Oncomine™ TCR Pan-Clonality Assay

Catalog Numbers A51044, A51562

Pub. No. MAN0019237 Rev. A.0

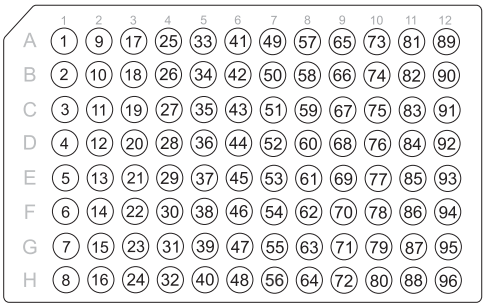
IMPORTANT! The target amplification cycling program and sample input vary with the sample type and assay. Performing the wrong procedure can result in suboptimal sequencing read depth and decreased number of clones detected.

Proceed to the target amplification procedure specific to the assay and sample type you are performing.

Guidelines for DNA isolation, quantification, and input

IMPORTANT! Sample input amount and the target amplification cycling program vary with sample type.

- Use kits described in the *Oncomine™ Human Immune Repertoire User Guide* (Pub. No. MAN0019231) for isolating high quality gDNA from research samples for use in library preparation.
- We recommend the Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) for quantifying human genomic DNA.
- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA. Use of these methods can lead to gross overestimation of the sample DNA concentration, under-seeding of the target amplification reaction, and low library yields.
- For best results, use blood that is collected in EDTA tubes that have been centrifuged to separate the plasma from cellular blood components as soon as possible after blood collection. We recommend the plastic BD Vacutainer® blood collection tube with K_2EDTA: BD Hemogard™ Closure. See the *Oncomine™ Human Immune Repertoire User Guide* (Pub. No. MAN0019231).
- Sample input amount and the target amplification cycling program vary with sample type. In general, DNA from sorted cell samples includes a greater fraction of relevant template molecules. The fraction of relevant molecules is higher when using PBMC or PBL compared to whole blood.
- Increasing the amount of DNA results in higher-quality libraries, especially when DNA quality or quantity is unknown.
- In general, library yield from high-quality DNA is greater than from degraded samples. Library yield is not indicative of sequencing performance.
- The Ion Torrent™ Dual Barcode Kit 1–96 is arrayed by column with barcode 1 in position A1, barcode 8 in position H1, and barcode 96 in position H12.



Set up DNA target amplification reactions

Prepare DNA target amplification reactions

1. Place a new 96-well plate in a pre-chilled 4°C cold block or on ice.
2. Dilute the dNTP mix (25 mM each) to 7.5 mM each.

Component	Volume
Nuclease-free Water	14 µL
dNTP Mix (25 mM each)	6 µL
Total volume	20 µL

3. Gently vortex the 5X Ion AmpliSeq™ HiFi Mix, centrifuge briefly to collect, then keep on ice.
4. For each sample, add the following components to each sample well. Prepare a target amplification master mix for multiple reactions.

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4.0 µL
5X Oncomine™ TCR Pan-Clonality Assay panel	4.0 µL
dNTP Mix (7.5 mM each dNTP, prepared in step 2)	2.0 µL
gDNA (200 ng–2.0 µg)[1]	≤10 µL
Nuclease-free Water	to 20 µL

[1] Substitute an equal volume of nuclease-free water or low TE to prepare a no-template control (NTC).

Note: Avoid using columns on the periphery of the plate.

5. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge briefly to collect droplets.

Amplify the targets

1. Place a MicroAmp™ Compression Pad on the plate, then load the plate into the thermal cycler.

2. Run the following program to amplify the target regions.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	95°C	2 minutes
28 Cycles[1]	Denature	95°C	30 seconds
	Anneal	60°C	45 seconds
	Extend	72°C	45 seconds
Hold	Final extension	72°C	10 minutes
Hold	—	10°C	Hold

[1] Increase number by +3 when starting from FFPE samples. Cycle number can be increased when input material quality or quantity is questionable.

STOPPING POINT Target amplification reactions can be stored at 10°C overnight on the thermal cycler. For longer duration, store at –20°C.

Partially digest the amplicons

IMPORTANT! Keep the plate on ice or in a pre-chilled 4°C cold block while preparing the reactions.

1. Thaw the FuPa Reagent (brown cap) on ice, gently vortex to mix, then centrifuge briefly to collect.

2. Add 2 µL of FuPa Reagent to each amplified sample. The total volume is ~22 µL.

   IMPORTANT! FuPa Reagent is viscous. Pipet slowly and mix thoroughly.

3. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge briefly to collect droplets.

4. Place a compression pad on the plate, load in the thermal cycler, then run the following program:

Temperature	Time
50°C	10 minutes
55°C	10 minutes
60°C	20 minutes
10°C	Hold (for up to 1 hour)

Proceed immediately to "Perform the ligation reaction".

IMPORTANT! Do not store the partially digested amplicons overnight.

Ligate adapters to the amplicons and purify

When sequencing multiple libraries on a single run, you *must* ligate a different barcode to each library.

IMPORTANT! When handling barcoded adapters, be careful to avoid cross contamination by changing gloves frequently and opening one tube at a time.

Perform the ligation reaction

IMPORTANT! When using Ion Torrent™ Dual Barcode Kit 1–96 adapters, we recommend that you remove the foil seal from only the specific wells being used to prevent potential cross-contamination. After adding dual barcode adapters to your reaction, cut a portion of a MicroAmp™ Clear Adhesive Film to size, then reseal the used wells. Each well of Ion Torrent™ Dual Barcode adapter is single use only.

1. If there is visible precipitate in the Switch Solution or the tube cap after thawing, vortex or pipet up and down at room temperature to resuspend before pipetting.

