

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

Derek Janssens<sup>1</sup>, Steven Henikoff<sup>1</sup>

<sup>1</sup>Fred Hutchinson Cancer Research Center

2 Works for me

[dx.doi.org/10.17504/protocols.io.bgztjx6n](https://dx.doi.org/10.17504/protocols.io.bgztjx6n)

protocols.io news

Human Cell Atlas Method Development Community

Derek Janssens

## 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.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Derek Janssens, Michael P Meers, Steven J Wu, Ekatarina Babaeva, Soheil Meshinch, Jay F Sarthy, Kami Ahmad, Steve Henikoff (2020) Automated CUT&Tag profiling of chromatin heterogeneity in mixed-lineage leukemia. bioRxiv, doi: <https://doi.org/10.1101/2020.10.06.328948>

## DOI

[dx.doi.org/10.17504/protocols.io.bgztjx6n](https://dx.doi.org/10.17504/protocols.io.bgztjx6n)

## PROTOCOL CITATION

Derek Janssens, Steven Henikoff 2020. AutoCUT&Tag: streamlined genome-wide profiling of chromatin proteins on a liquid handling robot. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.bgztjx6n>

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Derek Janssens, Michael P Meers, Steven J Wu, Ekatarina Babaeva, Soheil Meshinch, Jay F Sarthy, Kami Ahmad, Steve Henikoff (2020) Automated CUT&Tag profiling of chromatin heterogeneity in mixed-lineage leukemia. bioRxiv, doi: <https://doi.org/10.1101/2020.10.06.328948>

## KEYWORDS

CUT&Tag, AutoCUT&Tag, Chromatin, Chromatin Profiling, CUT&RUN, AutoCUT&RUN, High-Throughput

## LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## CREATED

May 31, 2020

## LAST MODIFIED

Oct 08, 2020

PROTOCOL INTEGER ID  
37651

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,  $\geq 1$  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	<ul style="list-style-type: none"> <li>Avoid using problematic wells.</li> <li>Contact Beckman Coulter technician, and possibly replace the P200 head.</li> </ul>
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.	<ul style="list-style-type: none"> <li>Adjust aspiration height in the Method.</li> <li>Adjust volumes for desired result as determined empirically.</li> <li>Contact Beckman Coulter technician and possibly recalibrate the machine.</li> </ul>
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.	<ul style="list-style-type: none"> <li>Run a positive control sample for an abundant epitope, e.g. H3K27me3.</li> <li>Ensure Biomek is pipetting accurately e.g. no Ethanol remains after 5 min Air Dry during Post-PCR Cleanup.</li> </ul>
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	<ul style="list-style-type: none"> <li>Ensure ConA beads are binding the nuclei by comparing the input to the the unbound fraction following binding.</li> <li>Increase nuclei to 100K per sample.</li> <li>Ensure aspirations from V-Bottom plates are not bottoming out and preventing transfers, or shearing reagents.</li> </ul>
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 $\geq$ 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.	<ul style="list-style-type: none"> <li>Ensure samples are suspended in 10 <math>\mu</math>L of 0.1 % SDS at the end of the CUT&amp;Tag Full Reaction Method.</li> <li>Alter PCR conditions to increase extension by (1) reducing the ramp rate (2) adding a 2 second 72°C extension step.</li> <li>Remake SDS and Triton X-100 solutions</li> <li>Perform an SDS vs. Triton X-100 titration on positive control samples.</li> </ul>
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.	<ul style="list-style-type: none"> <li>Ensure primary and secondary antibodies are compatible and protein A binds the secondary.</li> <li>Increase antibody concentration.</li> <li>Test antibody binding by adding a fluorescent secondary and imaging.</li> <li>Test antibody using standard CUT&amp;Tag or CUT&amp;RUN using digitonin permeabilized cells.</li> <li>Replace antibody.</li> </ul>

