High-throughput proteomic sample preparation using pressure cycling technology
基于压力循环技术制备高通量蛋白质组学样品

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Introduction

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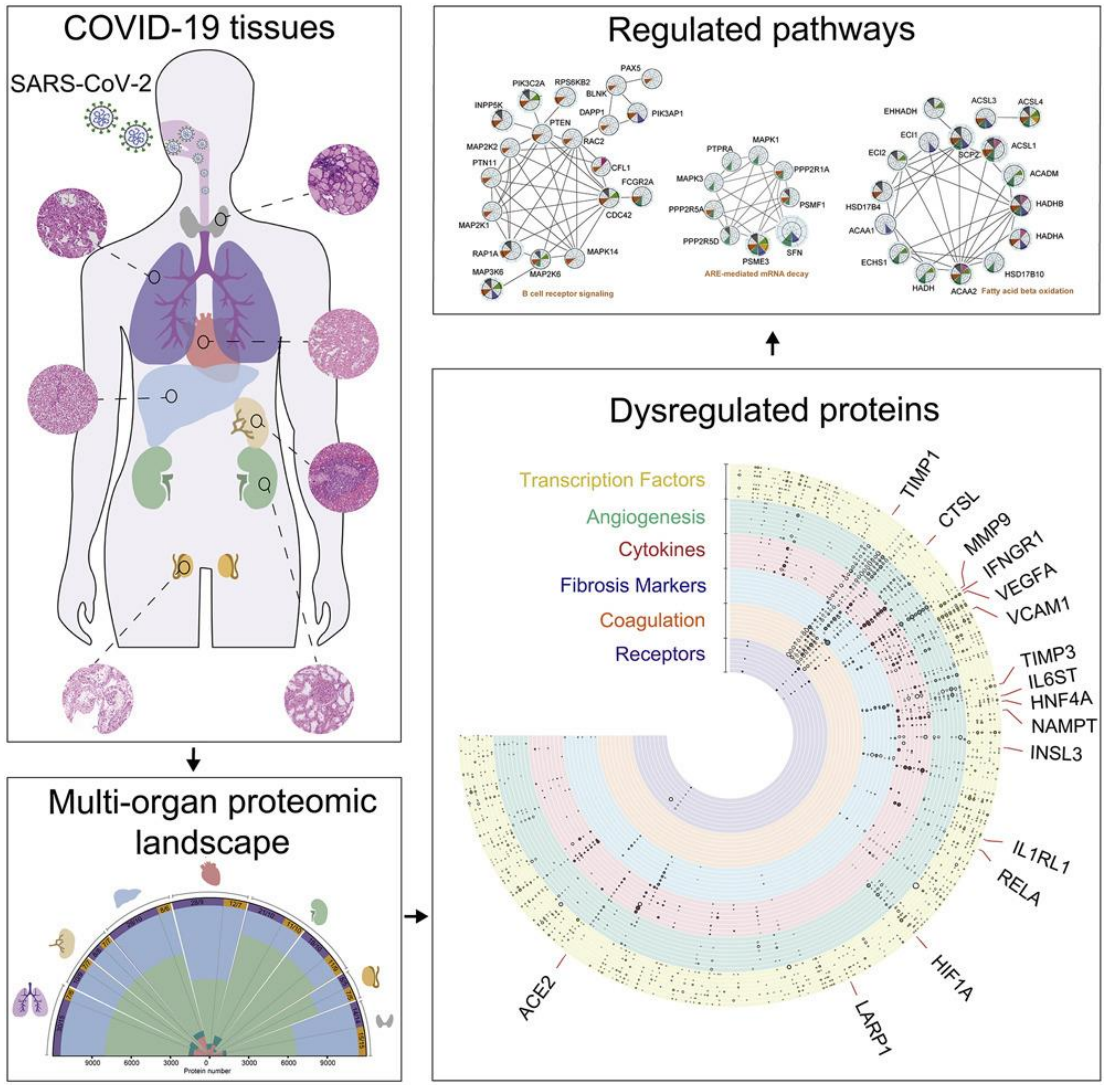
Clinical proteomics

