hours to reduce evaporation. One can also use another lid to protect this plate.

Prior to executing this protocol:

- The QIAseq Targeted DNA Panel (96) Part 2 protocol requires the Post Ligation Cleaned-up DNA from Part 1 as starting material.
- Prepare the reaction mix for Target Enrichment Master Mix and Universal PCR Mix. The volume must be adjusted accordingly.
- This protocol also requires a pre-filled fresh 80% ethanol plate containing 800ul/well.

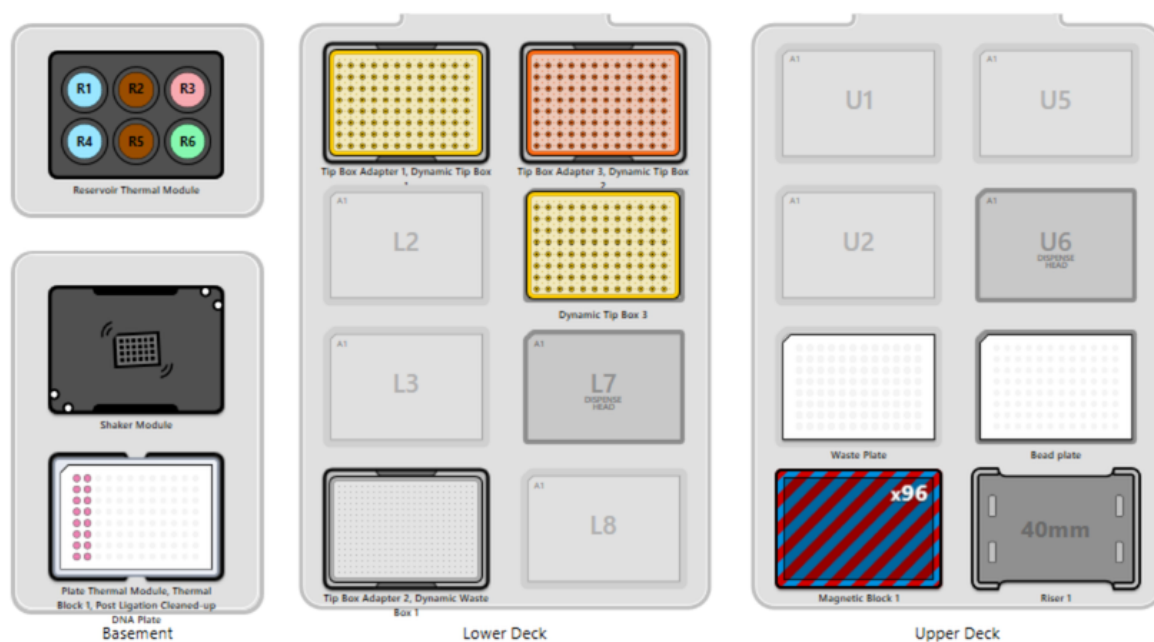


Figure 4: QIAseq® Targeted DNA Panel Part II initial deck layout.

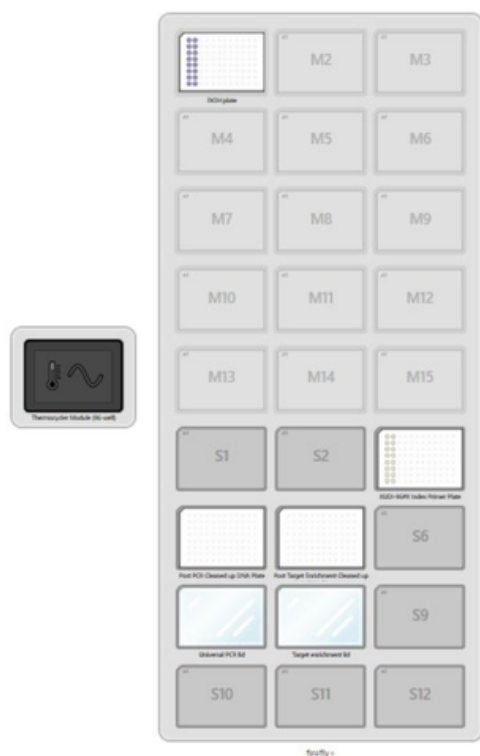


Figure 5: QIAseq® Targeted DNA Panel Part II plus module initial layout.

For questions regarding SPT Labtech's firefly, contact our support team through fireflysupport@sptlabtech.com

Appendix 1:

Consumables and Accessories

Supplier	Part Name	Part Number
SPT Labtech	dragonfly® discovery LDV Reservoirs	4150-07202 4150-
SPT Labtech	dragonfly® Reservoirs	07103
SPT Labtech	dragonfly® discovery Ultra Low Retention Syringes	4150-07208
SPT Labtech	dragonfly® discovery Syringes	4150-07200
SPT Labtech	firefly® Pipette Tips, 100µL, with Filters, Sterile	050-96-FF-AL-FS
SPT Labtech	firefly® Pipette Tips, 35µL, with Filters, Sterile	125-96-FF-AL-FS
SPT Labtech	Thermal Adapter for PCR Plate, 96	3276-01065
Eppendorf	Twin.tec, 96	30128648
Eppendorf	Deep well plate, 96	0030501209
Azenta	Azenta Auto-Sealing PCR Plate Lid	4ti-0291

Table 8. Overview of consumables and accessories required for processing 16 samples using QIAseq® Targeted DNA Panel on SPT Labtech's firefly indicating their part number.