## ■ BIOMEK PROGRAMMING

### Labware Type Editor:

Eppendorf 96 Well LoBind PCR Plate, Semi-skirted (PCR 96 Well Plate)																																					
Basic Info		<table border="1"> <thead> <tr> <th></th><th>X</th><th>Y</th><th></th></tr> </thead> <tbody> <tr> <td>Span:</td><td>12.426</td><td>8.397</td><td>cm</td></tr> <tr> <td>Height:</td><td>2.02</td><td></td><td>cm</td></tr> </tbody> </table>					X	Y		Span:	12.426	8.397	cm	Height:	2.02		cm																				
	X	Y																																			
Span:	12.426	8.397	cm																																		
Height:	2.02		cm																																		
Movement Info		<table border="1"> <thead> <tr> <th></th><th>X</th><th>Y</th><th>Z</th></tr> </thead> <tbody> <tr> <td>Gripper Offset:</td><td>0</td><td>0.15</td><td>0.8</td></tr> <tr> <td>Gripper Squeeze:</td><td>0.45</td><td></td><td>cm</td></tr> <tr> <td>Gripper Unsqueeze:</td><td>-1</td><td></td><td>cm</td></tr> <tr> <td>Speed Limit:</td><td>50</td><td></td><td>%</td></tr> </tbody> </table>					X	Y	Z	Gripper Offset:	0	0.15	0.8	Gripper Squeeze:	0.45		cm	Gripper Unsqueeze:	-1		cm	Speed Limit:	50		%												
	X	Y	Z																																		
Gripper Offset:	0	0.15	0.8																																		
Gripper Squeeze:	0.45		cm																																		
Gripper Unsqueeze:	-1		cm																																		
Speed Limit:	50		%																																		
Wells		<table border="1"> <thead> <tr> <th></th><th>X</th><th>Y</th><th></th></tr> </thead> <tbody> <tr> <td>Well Offset:</td><td>1.44</td><td>1.12</td><td>cm</td></tr> <tr> <td>Well Count:</td><td>12</td><td>8</td><td></td></tr> <tr> <td>Well Spacing:</td><td>0.9</td><td>0.9</td><td>cm</td></tr> <tr> <td>Max Volume:</td><td>235</td><td></td><td>µL</td></tr> </tbody> </table>					X	Y		Well Offset:	1.44	1.12	cm	Well Count:	12	8		Well Spacing:	0.9	0.9	cm	Max Volume:	235		µL												
	X	Y																																			
Well Offset:	1.44	1.12	cm																																		
Well Count:	12	8																																			
Well Spacing:	0.9	0.9	cm																																		
Max Volume:	235		µL																																		
Well Configuration		<table border="1"> <tbody> <tr> <td>Shape:</td><td>Round</td><td></td><td></td></tr> <tr> <td>Upper Radius:</td><td>0.34</td><td></td><td>cm</td></tr> <tr> <td>Lower Radius:</td><td>0.31</td><td></td><td>cm</td></tr> <tr> <td>Height:</td><td>0.5</td><td></td><td>cm</td></tr> <tr> <td>Bottom:</td><td>X</td><td></td><td></td></tr> <tr> <td>Shape:</td><td>Cone</td><td></td><td></td></tr> <tr> <td>Radius:</td><td>0.31</td><td></td><td>cm</td></tr> <tr> <td>Height:</td><td>1.4</td><td></td><td>cm</td></tr> </tbody> </table>				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
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		<table border="1"> <thead> <tr> <th></th><th>X</th><th>Y</th><th></th></tr> </thead> <tbody> <tr> <td>Span:</td><td>12.7762</td><td>8.5471</td><td>cm</td></tr> <tr> <td>Height:</td><td>1.4</td><td></td><td>cm</td></tr> </tbody> </table>					X	Y		Span:	12.7762	8.5471	cm	Height:	1.4		cm								
	X	Y																							
Span:	12.7762	8.5471	cm																						
Height:	1.4		cm																						
Stacking		<table border="1"> <thead> <tr> <th></th><th>X</th><th>Y</th><th>Z</th></tr> </thead> <tbody> <tr> <td>Stack Offset:</td><td>0</td><td>0</td><td>0.1</td><td>cm</td></tr> <tr> <td>Stacking Speed:</td><td>100</td><td></td><td>%</td><td></td></tr> </tbody> </table>					X	Y	Z	Stack Offset:	0	0	0.1	cm	Stacking Speed:	100		%							
	X	Y	Z																						
Stack Offset:	0	0	0.1	cm																					
Stacking Speed:	100		%																						
Stack Offsets Edit		<table border="1"> <tbody> <tr> <td>PCR 96 Well Plate:</td><td>X</td><td>Stack Offset X:</td><td>0</td><td>cm</td></tr> <tr> <td></td><td></td><td>Stack Offset Y:</td><td>0</td><td>cm</td></tr> <tr> <td></td><td></td><td>Stack Offset Z:</td><td>0.75</td><td>cm</td></tr> </tbody> </table>				PCR 96 Well Plate:	X	Stack Offset X:	0	cm			Stack Offset Y:	0	cm			Stack Offset Z:	0.75	cm					
PCR 96 Well Plate:	X	Stack Offset X:	0	cm																					
		Stack Offset Y:	0	cm																					
		Stack Offset Z:	0.75	cm																					
Wells		<table border="1"> <thead> <tr> <th></th><th>X</th><th>Y</th><th></th></tr> </thead> <tbody> <tr> <td>Well Offset:</td><td>1.438</td><td>1.123</td><td>cm</td></tr> <tr> <td>Well Count:</td><td>12</td><td>8</td><td></td></tr> <tr> <td>Well Spacing:</td><td>0.9</td><td>0.9</td><td>cm</td></tr> <tr> <td>Max Volume:</td><td>250</td><td></td><td>µL</td></tr> </tbody> </table>					X	Y		Well Offset:	1.438	1.123	cm	Well Count:	12	8		Well Spacing:	0.9	0.9	cm	Max Volume:	250		µL
	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		<table border="1"> <tbody> <tr> <td>Shape:</td><td>Round</td><td></td><td></td></tr> <tr> <td>Upper Radius:</td><td>0.3</td><td></td><td>cm</td></tr> <tr> <td>Lower Radius:</td><td>0.28</td><td></td><td>cm</td></tr> <tr> <td>Height:</td><td>1</td><td></td><td>cm</td></tr> <tr> <td>Bottom:</td><td>N/A</td><td></td><td></td></tr> </tbody> </table>				Shape:	Round			Upper Radius:	0.3		cm	Lower Radius:	0.28		cm	Height:	1		cm	Bottom:	N/A		
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			
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			
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			
Shape:	Rectangle				
Well Configuration	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 ms μL/s	Delay:	0	ms		
Delay:	0		Speed:	10	μL/s		
Speed:	25		Cutoff Velocity:	150	μL/s		
Blowout		Tip Touch					
Volume:	20	μL ms	Height:	-1	mm		
Delay:	5000		from:	Top			
Prewet		μL ms	Angle:	90			
Overage:	0		Speed:	100	%		
Delay:	0		Delay:	0	ms		
Wash		Sensitivity					
Default Cycles:	1	μL	Liquid Level Sensing				
Default Volume:	100%		N/A				

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

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

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

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

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

Tris-HCl-2						
Aspirate		Dispense				
Trailing Air Gap:	1	μL	Delay: 0 ms	0	ms	
	Delay:	0		10	μL/s	
	Speed:	1		150	μL/s	
Blowout		Tip Touch				
Volume:	20	μL	Height: -1 mm	from: Top		
	Delay:	5000				
Prewet		Angle: 90				
Overage:	0	μL	Speed: 100 %	Delay: 0 ms		
	Delay:	0				
Wash		Sensitivity				
Default Cycles:	1	μL	Liquid Level Sensing			
	Default Volume:	100%	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	
<b>Dispense Tab</b>		
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	
<b>Aspirate Tab</b>		
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	
<b>Dispense Tab</b>		
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	
<b>Aspirate Tab</b>		
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
Aspirate at:	2	mm from the Bottom
Dispense at:	2	mm from the Bottom
	5	times
	at	25 µL/s
	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

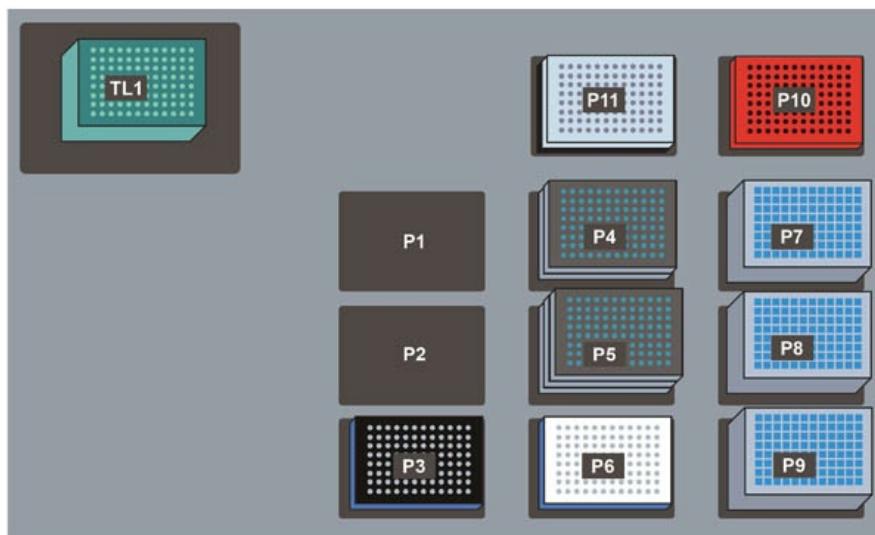
Dispense on Rack		
Pipetting Template:	Default Template	
<b>Dispense Tab</b>		
Move within the Well at:	25	% speed
Dispense at:	1	mm from the Bottom
N/A		Follow liquid level when aspirating or dispensing liquid
X		Touch tips on the sides of the wells
X		Blowout all leading air gaps
N/A		Mix after dispensing