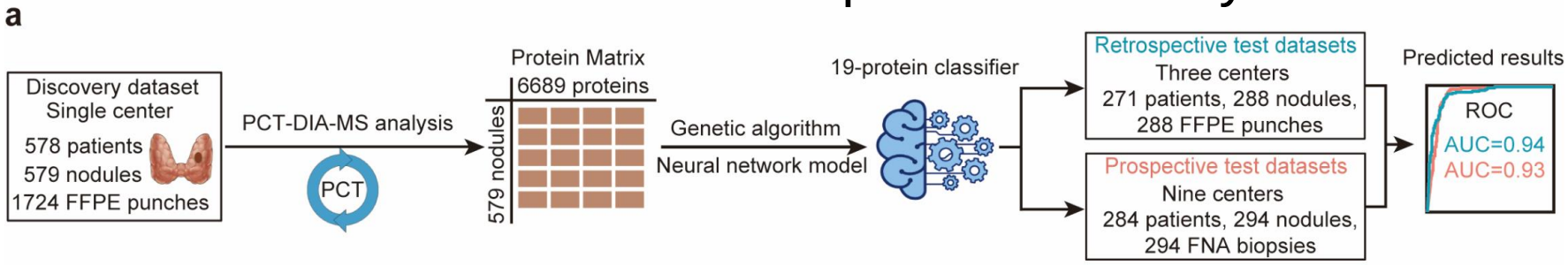


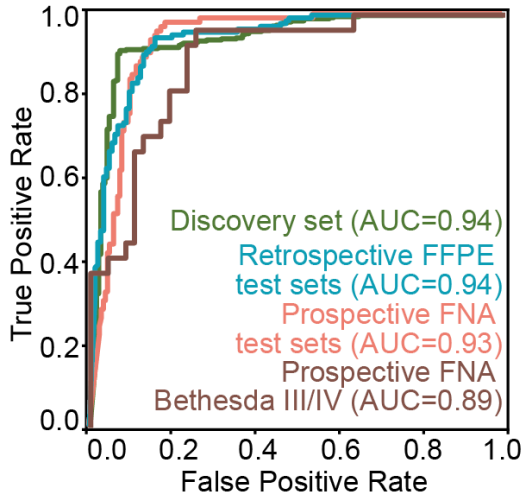
- 144 autopsy samples
- 7 organs
- 19 COVID-19 patients
- quantified 11,394 proteins
- depicts a multi-organ proteomic landscape of COVID-19 autopsies

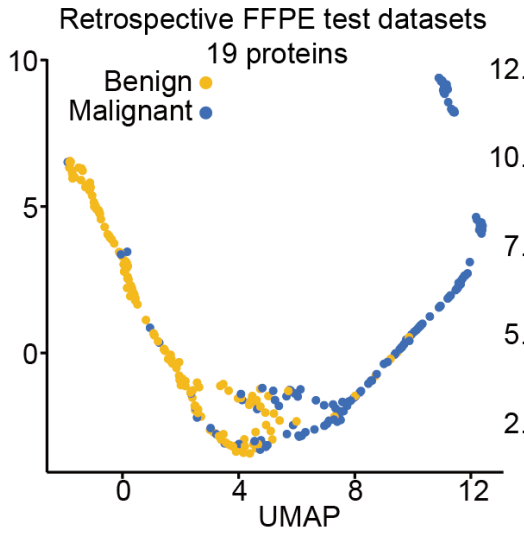
Nie, X. et al. Multi-organ proteomic landscape of COVID-19 autopsies. Cell 184, 775-791 e714,

