Illumina DNA Prep Checklist

illumina

Step 1: Tagment Genomic DNA

1. Add 2–30 µl DNA to a 96-well PCR plate.
2. If DNA volume < 30 µl, add nuclease-free water to bring the volume to 30 µl.
3. Vortex BLT vigorously to resuspend.
4. For each sample, combine the following volumes.
   - BLT (11 µl)
   - TB1 (11 µl)
5. Vortex the master mix to resuspend.
6. Divide the master mix volume into an 8-tube strip.
7. Transfer 20 µl to each well containing a sample.
8. Discard the 8-tube strip.
9. Pipette 10 times to resuspend.
10. Seal the plate, place on the thermal cycler, and run the TAG program.

Step 2: Post Tagmentation Cleanup

1. Add 10 µl TSB.
2. Slowly pipette 10 times to resuspend the beads.
3. Seal the plate, place on the thermal cycler, and run the PTC program.
4. Place the plate on the magnetic stand until liquid is clear.
5. Remove and discard supernatant.
6. Remove from the magnetic stand and 100 µl TWB.
7. Pipette to resuspend or shake at 1600 rpm for 1 minute
8. Repeat steps 4–7 two times for a total of 3 washes.
9. Seal the plate and place on the magnetic stand until the liquid is clear.

Step 3: Amplify Tagmented DNA

1. For each sample, combine the following volumes.
   - EPM (22 µl)
   - Nuclease-free water (22 µl)
2. Vortex, and then centrifuge at 280 × g for 10 seconds.
3. Remove and discard supernatant.
4. Remove from the magnet.
5. Add 40 µl master mix in each sample well.
6. Pipette 10 times or shake at 1600 rpm for 1 minute.
7. Seal the plate and centrifuge at 280 × g for 3 seconds.
8. Add the appropriate index adapters to each sample.
9. Pipette 10 times or shake at 1600 rpm for 1 minute.
10. Seal the plate, and then centrifuge at 280 × g for 30 seconds.
11. Place on the thermal cycler and run the BLT PCR program.

SAFE STOPPING POINT

If you are stopping, store at 2°C to 8°C for up to 3 days.

Step 4: Clean Up Libraries

1. Centrifuge at 280 × g for 1 minute.
2. Place on the magnetic stand until the liquid is clear.
3. Transfer 45 µl supernatant to a new midi plate.
4. Vortex and invert IPB to resuspend.
5. For standard DNA input, perform the following steps.
   a. Add 40 µl nuclease-free water.
   b. Add 45 µl IPB.

c. Pipette 10 times or shake at 1600 rpm for 1 minute.
d. Seal the plate and incubate for 5 minutes.
e. Place on the magnetic stand until the liquid is clear.
f. Vortex IPB (*undiluted* stock tube), and then add 15 µl to a *new* midi plate.
g. Transfer 125 µl supernatant to the new plate.
h. Pipette 10 times or shake at 1600 rpm for 1 minute.
i. Discard the first plate.

6. For small PCR amplicon input, perform the following steps.
   a. Add 81 µl IPB.
   b. Pipette 10 times or shake at 1600 rpm for 1 minute.
7. Incubate at room temperature for 5 minutes.
8. Place on the magnetic stand until the liquid is clear.
9. Remove and discard supernatant.
10. Wash two times with 200 µl fresh 80% EtOH.
11. Remove and discard residual EtOH.
12. Air-dry for 5 minutes.
13. Remove from the magnetic stand.
14. Add 32 µl RSB.
15. Pipette to resuspend.
16. Incubate at room temperature for 2 minutes.
17. Place the plate on the magnetic stand until the liquid is clear.
18. Transfer 30 µl supernatant to a new plate.

SAFE STOPPING POINT

If you are stopping, seal the plate with Microseal 'B' adhesive or Microseal 'F' foil seal, and store at -25°C to -15°C for up to 30 days.

Step 5: Check Library Quality (Optional)

1. Run 1 µL library or poopled libraries on one of the following instruments. Add 1 µl RSB to the library to achieve the 2 µl volume required for Fragment Analyzer.

Step 6: Dilute Libraries to the Starting Concentration

1. Calculate the molarity value of the library or pooled libraries.
2. Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration.

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
HiSeq X, HiSeq 4000, and HiSeq 3000	2–3	200–300
iSeq 100	2	200
MiniSeq	2	1.2–1.3
MiSeq (v3 reagents)	4	12

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
NextSeq 550 and NextSeq 500	2	1.2–1.3
NextSeq 2000	2	750
NovaSeq 6000	2	See document NovaSeq 6000 System Guide (document # 10000000019358)

3. Dilute libraries using RSB:
   - **Libraries quantified as a multiplexed library pool**—Dilute the pool to the starting concentration.
   - **Libraries quantified individually**—Dilute each library to the starting concentration. Add 10 µl of each diluted library to a tube.
4. Dilute to the final loading concentration.

Acronyms

Acronym	Definition
BLT Bead Linked Transposome	Bead Linked Transposome
CD	Combinatorial Dual
dsDNA	Double stranded DNA
EPM	Enhanced PCR Mix
EtOH	Ethanol
IPB	Illumina Purification Beads
PK1	Proteinase K
RSB	Resuspension Buffer
ssDNA	Single stranded DNA
TB1	Tagmentation Buffer 1
TSB	Tagment Stop Buffer
TWB	Tagment Wash Buffer
UD	Unique Dual