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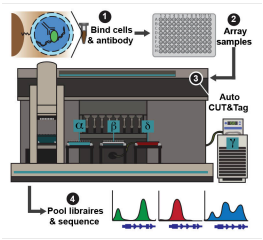
AutoCUT&Tag: streamlined genome-wide profiling of chromatin proteins on a liquid handling robot



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Human Cell Atlas Metho...

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We use this protocol and it's working

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Abstract

The CUT&Tag method is based on antibody tethering of the Tn5 transposase to profile the genome-wide occupancy of DNA-binding proteins, histone modifications and chromatin modifying proteins *in situ* starting from relatively low cell numbers (1-100K cells). Activation of tethered transposase integrates library adapters at the sites of bound chromatin factors. By taking advantage of protocol modifications that enable Tn5 release and PCR in the same sample reaction chamber, we adapted CUT&Tag to an automated 96 well format. We provide programs and equipment specifications for performing AutoCUT&Tag on a Beckman Coulter Biomek FX liquid handling robot equipped for magnetic separation and temperature control, but in principle the method could be easily translated to other liquid handling units. Using this automated protocol a single operator is able to generate up to 96 CUT&Tag libraries in a single day that are ready to be pooled and sequenced.

Guidelines

- **WORKFLOW**

Days 1-2 Cells to Libraries
Steps 1-8, prepare nuclei: 30 min
Steps 9-15, bind nuclei to beads and bind primary antibody: 2 hr-overnight
Steps 16-23, AutoCUT&Tag reaction: 4 hr
Steps 24-29, chromatin release and PCR amplification: 2 hr
Steps 30-35, post-PCR DNA cleanup: 1 hr
Days 3-5 Sequencing
Step 36-38, sequencing: 1-2 days
Day 6 (variable) Data processing and analysis
Step 39, ≥1 day

- **TROUBLESHOOTING**

Troubleshooting table			
Steps	**Problem**	**Possible reasons**	**Solutions**
Biomek Setup	Liquid volume is inconsistent between wells	The Biomek aspirate and or dispense function is imprecise from well to well potentially due to a clogged line or pump	• Avoid using problematic wells. • Contact Beckman Coulter technician, and possibly replace the P200 head.
Biomek Setup	Incorrect volume is being aspirated or dispensed in all wells	Aspiration is not occurring at the desired height within the well. The Biomek aspirate and or dispense function is not accurate.	• Adjust aspiration height in the Method. • Adjust volumes for desired result as determined empirically. • Contact Beckman Coulter technician and possibly recalibrate the machine.
37	No DNA is detected by Tape Station analysis	This indicates the reaction failed and could be due to (1) failed CUT&Tag reaction or (2) failed Library Prep.	• Run a positive control sample for an abundant epitope, *e.g.* H3K27me3. • Ensure Biomek is pipetting accurately e.g. no Ethanol remains after 5 min Air Dry during Post-PCR Cleanup.
37	Library yield for all samples is very low, and the fragment size distribution is as expected (i.e. mostly ~350bp mono-nucleosomal DNA)	This indicates the input may have been less than expected or material is being lost during processing on the Biomek	• Ensure ConA beads are binding the nuclei by comparing the input to the the unbound fraction following binding. • Increase nuclei to 100K per sample. • Ensure aspirations from V-Bottom plates are not bottoming out and preventing transfers, or shearing reagents.
37	Library yield for all samples is very low, and the fragment size distribution is skewed toward smaller sizes (i.e. concentration of 220 bp sub-nucleosomal DNA ≥ 350 mono-nucleosomal DNA)	This indicates an issue with the PCR extension and could be due to a bad ratio of SDS to Triton-x or to over-fixation of the nuclei.	• Ensure samples are suspended in 10 µL of 0.1 % SDS at the end of the CUT&Tag Full Reaction Method. • Alter PCR conditions to increase extension by (1) reducing the ramp rate (2) adding a 2 second 72°C extension step. • Remake SDS and Triton X-100 solutions • Perform an SDS vs. Triton X-100 titration on positive control samples.
39	Data quality from a sample of interest is poor or indistinguishable from the IgG control	This indicates the reaction failed possibly due to an antibody failure.	• Ensure primary and secondary antibodies are compatible and protein A binds the secondary. • Increase antibody concentration. • Test antibody binding by adding a fluorescent secondary and imaging. • Test antibody using standard CUT&Tag or CUT&RUN using digitonin permeabilized cells. • Replace antibody.

- **BIOMEK PROGRAMMING**

Labware Type Editor:

Eppendorf 96 Well LoBind PCR Plate, Semi-skirted (PCR 96 Well Plate)				
Basic Info		X	Y	
	Span:	12.426	8.397	cm
	Height:	2.02	cm	
Movement Info		X	Y	Z
	Gripper Offset:	0	0.15	0.8
	Gripper Squeeze:	0.45	cm	
	Gripper Unsqueeze:	-1	cm	
	Speed Limit:	50	%	
Wells		X	Y	
	Well Offset:	1.44	1.12	cm
	Well Count:	12	8	
	Well Spacing:	0.9	0.9	cm
Well Configuration	Max Volume:	235	µL	
	Shape:	Round		
	Upper Radius:	0.34	cm	
	Lower Radius:	0.31	cm	
	Height:	0.5	cm	
	Bottom:	X		
	Shape:	Cone		
	Radius:	0.31	cm	
	Height:	1.4	cm	

MicroAmp Support Base (PCR Plate Rack)						
Basic Info		X	Y			
	Span:	12.7762	8.5471	cm		
	Height:	1.4	cm			
Stacking		X	Y	Z		
	Stack Offset:	0	0	0.1	cm	
	Stacking Speed:	100	%			
Stack Offsets Edit	PCR 96 Well Plate:	X	Stack Offset X:	0	cm	
			Stack Offset Y:	0	cm	
			Stack Offset Z:	0.75	cm	
Wells		X	Y			
	Well Offset:	1.438	1.123	cm		
	Well Count:	12	8			
	Well Spacing:	0.9	0.9	cm		
	Max Volume:	250	µL			
Well Configuration	Shape:	Round				
	Upper Radius:	0.3	cm			
	Lower Radius:	0.28	cm			
	Height:	1	cm			
	Bottom:	N/A				

96S Super Magnet Plate (ALPAQUA Magnet Plate)						
Basic Info		X	Y			
	Span:	12.7762	8.5471	cm		
	Height:	1.6	cm			
Stacking		X	Y	Z		
	Stack Offset:	0	0	0.1	cm	
	Stacking Speed:	100	%			
Stack Offsets Edit	PCR 96 Well Plate:	X	Stack Offset X:	0	cm	
			Stack Offset Y:	0.05	cm	
			Stack Offset Z:	1.15	cm	
Wells		X	Y			
	Well Offset:	1.43764	1.12268	cm		
	Well Count:	12	8			
	Well Spacing:	0.9	0.9	cm		
	Max Volume:	170	µL			
Well Configuration	Shape:	Round				
	Upper Radius:	0.28	cm			
	Lower Radius:	0.28	cm			
	Height:	0.7	cm			
	Bottom:	N/A				

LE Magnet Plate (ALPAQUA LE Magnet Plate)					
Basic Info		X	Y		
	Span:	12.7762	8.5471	cm	
	Height:	1.3	cm		
Stacking		X	Y	Z	
	Stack Offset:	0	0	0.1	cm
	Stacking Speed:	100	%		
Stack Offsets Edit	PCR 96 Well Plate:	X	Stack Offset X:	0.05	cm
			Stack Offset Y:	0.05	cm
			Stack Offset Z:	1.275	cm
Wells		X	Y		
	Well Offset:	1.43764	1.12268	cm	
	Well Count:	12	8		
	Well Spacing:	0.9	0.9	cm	
	Max Volume:	170	µL		
Well Configuration	Shape:	Round			
	Upper Radius:	0.28	cm		
	Lower Radius:	0.28	cm		
	Height:	0.7	cm		
	Bottom:	N/A			

Aluminum Heat Block for PCR Plates (Cold Block)						
Basic Info		X	Y			
	Span:	12.7762	8.5471		cm	
	Height:	1.4	cm			
Stacking		X	Y	Z		
	Stack Offset:	0	0	0.1	cm	
	Stacking Speed:	100	%			
Stack Offsets Edit	PCR 96 Well Plate:	X	Stack Offset X:	0	cm	
			Stack Offset Y:	0	cm	
			Stack Offset Z:	0.45	cm	
Wells		X	Y			
	Well Offset:	1.43764	1.12268		cm	
	Well Count:	12	8			
	Well Spacing:	0.9	0.9		cm	
	Max Volume:	180	µL			
Well Configuration	Shape:	Round				
	Upper Radius:	0.3	cm			
	Lower Radius:	0.28	cm			
	Height:	0.5	cm			
	Bottom:	X				
	Shape:	Cone				
	Radius:	0.28	cm			
	Height:	0.7	cm			

96 Well Polystyrene V-Bottom Microplate (V-Bottom Plate)					
Basic Info		X	Y		
	Span:	12.78	8.56	cm	
	Height:	1.41	cm		
Movement Information					
		X	Y	Z	
	Gripper Offset:	0	0.2	0.5	cm
	Gripper Squeeze:	0.45	cm		
	Gripper Unsqueeze:	-1	cm		
	Speed Limit:	100	%		
	X	Use the gripper sensor, when available, to ensure the labware was gripped			
Stacking		X	Y	Z	
	Stack Offset:	0	0	0.129	cm
	Stacking Speed:	100	%		
	Allow Self-Stacking	X			
Secure Stacking Edit	V Bottom Plate:	X			
Wells		X	Y		
	Well Offset:	1.44	1.12	cm	
	Well Count:	12	8		
	Well Spacing:	0.9	0.9	cm	
	Max Volume:	215	µL		
Well Configuration	Shape:	Round			
	Upper Radius:	0.309	cm		
	Lower Radius:	0.2735	cm		
	Height:	0.75	cm		
	Bottom:	X			
	Shape:	Cone			
	Radius:	0.2735			
	Height:	0.2			

96 Deep Well 2 mL Plate (Deep Well Plate)				
Basic Info		X	Y	
	Span:	12.78	8.56	cm
	Height:	4.4	cm	
Wells		X	Y	
	Well Offset:	1.44	1.13	cm
	Well Count:	12	8	
	Well Spacing:	0.9	0.9	cm
	Max Volume:	2300	µL	
Well Configuration	Shape:	Rectangle		
		X	Y	
	Upper:	0.82	0.82	cm
	Lower:	0.737	0.737	cm
	Height:	3.732	cm	
	Bottom:	X		
	Shape:	Hemisphere		
	Radius:	0.3685	cm	

Biomek AP96 P250 barrier tips (AP96_200uL)				
Basic Info		X	Y	
	Span:	12.789	8.56	cm
	Height:	5.6	cm	
Tips		X	Y	
	Tip Offset:	1.447	1.132	cm
	Tip Count:	12	8	
	Tip Spacing:	0.9	0.9	cm
	Tip Load Z Offset:	-0.53	cm	
	Tip Unload Z Offset:	0	cm	
	Tip Type:	P200		

Liquid Type Editor:

Wash					
Aspirate			**Dispense**		
Trailing Air Gap:	1	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	25	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

TAPS LE					
Aspirate			**Dispense**		
Trailing Air Gap:	1	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	1	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

PCR Buffer					
Aspirate			**Dispense**		
Trailing Air Gap:	0	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	25	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

Tris-HCl-1					
Aspirate			**Dispense**		
Trailing Air Gap:	1	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	25	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

Ampure Wash					
Aspirate			**Dispense**		
Trailing Air Gap:	1	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	1	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

EtOH					
Aspirate			**Dispense**		
Trailing Air Gap:	1	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	25	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

Tris-HCl-2					
Aspirate			**Dispense**		
Trailing Air Gap:	1	µL	Delay:	0	ms
Delay:	0	ms	Speed:	10	µL/s
Speed:	1	µL/s	Cutoff Velocity:	150	µL/s
Blowout			**Tip Touch**		
Volume:	20	µL	Height:	-1	mm
Delay:	5000	ms	from:	Top	
Prewet			Angle:	90	
Overage:	0	µL	Speed:	100	%
Delay:	0	ms	Delay:	0	ms
Wash			**Sensitivity**		
Default Cycles:	1		Liquid Level Sensing		
Default Volume:	100%	µL	N/A		

Techniques:

Aspirate on Magnet		
Pipetting Template:	Default Template	
Aspirate Tab		
Move within the well at:	25	% speed
Aspirate at:	0	mm from the Bottom
N/A	Follow liquid level when aspirating or dispensing liquid	
N/A	Touch tips on the sides of wells	
N/A	Prewet the tips	
X	Aspirate a leading air gap for blowout	
N/A	Mix prior to aspirating liquid	
X	Aspirate a trailing air gap after leaving the liquid	

Dispense in Waste			
Pipetting Template:	Default Template		
Dispense Tab			
Move within the Well at:	100	% speed	
Dispense at:	35	mm from the	Bottom
N/A	Follow liquid level when aspirating or dispensing liquid		
X	Touch tips on the sides of the wells		
X	Blowout all leading air gaps		
N/A	Mix after dispensing		