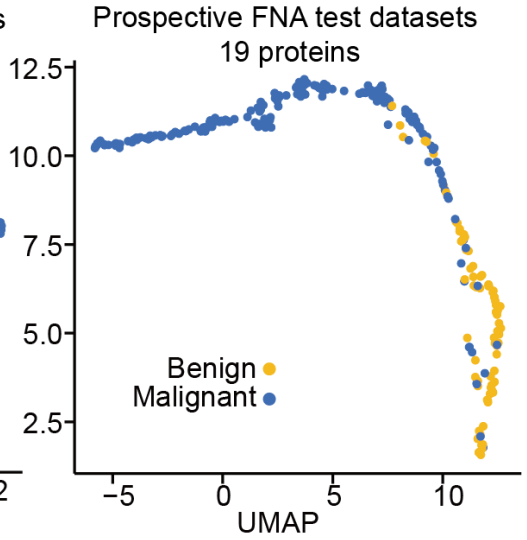
Clinical proteomics

Multicenter Clinical Evaluation of patients with thyroid nodules









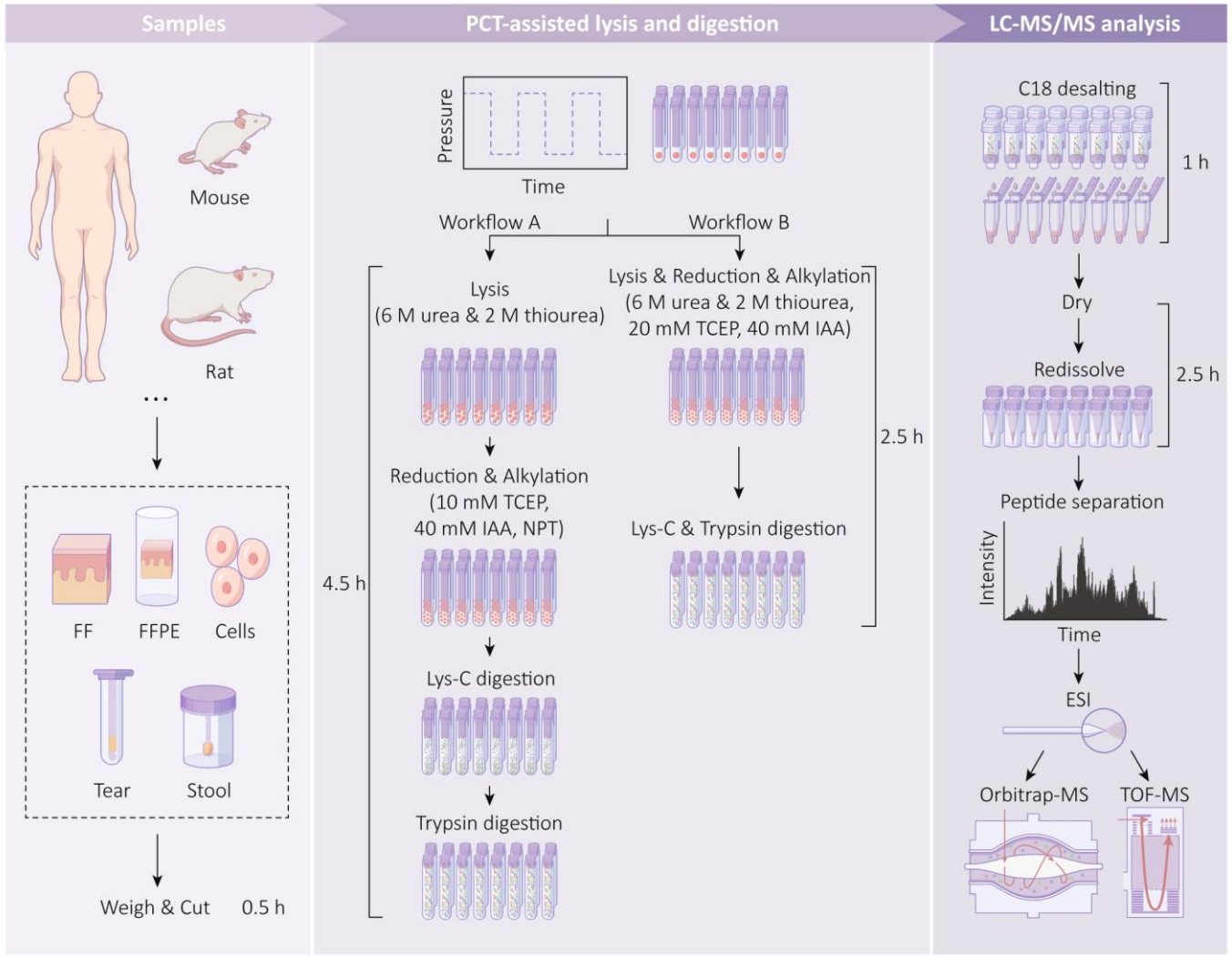
Retrospective sets
FFPE, 3 hospitals, 288 nodules

Prospective sets
FNA, 9 hospitals, 294 nodules

Sun, Y., Selvarajan, S., Zang, Z. et al. Artificial intelligence defines protein-based classification of thyroid nodules. Cell Discovery (2022).

