Supplemental Note. Reduced-input TdT Pacbio library preparation protocol.
For Pacific Biosciences SMRT sequencing, an experimental low DNA input protocol under development at Pacific Biosciences was used, which relies on terminal deoxynucleotidyl transferase (TdT) to add poly(dA) tails to the 3' ends of DNA fragments for priming and magnetic bead loading. DNA samples 60 (arm)-100 (foot) ng were randomly sheared to 10kb target size using G-tube (Covaris Inc.) and purified with 0.45x PacBio Ampure beads (Pac Bio part# 100-265-900) plus 10 min 42°C incubation at the elution step (This incubation step was included for subsequent Ampure purification steps during library preparation). Purified DNA was treated with NEB PreCR repair mix (NEB catlog# M0309) using manufacturer recommended protocol for 30 min at 37°C in 50µl reaction volume. Damage repaired DNA was then cooled on ice and treated with 5µl diluted NEB exoIII 1 min on ice (NEB catlog# M0206, diluted 10-fold in 1xNEBbuffer3). Exonuclease treated DNA was purified immediately with 0.6x PacBio Ampure beads and eluted with 14.5µl PacBio EB buffer. Promega TdT kit (Promega catalog# M1875) was used for adding poly-dA tail to the DNA template in a 40µl reaction with two modifications (Include 0.375xNEBbuffer3 in the reaction mixture and use 10µM dATP plus 2µM ddCTP as substrate). The TdT reaction mixture was incubated 1hr at 37 °C and terminated with 10µl of 0.5M EDTA. Final template DNA was purified with 0.45x PacBio Ampure beads and eluted with 15µl PacBio EB buffer. A spreadsheet is used for setting up SMRT sequencing with PacBio DNA/polymerase binding kit 2.0 and PacBio Magbead loading kit. The Pacific Biosciences RS II instrument was used to collect 120 min sequencing traces.

Additional materials and reagents required for SMRT sequencing:

- Poly-dA sequencing primer: TTTTTTTTTTmUmUmUmUmUmUmUmUTTTTT
- Heparin solution: Sigma H4784 (sodium salt from porcine intestinal mucosa), dissolved in ddH_2O at 20mg/ml concentration
- PacBio DNA/polymerase binding kit 2.0
- PacBio Magbead loading kit
- 5M KCl

	A	B	C
1	**TdT Sequencing calculator v5 (Please modify settings in red. Results are in blue, green, and orange) 01/30/2014**		
2	TdT library concentration (ng/µl)	6.76	(Use Qubit reading)
3	TdT library insert size (bases)	10000	
4	Number of chips to run	8	(1-8 cells)
5	On chip loading concentration (pM)	7.5	(On chip concentration = on plate concentration x 0.2)
6	itube concentration (pM)	60	
7	Stock Primer concentration (nM)	600	
8	Polymerase ratio	40	(Lower the ratio to 20 or 10 if the ZMW productivity distribution in sequencing run shows very high P2 and low P1)
9			
10	Library concentration (pM)	=ROUND(B2*0.000000001/B3/660/0.000001*1000000000000,2)	
11	Mag beads required (µl)	=37*(B4+1)	
12			
13	**Pol-DNA binding**		
14	ddH2O	=$B$23-B22-B20-B19-B18-B17-B16-B15	
15	Binding buffer	=$B$23/10	
16	Buffer A	=$B$23*190/5000	
17	DTT	=$B$23/10	
18	dNTP	=$B$23/10	
19	TdT-library	=$B$23*$B$6/$B$10	
20	Primer	=20*$B$23/$B$7	
21	**STOP: Incubate 30min on ice before adding polymerase.**		Following the steps below to dilute the P4 polymerase when 30min incubation is completed.
22	P4 polymerase dilute#2	=B8*$B$6*$B$23/8000	1. Dilute 1µl of P4 polymerase stock with 9µl of binding buffer (dilute#1).
23	Total volume (µl)	=B5*40*(B4+1)/B6	2. Take 1µl of the dilute#1 polymerase and dilute once again with 19µl of binding buffer (dilute#2).
24			
25	**Mag bead binding**		
26	Heparin	=$B$30/20	
27	BBB	=2*B28-2*B26	
28	BWB	=(B30+B26-B29)/3	
29	DNA-pol complex	=$B$5*40*(B4+1)/$B$6	
30	Total volume	=10*B4+10	
31	**STOP: Incubate 2min on ice before combining with beads.**		
32	washed Magbead (liquid removed)	=$B$11	
33			
34	**Mag bead washing**		
35	BBB	=19+9*(B4-1)	
36	BWB	=19+9*(B4-1)	
37	Final BBB volume for sample plate	=19+9*(B4-1)	