Aspirate from DWP			
Pipetting Template:	Default Template		
Aspirate Tab			
Move within the well at:	50	% speed	
Aspirate at:	2	mm from the	Bottom
N/A	Follow liquid level when aspirating or dispensing liquid		
N/A	Touch tips on the sides of wells		
N/A	Prewet the tips		
N/A	Aspirate a leading air gap for blowout		
N/A	Mix prior to aspirating liquid		
X	Aspirate a trailing air gap after leaving the liquid		

Dispense on Magnet			
Pipetting Template:	Default Template		
Dispense Tab			
Move within the Well at:	25	% speed	
Dispense at:	2	mm from the	Bottom
N/A	Follow liquid level when aspirating or dispensing liquid		
N/A	Touch tips on the sides of the wells		
N/A	Blowout all leading air gaps		
N/A	Mix after dispensing		

Mix and Aspirate from VBP							
Pipetting Template:	Default Template						
Aspirate Tab							
Move within the Well at:	50	% speed					
Dispense at:	1.5	mm from the	Bottom				
N/A	Follow liquid level when aspirating or dispensing liquid						
N/A	Touch tips on the sides of the wells						
N/A	Prewet the Tips						
N/A	Aspirate a leading air gap for blowout						
X	Mix after dispensing liquid						
Mix:	100	µL	5	times			
Aspirate at:	2	mm from the	Bottom	at	25	µL/s	
Dispense at:	2	mm from the	Bottom	at	25	µL/s	
X	Aspirate a trailing air gap after leaving the liquid						

Aspirate from VBP			
Pipetting Template:	Default Template		
Aspirate tab			
Move within the well at:	50	% speed	
Aspirate at:	0.5	mm from the	Bottom
N/A	Follow liquid level when aspirating or dispensing liquid		
N/A	Touch tips on the sides of wells		
N/A	Prewet the tips		
N/A	Aspirate a leading air gap for blowout		
N/A	Mix prior to aspirating liquid		
X	Aspirate a trailing air gap after leaving the liquid		

Dispense on Magnet-2						
Pipetting Template:	Default Template					
Dispense Tab						
Move within the Well at:	25	% speed				
Dispense at:	0.5	mm from the	Bottom			
N/A	Follow liquid level when aspirating or dispensing liquid					
N/A	Touch tips on the sides of the wells					
N/A	Blowout all leading air gaps					
N/A	Mix after dispensing					

Dispense and Mix-1						
Pipetting Template:	Default Template					
Dispense Tab						
Move within the Well at:	25	% speed				
Dispense at:	1	mm from the	Bottom			
N/A	Follow liquid level when aspirating or dispensing liquid					
X	Touch tips on the sides of the wells					
X	Blowout all leading air gaps					
X	Mix after dispensing liquid					
Mix:	150	µL	20	times		
Aspirate at:	2	mm from the	Bottom	at	25	µL/s
Dispense at:	2	mm from the	Bottom	at	25	µL/s

Dispense and Mix-2							
Pipetting Template:	Default Template						
Dispense Tab							
Move within the Well at:	25	% speed					
Dispense at:	1	mm from the	Bottom				
N/A	Follow liquid level when aspirating or dispensing liquid						
X	Touch tips on the sides of the wells						
X	Blowout all leading air gaps						
X	Mix after dispensing liquid						
Mix:	30	µL	20	times			
Aspirate at:	1	mm from the	Bottom	at	50	µL/s	
Dispense at:	1	mm from the	Bottom	at	50	µL/s	

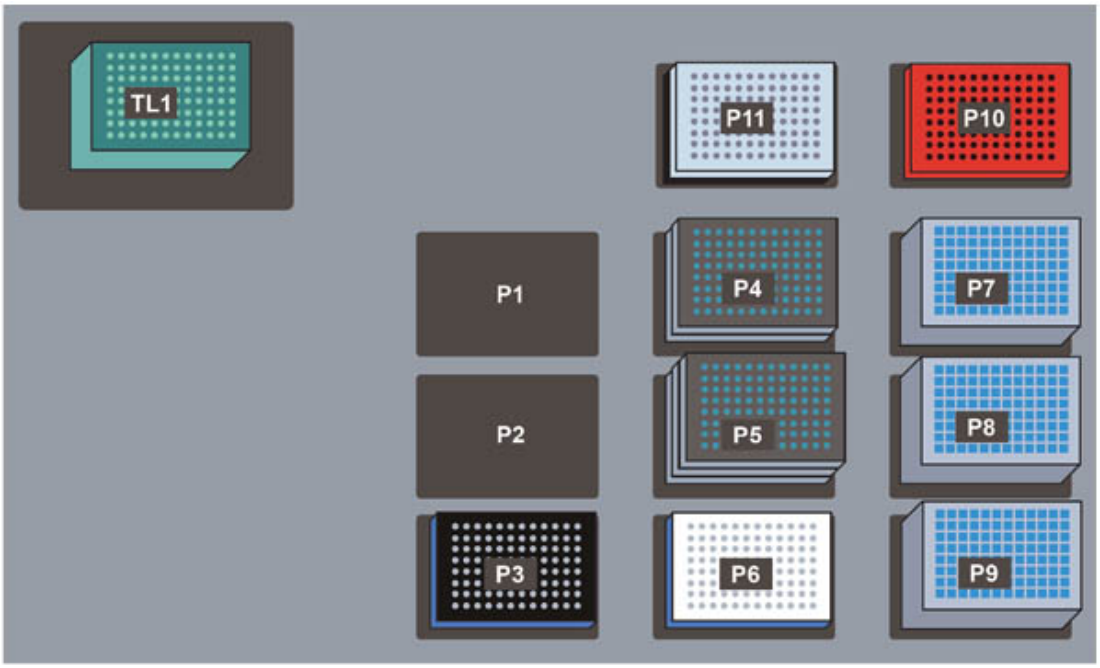
Dispense on Rack			
Pipetting Template:	Default Template		
Dispense Tab			
Move within the Well at:	25	% speed	
Dispense at:	1	mm from the	Bottom
N/A	Follow liquid level when aspirating or dispensing liquid		
X	Touch tips on the sides of the wells		
X	Blowout all leading air gaps		
N/A	Mix after dispensing		

Methods:

- **CUT&Tag Full Reaction**

1) Start

2) Instrument Setup



TL1: Fresh AP96 200 µL Tips (double click to increase the # of load times)
P3: ALPAQUA Magnet Plate
P4: V-Bottom Plate preloaded with 125 µL TAPS Buffer stacked on top of a V-Bottom Plate preloaded with 125 µL 0.1% SDS Release Buffer
P5: V-Bottom Plate preloaded with 125 µL Secondary Antibody Solution, stacked on top of a V-Bottom Plate preloaded with 125 µL pA-Tn5 Solution, stacked on top of a V-Bottom Plate preloaded with 125 µL Tagmentation Buffer
P6: ALPAQUA LE Magnet Plate
P7: Deep Well Plate preloaded with 1 mL of 300-Wash Buffer
P8: Deep Well Plate preloaded with 1 mL of Wash Buffer
P9: Deep Well Plate for receiving liquid waste
P10: Cold Block seated on a Cooling/Heating ALP routed to a Heating/Cooling Unit set to 37^{O}C
P11: PCR 96 Well Plate preloaded with up to 150 µL of conA bead-bound nuclei + primary antibody stacked on a PCR Plate Rack

3) Move Labware from P11 to P3			
Using pod:	Pod1		
Move labware from:	P11	to	P3
Move the topmost:	1	piece of labware from the stack	

4) Pause the whole system for 180 s				
Check: Pause	the whole system	for	180	s

5) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

6) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate on Magnet		

7) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense in Waste		

8) Aspirate from P8			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P8	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate from DWP		

9) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense on Magnet		

10) Pause the whole system for 30 s				
Check: Pause	the whole system	for	30	s

11) Repeat 6-10 to wash cells a second time

12) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	175	µL	
Technique:	Aspirate on Magnet		

13) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	175	µL	
Technique:	Dispense in Waste		

14) Aspirate from P5			
	Ensure tip height in well is 1.50 mm from bottom		
Labware Type:	V Bottom Plate	Pod:	Pod1
Position:	P5	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	100	µL	
Technique:	Mix & Aspirate from VBP		

15) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	100	µL	
Technique:	Dispense on Magnet		

16) Pause the whole system for 1350 s				
Check: Pause	the whole system	for	1350	s

17) Mix in P3							
		Ensure tip height in well is 0.00 mm from bottom					
Labware Type:	PCR 96 Well Plate	Pod	Pod1				
Position:	P3	N/A	Refresh tips				
Liquid Type:	Wash	Mix	5	times			
Volume:	50	µL					
Technique:	Custom						
Customize							
Pipetting Template:	Default Template						
Mix Tab							
Move within the well at:	25	% speed					
Aspirate at:	0	mm from the	Bottom	at	25	µL/s	
Dispense at:	0	mm from the	Bottom	at	25	µL/s	
N/A	Follow liquid level when aspirating or dispensing liquid						
N/A	Touch tips on the sides of the wells						
N/A	Aspirate a leading air gap prior to mix and blowout after mix is complete						

18) Pause the whole system for 1350 s				
Check: Pause	the whole system	for	1350	s

19) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

20) Move Labware from P5 to P2			
Using pod:	Pod1		
Move labware from:	P5	to	P2
Move the topmost:	1	piece of labware from the stack	

21) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

22) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	100	µL	
Technique:	Aspirate on Magnet		

23) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	100	µL	
Technique:	Dispense in Waste		

24) Aspirate from P8			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P8	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate from DWP		

25) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense on Magnet		

26) Pause the whole system for 30 s				
Check: Pause	the whole system	for	30	s

27) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate on Magnet		

28) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense in Waste		

29) Repeat 24-26 to Wash a second time.

30) Repeat 12-23 to bind pA-Tn5.

31) Aspirate from P7			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P7	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate from DWP		

32) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense on Magnet		

33) Pause the whole system for 30 s				
Check: Pause	the whole system	for	30	s

34) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate on Magnet		

35) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense in Waste		

36) Repeat 31-33 to Wash a second time.

37) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	160	µL	
Technique:	Aspirate on Magnet		

38) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	160	µL	
Technique:	Dispense in Waste		

39) Aspirate from P5			
	Ensure tip height in well is 1.50 mm from bottom		
Labware Type:	V Bottom Plate	Pod:	Pod1
Position:	P5	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	50	µL	
Technique:	Mix & Aspirate from VBP		

40) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	50	µL	
Technique:	Dispense on Magnet		

41) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

42) Move Labware from P3 to P10			
Using pod:	Pod1		
Move labware from:	P3	to	P10
Move the topmost:	1	piece of labware from the stack	

43) Pause the whole system for 3600 s				
Check: Pause	the whole system	for	3600	s

44) Move Labware from P10 to P3			
Using pod:	Pod1		
Move labware from:	P10	to	P3
Move the topmost:	1	piece of labware from the stack	

45) Pause the whole system for 180 s				
Check: Pause	the whole system	for	180	s

46) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

47) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	50	µL	
Technique:	Aspirate on Magnet		

48) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	50	µL	
Technique:	Dispense in Waste		

49) Aspirate from P8			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P8	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Aspirate from DWP		

50) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	150	µL	
Technique:	Dispense on Magnet		

51) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

52) Move Labware from P3 to P6			
Using pod:	Pod1		
Move labware from:	P3	to	P6
Move the topmost:	1	piece of labware from the stack	

53) Move Labware from P4 to P1			
Using pod:	Pod1		
Move labware from:	P4	to	P1
Move the topmost:	1	piece of labware from the stack	

54) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

55) Mix in P6						
		Ensure tip height in well is 5.00 mm from bottom				
Labware Type:	PCR 96 Well Plate	Pod	Pod1			
Position:	P3	N/A	Refresh tips			
Liquid Type:	Wash	Mix	5	times		
Volume:	50	µL				
Technique:	Custom					
Customize						
Pipetting Template:	Default Template					
Mix Tab						
Move within the well at:	100	% speed				
Aspirate at:	5	mm from the	Bottom	at	25	µL/s
Dispense at:	5	mm from the	Bottom	at	25	µL/s
N/A	Follow liquid level when aspirating or dispensing liquid					
N/A	Touch tips on the sides of the wells					
N/A	Aspirate a leading air gap prior to mix and blowout after mix is complete					

56) Pause the whole system for 180 s				
Check: Pause	the whole system	for	180	s

57) Aspirate from P6			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P6	N/A	Refresh tips
Liquid Type:	TAPS LE		
Volume:	155	µL	
Technique:	Aspirate on Magnet		

58) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	155	µL	
Technique:	Dispense in Waste		

59) Aspirate from P1			
	Ensure tip height in well is 0.50 mm from bottom		
Labware Type:	V Bottom Plate	Pod:	Pod1
Position:	P1	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	50	µL	
Technique:	Aspirate from VBP		

60) Dispense in P6			
	Ensure tip height in well is 0.50 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P6	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	50	µL	
Technique:	Dispense on Magnet-2		