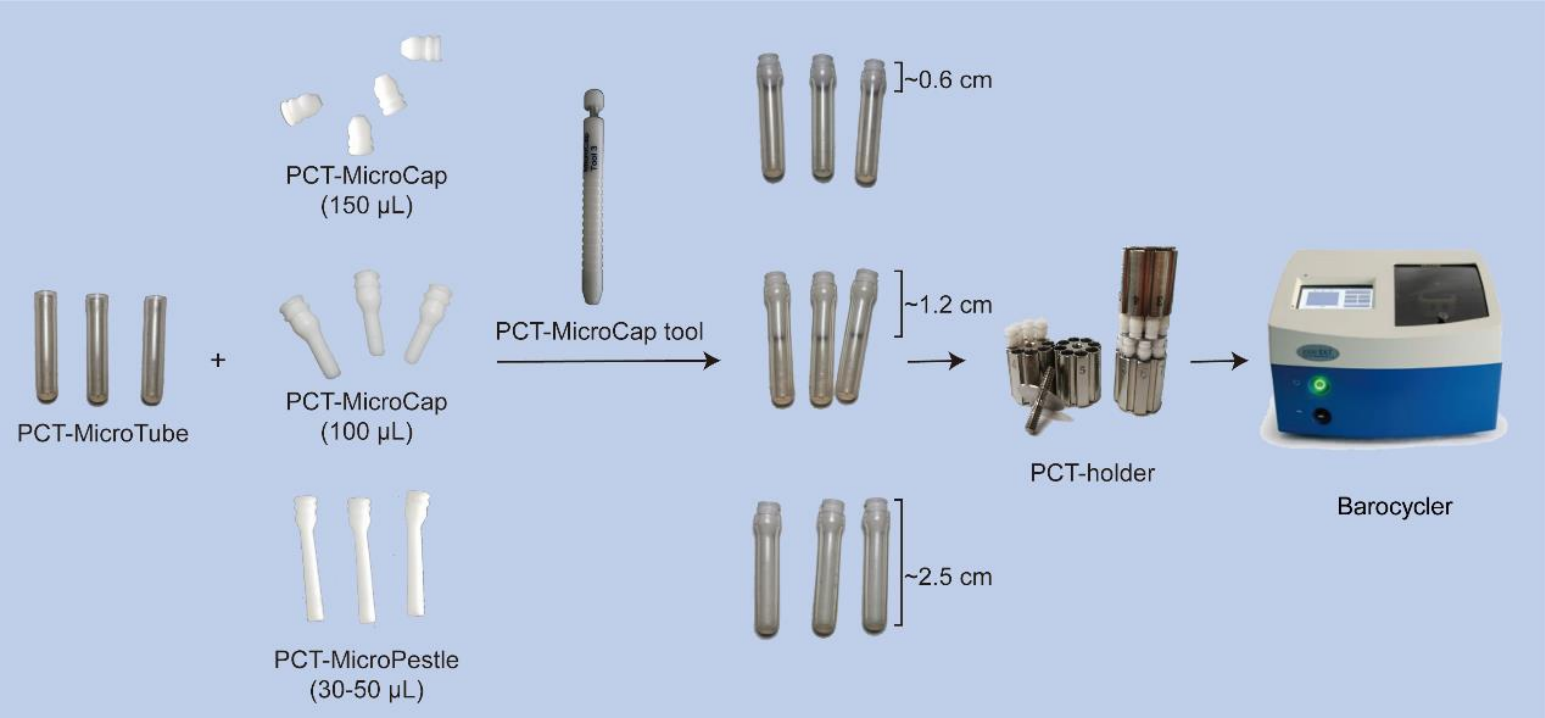
PCT-DIA/SWATH workflow

1. human, rat, mouse
2. FF, FFPE, cells, tear, stool
3. high-throughput and robustness
4. Potential for quantification of entire proteome



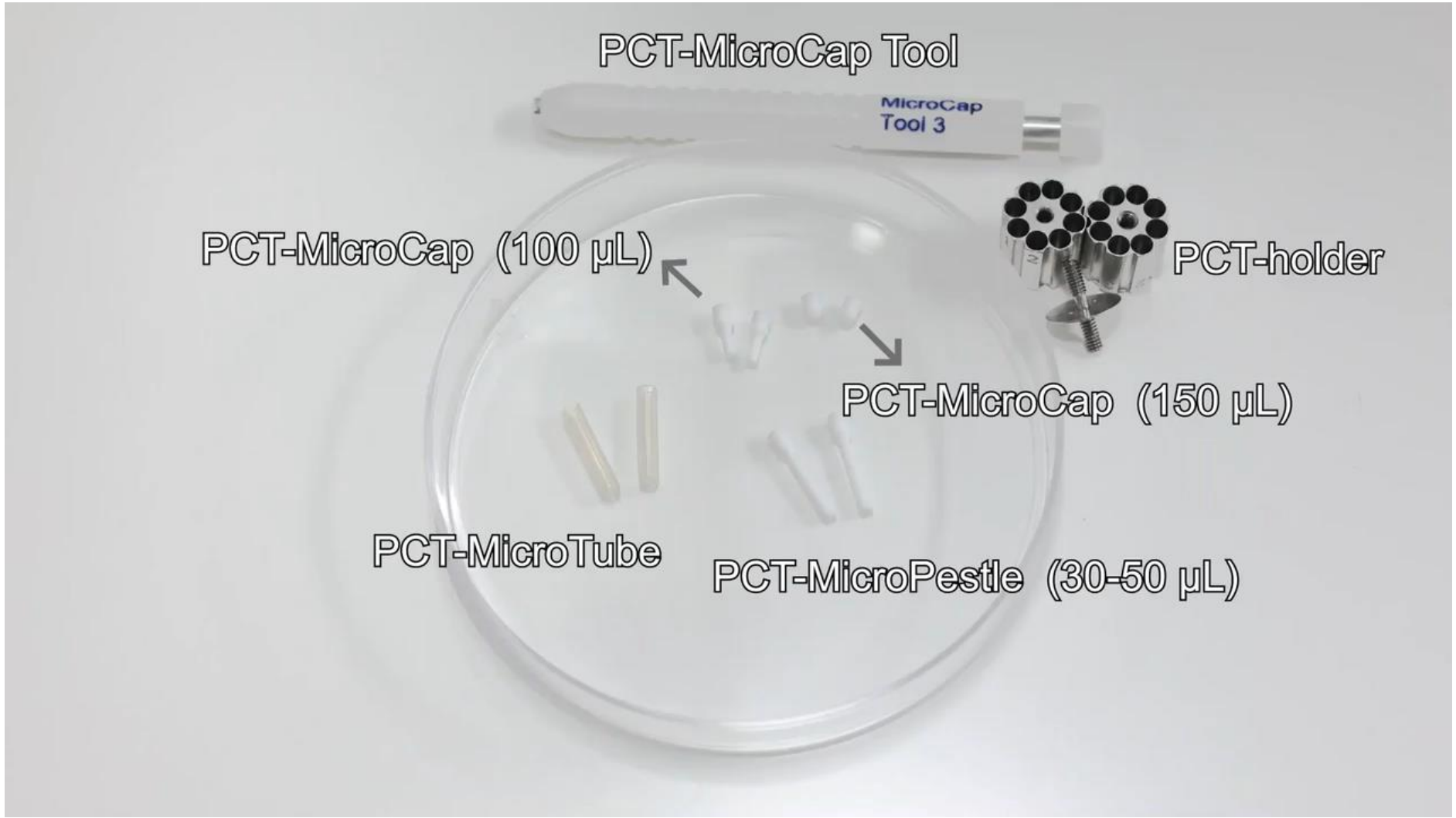
Cai, X., Xue, Z., Wu, C. et al. High-throughput proteomic sample preparation using pressure cycling technology. Nat Protoc (2022).