### 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 Tgmentation 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°C

P11: PCR 96 Well Plate preloaded with up to 150  $\mu$ L of conA bead-bound nuclei + primary antibody stacked on a PCR Plate Rack

3) Move Labware from P11 to P3			
Move labware from: Move the topmost:	Using pod:	Pod1	
	P11	to	P3
	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			
Labware Type:	Ensure tip height in well is 0.00 mm from bottom		
Position:	PCR 96 Well Plate	Pod:	Pod1
Liquid Type:	P3	N/A	Refresh tips
Volume:	Wash	$\mu$ L	
Technique:	150		
	Aspirate on Magnet		

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

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

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

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

**13) Dispense in P9**

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

**14) Aspirate from P5**

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

**15) Dispense in P3**

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

**16) Pause the whole system for 1350 s**

Check: Pause	the whole system	for	1350	s
--------------	------------------	-----	------	---

17) Mix in P3			
Labware Type:	Ensure tip height in well is 0.00 mm from bottom		
Position:	PCR 96 Well Plate	Pod	Pod1
Liquid Type:	P3	N/A	Refresh tips
Volume:	Wash	Mix	5 times
Technique:	50 $\mu\text{L}$		
Customize			
Pipetting Template:	Default Template		
Mix Tab			
Move within the well at:	25 % speed		
Aspirate at:	0 mm from the Bottom	at	25 $\mu\text{L}/\text{s}$
Dispense at:	0 mm from the Bottom	at	25 $\mu\text{L}/\text{s}$
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			
Labware Type:	Ensure tip height in well is 0.00 mm from bottom		
Position:	PCR 96 Well Plate	Pod:	Pod1
Liquid Type:	P3	N/A	Refresh tips
Volume:	Wash		
Technique:	100 $\mu\text{L}$		
	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			
Labware Type:	Ensure tip height in well is 2.00 mm from bottom		
Position:	Deep Well Plate	Pod:	Pod1
Liquid Type:	P7	N/A	Refresh tips
Volume:	Wash		
Technique:	150	μL	
	Aspirate from DWP		

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

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

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

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

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

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

38) Dispense in P9			
Labware Type:	Ensure tip height in well is 35.00 mm from bottom		
	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			
Labware Type:	Ensure tip height in well is 1.50 mm from bottom		
	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			
Labware Type:	Ensure tip height in well is 2.00 mm from bottom		
	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**

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

**48) Dispense in P9**

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

**49) Aspirate from P8**

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

**50) Dispense in P3**

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

**51) Unload tips to TL1**

Tips:	Unload
Location:	TL1
Pod:	Pod1

**52) Move Labware from P3 to P6**

Using pod:	Pod1	to P6
Move labware from:	P3	
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			
Labware Type:	PCR 96 Well Plate	Ensure tip height in well is 5.00 mm from bottom	
Position:	P3	Pod	Pod1
Liquid Type:	Wash	N/A	Refresh tips
Volume:	50	Mix	5 times
Technique:	Custom	µL	
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			
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			
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			
Labware Type:	Ensure tip height in well is 0.50 mm from bottom		
Position:	V Bottom Plate	Pod:	Pod1
Liquid Type:	P1	N/A	Refresh tips
Volume:	Wash		
Technique:	50	μL	
	Aspirate from VBP		

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

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

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

63) Dispense in P9			
Labware Type:	Ensure tip height in well is 35.00 mm from bottom		
Position:	Deep Well Plate	Pod:	Pod1
Liquid Type:	P9	N/A	Empty Tips
Volume:	Wash		
Technique:	60	μL	
	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 $\mu\text{L}$		
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 $\mu\text{L}/\text{s}$
Dispense at:	1.5 mm from the Bottom	at	25 $\mu\text{L}/\text{s}$
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 $\mu\text{L}$		
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 $\mu\text{L}$		
Technique:	Dispense on Magnet-2		

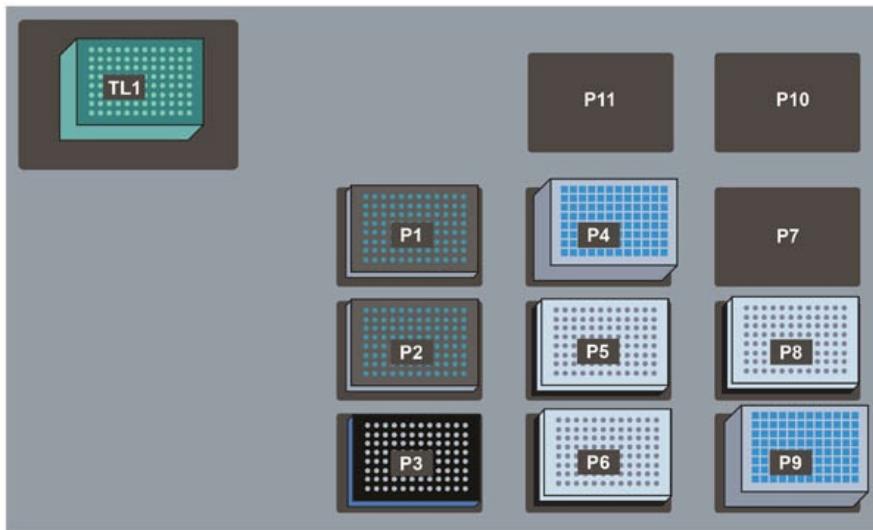
67) Unload tips to TL1		
Tips:	Unload	
Location:	TL1	
Pod:	Pod1	

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 µL 10 mM Tris-HCl pH 8 (used for washing tips)

P2: V-Bottom Plate preloaded with 100 µL 10 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			
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			
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			
Labware Type:	Ensure tip height in well is 7.00 mm from bottom		
Position:	PCR 96 Well Plate	Pod:	Pod1
N/A	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			
Labware Type:	Ensure tip height in well is 1.50 mm from bottom		
Position:	V-Bottom Plate	Pod:	Pod1
N/A	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			
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			
Labware Type:	Ensure tip height in well is 35.00 mm from bottom		
Position:	Deep Well Plate	Pod:	Pod1
N/A			Empty Tips
Liquid Type:	Ampure Wash		
Volume:	120	µL	
Technique:	Dispense in Waste		

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

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

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

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

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

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

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

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

26) Aspirate from P3			
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			
Labware Type:	Ensure tip height in well is 35.00 mm from bottom		
Position:	Deep Well Plate	Pod:	Pod1
Liquid Type:	P9	N/A	Empty Tips
Volume:	EtOH		
Technique:	155	μL	
	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			
Labware Type:	Ensure tip height in well is 0.50 mm from bottom		
Position:	V-Bottom Plate	Pod:	Pod1
Liquid Type:	P2	N/A	Refresh tips
Volume:	Tris-HCl-1		
Technique:	45	μL	
	Aspirate from VBP		