61) Pause the whole system for 30 s				
Check: Pause	the whole system	for	30	s

62) Aspirate from P6			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P6	N/A	Refresh tips
Liquid Type:	TAPS LE		
Volume:	60	µL	
Technique:	Aspirate on Magnet		

63) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	60	µL	
Technique:	Dispense in Waste		

64) Mix in P1							
		Ensure tip height in well is 1.50 mm from bottom					
Labware Type:	V Bottom Plate	Pod	Pod1				
Position:	P1	N/A	Refresh tips				
Liquid Type:	Wash	Mix	5	times			
Volume:	50	µL					
Technique:	Custom						
Customize							
Pipetting Template:	Default Template						
Mix Tab							
Move within the well at:	100	% speed					
Aspirate at:	1.5	mm from the	Bottom	at	25	µL/s	
Dispense at:	1.5	mm from the	Bottom	at	25	µL/s	
N/A	Follow liquid level when aspirating or dispensing liquid						
X	Touch tips on the sides of the wells						
X	Aspirate a leading air gap prior to mix and blowout after mix is complete						

65) Aspirate from P4			
	Ensure tip height in well is 1.50 mm from bottom		
Labware Type:	V Bottom Plate	Pod:	Pod1
Position:	P4	N/A	Refresh tips
Liquid Type:	Wash		
Volume:	10	µL	
Technique:	Mix & Aspirate from VBP		

66) Dispense in P6			
	Ensure tip height in well is 0.50 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P6	N/A	Empty Tips
Liquid Type:	Wash		
Volume:	10	µL	
Technique:	Dispense on Magnet-2		

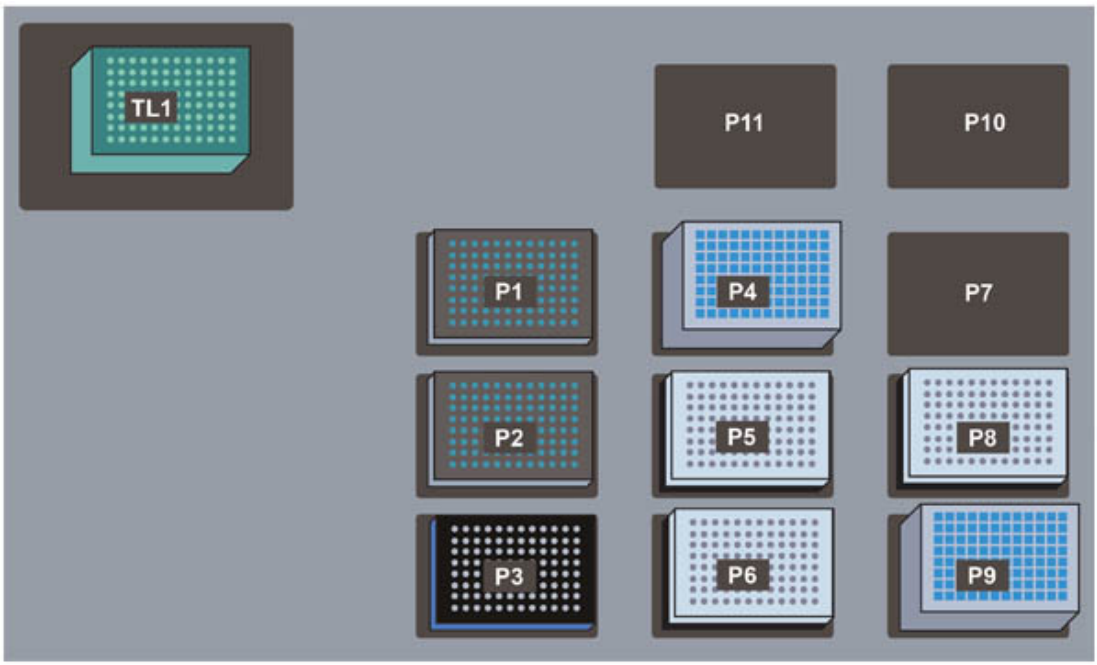
67) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

68) Finish
Check: Clear current instrument setup of all labware after the method completes
Check: Clear current devise setup of all labware after the method completes
Check: Unload disposable tips from all pods after the method completes
Check: Clear all global variables after the method completes

- **CUT&Tag Post-PCR Cleanup**

1) Start

2) Instrument Setup



TL1: Fresh AP96 200 µL Tips (double click to increase the # of load times)
P1: V-Bottom Plate preloaded with 100 µL10 mM Tris-HCl pH 8 (used for washing tips)
P2: V-Bottom Plate preloaded with 100 µL10 mM Tris-HCl pH 8 (used for DNA elution)
P3: ALPAQUA Magnet Plate
P4: Deep Well Plate preloaded with 1 mL 80% Ethanol
P5: PCR 96 Well Plate containing 100 µL of PCR product stacked on a PCR Plate Rack
P6: PCR 96 Well Plate preloaded with 130 µL of Ampure Beads stacked on a PCR Plate Rack
P8: PCR 96 Well Plate for accepting cleaned-up DNA stacked on a PCR Plate Rack
P9: Deep Well Plate for receiving liquid waste

3) Move Labware from P5 to P3			
Using pod:	Pod1		
Move labware from:	P5	to	P3
Move the topmost:	1	piece of labware from the stack	

4) Pause the whole system for 120 s				
Check: Pause	the whole system	for	120	s

5) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

6) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	PCR Buffer		
Volume:	105	µL	
Technique:	Aspirate on Magnet		

7) Dispense in P6			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P6	N/A	Empty Tips
Liquid Type:	PCR Buffer		
Volume:	105	µL	
Technique:	Dispense and Mix-1		

8) Mix in P6							
		Ensure tip height in well is 7.00 mm from bottom					
Labware Type:	PCR 96 Well Plate	Pod:	Pod1				
Position:	P6	N/A	Refresh tips				
Liquid Type:	Well Contents	Mix:	10	times			
Volume:	150	µL					
Technique:	Custom						
Customize							
Pipetting Template:	Default Template						
Mix Tab							
Move within the well at:	100	% speed					
Aspirate at:	7	mm from the	Bottom	at	25	µL/s	
Dispense at:	7	mm from the	Bottom	at	25	µL/s	
N/A	Follow liquid level when aspirating or dispensing liquid						
X	Touch tips on the sides of the wells						
X	Aspirate a leading air gap prior to mix and blowout after mix is complete						

9) Mix in P1						
		Ensure tip height in well is 1.50 mm from bottom				
Labware Type:	V-Bottom Plate	Pod:	Pod1			
Position:	P1	N/A	Refresh tips			
Liquid Type:	Tris-HCl-1	Mix:	5	times		
Volume:	50	µL				
Technique:	Custom					
Customize						
Pipetting Template:	Default Template					
Mix Tab						
Move within the well at:	25	% speed				
Aspirate at:	1.5	mm from the	Bottom	at	25	µL/s
Dispense at:	1.5	mm from the	Bottom	at	25	µL/s
N/A	Follow liquid level when aspirating or dispensing liquid					
X	Touch tips on the sides of the wells					
N/A	Aspirate a leading air gap prior to mix and blowout after mix is complete					

10) Pause the whole system for 300 s				
Check: Pause	the whole system	for	300	s

11) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

12) Move Labware from P3 to P5			
Using pod:	Pod1		
Move labware from:	P3	to	P5
Move the topmost:	1	piece of labware from the stack	

13) Move Labware from P6 to P3			
Using pod:	Pod1		
Move labware from:	P6	to	P3
Move the topmost:	1	piece of labware from the stack	

14) Pause the whole system for 180 s				
Check: Pause	the whole system	for	180	s

15) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

16) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Ampure Wash		
Volume:	120	µL	
Technique:	Aspirate on Magnet		

17) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Ampure Wash		
Volume:	120	µL	
Technique:	Dispense in Waste		

18) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Ampure Wash		
Volume:	110	µL	
Technique:	Aspirate on Magnet		

19) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	Ampure Wash		
Volume:	110	µL	
Technique:	Dispense in Waste		

20) Aspirate from P4			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P4	N/A	Refresh tips
Liquid Type:	EtOH		
Volume:	150	µL	
Technique:	Aspirate from DWP		

21) Dispense in P3			
	Ensure tip height in well is 2.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Empty Tips
Liquid Type:	EtOH		
Volume:	150	µL	
Technique:	Dispense on Magnet		

22) Pause the whole system for 30 s			
Check: Pause	the whole system	for	30 s

23) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	EtOH		
Volume:	150	µL	
Technique:	Aspirate on Magnet		

24) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	EtOH		
Volume:	150	µL	
Technique:	Dispense in Waste		

25) Repeat Steps 20-22 to wash bead-bound DNA a second time with Ethanol

26) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	EtOH		
Volume:	155	µL	
Technique:	Aspirate on Magnet		

27) Dispense in P9			
	Ensure tip height in well is 35.00 mm from bottom		
Labware Type:	Deep Well Plate	Pod:	Pod1
Position:	P9	N/A	Empty Tips
Liquid Type:	EtOH		
Volume:	155	µL	
Technique:	Dispense in Waste		

28) Pause the whole system for 300 s				
Check: Pause	the whole system	for	300	s

29) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

30) Move Labware from P3 to P6			
Using pod:	Pod1		
Move labware from:	P3	to	P6
Move the topmost:	1	piece of labware from the stack	

31) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

32) Aspirate from P2			
	Ensure tip height in well is 0.50 mm from bottom		
Labware Type:	V-Bottom Plate	Pod:	Pod1
Position:	P2	N/A	Refresh tips
Liquid Type:	Tris-HCl-1		
Volume:	45	µL	
Technique:	Aspirate from VBP		

33) Dispense in P6			
	Ensure tip height in well is 1.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P6	N/A	Empty Tips
Liquid Type:	Tris-HCl-1		
Volume:	45	µL	
Technique:	Dispense and Mix-2		

34) Mix in P1						
		Ensure tip height in well is 1.50 mm from bottom				
Labware Type:	V-Bottom Plate	Pod:	Pod1			
Position:	P1	N/A	Refresh tips			
Liquid Type:	Tris-HCl-1	Mix:	5	times		
Volume:	50	µL				
Technique:	Custom					
Customize						
Pipetting Template:	Default Template					
Mix Tab						
Move within the well at:	25	% speed				
Aspirate at:	1.5	mm from the	Bottom	at	25	µL/s
Dispense at:	1.5	mm from the	Bottom	at	25	µL/s
N/A	Follow liquid level when aspirating or dispensing liquid					
X	Touch tips on the sides of the wells					
N/A	Aspirate a leading air gap prior to mix and blowout after mix is complete					

35) Pause the whole system for 180 s				
Check: Pause	the whole system	for	180	s

36) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

37) Move Labware from P6 to P3			
Using pod:	Pod1		
Move labware from:	P6	to	P3
Move the topmost:	1	piece of labware from the stack	

38) Pause the whole system for 120 s				
Check: Pause	the whole system	for	120	s

39) Load tips from TL1	
Tips:	Load
Location:	TL1
Pod:	Pod1

40) Aspirate from P3			
	Ensure tip height in well is 0.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P3	N/A	Refresh tips
Liquid Type:	Tris-HCl-2		
Volume:	40	µL	
Technique:	Aspirate on Magnet		

41) Dispense in P8			
	Ensure tip height in well is 1.00 mm from bottom		
Labware Type:	PCR 96 Well Plate	Pod:	Pod1
Position:	P8	N/A	Empty Tips
Liquid Type:	Tris-HCl-2		
Volume:	40	µL	
Technique:	Dispense on Rack		

42) Unload tips to TL1	
Tips:	Unload
Location:	TL1
Pod:	Pod1

43) Finish
Check: Clear current instrument setup of all labware after the method completes
Check: Clear current devise setup of all labware after the method completes
Check: Unload disposable tips from all pods after the method completes
Check: Clear all global variables after the method completes

