

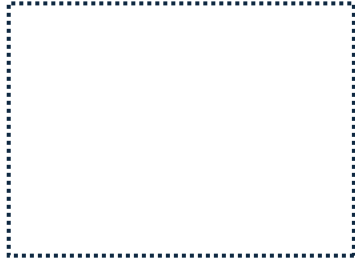
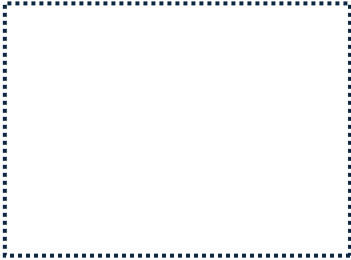
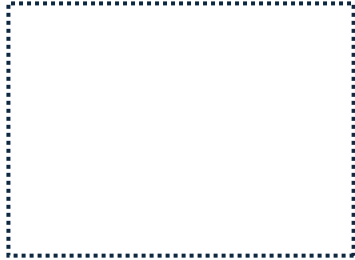
A

B

C

D

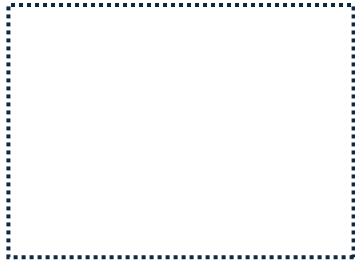
E



1

T:

P:



2

T:

P:

Slide ID:

Date:

Notes:

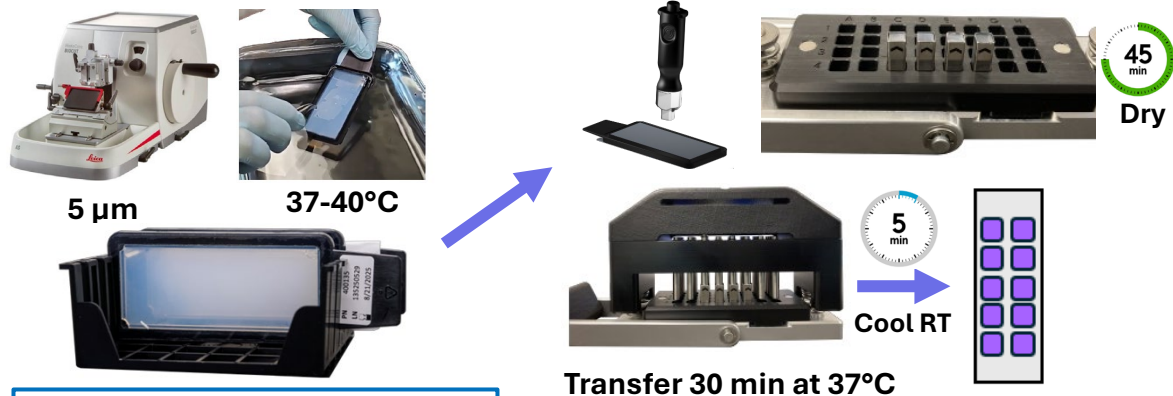
Experiment Name:

A	B	C	D	E	F	G	H		
								1	T: P:
								2	T: P:
								3	T: P:
								4	T: P:

Slide ID:

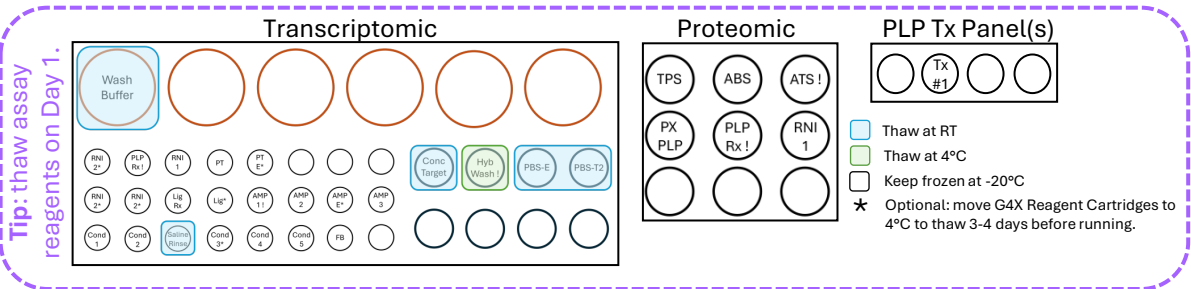
Date:	Experiment Name:
Notes:	