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

34) Mix in P1			
Labware Type:	Ensure tip height in well is 1.50 mm from bottom		
Position:	V-Bottom Plate	Pod:	Pod1
Liquid Type:	P1	N/A	Refresh tips
Volume:	Tris-HCl-1	Mix:	5 times
Technique:	50	μL	
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			
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			
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

NAME	CATALOG #	VENDOR
Glycerol	G5516	
Triton(R) X-100 100ml	H5142	Promega
Microtube, 1.5ml, 1000/bag	V1231	Promega
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)	203734	Sigma Aldrich
1 M Calcium Chloride (CaCl2)	BP510	Fisher Scientific
1 M Potassium Chloride (KCl)	P3911	Sigma Aldrich
0.5 M Ethylenediaminetetraacetic acid (EDTA)	3002E	Research Organics
2 M Spermidine	S2501	Sigma Aldrich
Agencourt AMPure XP magnetic beads	A63880	Beckman Coulter
10% Sodium dodecyl sulfate (SDS)	L4509	Sigma Aldrich

NAME	CATALOG #	VENDOR
Ethanol	2716	Decon Labs
Concanavalin-coated magnetic beads	BP531	Bangs Laboratories
1M HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid pH 7.5	H3375	Sigma Aldrich
1 M HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid pH 7.9	H3375	Sigma Aldrich
Roche Complete Protease Inhibitor EDTA-Free tablets	5056489001	Sigma Aldrich
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	022625004	Eppendorf Centrifuge
Centrifuge 5424 R refrigerated with Rotor FA-45-24-11 rotary knobs 120 V/50 – 60 Hz (US)	5404000537	Eppendorf Centrifuge
MACSiMAG Separator	130-092-168	Miltenyi Biotec
0.6 mL low-retention microcentrifuge tubes	3446	Thermo Fisher Scientific
BD Clay Adams™ Nutator Mixer BD Diagnostics	15172-203	Vwr
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 x 1 Tip Loader ALP	C02867	Beckman Coulter
Three 1 x 3 Static ALPs	B87478	Beckman Coulter
One Single Position Cooling/Heating ALP	719361	Beckman Coulter
96S Super Magnet Plate	A001322	Alpaqua
Aluminum Heat Block Insert for PCR Plates	VP741I6A	V&P Scientific
Recirculating Cooling Unit filled with antifreeze (e.g Thermo Neslab RTE-7 Digital One Recirculating Chiller Mfr # 271103200000)		
MicroAmp Support Bases	N801-0531	Thermofisher
96 well LoBind PCR plates Semi-skirted	0030129504	Eppendorf
Biomek AP96 P250 Pre-Sterile Tips with barrier	717253	Beckman Coulter
96 well Polystyrene V-Bottom Microplates	651101	greiner bio-one
• MASTERBLOCKTM 96 Deep Well Conical Bottom 2 mL Storage Plates	780271	greiner bio-one
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)	S271	Fisher Scientific
Glycine	G46	Fisher Scientific
Protein A-Tn5 (pA-Tn5) fusion protein (plasmid for protein prep available).	124601	addgene
KAPA HiFi PCR Kit	KK2102	Kapa Biosystems
AlumaSeal(R) 96 film	Z721549-100EA	Sigma Aldrich
100-1200 µL 8-Channel Multi Pipette (e.g. Rainin 17014496)		

NAME	CATALOG #	VENDOR
One 1X1 Static ALP	B87477	Beckman Coulter
LE Magnet Plate	A000350	Alpaqua
16% Formaldehyde Solution (w/v) Methanol-free	28906	Thermo Scientific
1 M Magnesium Chloride (MgCl2)	M8266	Sigma Aldrich

#### MATERIALS TEXT

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 TCGTCGGCAGCGTCAGATGTGATAAGAGACAG
- Mosaic end\_Adapter B GTCTCGTGGGCTCGGAGATGTGATAAGAGACAG
  
- 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	AATGATAACGGCGACCACCG AGATCTACACTAGATCGCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.2_CTCTCTAT	AATGATAACGGCGACCACCG AGATCTACACCTCTCTATTG GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.3_TATCCTCT	AATGATAACGGCGACCACCG AGATCTACACTATCCTCTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.4_AGAGTAGA	AATGATAACGGCGACCACCG AGATCTACACAGAGTAGAT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.5_GTAAGGAG	AATGATAACGGCGACCACCG AGATCTACACGTAAGGAGT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.6_ACTGCATA	AATGATAACGGCGACCACCG AGATCTACACACTGCATATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.7_AAGGAGTA	AATGATAACGGCGACCACCG AGATCTACACAAGGAGTAT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.8_CTAAGCCT	AATGATAACGGCGACCACCG AGATCTACACCTAACGCCTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.9_TGGAAATC	AATGATAACGGCGACCACCG AGATCTACACTGGAAATCTC GTCGGCAGCGTCAGATGTG TAT

v2_Ad1.10_AACATGAT	AATGATAACGGCGACCACCG AGATCTACACAACATGATTG GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.11_TGATGAAA	AATGATAACGGCGACCACCG AGATCTACACTGATGAAATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.12_GTCGGACT	AATGATAACGGCGACCACCG AGATCTACACGTCGGACTT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.13_TTTCTAGC	AATGATAACGGCGACCACCG AGATCTACACTTCTAGCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.14_TAACCAAG	AATGATAACGGCGACCACCG AGATCTACACTAACCAAGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.15_GTGTATCG	AATGATAACGGCGACCACCG AGATCTACACGTGTATCGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.16_TCCATCAA	AATGATAACGGCGACCACCG AGATCTACACTCCATCAATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.17_TTCGTGCA	AATGATAACGGCGACCACCG AGATCTACACTTGTGCATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.18_AGGTTGCC	AATGATAACGGCGACCACCG AGATCTACACAGGTTGCC CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.19_CCTTATGT	AATGATAACGGCGACCACCG AGATCTACACCCTTATGTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.20_CAGCAACG	AATGATAACGGCGACCACCG AGATCTACACCAGCAACGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.21_GGTTCAAT	AATGATAACGGCGACCACCG AGATCTACACGGTTCAATT GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.22_ACATTCGT	AATGATAACGGCGACCACCG AGATCTACACACATTGTT GTCGGCAGCGTCAGATGTG TAT