Materials

MATERIALS

- Glycerol **Catalog #**G5516
- Triton(R) X-100 100ml **Promega Catalog #**H5142
- Microtube, 1.5ml, 1000/bag **Promega Catalog #**V1231
- Positive control antibody to an abundant epitope, e.g. α-H3K27me3 rabbit monoclonal antibody (Cell Signaling Technology, cat. no. 9733)
- Negative control antibody to an absent epitope, e.g. guinea pig α-rabbit antibody
- 1 M Manganese Chloride (MnCl2) **Sigma Aldrich Catalog #**203734
- 1 M Calcium Chloride (CaCl2) **Fisher Scientific Catalog #**BP510
- 1 M Potassium Chloride (KCl) **Sigma Aldrich Catalog #**P3911
- 0.5 M Ethylenediaminetetraacetic acid (EDTA) **Research Organics Catalog #**3002E
- 2 M Spermidine **Sigma Aldrich Catalog #**S2501
- Agencourt AMPure XP magnetic beads **Beckman Coulter Catalog #**A63880
- 10% Sodium dodecyl sulfate (SDS) **Sigma Aldrich Catalog #**L4509
- Ethanol **Decon Labs Catalog #**2716
- Concanavalin-coated magnetic beads **Bangs Laboratories Catalog #**BP531
- 1M HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH 7.5 **Sigma Aldrich Catalog #**H3375
- 1 M HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH 7.9 **Sigma Aldrich Catalog #**H3375
- Roche Complete Protease Inhibitor EDTA-Free tablets **Sigma Aldrich Catalog #**5056489001
- Cell suspension. We have used human K562 and H1 cells as well as several human brain tumor lines propagated both in cell culture as well as in the brains of immuno-compromised mice before being resected and frozen Drosophila S2 cells and dissected Drosophila tissues such as brains and imaginal disks and spheroplasted yeast.
- Centrifuge 5810 swinging bucket **Eppendorf Centrifuge Catalog #**022625004
- Centrifuge 5424 R refrigerated with Rotor FA-45-24-11 rotary knobs 120 V/50–60 Hz (US) **Eppendorf Centrifuge Catalog #**5404000537
- MACSiMAG Separator **Miltenyi Biotec Catalog #**130-092-168
- 0.6 mL low-retention microcentrifuge tubes **Thermo Fisher Scientific Catalog #**3446
- BD Clay Adams™ Nutator Mixer BD Diagnostics **Vwr Catalog #**15172-203
- Capillary electrophoresis instrument (e.g. Agilent Tapestation 4200)
- Thermal cycler with 3 degree C/sec ramp rate that is compatible with a 96 well PCR plate

Biomek FX or FXP equipped with a 96-channel pod and P200 head **Beckman Coulter**

One 1 × 1 Tip Loader ALP **Beckman Coulter Catalog #**C02867

Three 1 × 3 Static ALPs **Beckman Coulter Catalog #**B87478

One Single Position Cooling/Heating ALP **Beckman Coulter Catalog #**719361

96S Super Magnet Plate **Alpaqua Catalog #**A001322

Aluminum Heat Block Insert for PCR Plates **V&P Scientific Catalog #**VP741I6A

Recirculating Cooling Unit filled with antifreeze (e.g Thermo Neslab RTE-7 Digital One Recirculating Chiller Mfr # 271103200000)

MicroAmp Support Bases **Thermofisher Catalog #**N801-0531

96 well LoBind PCR plates Semi-skirted **Eppendorf Catalog #**0030129504

Biomek AP96 P250 Pre-Sterile Tips with barrier **Beckman Coulter Catalog #**717253

96 well Polystyrene V-Bottom Microplates **greiner bio-one Catalog #**651101

• MASTERBLOCKTM 96 Deep Well Conical Bottom 2 mL Storage Plates **greiner bio-one Catalog #**780271

2-20 µL 8-Channel Multi Pipette (e.g. Rainin 17013803)

20-200 µL 8-Channel Multi Pipette (e.g. Rainin 17013805)

Reagent Reservoirs (e.g. Thermo Scientific 8095)

Sodium Chloride (NaCl) **Fisher Scientific Catalog #**S271

Glycine **Fisher Scientific Catalog #**G46

Protein A-Tn5 (pA-Tn5) fusion protein (plasmid for protein prep available). **addgene Catalog #**124601

KAPA HiFi PCR Kit **Kapa Biosystems Catalog #**KK2102

AlumaSeal(R) 96 film **Sigma Aldrich Catalog #**Z721549-100EA

100-1200 µL 8-Channel Multi Pipette (e.g. Rainin 17014496)

One 1X1 Static ALP **Beckman Coulter Catalog #**B87477

LE Magnet Plate **Alpaqua Catalog #**A000350

16% Formaldehyde Solution (w/v) Methanol-free **Thermo Scientific Catalog #**28906

1 M Magnesium Chloride (MgCl2) **Sigma Aldrich Catalog #**M8266

Prior to use the pA-Tn5 protein must be loaded with double-stranded adapters with 19mer Tn5 mosaic ends (Sequence information was derived from Picelli, S. et al. Genome Res 24, 2033-2040 (2014), and ordered through Eurofins, 100 µM in TE buffer)

- Mosaic end_reverse [PHO]CTGTCTCTTATACACATCT
- Mosaic end_Adapter A TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
- Mosaic end_Adapter B GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

- PCR primers: i5 primer and i7 primers with unique barcodes [Buenrostro, J.D. et al. Nature 523:486 (2015)] brought up in in 10 mM Tris pH 8. Standard salt-free primers may be used. Do not use Nextera primers..

	Custom Barcodes Adapter 1 (index i5):	
	v2_Ad1.1_TAGATCGC	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.2_CTCTCTAT	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.3_TATCCTCT	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.4_AGAGTAGA	AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.5_GTAAGGAG	AATGATACGGCGACCACCGAG

		ATCTAC ACGTA AGGAG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.6_ACTGCATA	AATGAT ACGGC GACCA CCGAG ATCTAC ACACT GCATAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.7_AAGGAGTA	AATGAT ACGGC GACCA CCGAG ATCTAC ACAAG GAGTAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.8_CTAAGCCT	AATGAT ACGGC GACCA CCGAG ATCTAC ACCTA AGCCT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.9_TGGAAATC	AATGAT ACGGC GACCA CCGAG ATCTAC ACTGG AAATC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.10_AACATGAT	AATGAT ACGGC GACCA CCGAG ATCTAC

		ACAAC ATGATT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.11_TGATGAAA	AATGAT ACGGC GACCA CCGAG ATCTAC ACTGA TGAAA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.12_GTCGGACT	AATGAT ACGGC GACCA CCGAG ATCTAC ACGTC GGACT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.13_TTTCTAGC	AATGAT ACGGC GACCA CCGAG ATCTAC ACTTT CTAGC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.14_TAACCAAG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTAA CCAAG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.15_GTGTATCG	AATGAT ACGGC GACCA CCGAG ATCTAC ACGTG

		TATCGT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.16_TCCATCAA	AATGAT ACGGC GACCA CCGAG ATCTAC ACTCC ATCAAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.17_TTCGTGCA	AATGAT ACGGC GACCA CCGAG ATCTAC ACTTC GTGCA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.18_AGGTTGCC	AATGAT ACGGC GACCA CCGAG ATCTAC ACAGG TTGCC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.19_CCTTATGT	AATGAT ACGGC GACCA CCGAG ATCTAC ACCCT TATGTT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.20_CAGCAACG	AATGAT ACGGC GACCA CCGAG ATCTAC ACCAG CAACG

		TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.21_GGTTCAAT	AATGAT ACGGC GACCA CCGAG ATCTAC ACGGT TCAATT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.22_ACATTCGT	AATGAT ACGGC GACCA CCGAG ATCTAC ACACA TTCGT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.23_GATTCCCA	AATGAT ACGGC GACCA CCGAG ATCTAC ACGAT TCCCA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.24_CGGACTGC	AATGAT ACGGC GACCA CCGAG ATCTAC ACCGG ACTGC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.25_AGCCGTTC	AATGAT ACGGC GACCA CCGAG ATCTAC ACAGC CGTTC TCGTC

		GGCAG CGTCA GATGT GTAT
	v2_Ad1.26_ATTGGGTC	AATGAT ACGGC GACCA CCGAG ATCTAC ACATT GGGTC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.27_TGCATACT	AATGAT ACGGC GACCA CCGAG ATCTAC ACTGC ATACTT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.28_GGGCTTGG	AATGAT ACGGC GACCA CCGAG ATCTAC ACGGG CTTGG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.29_GACGTGGC	AATGAT ACGGC GACCA CCGAG ATCTAC ACGAC GTGGC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.30_GCAAATTT	AATGAT ACGGC GACCA CCGAG ATCTAC ACGCA AATTTT CGTCG GCAGC

		GTCAG ATGTGT AT
	v2_Ad1.31_GCAGCCTC	AATGAT ACGGC GACCA CCGAG ATCTAC ACGCA GCCTC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.32_TCCGAGTT	AATGAT ACGGC GACCA CCGAG ATCTAC ACTCC GAGTT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.33_GCATTAAG	AATGAT ACGGC GACCA CCGAG ATCTAC ACGCA TTAAGT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.34_ACGATAAC	AATGAT ACGGC GACCA CCGAG ATCTAC ACACG ATAACT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.35_CCTGCGGG	AATGAT ACGGC GACCA CCGAG ATCTAC ACCCT GCGGG TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.36_TGATTGTT	AATGAT ACGGC GACCA CCGAG ATCTAC ACTGA TTGTTT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.37_GGCACGGA	AATGAT ACGGC GACCA CCGAG ATCTAC ACGGC ACGGA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.38_GATCATTC	AATGAT ACGGC GACCA CCGAG ATCTAC ACGAT CATTC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.39_ATGGTCAT	AATGAT ACGGC GACCA CCGAG ATCTAC ACATG GTCAT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.40_CGTACCAA	AATGAT ACGGC GACCA CCGAG ATCTAC ACCGT ACCAA TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.41_CCAGTTTA	AATGAT ACGGC GACCA CCGAG ATCTAC ACCCA GTTTAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.42_ACCGGCCC	AATGAT ACGGC GACCA CCGAG ATCTAC ACACC GGCCC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.43_CTAGAAGT	AATGAT ACGGC GACCA CCGAG ATCTAC ACCTA GAAGT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.44_CGCCAGAT	AATGAT ACGGC GACCA CCGAG ATCTAC ACCGC CAGAT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.45_TCACATGG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTCA CATGG TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.46_GAACTCGA	AATGAT ACGGC GACCA CCGAG ATCTAC ACGAA CTCGA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.47_CCACCGTT	AATGAT ACGGC GACCA CCGAG ATCTAC ACCCA CCGTT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.48_TAAGTTAC	AATGAT ACGGC GACCA CCGAG ATCTAC ACTAA GTTAC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.49_GAGACGTG	AATGAT ACGGC GACCA CCGAG ATCTAC ACGAG ACGTG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.50_TTGCCTAA	AATGAT ACGGC GACCA CCGAG ATCTAC ACTTG CCTAA TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.51_TTAACTTG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTTA ACTTG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.52_CTTTAACA	AATGAT ACGGC GACCA CCGAG ATCTAC ACCTT TAACAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.53_CGTAGACC	AATGAT ACGGC GACCA CCGAG ATCTAC ACCGT AGACC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.54_TATTTGCG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTATT TGCGT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.55_ATCCAGGA	AATGAT ACGGC GACCA CCGAG ATCTAC ACATC CAGGA TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.56_TGTTCCTG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTGT TCCTG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.57_ACGCGCAG	AATGAT ACGGC GACCA CCGAG ATCTAC ACACG CGCAG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.58_TCTGGCGA	AATGAT ACGGC GACCA CCGAG ATCTAC ACTCT GGCGA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.59_AATCTACA	AATGAT ACGGC GACCA CCGAG ATCTAC ACAAT CTACAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.60_TACTGACC	AATGAT ACGGC GACCA CCGAG ATCTAC ACTAC TGACC TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.61_CGATAGGG	AATGAT ACGGC GACCA CCGAG ATCTAC ACCGA TAGGG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.62_ACTTAGAA	AATGAT ACGGC GACCA CCGAG ATCTAC ACACT TAGAAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.63_AGAGATCT	AATGAT ACGGC GACCA CCGAG ATCTAC ACAGA GATCT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.64_GGTGAAGG	AATGAT ACGGC GACCA CCGAG ATCTAC ACGGT GAAGG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.65_ATCGAATG	AATGAT ACGGC GACCA CCGAG ATCTAC ACATC GAATG TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.66_TCAAGAGC	AATGAT ACGGC GACCA CCGAG ATCTAC ACTCA AGAGC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.67_GCCCACGT	AATGAT ACGGC GACCA CCGAG ATCTAC ACGCC CACGT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.68_TGGGCGGT	AATGAT ACGGC GACCA CCGAG ATCTAC ACTGG GCGGT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.69_CCCTTGGA	AATGAT ACGGC GACCA CCGAG ATCTAC ACCCC TTGGA TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.70_ATTACCGT	AATGAT ACGGC GACCA CCGAG ATCTAC ACATTA CCGTT CGTCG GCAGC GTCAG

		ATGTGTAT
	v2_Ad1.71_AGTCCGAG	AATGATACGGCGACCACCGAGATCTACACAGTCCGAGTCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.72_ACTTGTTG	AATGATACGGCGACCACCGAGATCTACACACTTGTTGTCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.73_GTAATACA	AATGATACGGCGACCACCGAGATCTACACGTAATACATCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.74_GGCGTCTA	AATGATACGGCGACCACCGAGATCTACACGGCGTCTATCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.75_GCGCTGCT	AATGATACGGCGACCACCGAGATCTACACGCGCTGCTTCGTCGGCAGCGTCA