PCT introduction



- periodic pressure oscillations
- alternating ultrahigh (up to 45,000 psi) and mild pressure
- promotes tissue lysis and enzymatic reactions

PCT introduction



Development of PCT workflow

2015

Evaluated the minimal sample amount requirement
50 000 human cells and 0.2-0.5 mg wet mouse and human tissues

Shao, S. et al. *Proteomics* 15, 3711-3721, (2015)

2018

Allowed for the simultaneous processing of 16 samples

Zhu, Y. & Guo, T. *Methods Mol Biol* 1788, 279-287, (2018).

2020

Gao, H. et al. *J Proteome Res* 19, 1982-1990, (2020).

2015

Processing fresh frozen (FF) tissue biopsies allows for the preparation of up to 6 samples within 6-8 hrs

Guo, T. et al. *Nat Med* 21, 407-413 (2015).

2016

Developed a MicroPestle device that increased the yield of extracted peptides by 20%-40%

Shao, S. et al. *J Proteome Res* 15, 1821-1829, (2016).

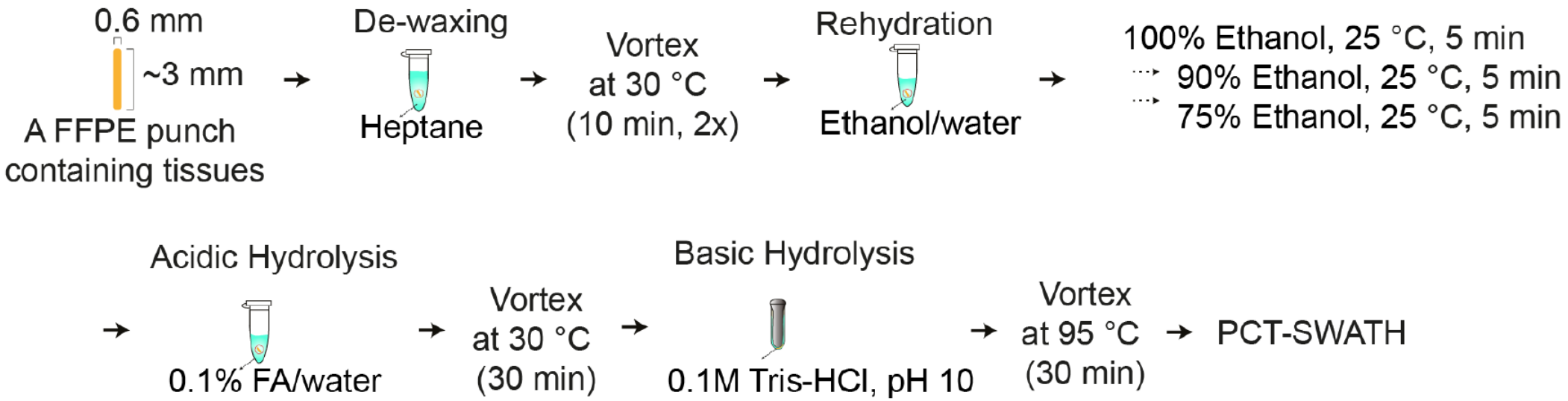
2019

Optimized to permit analysis of formalin-fixed, paraffin-embedded (FFPE) tissues

Zhu, Y. et al. *Mol Oncol* 13, 2305-2328, (2019).