v2_Ad1.23_GATTCCC	AATGATAACGGCGACCACCG AGATCTACACGATTCCCATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.24_CGGACTGC	AATGATAACGGCGACCACCG AGATCTACACCGGACTGCT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.25_AGCCGTTC	AATGATAACGGCGACCACCG AGATCTACACAGCGTTCTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.26_ATTGGGTC	AATGATAACGGCGACCACCG AGATCTACACATTGGTCT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.27_TGCATACT	AATGATAACGGCGACCACCG AGATCTACACTGCATACTTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.28_GGGCTTGG	AATGATAACGGCGACCACCG AGATCTACACGGGCTTGGT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.29_GACGTGGC	AATGATAACGGCGACCACCG AGATCTACACGACGTGGCT CGTCCGCAGCGTCAGATGT GTAT
v2_Ad1.30_GCAAATT	AATGATAACGGCGACCACCG AGATCTACACGCAAATTTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.31_GCAGCCTC	AATGATAACGGCGACCACCG AGATCTACACGCAGCCTCTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.32_TCCGAGTT	AATGATAACGGCGACCACCG AGATCTACACTCCGAGTTTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.33_GCATTAAG	AATGATAACGGCGACCACCG AGATCTACACGCATTAAGTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.34_ACGATAAC	AATGATAACGGCGACCACCG AGATCTACACACGATAACTC GTCGGCAGCGTCAGATGT TAT
v2_Ad1.35_CCTGCGGG	AATGATAACGGCGACCACCG AGATCTACACCTCGGGGT CGTCCGCAGCGTCAGATGT GTAT

v2_Ad1.36_TGATTGTT	AATGATAACGGCGACCACCG AGATCTACACTGATTGTTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.37_GGCACGGA	AATGATAACGGCGACCACCG AGATCTACACGGCACGGAT CGTGGCAGCGTCAGATGTG GTAT
v2_Ad1.38_GATCATTC	AATGATAACGGCGACCACCG AGATCTACACGATCATTCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.39_ATGGTCAT	AATGATAACGGCGACCACCG AGATCTACACATGGTCATTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.40_CGTACCAA	AATGATAACGGCGACCACCG AGATCTACACCGTACCAATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.41_CCAGTTA	AATGATAACGGCGACCACCG AGATCTACACCCAGTTATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.42_ACCGGCCC	AATGATAACGGCGACCACCG AGATCTACACACCGGCCCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.43_CTAGAAGT	AATGATAACGGCGACCACCG AGATCTACACCTAGAAGTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.44_CGCCAGAT	AATGATAACGGCGACCACCG AGATCTACACGCCAGATTG GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.45_TCACATGG	AATGATAACGGCGACCACCG AGATCTACACTCACATGGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.46_GAACTCGA	AATGATAACGGCGACCACCG AGATCTACACGAACTCGATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.47_CCACCGTT	AATGATAACGGCGACCACCG AGATCTACACCCACCGTTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.48_TAAGTTAC	AATGATAACGGCGACCACCG AGATCTACACTAAGTTACTC GTCGGCAGCGTCAGATGTG TAT

v2_Ad1.49_GAGACGTG	AATGATAACGGCGACCACCG AGATCTACACGAGACGTGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.50_TTGCCTAA	AATGATAACGGCGACCACCG AGATCTACACTTGCTAATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.51_TTAACTTG	AATGATAACGGCGACCACCG AGATCTACACTTAACCTGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.52_CTTTAACA	AATGATAACGGCGACCACCG AGATCTACACCTTAACATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.53_CGTAGACC	AATGATAACGGCGACCACCG AGATCTACACCGTAGACCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.54_TATTTGCG	AATGATAACGGCGACCACCG AGATCTACACTATTGCGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.55_ATCCAGGA	AATGATAACGGCGACCACCG AGATCTACACATCCAGGATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.56_TGTTCCCTG	AATGATAACGGCGACCACCG AGATCTACACTGTTCCCTGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.57_ACGCGCAG	AATGATAACGGCGACCACCG AGATCTACACACCGCAGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.58_TCTGGCGA	AATGATAACGGCGACCACCG AGATCTACACTCTGGCGAT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.59_AATCTACA	AATGATAACGGCGACCACCG AGATCTACACAATCTACATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.60_TACTGACC	AATGATAACGGCGACCACCG AGATCTACACTACTGACCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.61(CGATAGGG	AATGATAACGGCGACCACCG AGATCTACACCGATAGGGT CGTCGGCAGCGTCAGATGT GTAT

v2_Ad1.62_ACTTAGAA	AATGATAACGGCGACCACCG AGATCTACACACTTGAATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.63_AGAGATCT	AATGATAACGGCGACCACCG AGATCTACACAGAGATCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.64_GGTGAAGG	AATGATAACGGCGACCACCG AGATCTACACGGTAAGGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.65_ATCGAATG	AATGATAACGGCGACCACCG AGATCTACACATCGAATGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.66_TCAAGAGC	AATGATAACGGCGACCACCG AGATCTACACTCAAGAGCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.67_GCCCACGT	AATGATAACGGCGACCACCG AGATCTACACGCCACGTT GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.68_TGGGCGGT	AATGATAACGGCGACCACCG AGATCTACACTGGGCGGTT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.69_CCCTTGGA	AATGATAACGGCGACCACCG AGATCTACACCCCTGGATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.70_ATTACCGT	AATGATAACGGCGACCACCG AGATCTACACATTACCGTT GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.71_AGTCCGAG	AATGATAACGGCGACCACCG AGATCTACACAGTCGAGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.72_ACTTGTG	AATGATAACGGCGACCACCG AGATCTACACACTTGTGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.73_GTAATACA	AATGATAACGGCGACCACCG AGATCTACACGTAAACATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.74_GGCGTCTA	AATGATAACGGCGACCACCG AGATCTACACGGGTCTAT CGTCGGCAGCGTCAGATGT GTAT

v2_Ad1.75_GCGCTGCT	AATGATAACGGCACCACCG AGATCTACACGCCTGCTT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.76_GTGCCATT	AATGATAACGGCACCACCG AGATCTACACGTGCCATTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.77_TAGGTATG	AATGATAACGGCACCACCG AGATCTACACTAGGTATGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.78_AACACCTA	AATGATAACGGCACCACCG AGATCTACACAACACCTATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.79_CTCCGAAC	AATGATAACGGCACCACCG AGATCTACACCTCCGAACTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.80_CAACGGCA	AATGATAACGGCACCACCG AGATCTACACCAACGGCATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.81_CAAATGTAG	AATGATAACGGCACCACCG AGATCTACACCAATGTAGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.82_GGCTACCC	AATGATAACGGCACCACCG AGATCTACACGGCTACCCCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.83_AAAGTCCG	AATGATAACGGCACCACCG AGATCTACACAAAGTCCGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.84_TTCCCGGG	AATGATAACGGCACCACCG AGATCTACACTTCGCGGT CGTCGGCAGCGTCAGATGT GTAT
v2_Ad1.85_AGGCACTT	AATGATAACGGCACCACCG AGATCTACACAGGCACTTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.86_CTTCAGTG	AATGATAACGGCACCACCG AGATCTACACCTTCAGTGTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.87_GCCGGTAG	AATGATAACGGCACCACCG AGATCTACACGCCGGTAGT CGTCGGCAGCGTCAGATGT GTAT