		GATGT GTAT
	v2_Ad1.76_GTGCCATT	AATGAT ACGGC GACCA CCGAG ATCTAC ACGTG CCATT TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.77_TAGGTATG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTAG GTATGT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.78_AACACCTA	AATGAT ACGGC GACCA CCGAG ATCTAC ACAAC ACCTAT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.79_CTCCGAAC	AATGAT ACGGC GACCA CCGAG ATCTAC ACCTC CGAAC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.80_CAACGGCA	AATGAT ACGGC GACCA CCGAG ATCTAC ACCAA CGGCA TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.81_CAATGTAG	AATGAT ACGGC GACCA CCGAG ATCTAC ACCAA TGTAGT CGTCG GCAGC GTCAG ATGTGT AT
	v2_Ad1.82_GGCTACCC	AATGAT ACGGC GACCA CCGAG ATCTAC ACGGC TACCC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.83_AAAGTCCG	AATGAT ACGGC GACCA CCGAG ATCTAC ACAAA GTCCG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.84_TTCCGCGG	AATGAT ACGGC GACCA CCGAG ATCTAC ACTTC CGCGG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.85_AGGCACTT	AATGAT ACGGC GACCA CCGAG ATCTAC ACAGG CACTT TCGTC GGCAG CGTCA

		GATGT GTAT
	v2_Ad1.86_CTTCAGTG	AATGAT ACGGC GACCA CCGAG ATCTAC ACCTT CAGTG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.87_GCCGGTAG	AATGAT ACGGC GACCA CCGAG ATCTAC ACGCC GGTAG TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.88_TTCAATCC	AATGAT ACGGC GACCA CCGAG ATCTAC ACTTC AATCC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.89_CCACACAC	AATGAT ACGGC GACCA CCGAG ATCTAC ACCCA CACAC TCGTC GGCAG CGTCA GATGT GTAT
	v2_Ad1.90_ATATTATC	AATGAT ACGGC GACCA CCGAG ATCTAC ACATAT TATCTC GTCGG CAGCG TCAGA

		TGTGTAT
	v2_Ad1.91_CCGAAGCA	AATGATACGGCGACCACCGAGATCTACACCCGAAGCATCGTCGGCAGCGTCAGATGTGTAT
	v2_Ad1.92_GTATCGGT	AATGATACGGCGACCACCGAGATCTACACGTATCGGTTCGTCGGCAGCGTCAGATGTGTAT
	Custom Barcodes Adapter 2 (index i7):	
	v2_Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTG
	v2_Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGTG
	v2_Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGG

		GCTCG GAGAT GTG
	v2_Ad2.4_TCCTGAGC	CAAGC AGAAG ACGGC ATACG AGATG CTCAG GAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.5_GGACTCCT	CAAGC AGAAG ACGGC ATACG AGATA GGAGT CCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.6_TAGGCATG	CAAGC AGAAG ACGGC ATACG AGATCA TGCCT AGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.7_CTCTCTAC	CAAGC AGAAG ACGGC ATACG AGATGT AGAGA GGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.8_CAGAGAGG	CAAGC AGAAG ACGGC ATACG AGATC CTCTC TGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.9_GCTACGCT	CAAGC AGAAG

		ACGGC ATACG AGATA GCGTA GCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.10_CGAGGCTG	CAAGC AGAAG ACGGC ATACG AGATC AGCCT CGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.11_AAGAGGCA	CAAGC AGAAG ACGGC ATACG AGATT GCCTC TTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.12_GTAGAGGA	CAAGC AGAAG ACGGC ATACG AGATT CCTCT ACGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.13_TGGATCTG	CAAGC AGAAG ACGGC ATACG AGATC AGATC CAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.14_CCGTTTGT	CAAGC AGAAG ACGGC ATACG AGATA CAAAC GGGTC TCGTG

		GGCTC GGAGA TGTG
	v2_Ad2.15_TGCTGGGT	CAAGC AGAAG ACGGC ATACG AGATA CCCAG CAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.16_AGGTTGGG	CAAGC AGAAG ACGGC ATACG AGATC CCAAC CTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.17_GTGTGGTG	CAAGC AGAAG ACGGC ATACG AGATC ACCAC ACGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.18_TGGGTTTC	CAAGC AGAAG ACGGC ATACG AGATG AAACC CAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.19_TGGTCACA	CAAGC AGAAG ACGGC ATACG AGATT GTGAC CAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.20_TTGACCCT	CAAGC AGAAG

		ACGGC ATACG AGATA GGGTC AAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.21_CGCGGACA	CAAGC AGAAG ACGGC ATACG AGATT GTCCG CGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.22_TTCCATAT	CAAGC AGAAG ACGGC ATACG AGATAT ATGGA AGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.23_AATTCGTT	CAAGC AGAAG ACGGC ATACG AGATA ACGAA TTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.24_GGCGTCGA	CAAGC AGAAG ACGGC ATACG AGATT CGACG CCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.25_ACAAAGTG	CAAGC AGAAG ACGGC ATACG AGATC ACTTT GTGTC TCGTG

		GGCTC GGAGA TGTG
	v2_Ad2.26_TACTTGAA	CAAGC AGAAG ACGGC ATACG AGATTT CAAGT AGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.27_GTGATAGC	CAAGC AGAAG ACGGC ATACG AGATG CTATCA CGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.28_AGTAGATT	CAAGC AGAAG ACGGC ATACG AGATAA TCTAC TGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.29_ATTGCCGG	CAAGC AGAAG ACGGC ATACG AGATC CGGCA ATGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.30_TTGCTAAG	CAAGC AGAAG ACGGC ATACG AGATC TTAGC AAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.31_ATAAGTTA	CAAGC AGAAG

		ACGGC ATACG AGATTA ACTTAT GTCTC GTGGG CTCGG AGATG TG
	v2_Ad2.32_ATCACTCG	CAAGC AGAAG ACGGC ATACG AGATC GAGTG ATGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.33_GTTAACAG	CAAGC AGAAG ACGGC ATACG AGATC TGTTA ACGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.34_AATGGTAG	CAAGC AGAAG ACGGC ATACG AGATC TACCAT TGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.35_GAGCACGT	CAAGC AGAAG ACGGC ATACG AGATA CGTGC TCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.36_TTTCGTCA	CAAGC AGAAG ACGGC ATACG AGATT GACGA AAGTC TCGTG

		GGCTC GGAGA TGTG
	v2_Ad2.37_CAAGAATT	CAAGC AGAAG ACGGC ATACG AGATAA TTCTT GGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.38_GAAATGCC	CAAGC AGAAG ACGGC ATACG AGATG GCATT TCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.39_AACGCCAT	CAAGC AGAAG ACGGC ATACG AGATAT GGCGT TGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.40_CCTCGCAG	CAAGC AGAAG ACGGC ATACG AGATC TGCGA GGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.41_TACACCTC	CAAGC AGAAG ACGGC ATACG AGATG AGGTG TAGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.42_GGTCATTT	CAAGC AGAAG

		ACGGC ATACG AGATA AATGA CCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.43_CAATCTTA	CAAGC AGAAG ACGGC ATACG AGATTA AGATT GGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.44_TGTGCCTT	CAAGC AGAAG ACGGC ATACG AGATA AGGCA CAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.45_TCTTATTA	CAAGC AGAAG ACGGC ATACG AGATTA ATAAG AGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.46_GACTTAGT	CAAGC AGAAG ACGGC ATACG AGATA CTAAG TCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.47_AGACCAGC	CAAGC AGAAG ACGGC ATACG AGATG CTGGT CTGTC TCGTG

		GGCTC GGAGA TGTG
	v2_Ad2.48_AAATACAG	CAAGC AGAAG ACGGC ATACG AGATC TGTATT TGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.49_TTATGAAA	CAAGC AGAAG ACGGC ATACG AGATTT TCATAA GTCTC GTGGG CTCGG AGATG TG
	v2_Ad2.50_CTTGGGTC	CAAGC AGAAG ACGGC ATACG AGATG ACCCA AGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.51_CCAAATAA	CAAGC AGAAG ACGGC ATACG AGATTT ATTTG GGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.52_GCGTTAAA	CAAGC AGAAG ACGGC ATACG AGATTT TAACG CGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.53_CATCCTGT	CAAGC AGAAG

		ACGGC ATACG AGATA CAGGA TGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.54_GGAGTAAG	CAAGC AGAAG ACGGC ATACG AGATC TTACT CCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.55_GACGCTCC	CAAGC AGAAG ACGGC ATACG AGATG GAGCG TCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.56_TTCGCGGC	CAAGC AGAAG ACGGC ATACG AGATG CCGCG AAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.57_CGGTTCCC	CAAGC AGAAG ACGGC ATACG AGATG GGAAC CGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.58_ACCGGCTA	CAAGC AGAAG ACGGC ATACG AGATTA GCCGG TGTCT CGTGG

		GCTCG GAGAT GTG
	v2_Ad2.59_CTCATGGG	CAAGC AGAAG ACGGC ATACG AGATC CCATG AGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.60_TTTAATGC	CAAGC AGAAG ACGGC ATACG AGATG CATTA AAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.61_AAACGGTC	CAAGC AGAAG ACGGC ATACG AGATG ACCGT TTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.62_GATCCAAA	CAAGC AGAAG ACGGC ATACG AGATTT TGGAT CGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.63_ATGATGAT	CAAGC AGAAG ACGGC ATACG AGATAT CATCAT GTCTC GTGGG CTCGG AGATG TG
	v2_Ad2.64_CCAACACG	CAAGC AGAAG

		ACGGC ATACG AGATC GTGTT GGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.65_TAACAACA	CAAGC AGAAG ACGGC ATACG AGATT GTTGT TAGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.66_GGTAAACC	CAAGC AGAAG ACGGC ATACG AGATG GTTTA CCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.67_CATCGACC	CAAGC AGAAG ACGGC ATACG AGATG GTCGA TGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.68_ATGGGAAC	CAAGC AGAAG ACGGC ATACG AGATG TTCCC ATGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.69_CGGCCAAT	CAAGC AGAAG ACGGC ATACG AGATAT TGGCC GGTCT CGTGG

		GCTCG GAGAT GTG
	v2_Ad2.70_GGGAATGA	CAAGC AGAAG ACGGC ATACG AGATT CATTC CCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.71_GTATTCGG	CAAGC AGAAG ACGGC ATACG AGATC CGAAT ACGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.72_TCAGCTAT	CAAGC AGAAG ACGGC ATACG AGATAT AGCTG AGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.73_ATTTATCT	CAAGC AGAAG ACGGC ATACG AGATA GATAAA TGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.74_ACAGTTGC	CAAGC AGAAG ACGGC ATACG AGATG CAACT GTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.75_CCCGAGAT	CAAGC AGAAG

		ACGGC ATACG AGATAT CTCGG GGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.76_TAATGTCT	CAAGC AGAAG ACGGC ATACG AGATA GACAT TAGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.77_GCCAATTC	CAAGC AGAAG ACGGC ATACG AGATG AATTG GCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.78_CGCCGTGC	CAAGC AGAAG ACGGC ATACG AGATG CACGG CGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.79_CTGACCGA	CAAGC AGAAG ACGGC ATACG AGATT CGGTC AGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.80_CATTTCGA	CAAGC AGAAG ACGGC ATACG AGATT CGAAA TGGTC TCGTG

		GGCTC GGAGA TGTG
	v2_Ad2.81_GCTTGCCA	CAAGC AGAAG ACGGC ATACG AGATT GGCAA GCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.82_TTCTACCA	CAAGC AGAAG ACGGC ATACG AGATT GGTAG AAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.83_ACGTGACG	CAAGC AGAAG ACGGC ATACG AGATC GTCAC GTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.84_TGTCCGCG	CAAGC AGAAG ACGGC ATACG AGATC GCGGA CAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.85_TTAAACTT	CAAGC AGAAG ACGGC ATACG AGATA AGTTTA AGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.86_ACCACAAC	CAAGC AGAAG

		ACGGC ATACG AGATG TTGTG GTGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.87_GCCTCTGG	CAAGC AGAAG ACGGC ATACG AGATC CAGAG GCGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.88_TCGCCCAC	CAAGC AGAAG ACGGC ATACG AGATG TGGGC GAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.89_CACTAGGC	CAAGC AGAAG ACGGC ATACG AGATG CCTAG TGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.90_TCGAAGCC	CAAGC AGAAG ACGGC ATACG AGATG GCTTC GAGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.91_GCATGTAC	CAAGC AGAAG ACGGC ATACG AGATGT ACATG CGTCT CGTGG

		GCTCG GAGAT GTG
	v2_Ad2.92_GTTCGAGT	CAAGC AGAAG ACGGC ATACG AGATA CTCGA ACGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.93_CCGGGCGC	CAAGC AGAAG ACGGC ATACG AGATG CGCCC GGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.94_AGATTTAA	CAAGC AGAAG ACGGC ATACG AGATTT AAATC TGTCT CGTGG GCTCG GAGAT GTG
	v2_Ad2.95_CACCATTG	CAAGC AGAAG ACGGC ATACG AGATC AATGG TGGTC TCGTG GGCTC GGAGA TGTG
	v2_Ad2.96_AATAAGAC	CAAGC AGAAG ACGGC ATACG AGATG TCTTAT TGTCT CGTGG GCTCG GAGAT GTG