Pretreatment

FFPE pretreatment



Zhu, Y. et al. High-throughput proteomic analysis of FFPE tissue samples facilitates tumor stratification. Mol Oncol 13, 2305-2328

FF (embedded in OCT) pretreatment

1. Add 1 mL of 70% ethanol and wash the tissue by vortexing on a Thermo Shaker at 800 rpm for 30 s at 25 ℃. Discard the supernatant.
2. Add 1 mL of 100% water and wash the tissue by vortexing on a Thermo Shaker at 800 rpm for 30 s at 25 ℃. Discard the supernatant.
3. Add 1 mL of 70% ethanol and wash the tissue by vortexing on a Thermo Shaker at 800 rpm for 5 min at 25 ℃. Discard the supernatant. Repeat this step once.
4. Add 1 mL of 85% ethanol and wash the tissue by vortexing on a Thermo Shaker at 800 rpm for 5 min at 25 ℃. Discard the supernatant. Repeat this step once.
5. Add 1 mL of 100% ethanol and wash the tissue by vortexing on a Thermo Shaker at 800 rpm for 5 min at 25 ℃. Discard the supernatant. Repeat this step once.

Cells and feces pretreatment

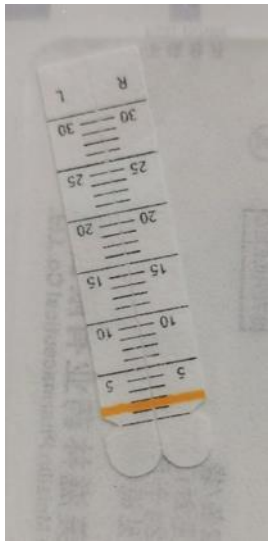
Cell

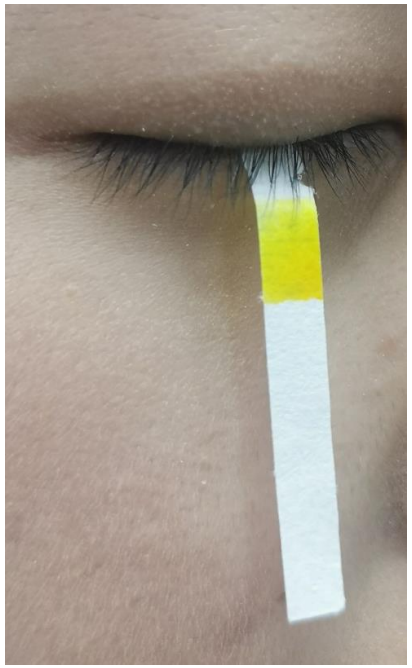
1. Wash cell pellets with 4 ℃ PBS. Centrifuge at 300 g, 4 ℃ for 5 min. Discard the supernatant. Repeat three times.
2. Add 30 µL of lysis buffer (6 M urea and 2 M thiourea), vortex for 30 s by pipetting up and down gently. Transfer the cell suspension to a PCT-MicroTube using pipettes.

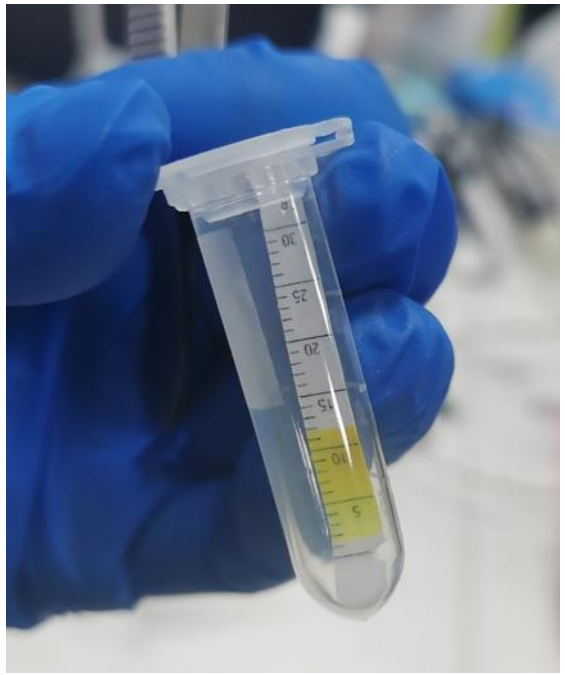
Feces

1. Wash 200 mg ~ 300 mg feces with 500 µL of 4 ℃ PBS, centrifuge at 500 g for 5 minutes at 4 ℃, and collect the supernatant. Repeat three times. Combine all the supernatant together.
2. Add 100 µL of the supernatant to a PCT-MicroTube. Place the PCT-MicroTube into a 1.5-mL tube, and centrifuge at 20,000 g for 20 minutes at 4 ℃. Discard the supernatant and retain the precipitate.