v2_Ad1.88_TTCAATCC	AATGATAACGGCGACCACCG AGATCTACACTTCAATCCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.89_CCACACAC	AATGATAACGGCGACCACCG AGATCTACACCCACACACTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.90_ATATTATC	AATGATAACGGCGACCACCG AGATCTACACATATTATCTC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.91_CCGAAGCA	AATGATAACGGCGACCACCG AGATCTACACCCGAAGCATC GTCGGCAGCGTCAGATGTG TAT
v2_Ad1.92_GTATCGGT	AATGATAACGGCGACCACCG AGATCTACACGTATCGGTT CGTCGGCAGCGTCAGATGTG GTAT
Custom Barcodes Adapter 2 (index i7):	
v2_Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATAC GAGATTGCCTTAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATAC GAGATCTAGTACGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATAC GAGATTCTGCCTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATAC GAGATGCTAGGAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATAC GAGATAGGAGTCCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATAC GAGATCATGCCTAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATAC GAGATGTAGAGAGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATAC GAGATCCTCTGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATAC GAGATAGCGTAGCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATAC GAGATCAGCCTCGGTCTCG TGGGCTCGGAGATGTG

v2_Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATAC GAGATTGCCTTTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATAC GAGATTCCCTACGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.13_TGGATCTG	CAAGCAGAAGACGGCATAC GAGATCAGATCCAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.14_CCGTTGT	CAAGCAGAAGACGGCATAC GAGATACAAACGGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.15_TGCTGGGT	CAAGCAGAAGACGGCATAC GAGATACCCACCTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.16_AGGTTGGG	CAAGCAGAAGACGGCATAC GAGATCCAACCTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.17_GTGTGGTG	CAAGCAGAAGACGGCATAC GAGATCACCAACCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.18_TGGGTTTC	CAAGCAGAAGACGGCATAC GAGATGAAACCCAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.19_TGGTCACA	CAAGCAGAAGACGGCATAC GAGATTGTGACCAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.20_TTGACCCT	CAAGCAGAAGACGGCATAC GAGATAGGGTCAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.21_CGC GGACA	CAAGCAGAAGACGGCATAC GAGATTGTCCGCGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.22_TTCCATAT	CAAGCAGAAGACGGCATAC GAGATATATGGAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.23_AATTCGTT	CAAGCAGAAGACGGCATAC GAGATAACGAATTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.24_GGC GTCGA	CAAGCAGAAGACGGCATAC GAGATTGACGCCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.25_AC AAAGTG	CAAGCAGAAGACGGCATAC GAGATCACTTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.26_TACTTGAA	CAAGCAGAAGACGGCATAC GAGATTCAAGTAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.27_GTGATAGC	CAAGCAGAAGACGGCATAC GAGATGCTATCACGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.28_ AGTAGATT	CAAGCAGAAGACGGCATAC GAGATAATCTACTGTCTCG TGGGCTCGGAGATGTG

v2_Ad2.29_ATTGCCGG	CAAGCAGAAGACGGCATAC GAGATCCGGCAATGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.30_TTGCTAAG	CAAGCAGAAGACGGCATAC GAGATCTTAGCAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.31_ATAAGTTA	CAAGCAGAAGACGGCATAC GAGATTAACATTATGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.32_ATCACTCG	CAAGCAGAAGACGGCATAC GAGATCGAGTGATGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.33_GTTAACAG	CAAGCAGAAGACGGCATAC GAGATCTGTTAACGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.34_AATGGTAG	CAAGCAGAAGACGGCATAC GAGATCTACCATTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.35_GAGCACGT	CAAGCAGAAGACGGCATAC GAGATACGTGCTCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.36_TTTCGTCA	CAAGCAGAAGACGGCATAC GAGATTGACGAAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.37_CAAAGAATT	CAAGCAGAAGACGGCATAC GAGATAATTCTGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.38_GAAATGCC	CAAGCAGAAGACGGCATAC GAGATGGCATTGCTCTCG TGGGCTCGGAGATGTG
v2_Ad2.39_AACGCCAT	CAAGCAGAAGACGGCATAC GAGATATGGCGTTGCTCG TGGGCTCGGAGATGTG
v2_Ad2.40_CCTCGCAG	CAAGCAGAAGACGGCATAC GAGATCTGCGAGGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.41_TACACCTC	CAAGCAGAAGACGGCATAC GAGATGAGGTGTAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.42_GGTCATT	CAAGCAGAAGACGGCATAC GAGATAATGACCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.43_CAACTTTA	CAAGCAGAAGACGGCATAC GAGATTAAGATTGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.44_TGTGCCTT	CAAGCAGAAGACGGCATAC GAGATAAGGCACAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.45_TCTTATT	CAAGCAGAAGACGGCATAC GAGATTAATAAGAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.46_GACTTAGT	CAAGCAGAAGACGGCATAC GAGATACTAAGTCGTCTCG TGGGCTCGGAGATGTG