1. Section Tissue & Transfer ROI



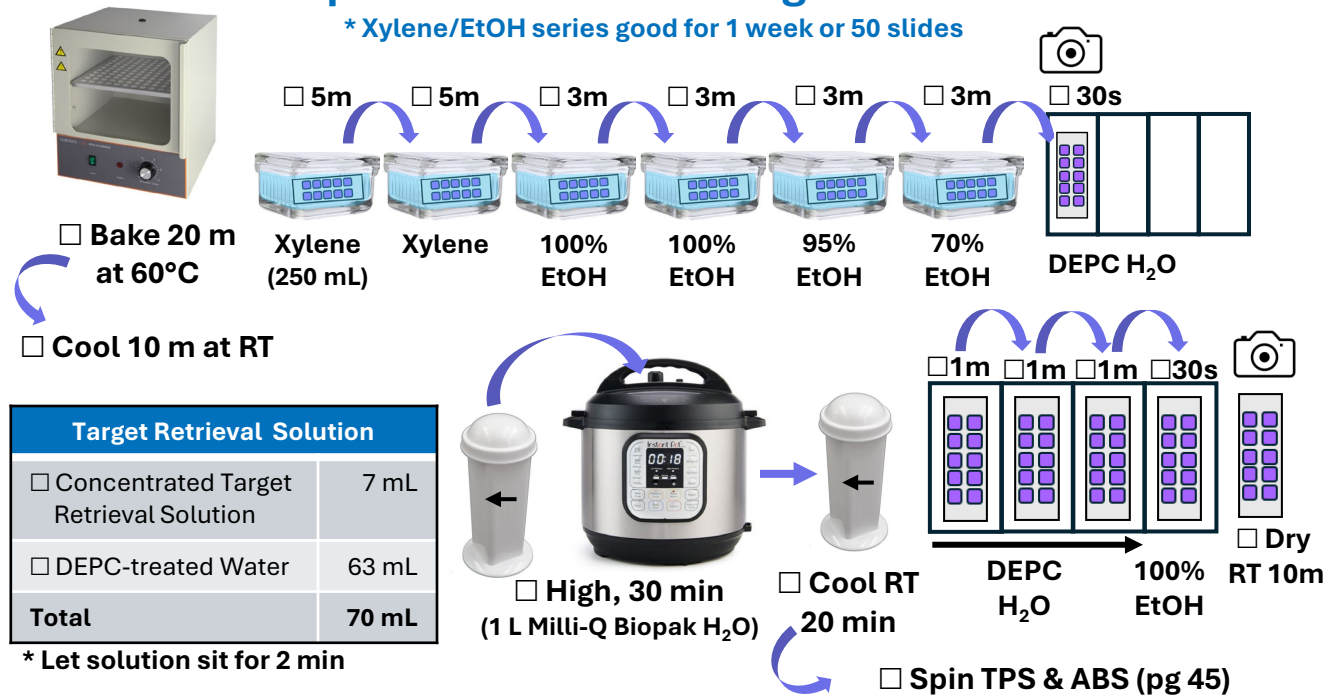
Dry for ~15 min RT → 4°C (1 mo)
or
Dry for 60 min RT and continue

Store slide at RT with desiccant packet for up to 1 month.



2. Deparaffinization & Antigen Retrieval

* Xylene/EtOH series good for 1 week or 50 slides

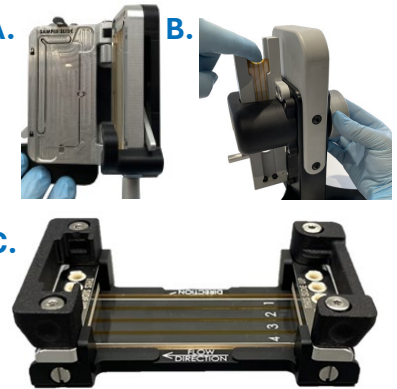


Target Retrieval Solution	
Concentrated Target Retrieval Solution	7 mL
DEPC-treated Water	63 mL
Total	70 mL

* Let solution sit for 2 min

4. Antibody Binding & Padlock Probe Hybridization

3. Prepare Flow Cell & Install Pipette Aid



Tissue Passivation (TP)	1 FC
□ Tissue Passivation Solution	1247.0 µl
□ RNase Inhibitor 2	12.5 µl
Total	1259.5 µl
Antibody Binding (AB)	1 FC
□ Antibody Binding Solution	330.0 µl
□ RNase Inhibitor 2	3.3 µl
Total	333.3 µl

Transcript (Tx) PLP Working Mix	1 FC
□ PLP Reaction Buffer	331.6 µl
□ Transcript Panel	63.0 µl
□ RNase Inhibitor 1	31.5 µl
□ DEPC-treated H ₂ O	203.9 µl
Total	630.0 µl

Wash Buffer	Wash Buffer	TP	AB	TP	Wash Buffer
500µl 1000µl	500µl 1000µl	✓ 70µl 150µl	✓ 70µl 150µl	✓ 70µl 150µl	200µl 400µl
□ RT --	□ RT --	□ RT 30m	□ 37°C 2h	□ 37°C 15m	□ RT 5m
Wash Buffer	ATS	Wash Buffer	Wash Buffer	HYB Wash Buffer	Tx PLP Working Mix
200µl 400µl	✓ 70µl 150µl	✓ 200µl 400µl	✓ 200µl 400µl	500µl 1000µl	✓ 70µl 150µl
□ RT 5m	□ 45°C 10m	□ RT 5m	□ RT 5m	□ RT --	□ 45°C ON