Tear strip pretreatment







Cut 3 mm*3 mm

PCT

PCT procedures

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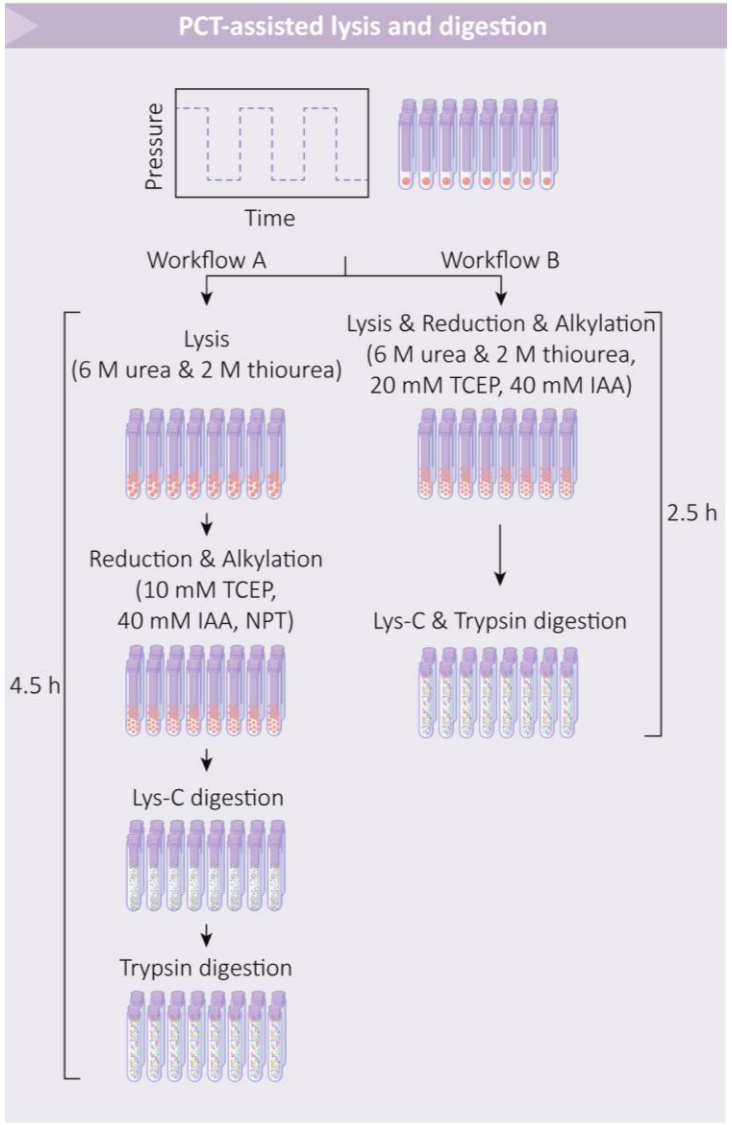
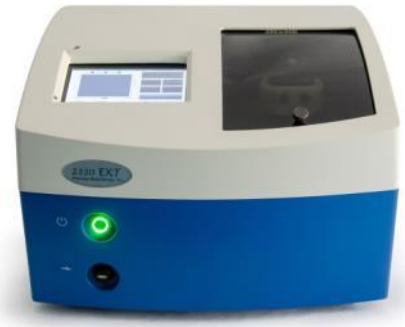
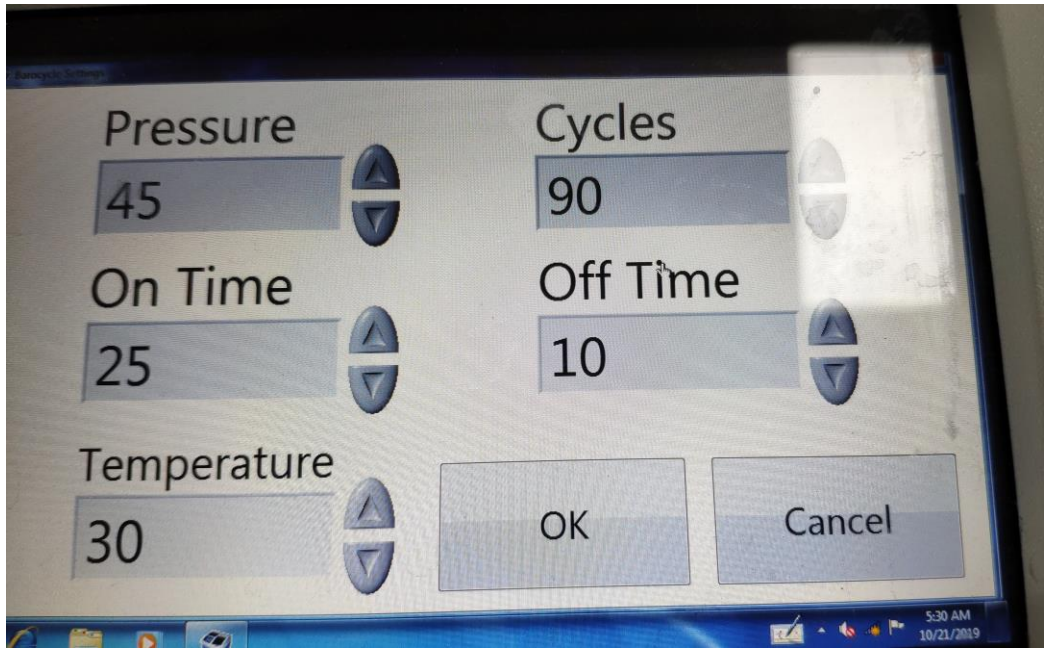
PCT procedures