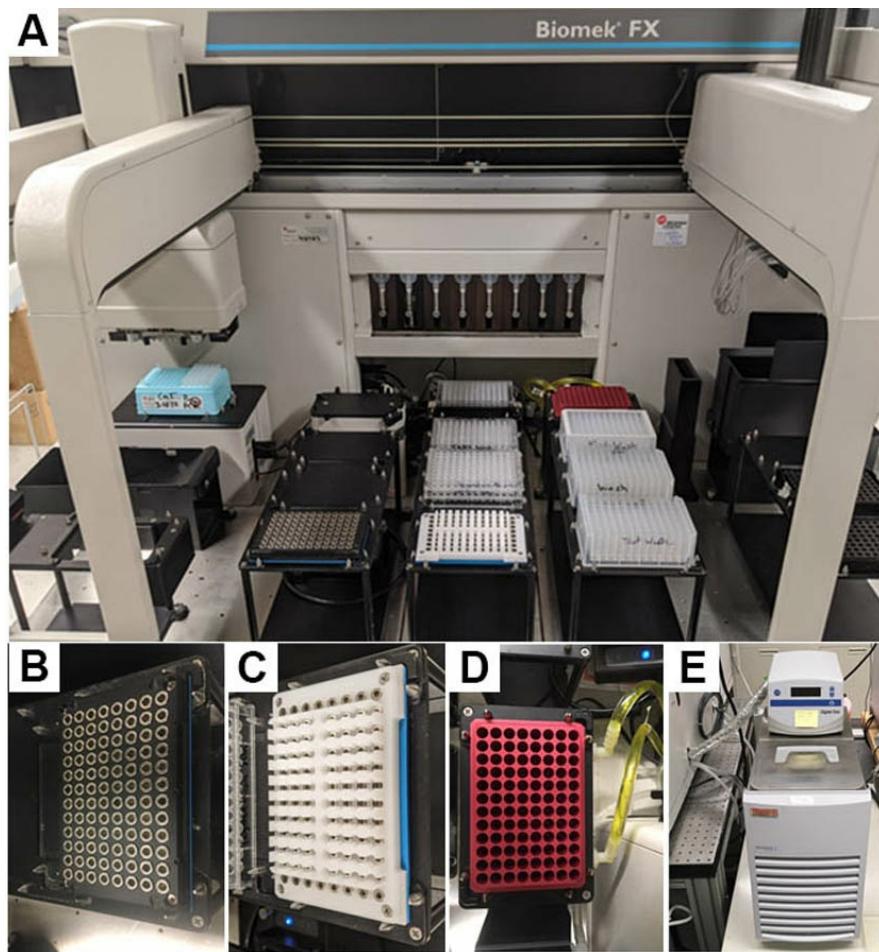
v2_Ad2.47_AGACCAGC	CAAGCAGAAGACGGCATAC GAGATGCTGGTCTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.48_AAATACAG	CAAGCAGAAGACGGCATAC GAGATCTGTATTTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.49_TTATGAAA	CAAGCAGAAGACGGCATAC GAGATTTCTAAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.50_CTTGGGTC	CAAGCAGAAGACGGCATAC GAGATGACCCAAGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.51_CCAAATAA	CAAGCAGAAGACGGCATAC GAGATTTATTGGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.52_GCGTTAAA	CAAGCAGAAGACGGCATAC GAGATTTAACCGCTCTCG TGGGCTCGGAGATGTG
v2_Ad2.53_CATCCTGT	CAAGCAGAAGACGGCATAC GAGATACAGGATGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.54_GGAGTAAG	CAAGCAGAAGACGGCATAC GAGATCTTACTCCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.55_GACGCTCC	CAAGCAGAAGACGGCATAC GAGATGGAGCGTCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.56_TTCGCGGC	CAAGCAGAAGACGGCATAC GAGATGCCGCGAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.57_CGGTTCCC	CAAGCAGAAGACGGCATAC GAGATGGGAACCGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.58_ACCGGCTA	CAAGCAGAAGACGGCATAC GAGATTAGCCGGTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.59_CTCATGGG	CAAGCAGAAGACGGCATAC GAGATCCCAGGAGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.60_TTTAATGC	CAAGCAGAAGACGGCATAC GAGATGCATTAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.61_AAACGGTC	CAAGCAGAAGACGGCATAC GAGATGACCGTTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.62_GATCCAAA	CAAGCAGAAGACGGCATAC GAGATTTGGATCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.63_ATGATGAT	CAAGCAGAAGACGGCATAC GAGATATCATCATGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.64_CCAACACG	CAAGCAGAAGACGGCATAC GAGATCGTTGGGTCTCG TGGGCTCGGAGATGTG

v2_Ad2.65_TAACAACA	CAAGCAGAAGACGGCATAC GAGATTGTTGTTAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.66_GGTAAACC	CAAGCAGAAGACGGCATAC GAGATGGTTTACCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.67_CATCGACC	CAAGCAGAAGACGGCATAC GAGATGGTCGATGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.68_ATGGGAAC	CAAGCAGAAGACGGCATAC GAGATGTTCCCATGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.69_CGGCCAAT	CAAGCAGAAGACGGCATAC GAGATATTGGCCGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.70_GGGAATGA	CAAGCAGAAGACGGCATAC GAGATTCAATTCCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.71_GTATTCGG	CAAGCAGAAGACGGCATAC GAGATCCGAATACGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.72_TCAGCTAT	CAAGCAGAAGACGGCATAC GAGATATAAGCTGAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.73_ATTTATCT	CAAGCAGAAGACGGCATAC GAGATAGATAAAATGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.74_ACAGTTGC	CAAGCAGAAGACGGCATAC GAGATGCAACTGTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.75_CCCGAGAT	CAAGCAGAAGACGGCATAC GAGATATCTCGGGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.76_TAATGTCT	CAAGCAGAAGACGGCATAC GAGATAGACATTAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.77_GCCAATT	CAAGCAGAAGACGGCATAC GAGATGAATTGGCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.78_CGCCGTGC	CAAGCAGAAGACGGCATAC GAGATGCACGGCGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.79_CTGACCGA	CAAGCAGAAGACGGCATAC GAGATTCGGTCAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.80_CATTTCGA	CAAGCAGAAGACGGCATAC GAGATTCGAAATGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.81_GCTTGCCA	CAAGCAGAAGACGGCATAC GAGATTGGCAAGCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.82_TTCTACCA	CAAGCAGAAGACGGCATAC GAGATTGGTAGAAGTCTCG TGGGCTCGGAGATGTG

v2_Ad2.83_ACGTGACG	CAAGCAGAAGACGGCATAC GAGATCGTCACGTGCTCG TGGGCTCGGAGATGTG
v2_Ad2.84_TGTCCGCG	CAAGCAGAAGACGGCATAC GAGATCGCGGACAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.85_TTAAACTT	CAAGCAGAAGACGGCATAC GAGATAAGTTTAAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.86_ACCACAAC	CAAGCAGAAGACGGCATAC GAGATGTTGTGGTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.87_GCCTCTGG	CAAGCAGAAGACGGCATAC GAGATCCAGAGGCCTCG TGGGCTCGGAGATGTG
v2_Ad2.88_TCGCCCAC	CAAGCAGAAGACGGCATAC GAGATGTGGCGAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.89_CACTAGGC	CAAGCAGAAGACGGCATAC GAGATGCCAGTGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.90_TCGAAGCC	CAAGCAGAAGACGGCATAC GAGATGGCTTCGAGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.91_GCATGTAC	CAAGCAGAAGACGGCATAC GAGATGTACATGCGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.92_GTTCGAGT	CAAGCAGAAGACGGCATAC GAGATACTCGAACGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.93_CCGGGCGC	CAAGCAGAAGACGGCATAC GAGATGCGCCGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.94_AGATTAA	CAAGCAGAAGACGGCATAC GAGATTTAAATCTGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.95_CACCATTG	CAAGCAGAAGACGGCATAC GAGATCAATGGTGGTCTCG TGGGCTCGGAGATGTG
v2_Ad2.96_AATAAGAC	CAAGCAGAAGACGGCATAC GAGATGTCTTATTGTCTCG TGGGCTCGGAGATGTG

## 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.



**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°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 x 3 Static ALPs (P1-P9), one 1 x 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 <u>1</u> Coordinates	-7.296	-9.398	-15.676	Position Framed
Pod <u>2</u> 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 <u>1</u> Coordinates	24.712	7.265	-15.692	Position Framed
Pod <u>2</u> Coordinates	N/A	N/A	N/A	Position Framed
Name	P2	ALP Type: OneByThree		
	X(cm)	Y(cm)	Z(cm)	Precision
Pod <u>1</u> Coordinates	24.707	18.67	-15.677	ALP Framed
Pod <u>2</u> Coordinates	N/A	N/A	N/A	Position Framed
Name	P3	ALP Type: OneByThree		
	X(cm)	Y(cm)	Z(cm)	Precision
Pod <u>1</u> Coordinates	24.726	30.126	-15.683	Position Framed
Pod <u>2</u> 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 <u>1</u> Coordinates	43.773	7.313	-15.685	Position Framed
Pod <u>2</u> Coordinates	N/A	N/A	N/A	Position Framed
Name	P5	ALP Type: OneByThree		
	X(cm)	Y(cm)	Z(cm)	Precision
Pod <u>1</u> Coordinates	43.765	18.753	-15.673	Position Framed
Pod <u>2</u> Coordinates	N/A	N/A	N/A	Position Framed
Name	P6	ALP Type: OneByThree		
	X(cm)	Y(cm)	Z(cm)	Precision
Pod <u>1</u> Coordinates	43.762	30.191	-15.678	Position Framed
Pod <u>2</u> 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
	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
	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
	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
	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
	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<sub>2</sub>O 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 °C for up to 6 months.