Before start

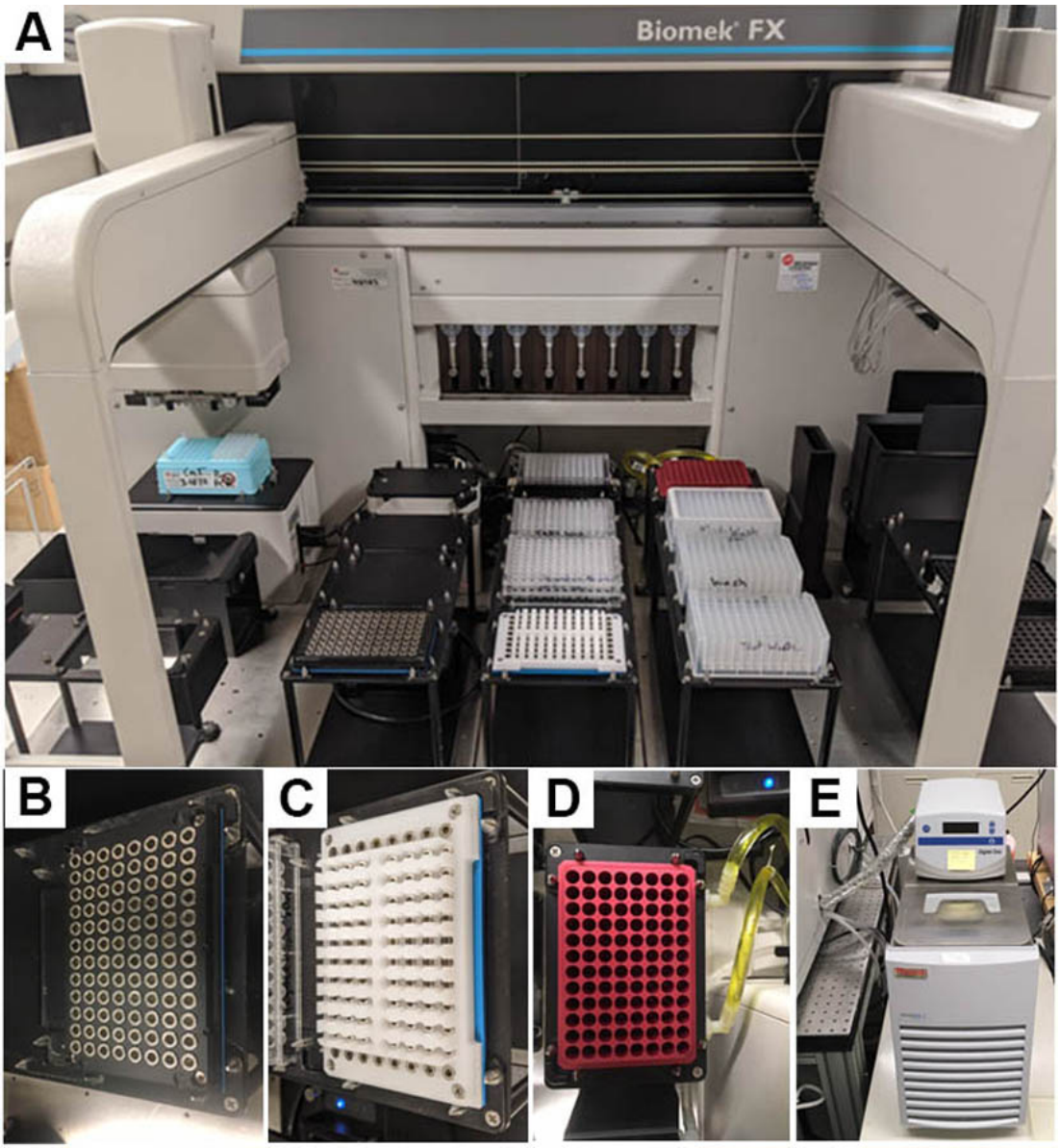


Figure 1: Biomek Deck Setup for AutoCUT&Tag. (A) Photo of the Biomek Deck setup in the Fred Hutch Genomics Shared Resources facility. AutoCUT&Tag uses the 96-channel head shown as Pod1, the Tip Loader Automated Labware Positioner (ALP) (back left), three 1X3 Stationary ALPS and one 1X1 Stationary ALP (middle), and a Single Position Cooling/Heating ALP (back right). (B) Photo of the ALPAQUA Magnetic Plate used for magnetic separation during wash steps. (C) Photo of the ALPAQUA LE Magnetic Plate used for magnetic separation and resuspension in low volumes. (D) Photo of the Aluminum Chiller Block used for temperature control during the tagmentation reaction. Note the tubing that routes antifreeze from the cooling unit to the Cooling/Heating ALP. (E) Photo of the recirculating cooling unit used to heat the Aluminum Block to 37^{o}C.

- **BIOMEK PROGRAMMING**

1) Set up the Biomek Deck. You will need a 96-channel pod and P200 head installed as Pod1, as well as one Tip Loader Automated Labware Positioner (ALP) (TL1), three 1 × 3 Static ALPs (P1-P9), one 1 × 1 Static ALP (P11), and one Single Position Cooling/Heating ALP routed to a circulating Cooling Unit filled with anti-freeze (P10). ALPs can be installed in the positions indicated below, or according to user preference, however this will require changing the ALP settings. To perform the tagmentation step at 37 degrees celsius, a Static Peltier ALP equipped with a 96 well adapter can be used in place of the Single Position Cooling/Heating ALP (P10). To set up the deck layout open the Deck Editor under the Instrument tab. Create new deck named “AutoCUT&Tag” that includes all of the equipment installed on your instrument. The following chart indicates the minimum number of ALP positions for AutoCUT&Tag, but there is no need to delete additional unused equipment from your deck layout, the specific X, Y, and Z coordinates of these ALP positions are provided as an example, and are subject to change based on the custom framing of your deck:

Tip Loader ALP Position Properties					
Name	TL1			ALP Type:	TipLoader
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		-7.296	-9.398	-15.676	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Deck Framed

1 X 3 Static ALP (1)					
Name	P1			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		24.712	7.265	-15.692	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed
Name	P2			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		24.707	18.67	-15.677	ALP Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed
Name	P3			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		24.726	30.126	-15.683	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed

1 X 3 Static ALP (2)					
Name	P4			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		43.773	7.313	-15.685	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed
Name	P5			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		43.765	18.753	-15.673	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed
Name	P6			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		43.762	30.191	-15.678	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed

1 X 3 Static ALP (3)					
Name	P7			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		62.869	7.296	-15.663	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed
Name	P8			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		62.851	18.741	-15.673	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed
Name	P9			ALP Type:	OneByThree
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		62.831	30.168	-15.68	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed

Single Position Cooling/Heating ALP Position Properties					
Name	P10			ALP Type:	OneByOne
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		62.805	-6.682	-15.676	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Deck Framed

1 X 1 Static ALP					
Name	P11			ALP Type:	OneByOne
		X(cm)	Y(cm)	Z(cm)	Precision
Pod 1 Coordinates		43.767	-6.711	-15.681	Position Framed
Pod 2 Coordinates		N/A	N/A	N/A	Position Framed

2) Input the AutoCUT&Tag Methods. Start a new project named "AutoCUT&Tag." This will avoid overwritting other equipment etc. that has the same name.

Option 1: The various Labware, Liquid Types, Techniques and Methods for AutoCUT&Tag are available below for download. The files can be imported into the AutoCUT&Tag project. Be sure to check the Instrument Setup. If your deck layout varies from what is suggested above you may need to "map to an existing deck," and confirm all

the labware shows up at the correct positions according to the Instrument Setup diagrams provided in Figures 1&2 below as well as in the Guidelines section (e.g. AP96_200uL at TL1).

- Note: These methods files were generated using Biomek v4, and cannot be imported into Biomek v5 and are unlikely to be backwards compatible with Biomek v3.

CUT&Tag_Full_Reaction.bmf

CUT&Tag_Post-PCR_Cleanup.bmf

Option 2: The Labware, Liquid Types, Techniques and Methods for AutoCUT&Tag can be input manually according to the specifications listed in the Guidelines section.

3) Calibrate, Center and Zero the Labware. The equipment specs provided should be empirically tested on your specific instrument.

Step 1: To ensure each of the plate types is properly centered and zeroed, a short method should be run in which the Biomek transfers liquid from each of the plate types with the aspirate and dispense height set at 0.5 mm from the bottom. Run the method using single step mode to carefully examine the tip heights within the wells in each of the plate types (i.e PCR 96 Well Plate, Deep Well Plate, and the V-Bottom Plate). The PCR 96 Well Plate should be centered and zeroed while stacked on the PCR Plate Rack, ALPAQUA Magnet Plate, ALPAQUA LE Magnet Plate and Cold Block. If necessary the Stack Offset X, Y, and Z parameter can be adjusted for each type of Rack independently. Properly zeroing the PCR 96 Well Plate on the ALPAQUA Magnet Plate and ALPAQUA LE Magnet Plate should engage the spring without causing the stand to bottom out. Reducing the movement speed within the well during these initial calibration steps will minimize the possibility of the instrument becoming damaged if a crash occurs.

Step 2: New users should also ensure the plate movements steps are robust by setting up a short method in which the Biomek moves the PCR 96 Well Plate between each of the stacked positions (i.e. PCR Plate Rack to the ALPAQUA Magnet Plate to the Cold Block to the ALPAQUA LE Magnet Plate to the PCR Plate Rack). In the event of PCR 96 Well Plate movement issues adjustments should be made to the Gripper Offsets using the Labware Type Editor for the PCR 96 Well Plate in the Movement Info section. The 96 well LoBind PCR plates, Semi-skirted (Eppendorf # 0030129504) are highly recommended because their dimensions are consistent enough from plate to plate to ensure the Biomek will also move and stack the plates in a consistent manner. To prevent the PCR Plate Racks from catching and being carried along with the PCR 96 Well Plate during movement steps the PCR Plate Racks can be taped down to the stationary ALPs (this issue has not been observed for the ALPAQUA Magnet Stand or the Cold Block).

Step 3: Prior to performing an AutoCUT&Tag reaction on biological samples it is recommended to test each method by pre-loading the labware with H_2O containing a small amount of food coloring to improve visibility.

Finally, the operator should remain present throughout each method for the first several runs to ensure the instrument performs as expected. In the event of a mishap the operator can then pause or stop the procedure and intervene before the experiment is compromised.

REAGENT SETUP

Binding buffer: Mix 20 mL of Binding Buffer in a 50 mL conical tube. Store the buffer at 4 ^{O}C for up to 6 months.

Binding Buffer		
Component	**Amount**	**Final concentration**
ddH_2O	19.36 mL	-
1M HEPES-KOH pH 7.9	400 µL	20 mM
1M KCl	200 µL	10 mM
1M $CaCl_2$	20 µL	1 mM
1M $MnCl_2$	20 µL	1 mM

Activate Concanavalin A-coated beads in Binding Buffer: Gently resuspend and withdraw enough of the bead suspension such that there will be 5-10 µL for each final sample. Transfer Concanavalin A-coated beads into 1 mL Binding Buffer in a 2 mL tube. Place tube on a magnet stand to clear (30 s to 2 min). Withdraw the liquid and remove from the magnet stand. Add 1 mL Binding Buffer, mix by inversion or gentle pipetting, remove liquid from the cap and side with a quick pulse on a micro-centrifuge. Place tube on a magnet stand to clear (30 s to 2 min). Withdraw the liquid, then wash Concanavalin A-coated beads a second time with 1 mL of Binding Buffer. After removing liquid from the second wash on a magnet stand, resuspend in a volume of Binding Buffer equal to the initial volume of bead suspension (5-10 µL per final sample).

Wash Buffer: 300 mL of Wash Buffer is sufficient to prep all the necessary reagents for up to 96 AutoCUT&Tag reactions. This buffer can be stored at 4^{O}C for up to 1 week, however, Roche Complete Protease Inhibitor tablet should be added fresh on the day of use.

- Note: A concentration of salt that is in the physiological range avoids stress when washing the cells and mixing with beads. Spermidine in the wash buffer is intended to compensate for removal of Mg2+ during incubation in the Antibody Buffer, which might otherwise affect chromatin properties.

Wash Buffer		
Component	**Amount per 96 samples**	**Final**
ddH_2O	284 mL	-
1M HEPES pH 7.5	6 mL	20 mM
5 M NaCl	9 mL	150 mM
2 M Spermidine	75 µL	0.5 mM
Roche Complete Protease Inhibitor EDTA-Free	6 tablet	-

Antibody Buffer: For up to 96 reactions prepare 12 mL Antibody Buffer.

- Note: The presence of EDTA during antibody treatment removes excess divalent cations used to activate the Concanavalin A-coated beads, as well as endogenous cations from the cells of interest. This serves to halt metabolic processes, stop endogenous DNAse activity, and prevent carry-over of Ca2+ from the Binding Buffer that might prematurely initiate strand cleavage after addition of pA-MNase. Washing out the EDTA before pA-MNase addition avoids inactivating the enzyme.

Antibody Buffer (100 µL per Sample)		
Component	**Amount per 96 samples**	**Final**
Wash Buffer	10 mL	-
0.5 M EDTA	40 µL	2 mM

NE 1 Buffer: If working directly from fresh cells or tissue prepare 25 mL NE 1 Buffer and store on ice. If starting with previously prepared cryopreserved nuclei NE 1 Buffer will not be necessary.