Adjust heat block temperature after step

Discard & replace pipette aid
Plugs after ON incubation

5. Ligation & Amplification

HYB Wash Buffer		HYB Wash Buffer		Wash Buffer		Wash Buffer		PLP Treatment Priming Buffer		PLP Treatment Working Mix		Wash Buffer		HYB Wash Buffer		Pr PLP Working Mix		Wash Buffer		Ligation Priming	
<input type="checkbox"/>	200µl 400µl	<input type="checkbox"/>	200µl 400µl	<input type="checkbox"/>	200µl 400µl	<input type="checkbox"/>	200µl 400µl	<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl	<input checked="" type="checkbox"/>	70µl 150µl	<input type="checkbox"/>	500µl 1000µl	<input checked="" type="checkbox"/>	70µl 150µl
<input type="checkbox"/> 45°C 5m		<input type="checkbox"/> 45°C 5m		<input type="checkbox"/> RT 5m		<input type="checkbox"/> RT 5m		<input type="checkbox"/> RT --		<input type="checkbox"/> 45°C 15m		<input type="checkbox"/> RT --		<input type="checkbox"/> RT --		<input type="checkbox"/> 45°C 30m		<input type="checkbox"/> RT --		<input type="checkbox"/> RT --	
Ligation Working		Wash Buffer		HYB Wash Buffer		AMP 1		HYB Wash Buffer		HYB Wash Buffer		Wash Buffer		AMP 2		AMP 2		AMP 3		PBS-E	
<input checked="" type="checkbox"/>	70µl 150µl	<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl	<input checked="" type="checkbox"/>	70µl 150µl	<input type="checkbox"/>	200µl 400µl	<input type="checkbox"/>	200µl 400µl	<input type="checkbox"/>	500µl 1000µl	<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input type="checkbox"/>	500µl 1000µl
<input type="checkbox"/> 37°C 30m		<input type="checkbox"/> RT --		<input type="checkbox"/> RT --		<input type="checkbox"/> 37°C 10m		<input type="checkbox"/> 37°C 5m		<input type="checkbox"/> 37°C 5m		<input type="checkbox"/> RT --		<input type="checkbox"/> 33°C 15m		<input type="checkbox"/> 33°C 15m		<input type="checkbox"/> 37°C 3h		<input type="checkbox"/> RT --	

PLP Priming Buffer	1 FC
<input type="checkbox"/> PLP Conditioning Buffer	623.7 µl
<input type="checkbox"/> RNase Inhibitor 2	6.3 µl
Total	630.0 µl

PLP Treatment Mix	1 FC
<input type="checkbox"/> PLP Conditioning Buffer	604.8 µl
<input type="checkbox"/> RNase Inhibitor 2	6.3 µl
<input type="checkbox"/> PLP Conditioning 2 Spike-In	18.9 µl
Total	630.0 µl

Ligation Priming	1 FC
<input type="checkbox"/> Ligation Reaction Buffer	623.7 µl
<input type="checkbox"/> RNase Inhibitor 2	6.3 µl
Total	630.0 µl

Ligation Working	1 FC
<input type="checkbox"/> Ligation Reaction Buffer	603.5 µl
<input type="checkbox"/> RNase Inhibitor 2	6.3 µl
<input type="checkbox"/> Ligase	20.2 µl
Total	630.0 µl

Store at 4°C in PBS-E or continue to Conditioning 1

AMP 3	1 FC
<input type="checkbox"/> AMP 3 Buffer	623.7 µl
<input type="checkbox"/> RNase Inhibitor 2	6.3 µl
Total	630.0 µl

Adjust heat block temperature
Accurate execution and timing is critical

Conditioning Solution 1	1 FC
<input type="checkbox"/> Concentrated Conditioning 1	63.0 µl
<input type="checkbox"/> PBS-T2	567.0 µl
Total	630.0 µl

Conditioning Solution 5	1 FC
<input type="checkbox"/> Conditioning 5 Solid	--
<input type="checkbox"/> PBS-T2	1600.0 µl
Total	1600.0 µl

6. Sequencing Conditioning

Conditioning Solution 1	Wash Buffer	Conditioning Solution 2	Wash Buffer	Saline Rinse	Wash Buffer
<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input type="checkbox"/>	200µl 400µl
<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl
<input type="checkbox"/> RT 1h		<input type="checkbox"/> RT --		<input type="checkbox"/> RT --	
<input type="checkbox"/> RT 30m		<input type="checkbox"/> RT --		<input type="checkbox"/> 60°C 10m	

Store at 4°C in Wash Buffer or continue to Conditioning 3

4°C → 2 weeks or continue to G4X for sequencing

Adjust heat block temperature after step

Conditioning Solution 3	Wash Buffer	Conditioning Solution 4	Wash Buffer	Conditioning Solution 5	Wash Buffer	Focusing Beads	Wash Buffer	Wash Buffer	
<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	70µl 150µl	<input checked="" type="checkbox"/>	500µl 1000µl
<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl	<input type="checkbox"/>	500µl 1000µl
<input type="checkbox"/> 60°C 15m		<input type="checkbox"/> RT --		<input type="checkbox"/> 60°C 15m		<input type="checkbox"/> RT --		<input type="checkbox"/> RT --	

7. Framing & G4X Run



Thaw G4X Reagent Cartridges & Quenching Solution before run