Binding Buffer		
Component	Amount	Final concentration
ddH <sub>2</sub> O	19.36 mL	-
1M HEPES-KOH pH 7.9	400 µL	20 mM
1M KCl	200 µL	10 mM
1M CaCl <sub>2</sub>	20 µL	1 mM
1M MnCl <sub>2</sub>	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°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 Mg<sup>2+</sup> during incubation in

the Antibody Buffer, which might otherwise affect chromatin properties.

Wash Buffer		
Component	Amount per 96 samples	Final
ddH <sub>2</sub> O	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 Ca<sup>2+</sup> 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 <sub>2</sub> O	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°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.

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>A</b>	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>B</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
<b>C</b>	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
<b>D</b>	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
<b>E</b>	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
<b>F</b>	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
<b>G</b>	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
<b>H</b>	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  $\leq$  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°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°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°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 MgCl <sub>2</sub>	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 ddH <sub>2</sub> O from a 10 % stock)	1200	45	1245	0.1 %

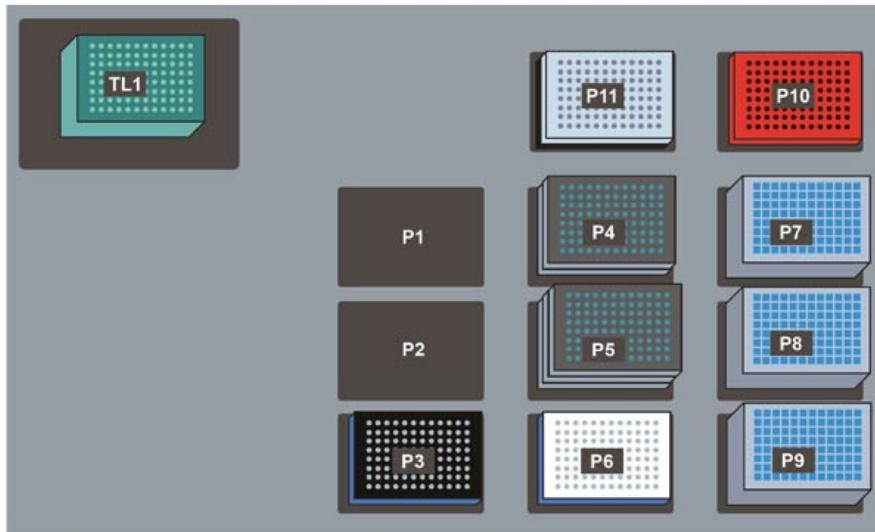
- **Note:** Store Wash Buffer, Secondary Antibody Solution, 300 Wash Buffer, pA-Tn5 Solution, and Tagmentation Buffer on ice. Keep 0.1 % SDS Release Buffer, and 10 mM TAPS Buffer pH 8.5 at Room Temp.

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  $\mu$ L Tips (double click to increase the # of load times)

P3: ALPAQUA Magnet Plate

P4: V-Bottom Plate preloaded with 125  $\mu$ L TAPS Buffer stacked on top of a V-Bottom Plate preloaded with 125  $\mu$ L 0.1% SDS Release Buffer

P5: V-Bottom Plate preloaded with 125  $\mu$ L Secondary Antibody Solution, stacked on top of a V-Bottom Plate preloaded with 125  $\mu$ L pA-Tn5 Solution, stacked on top of a V-Bottom Plate preloaded with 125  $\mu$ L Tgmentation 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°C

P11: PCR 96 Well Plate preloaded with up to 150  $\mu$ L 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  $\mu$ L 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°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 Tgmentation Buffer into a reservoir and dispense 125  $\mu$ L 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°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°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
ddH <sub>2</sub> O	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°C incubation, remove the PCR 96 Well Plate containing the samples and allow to cool to room temp (~ 5 min). Then remove and discarded 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:

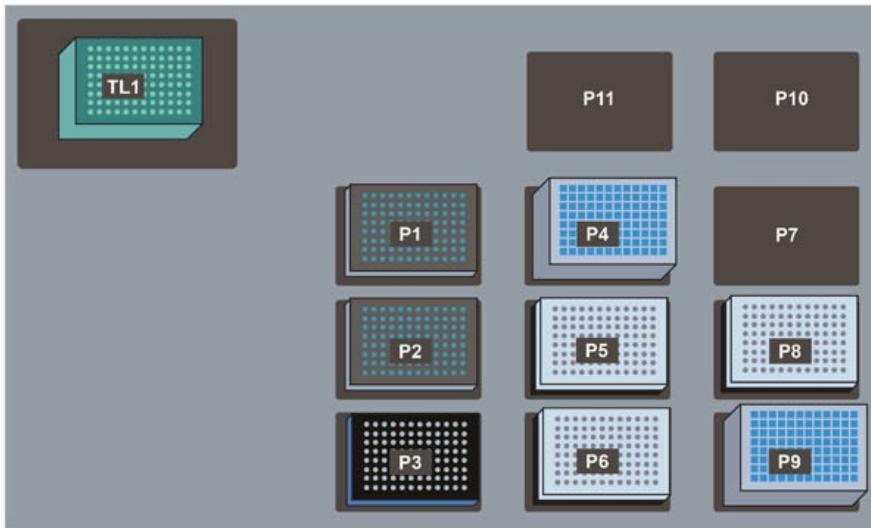
PCR Program				
Cycle number	Denature	Anneal	Extend	Final
1			58° C, 5 min	
2			72° C, 10 min	
3	98° C, 45 s			
4–17	98° C, 15 s	60° C, 10 s		
18			72° C, 1 min	
19				8° C, hold

- **Note:** Libraries should be amplified with 12-14 PCR cycles, preferably with a 10 s 60°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°C overnight or stored at 4°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  $\mu\text{L}$  Tips (double click to increase the # of load times)
- P1: V-Bottom Plate preloaded with 100  $\mu\text{L}$  10 mM Tris-HCl pH 8 (used for washing tips)
- P2: V-Bottom Plate preloaded with 100  $\mu\text{L}$  10 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  $\mu\text{L}$  of PCR product stacked on a PCR Plate Rack
- P6: PCR 96 Well Plate preloaded with 130  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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.

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°C indefinitely.

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

- **Note:** Library 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 25x25 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