NE 1 Buffer		
Component	**Volume**	**Final**
ddH_2O	25 mL	-
1M HEPES-KOH pH 7.9	500 µL	20 mM
1 M KCl	250 µL	10 mM
2 M Spermidine	6.25 µL	0.5 mM
12.5 % Triton X-100	200 µL	0.1 %
Glycerol	5 mL	20 %
Roche Complete Protease Inhibitor EDTA-Free	1/2 tablet	-

10 µM i5/i7 PCR Primer Plate: Dilute one i5 and one i7 primer to 10 µM each in 10 mM Tris pH 8 in each well of a PCR 96 well plate. Then seal and freeze at -20^{o}C. The example provided here uses a unique i7 primer for each sample, and includes sufficient i5 diversity to allow the samples to be pooled for a dual-index sequencing reaction.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.74 2.01	1.82 2.09	1.74 2.17	1.82 2.25	1.74 2.33	1.82 2.41	1.74 2.49	1.82 2.57	1.74 2.65	1.82 2.73	1.74 2.81	1.82 2.89
B	1.75 2.02	1.85 2.10	1.75 2.18	1.85 2.26	1.75 2.34	1.85 2.42	1.75 2.50	1.85 2.58	1.75 2.66	1.85 2.74	1.75 2.82	1.85 2.90
C	1.76 2.03	1.86 2.11	1.76 2.19	1.86 2.27	1.76 2.35	1.86 2.43	1.76 2.51	1.86 2.59	1.76 2.67	1.86 2.75	1.76 2.83	1.86 2.91
D	1.77 2.04	1.87 2.12	1.77 2.20	1.87 2.28	1.77 2.36	1.87 2.44	1.77 2.52	1.87 2.60	1.77 2.68	1.87 2.76	1.77 2.84	1.87 2.92
E	1.78 2.05	1.88 2.13	1.78 2.21	1.88 2.29	1.78 2.37	1.88 2.45	1.78 2.53	1.88 2.61	1.78 2.69	1.88 2.77	1.78 2.85	1.88 2.93
F	1.79 2.06	1.90 2.14	1.79 2.22	1.90 2.30	1.79 2.38	1.90 2.46	1.79 2.54	1.90 2.62	1.79 2.70	1.90 2.78	1.79 2.86	1.90 2.94
G	1.80 2.07	1.91 2.15	1.80 2.23	1.91 2.31	1.80 2.39	1.91 2.47	1.80 2.55	1.91 2.63	1.80 2.71	1.91 2.79	1.80 2.87	1.91 2.95
H	1.81 2.08	1.92 2.16	1.81 2.24	1.92 2.32	1.81 2.40	1.92 2.48	1.81 2.56	1.92 2.64	1.81 2.72	1.92 2.80	1.81 2.88	1.92 2.96

Prepare Nuclei

30m

1 Obtain cells of interest and centrifuge at 600XG for 3 min in a 1.5 mL microfuge tube (if prepping nuclei from ≤ 1 million cells) or in a 15 mL conical tube if preparing cells in bulk.

- Note: If starting with tissue, use either manual or enzymatic dissociate to achieve a near single cell suspension prior to starting the nuclei prep.

2 Carefully remove the liquid and resuspend cells in 1 mL cold NE 1 Buffer per 1 million cells by gentle pipetting or flicking the tube.

3 Incubate on ice for 10 min to lyse the cell membrane and release the nuclei.

4 Centrifuge at 600XG for 3 min and carefully remove the liquid.

5 Resuspend nuclei in 1 mL Wash Buffer per 1 million cells.

- **PAUSE POINT:** Native nuclei can be cryopreserved in 10% (vol/vol) DMSO using a Mr. Frosty isopropyl alcohol chamber and then stored at -80^{o}C for later use.

6 Optional: To lightly cross-link the nuclei add 16% formaldehyde to 0.1% (e.g. 6.25 µL 16% formaldehyde to 1 mL of Wash Buffer). Stop cross-linking after 2 min by addition of 2.5 M Glycine to a final concentration of 75 mM (e.g. 30 µL of 2.5 M Glycine to 1 mL of Wash Buffer).

7 Centrifuge at 1200XG for 3 min and carefully remove the liquid.

- **Note:** Once nuclei are cross-linked the pellet often becomes difficult to see. Keeping track of the orientation of the tube in the centrifuge (e.g. "elbows out") can help to anticipate the location of the pellet in the tube.

8 Resuspend lightly cross-linked nuclei in 1 mL Wash Buffer per 1 million cells.

- **PAUSE POINT:** Lightly cross-linked nuclei can be cryopreserved in 10% (vol/vol) DMSO using a Mr. Frosty isopropyl alcohol chamber and then stored at -80^{o}C for later use.

Bind nuclei to beads and bind primary antibody

2h

9 While gently vortexing the nuclei (~1100 rpm), add 5-10 µL of the Concanavalin A coated-bead suspension per sample in a dropwise manner.

Note: Using more than ~100,000 nuclei or >10 µL Con A beads per sample may inhibit the PCR.

10 Place on tube nutator at room temperature for 5-10 min.

11 Mix well by vigorous inversion to ensure the bead-bound nuclei are in a homogenous suspension and divide into aliquots in 0.6-mL low-bind tubes, one for each antibody to be used.

12 Place on the magnet stand to clear and pull off and discard the liquid.

13 Resuspend ConA bead bound nuclei in 100 µL of the Antibody Buffer per sample. Tap to dislodge the beads that are stuck to the side.

14 Mix in the primary antibody to a final concentration of 1:100 or to the manufacturer's recommended concentration for immunofluorescence.

CRITICAL STEP: To evaluate success of the procedure without requiring sequencing, include in parallel a positive control antibody (e.g. anti-H3K27me3) and a negative control antibody (e.g.anti-mouse IgG).

15 Place on the tube nutator at room temperature for 1-2 hr.

- **PAUSE POINT:** Antibody incubation may proceed overnight at 4^{o}C.

AutoCUT&Tag Reaction

4h

16

If Wash Buffer was prepared more than 24 hrs prior, add fresh Roche Complete Protease Inhibitor tablets (1/50 mL) and allow to dissolve (~5 min). Then prepare the following solutions:

Secondary Antibody Solution (125 µL per Sample)				
Component	**Amount per 96 samples (µL)**	**Added for Reservoir (µL)**	**Final volume per 96 samples (µL)**	**Final concentration**
Wash Buffer	11880	445.5	12325.5	
Guinea Pig anti-Rabbit IgG	120	4.5	124.5	1 X

- **CRITICAL STEP:** If a primary antibody was used from any species other than Rabbit, an additional Secondary Antibody Solution will need to be prepared to include 125 µL per sample (1:100 dilution of secondary antibody). Make sure the secondary antibody that is used is compatible with Protein-A binding (e.g. Rabbit anti-Mouse IgG works well because Protein A has a high affinity for Rabbit IgG, however Goat anti-Mouse IgG is problematic because Protein A has little to no affinity for Goat IgG).

300 Wash Buffer		
Component	**Amount per 96 samples**	**Final**
Wash Buffer	135.8 mL	-
5 M NaCl	4.2 mL	150 + 150 = 300 mM

pA-Tn5 Solution (125 µL per Sample)				
Component	**Amount per 96 samples (µL)**	**Added for Reservoir (µL)**	**Final volume per 96 samples (µL)**	**Final concentration**
300 Wash Buffer	11880	445.5	12325.5	
pA-Tn5 (100X)	120	4.5	124.5	1 X

Tagmentation Buffer (125 µL per Sample)				
Component	Amount per 96 samples (µL)	Added for Reservoir (µL)	Final volume per 96 samples (µL)	Final concentration
300 Wash Buffer	11880	445.5	12325.5	
1 M MgCl2	120	4.5	124.5	10 mM

0.1 % SDS Release Buffer (125 µL per Sample)				
Component	Amount per 96 samples (µL)	Added for Reservoir (µL)	Final volume per 96 samples (µL)	Final concentration
10 mM TAPS pH 8.5	10800	405	11205	
1 % SDS (diluted 1:10 in ddH20 from a 10 % stock)	1200	45	1245	0.1 %

- **Note:** Store Wash Buffer, Secondary Antibody Solution, 300 Wash Buffer, pA-Tn5 Solution, and Tagmentation Buffer on ice. Keep 0.1 % SDS Release Buffer, and 10 mM TAPS Buffer pH 8.5 at Room Temp.

17 Select the Pre-Programmed AutoCUT&Tag Project on the Biomek and open the CUT&Tag Full Reaction Method file (described in the ***BEFORE STARTING*** and ***Guidelines*** sections).

- **CRITICAL STEP:** Performing the steps up until this point by hand increases the versatility of the platform, allowing individual users to prepare nuclei from their cells or tissue of interest and bind any antibody of their choosing. Because the antibody incubation is not time sensitive, samples from multiple users can be synchronized at this step and arrayed on a single plate, allowing the remaining steps to be performed in unison on the Biomek by a single operator.

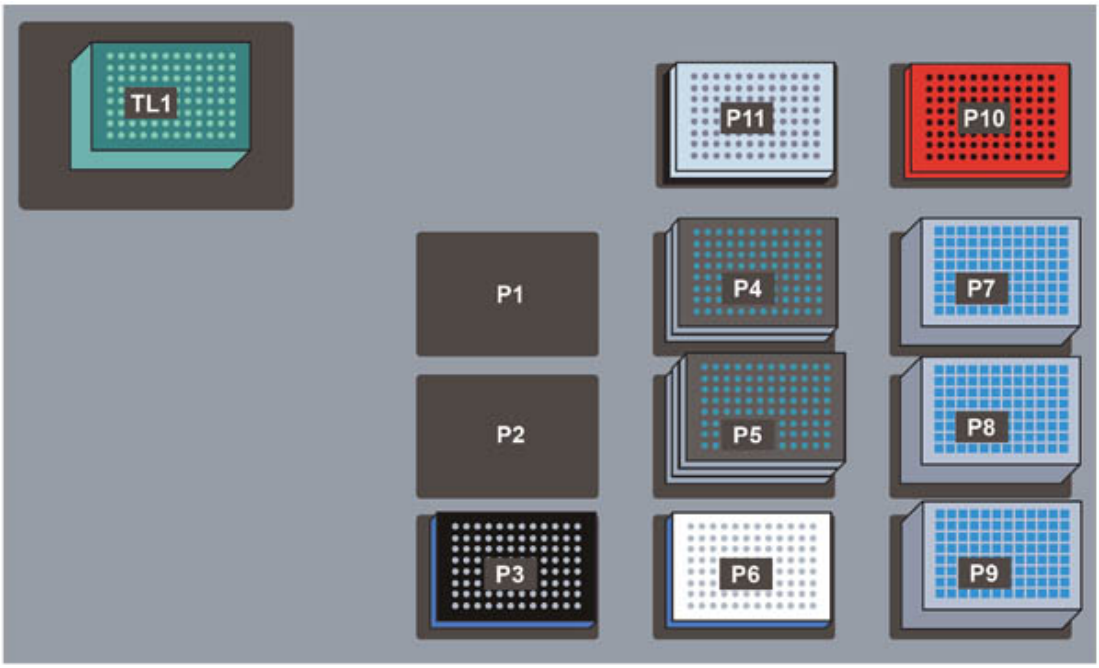


Figure 2: Biomek Deck Setup for CUT&Tag Full Reaction.

TL1: Fresh AP96 200 µL Tips (double click to increase the # of load times)
P3: ALPAQUA Magnet Plate
P4: V-Bottom Plate preloaded with 125 µL TAPS Buffer stacked on top of a V-Bottom Plate preloaded with 125 µL 0.1% SDS Release Buffer
P5: V-Bottom Plate preloaded with 125 µL Secondary Antibody Solution, stacked on top of a V-Bottom Plate preloaded with 125 µL pA-Tn5 Solution, stacked on top of a V-Bottom Plate preloaded with 125 µL Tagmentation Buffer
P6: ALPAQUA LE Magnet Plate
P7: Deep Well Plate preloaded with 1 mL of 300-Wash Buffer
P8: Deep Well Plate preloaded with 1 mL of Wash Buffer
P9: Deep Well Plate for receiving liquid waste
P10: Cold Block seated on a Cooling/Heating ALP routed to a Heating/Cooling Unit set to 37^{O}C
P11: PCR 96 Well Plate preloaded with up to 150 µL of conA bead-bound nuclei + primary antibody stacked on a PCR Plate Rack

18 Remove the seal and lid and place a fresh box of AP96 200 µL Tips on the Tip Loading ALP at position TL1 on the Biomek Deck. Place the ALPAQUA Magnet Plate at position P3 and the ALPAQUA LE Magnet Plate at position P6 on the Biomek Deck. Place an empty labeled Deep Well Plate at position P9 on the Biomek Deck for collecting liquid waste. Set the Heating/Cooling Unit to 37^{O}C.

