FILE S1.

Procedure:

Step 1 - Annealing of the linker.

Oligo sequences (from Picelli et al. 2014):

FC121-1030 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
FC121-1031 5'-GTCTCGTGGGCTCGGAGA TGTGTATAAGAGACAG-3'

Tn5MERev 5'-[phos]CTGTCTCTTATACACATCT-3'

1 Resuspend lyophilized oligos at 100uM in annealing buffer
2 Combine fwd and rev linkers 1:1 (FC121-1030 + Tn5MERev and FC121-1031 + Tn5MERev)

3 Anneal in thermocycler:

Cycle step	**Temperature (°C)**	**Time**
Denaturation	95	5 min
Cooling ramp 1	95 down to 65	0.1°C/s
Incubation	65	5 min
Cooling ramp 2	65 down to 4	0.1°C/s

store annealed linker in small aliquots of 5-10ul at -20°C

Side note - Tn5 linker complex assembly for optimized activity.

Example for mixing of Tn5 and linkers:
2.0 µl Tn5 protein (0.5 mg/ml – 9.36 uM)
0.5 µl linker A (1030-Tn5MERev annealed oligo)
0.5 µl linker B (1031-Tn5MERev annealed oligo)
8.0 µl Buffer A
11 µl total volume

Note: this is already a 5.5x dilution of the Tn5 protein.

Final buffer composition (approx.)
Storage buffer of Tn5 (20 mM Tris pH 7.4, 800 mM NaCl, 50% Glycerol)
Salt and Glycerol were diluted 5.5 times (2 µl in total volume of 11 µl)
Final Buffer:
20 mM Tris pH 7.4
145 mM NaCl
9 % Glycerol
This buffer favors the complex formation between the Tn5 and linkers, as high salt concentration (800 mM NaCl) will inhibit interaction between Tn5 and linkers.

*Further dilutions of the Tn5 are made with **Buffer B**, which is similar in composition to the buffer in which the Tn5-linker complexes are assembled. Final Tn5 dilutions of 1:10 to 1:50 work well. We recommend testing a dilution series for your application of interest to identify the preferred fragment size spectrum.*

Calculations:
2.0 µl of Tn5 (0.5 mg/ml) - 9.36 µM = 18.72 pmol
1 µl of linker mix - 50 µM = 50 pmol

Ratio:
Tn5:linkers = 1 : 2.67

Comment:
This ratio favors all Tn5 dimer molecules to be occupied by two linkers. Providing a slight excess of the linkers shifts the equilibrium to the fully saturated Tn5-linker complex.

Step 2 - Tn5 loading.
Thaw annealed linker on ice!
Avoid freeze-thaw cycles of Tn5 (aliquot stocks)

1) Add 0.5 ul of each linker (from above) to 2 ul Tn5 (0.5 mg/ml) stock and 8ul of Buffer A
2) Mix well and incubate at 23°C for 30 – 60 min at 300 rpm (thermomixer)
3) Dilute the Tn5 in Buffer B to the final desired dilution

Tn5 is ready to use!

If ideal dilution for desired fragment size is not known, make different final dilutions of Tn5 (e.g 1:10, 1:20, 1:50) and run tests for fragment size.

Step 3 - Cell wall digestion.
Use up to 100ul of saturated overnight culture (between 250,000 and ~2Mio cells final works well)
1) Spin down cells and resuspend in 25 ul 300U/ml Zymolyase (dilute Zymolyase in nuclease-free water)
2) Incubate 30 min at 37°C, inactivate 10 min at 95°C

Step 4 - Tagmentation.

Important: pre-heat block to desired temperature and make sure to exactly adhere to the incubation time (put samples on ice immediately after tagmentation).

Note: Tagmentation can be done at 37°C or 55°C. Fragment size spectrum can be adjusted by changing incubation temperature, time and the dilution factor of the loaded Tn5.

Tagmentation mix:
- 1.25 ul zymolyased cells
- 1.25 ul Tn5 dilution
- 2.5 ul tagmentation buffer + 20% DMF

mix well, spin down and incubate in pre-heated thermocycler

incubate 3 min at 55°C
put sample on ice
add 1.25 ul 0.2% SDS immediately
mix well by vortexing, spin down and incubate 5 min at room temperature

Step 5 Version A - Nucleosome release via Proteinase K.
1.75 ul 0.4 mg/ml Proteinase K
6.25 ul tagmented cells

incubate 30 min at 50°C, 15 min at 65°C

optional pause point: protocol can be stopped here and samples frozen at -20C

Step 5 Version B - Nucleosome release via salt.
3.75 ul 3M NaOAc
6.25 ul tagmented cells

incubate 60 min at room temperature.

Step 6 - 1.8X AMPure beads cleanup (5 min incubation of sample with beads at room temperature, two washes with 70% ethanol), elute in 6.25 ul nuclease-free water

Step 7 - PCR.
The oligos used in the PCR contain the sequencing adapter (grafting primer and index barcode)
1) Mix:

6.25 ul sample
6.75 ul 2x KAPA HiFi mix
0.75 ul DMSO
1.25 ul Index primer 1
1.25 ul Index primer 2

PCR program:

72°C	3 min	
95°C	30 s	
95°C	10 s	
55°C	30 s	x 12 cycles
72°C	60 s	
72°C	5 min	
10°C	hold	

Step 8 - clean up with 0.8x AmPure beads (to filter out small fragments), or 1.8x (recover everything), elute in 10 ul EB or nuclease-free water

Step 9 - QC dsDNA HS Qubit measurement and Bioanalyzer (Qubit yield should be minimum 1 ng/ul when starting from 100 ul saturated culture)

Buffers:

Annealing buffer:
40mM Tris pH 8
50mM NaCl

Buffer A:
20 mM Tris pH 7.5

Buffer B:
20 mM Tris pH 7.5
150 mM NaCl

Tagmentation buffer 2 (TB2, 100ml, in H_20, store at room temperature):
2ml 1M Tris (final 16 mM)
1ml 1M $MgCl_2$ (final 8 mM)
adjust pH to 7.6 with 100% acetic acid
Always fresh: add 20% (v/v) DMF