Fragment DNA

- ☐1 Quantify gDNA.
- ☐2 Normalize gDNA with RSB in a final volume of 15 µl in a new plate:
  - For a 350 bp insert size—1.67 ng/µl of sample
  - For a 550 bp insert size—5 ng/µl of sample
- ☐3 Transfer 15 µl DNA to a microTUBE-15.
- ☐4 Centrifuge at 3000 × g for 1 minute.
- ☐5 Fragment the DNA on a Covaris.

Table 1 Covaris S220 or E220 Settings

Setting	350 bp Insert	550 bp Insert
Duty factor	20%	
Peak Incident Power	18 W	
Cycles per burst	50	
Duration	45 seconds	22 seconds
Temperature	20°C	
Water Level—S220	15	
Water Level—E220	10	

Table 2 Covaris M220 Settings

Setting	350 bp Insert	550 bp Insert
Duty factor	20%	
Peak Incident Power	30 W	
Cycles per burst	50	
Duration	42 seconds	23 seconds
Temperature	20°C	

- ☐6 Centrifuge at 600 × g for 5 seconds.
- ☐7 Transfer 15 µl DNA to a new plate.

SAFE STOPPING POINT

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

Prepare Samples for Loading

- ☐1 Add 1 of the following to a new 1.5 ml microcentrifuge tube:
  - For a 350 bp insert size—900 µl SC350
  - For a 550 bp insert size—700 µl SC550
- ☐2 Vortex DMB.
- ☐3 Add 100 µl DMB. Vortex for 5 seconds.
- ☐4 Pour the DMB and SC mixture into a new reservoir.
- ☐5 Add 35 µl DMB and SC mixture to the sample plate. Pipette to mix.
- ☐6 Shake or vortex at 1400 rpm for 12 minutes.

Set Up Run and Load Library Card

- ☐1 Vortex the reagent plate for 3 seconds.
- ☐2 Centrifuge at 600 × g for 5 seconds.
- ☐3 Select **Prepare Libraries**.
- ☐4 Select a protocol or run. Select **Next**.
- ☐5 Configure the run. Select **Next**.
- ☐6 Review the run and sample information. Select **Next**.
- ☐7 Enter tracking information. Select **Next**.
- ☐8 Place the library card on the stage.
- ☐9 Close the door. Select **Verify Library Card**.
- ☐10 Place the guide on the library card.
- ☐11 Load the oil.
- ☐12 Transfer 45 µl of samples 1–8 and 9–16.
- ☐13 Add 45 µl RSB to empty sample wells.
- ☐14 Transfer 125 µl of reagents i–iv and v–vii.
- ☐15 Vortex DMB.
- ☐16 Add 60 µl DMB to reagent well viii.
- ☐17 Transfer 15 µl of reagents 1–4 and 5–8.
- ☐18 Transfer 5 µl of reagents a–d and e–h.
- ☐19 Transfer 3 µl of adapters A–H and I–P.
- ☐20 Remove the guide.
- ☐21 Close the door. Select **Start Run**.
- ☐22 When the run is complete, select **Next**.

Unload Libraries

- ☐1 Add 10 µl RSB to a new plate.
- ☐2 Place the guide on the library card.
- ☐3 Transfer 20 µl from 1L–8L and 9L–16L to the plate. Pipette to mix.
- ☐4 Centrifuge briefly.
- ☐5 Transfer from plate wells 1–8 and 9–16 to the library separation tubes.
- ☐6 Let stand for 10 seconds.
- ☐7 Transfer from library separation tubes 1–8 and 9–16 to a new plate.
- ☐8 Remove the library card and guide.
- ☐9 Select **Home**.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Pool Libraries

- ☐1 Determine the number of samples to combine.
- ☐2 Transfer 5 µl to a single well of a new plate. Pipette to mix.
- ☐3 Proceed to cluster generation.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Acronyms

Acronym	Definition
DMB	Digital Microfluidics Beads
RSB	Resuspension Buffer
SC350	Sample Concentration Solution 350
SC550	Sample Concentration Solution 550