19 Transfer Wash Buffer into a reservoir and dispense 1 mL into each of the active wells of a labeled Deep Well Plate using a Multi-Channel Pipette and place at position P8 on the Biomek deck. Transfer 300 Wash Buffer into a reservoir and dispense 1 mL into each of the active wells of a labeled Deep Well Plate using a Multi-Channel Pipette and place at position P7 on the Biomek deck.

20 Transfer Tagmentation Buffer into a reservoir and dispense 125 µL into each of the active wells of a labeled V-Bottom Plate using a Multi-Channel Pipette and place at position P5 on the Biomek deck (Bottom). Transfer pA-Tn5 Solution into a reservoir and dispense 125 µL into each of the active wells of a labeled V-Bottom Plate using a Multi-Channel Pipette and stack on top of the Tagmentation Buffer V-Bottom Plate at position P5 on the Biomek deck (Middle). Transfer Secondary Antibody Solution into a reservoir and dispense 125 µL into each of the active wells of a labeled V-Bottom Plate using a Multi-Channel Pipette and stack on top of the pA-Tn5 Solution V-Bottom Plate and Tagmentation Buffer V-Bottom Plate at position P5 on the Biomek deck (Top).

- **CRITICAL STEP:** Make sure to load the Secondary Antibody Solution that is compatible with the species of primary antibody used for each to samples into the corresponding wells of the Secondary Antibody Solution V-Bottom Plate. Depending on sample numbers a single channel pipette may be necessary at this step.

21 Transfer 0.1% SDS Release Buffer into a reservoir and dispense 125 µL into each of the active wells of a labeled V-Bottom Plate using a Multi-Channel Pipette and place at position P4 on the Biomek deck (Bottom). Transfer 10 mM TAPS Buffer pH 8.5 into a reservoir and dispense 125 µL into each of the active wells of a labeled V-Bottom Plate using a Multi-Channel Pipette and stack on top of the 0.1 % SDS Release Buffer V-Bottom Plate at position P4 on the Biomek deck (Top).

22 Using wide bore 200 µL tips, resuspend Concanavalin A bead-bound nuclei + Antibodies and array them in a PCR 96 Well Plate. Be sure to record the position of each sample in the plate and stack it on a PCR Plate Rack at position P11 on the Biomek deck.

- **Note:** To prevent the PCR Plate Racks from catching and being carried along with the PCR 96 Well Plate during movement steps, the PCR Plate Racks can be taped down to the stationary ALPs (this issue has not been observed for the ALPAQUA Magnet Stand or the Cold Block).

23 Start the CUT&Tag Full Reaction Method on the Biomek.

- **Note:** The full method takes roughly 3 hrs, but the operator should check in periodically to make sure things are progressing smoothly.

Chromatin Release and PCR Amplification

2h

24 Upon completion of the CUT&Tag Full Reaction program, immediately remove the PCR 96 Well Plate containing the ConA bead bound tagmented nuclei in 10 µL 0.1% SDS Release Buffer. Seal with an AlumaSeal 96 cover, and place in a ThermoCycler set to 58^{o}C with heated lid for 1 hr.

- **Note:** The excess liquid should be emptied from V-Bottom and Deep Well Plates. This labware can then be thoroughly washed with DI water (fill and rinse 5-10 times), allowed to air dry overnight, and then stored for reuse in subsequent experiments.

25 During the 1 hr 58^{O}C incubation thaw out the 10 µM i5/i7 PCR Primer Plate and PCR Reagents and prepare the following solutions:

KAPA PCR Master Mix (36 µL per Sample)				
Component	Amount per 96 samples (µL)	Added for Reservoir (µL)	Final volume per 96 samples (µL)	Final Concentration
5X KAPA buffer	1920	250	2170	~2.8 X
ddH2O	1056	137.5	1193.5	-
10 mM dNTPs	288	37.5	325.5	833 µM
KAPA HIFI polymerase	192	25	217	-

Note: To allow 3' extension of the tagmented DNA at the start of the PCR, avoid using HOT START polymerase.

0.15 % Triton X-100 Solution (54 µL per Sample)				
Component	Amount per 96 samples (µL)	Added for Reservoir (µL)	Final volume per 96 samples (µL)	Final concentration
ddH2O	5137.6	444.6	5582.2	
12.5 % Triton X-100	62.4	5.4	67.8	0.15 %

26 Upon completion of the 1 hr 58^{O}C incubation, remove the PCR 96 Well Plate containing the samples and allow to cool to room temp (~ 5 min). Then remove and discared the AlumaSeal 96 Cover. Transfer 0.15 % Triton X-100 Solution into a reservoir and using a Multi-Channel Pipette dispense and mix 54 µL into each sample in the PCR 96 Well Plate (pipette up and down 5-10 times).

27 Uncover the 10 µM i5/i7 PCR Primer Plate while being careful to avoid cross-contamination of barcoded adapters between wells. Using a Multi-Channel Pipette dispense and mix 4 µL 10 µM i5/i7 PCR Primer solution into each sample in the PCR 96

Well Plate (pipette up and down 5-10 times). Then reseal and freeze the 10 µM i5/i7 PCR Primer Plate.

- **CRITICAL STEP:** To allow sample multiplexing for sequencing in a single reaction, use each i5/i7 combination only once, and keep track of which combination of i5/i7 primers corresponds to each sample.

28 Transfer KAPA PCR Master Mix into a reservoir and using a Multi-Channel Pipette dispense and mix 36 µL into each sample in the PCR 96 Well Plate (pipette up and down 5-10 times).

29 Seal the PCR 96 Well Plate plate using an AlumaSeal 96 Cover, place in thermocycler and run the following program with heated lid:

PCR Program				
Cycle number	Denature	Anneal	Extend	Final
1			58° C, 5 min	
2			72° C, 10 min	
3	98° C, 45 s			
4–17	98° C, 15 s	60° C, 10 s		
18			72° C, 1 min	
19				8° C, hold

- **Note:** Libraries should be amplified with 12-14 PCR cycles, preferably with a 10 s 60^{O}C combined annealing/extension step to minimize the contribution of large DNA fragments to the library. The cycle times are based on using a conventional Peltier cycler (e.g. BioRad/MJ PTC200), in which the ramping times (3 °C/sec) are sufficient for annealing and extension to occur. Therefore, the use of a rapid cycler with a higher ramping rate will require either reducing the ramping time or other adjustments to ensure annealing and extension.
- **PAUSE POINT:** PCR amplified Libraries can be held at 8^{O}C overnight or stored at 4^{O}C indefinitely.

Post-PCR DNA Cleanup

1h

30 Select the Pre-Programmed AutoCUT&Tag Project on the Biomek and open the CUT&Tag Post-PCR Cleanup Method file (described in the ***BEFORE STARTING*** and ***Guidelines*** sections).

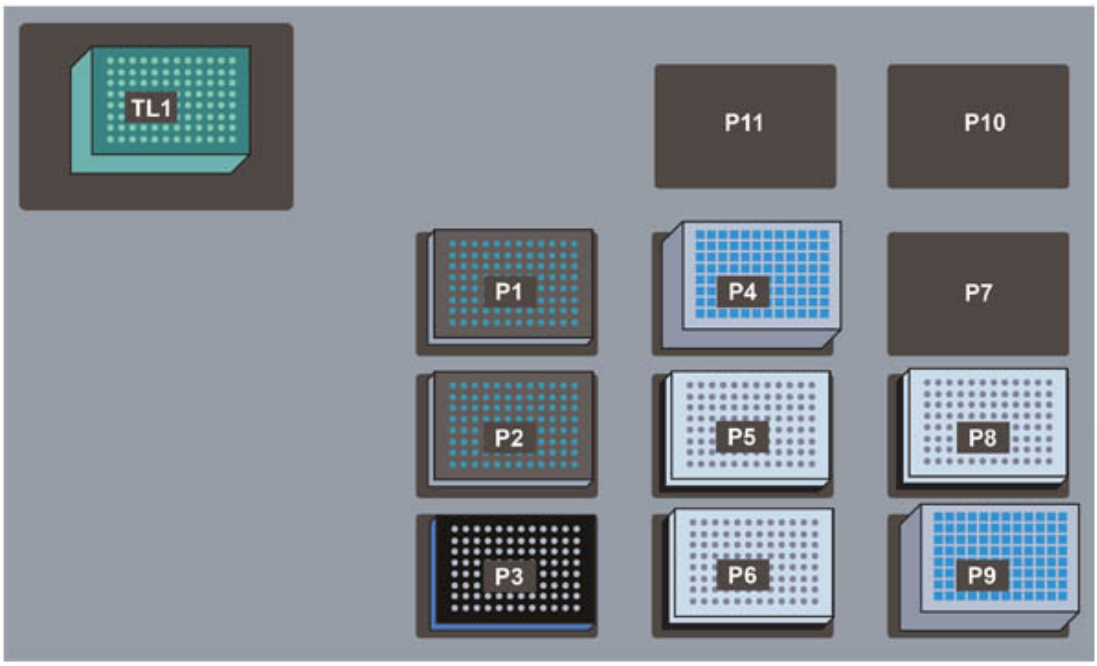


Figure 3: Biomek Deck Setup for CUT&Tag Post PCR Cleanup.
TL1: Fresh AP96 200 µL Tips (double click to increase the # of load times)
P1: V-Bottom Plate preloaded with 100 µL10 mM Tris-HCl pH 8 (used for washing tips)
P2: V-Bottom Plate preloaded with 100 µL10 mM Tris-HCl pH 8 (used for DNA elution)
P3: ALPAQUA Magnet Plate
P4: Deep Well Plate preloaded with 1 mL 80% Ethanol
P5: PCR 96 Well Plate containing 100 µL of PCR product stacked on a PCR Plate Rack
P6: PCR 96 Well Plate preloaded with 130 µL of Ampure Beads stacked on a PCR Plate Rack
P8: PCR 96 Well Plate for accepting cleaned-up DNA stacked on a PCR Plate Rack
P9: Deep Well Plate for receiving liquid waste

31 Remove the seal and Lid and place a fresh box of AP96 200 µL Tips at TL1, a labeled Deep Well Plate for accepting liquid waste at position P9, and a fresh PCR 96 Well Plate stacked on a PCR Plate Rack for accepting clean CUT&Tag DNA Libraries at position P8 on the Biomek deck.

32 Remove Ampure Bead Slurry from refrigerator, resuspend beads by vortexing and allow to equilibrate to room temperature. Using a Reservoir and Multi-Channel Pipette distribute 130 µL of the Ampure Bead Slurry into the active wells of a PCR 96 Well Plate and stack on a PCR Plate Rack positioned at P6 on the Biomek deck.

33 Upon completion of the PCR Amplification Reaction, remove the PCR 96 Well Plate containing the samples and allow to cool to room temp (~ 5 min). Then remove and discard the AlumaSeal 96 Cover and stack on a PCR Plate Rack positioned at P5 on the Biomek deck.

34 Using a Reservoir and Multi-Channel Pipette dispense 1 mL of 80% Ethanol into each of the active wells of a labeled Deep Well Plate and place at position P4 on the Biomek

Deck. Distribute 100 µL of 10mM Tris-HCl pH 8 into the active wells of two V-Bottom Plates (one for tip washes and one for DNA elution) and place at positions P1 and P2 on the Biomek deck.

35 Start the CUT&Tag Post-PCR Cleanup Method.

Sequencing

2d

36 Upon completion of the CUT&Tag Post-PCR Cleanup program, remove the PCR 96 Well Plate containing clean CUT&Tag Libraries (position P8) from the Biomek deck and seal with an AlumaSeal 96 cover for subsequent analysis.

- **Note:** The excess liquid should be emptied from V-Bottom and Deep Well Plates. This labware can then be thoroughly washed with DI water (fill and rinse 5-10 times), allowed to air dry overnight, and then stored for reuse in subsequent experiments.
- **PAUSE POINT:** Libraries can be stored at 4^{o}C indefinitely.

37 Determine the size distribution and concentration of libraries by Agilent 4200 TapeStation analysis.

- **Note:** Libary concentration can be determined using the regions tool in the TapeStation analysis software. We recommend limiting the region to 170-625 bp, which corresponds to the subnucleosomal to di-nucleosomal fragment sizes that will account for the majority of sequencing reads.

? TROUBLESHOOTING

38 Pool indexed CUT&Tag libraries and perform paired-end Illumina sequencing following the manufacturer's instructions.

- **CRITICAL STEP:** Because of the very low background with CUT&Tag, typically 2.5 million paired-end reads suffices for transcription factors or nucleosome modifications, even for the human genome. For maximum economy, we mix up to 48 barcoded samples per lane at equimolar concentration (provided a similar number of reads is desired for each sample) and perform paired-end 25×25 bp sequencing on a 2-lane flow cell. Single-end sequencing is not recommended for CUT&Tag, as it sacrifices resolution.

Data Processing and Analysis

39 We align paired-end reads using Bowtie2 version 2.4.1 with options: --end-to-end --very-sensitive --no-mixed --no-discordant -q --phred33 -I 10 -X 700. For mapping E.Coli

spike-in fragments, we also use the --no-overlap --no-dovetail options to avoid cross-mapping of the experimental genome to that of the spike-in DNA.

? TROUBLESHOOTING