2. Briefly centrifuge the sample plate to collect droplets, then carefully remove the plate seal.

   Note: Centrifuge the plate of dual barcode adapters at the same time, to collect the contents.

3. Add the following components in the order listed to each well containing digested amplicons.

   IMPORTANT! Add the DNA Ligase last. Do not combine DNA Ligase and barcode adapters before adding to the digested amplicons.

Order of addition	Component	Volume
1	Switch Solution (yellow cap)	4 µL
2	Ion Torrent™ Dual Barcode Kit 1–96 adapters	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume**	**~30 µL**

4. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets.

5. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

Temperature	Time
22°C	30 minutes
68°C	5 minutes
72°C	5 minutes
10°C	Hold (for up to 24 hours)

Purify the library

1. Prepare 70% ethanol (350 µL × # of samples) fresh daily.
2. Briefly centrifuge the plate to collect the contents in the bottom of the wells.
3. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.
4. Incubate the mixture for 5 minutes at room temperature.
5. Place the plate in a magnetic rack such as the DynaMag™-96 Side Magnet, then incubate for 2 minutes or until the solution clears. Carefully remove, then discard the supernatant without disturbing the pellet.
6. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet to wash the beads. Carefully remove, then discard the supernatant without disturbing the pellet.
7. Repeat step 6 for a second wash.
8. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 2–5 minutes.

Elute the library

1. Remove the plate with purified libraries from the plate magnet, then **add 50 µL of Low TE** to the pellet to disperse the beads.
2. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets.
3. Incubate at room temperature for at least 2 minutes.
4. Place the plate on the magnet for at least 2 minutes.
5. Prepare a 100-fold dilution for quantification. Remove 2 µL of supernatant, containing the library, then combine with 198 µL of Nuclease-free Water.

Quantify the library by qPCR and calculate the dilution factor

Determine the concentration of each library by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). Libraries that have not undergone a second round of amplification typically have yields of 50–500 pM. However, yield is not indicative of library quality. After quantification, determine the dilution factor that results in a concentration of ~25 pM. Analyze each sample, standard, and negative control in duplicate reactions.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Ion Control Library (~68 pM, from the Ion Library TaqMan™ Quantitation Kit) at 6.8 pM, 0.68 pM, and 0.068 pM. Mark these tubes as standards, then use these concentrations in the qPCR instrument software.
2. Calculate, then prepare the required volume of PCR reaction mix for duplicate reactions of each library sample, standard, and NTC using the following table. Include a 5–10% overage to accommodate pipetting errors.

Component	Volume per reaction	
	96-well plate	384-well plate
Ion Library qPCR Master Mix, 2X	10 µL	5 µL
Ion Library TaqMan™ Quantitation Assay, 20X	1 µL	0.5 µL
Total	**11 µL**	**5.5 µL**

3. In a MicroAmp™ Optical Reaction Plate , set up duplicate PCR reactions for each sample, standard, and NTC. Add the following components to each well.

Component	Volume per reaction	
	96-well plate	384-well plate
PCR Reaction Mix	11 µL	5.5 µL
1:100 dilution of the sample[1]	9 µL	4.5 µL

[1] Substitute E. coli DH10B standards prepared in step 1 for standards. Substitute nuclease-free water for NTC.

4. Seal the plate with a MicroAmp™ Optical Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets.
5. Program your Real-Time PCR instrument.

   Note: The fast cycling program was developed using the StepOnePlus™ Real-Time PCR System in **Fast** mode.

   a. Enter the concentrations of the control library standards.
   b. Select ROX™ Reference Dye as the passive reference dye.
   c. Select a reaction volume of 20 µL.
   d. Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.

Plate	Mode	Stage	Temp.	Time
96-well Standard OR 384-well Standard	Std	Hold	50°C	2 min
		Hold	95°C	2 min
		Cycle (40 cycles)	95°C	15 sec
			60°C	1 min
48- / 96-well Fast OR 384-well Standard	Fast	Hold	50°C	2 min
		Hold	95°C	20 sec
		Cycle (40 cycles)	95°C	1 sec
			60°C	20 sec

6. Following qPCR, calculate the average concentration of the undiluted library by multiplying the determined concentration × 100.

7. Based on the calculated library concentration, determine the dilution that results in a concentration of ~25 pM for template preparation on the Ion Chef™ System.

   Note:
   - Good results have been observed with libraries ≤25 pM. See the *Oncomine™ Human Immune Repertoire User Guide* (Pub. No.MAN0019231) for information on combining libraries, templating and sequencing.
   - Libraries that yield significantly <25 pM as determined by qPCR can be rescued with library amplification. For more information, see the *Oncomine™ Human Immune Repertoire User Guide* (Pub. No. MAN0019231).
   - If short off-target, possible primer-dimer or adapter-dimer products are observed in the histogram data, perform rescue amplification (see the *Oncomine™ Human Immune Repertoire User Guide* (Pub. No. MAN0019231)) and re-purify the library with Agencourt™ AMPure™ XP Reagent twice (1.0X sample volume) and elute in a final volume of 30–50 µL.
   - Dilute libraries to 50 pM if sequencing on an Ion 550™ Chip.

   For example:
   - The undiluted library concentration is 300 pM.
   - The dilution factor is 300 pM/25 pM = 12.
   - Therefore, 10 µL of library that is mixed with 110 µL of Low TE (1:12 dilution) yields approximately 25 pM.

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C. We recommend transferring the quantified library to an RNase-free microcentrifuge tube for long-term storage. Alternatively, transfer the library to a new well and seal the plate with a new MicroAmp™ Clear Adhesive Film for long-term storage.

Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, California 94566 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0019237

Revision	Date	Description
A.0	24 September 2021	New quick reference guide for Oncomine™ TCR Pan-Clonality Assay library preparation.