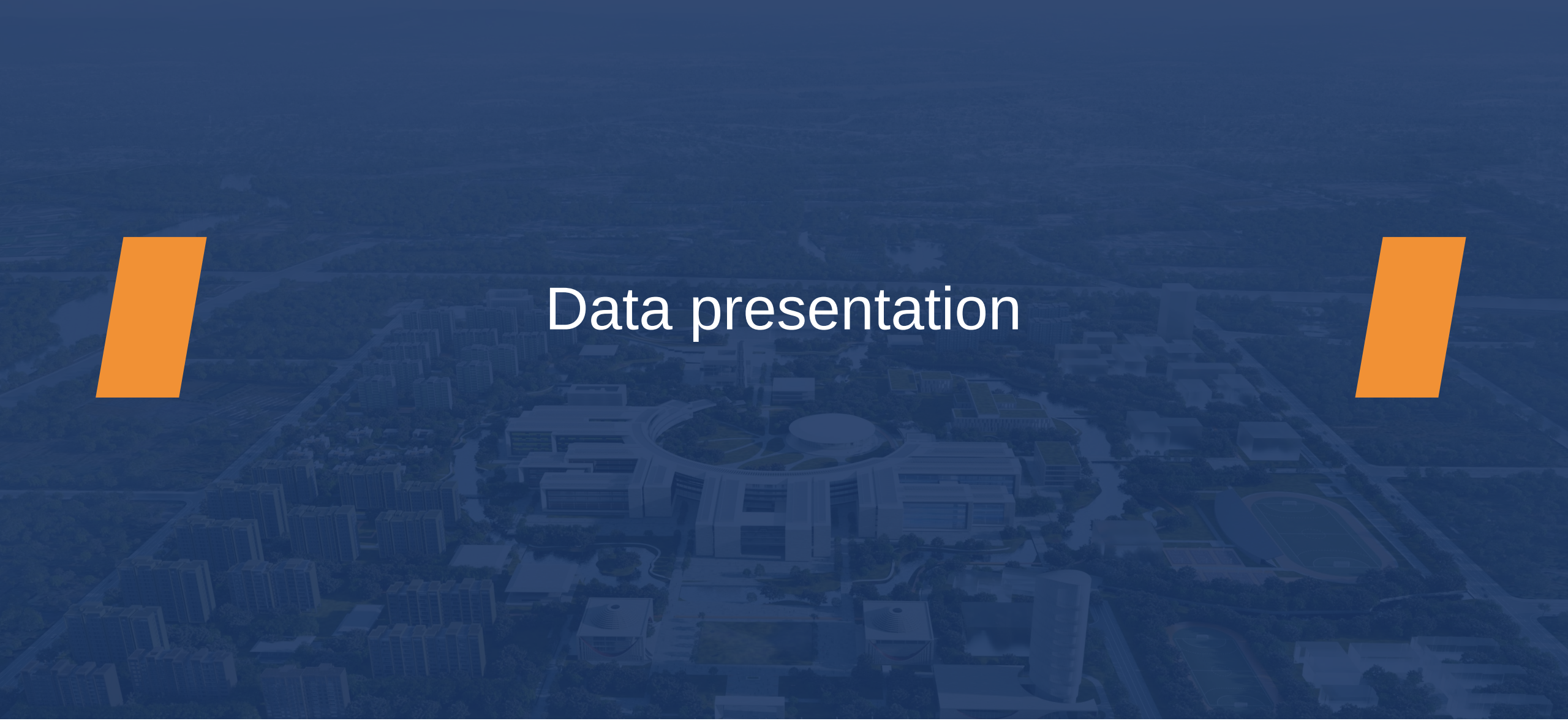






Troubleshooting

	Problem	Possible reason	Solution
	PCT-MicroPestle or PCT-MicroCap cannot be closed properly	The volume of the solution exceeds the limit of the PCT-MicroTube. This most likely occurs in cell samples when PBS is not completely removed.	Remove some buffer. For cell samples, minimize the residual PBS solution.
	PCT holder cannot be screwed properly	Frayed edges for PCT-MicroPestle or PCT-MicroCap due to excessive use.	Replace with a new PCT-MicroPestle or PCT-MicroCap.
		PCT-MicroPestles or PCT-MicroCaps are not closed tightly.	Replace with a new PCT-MicroPestle or PCT-MicroCap
		The thread for the PCT holder is damaged.	Replace it with a new PCT holder
		The PCT-MicroTubes are not placed in balance or there is an asymmetric number of samples in the upper and lower parts of the PCT holder.	Adjust the number of samples in upper and lower parts of the PCT holder.



Data presentation

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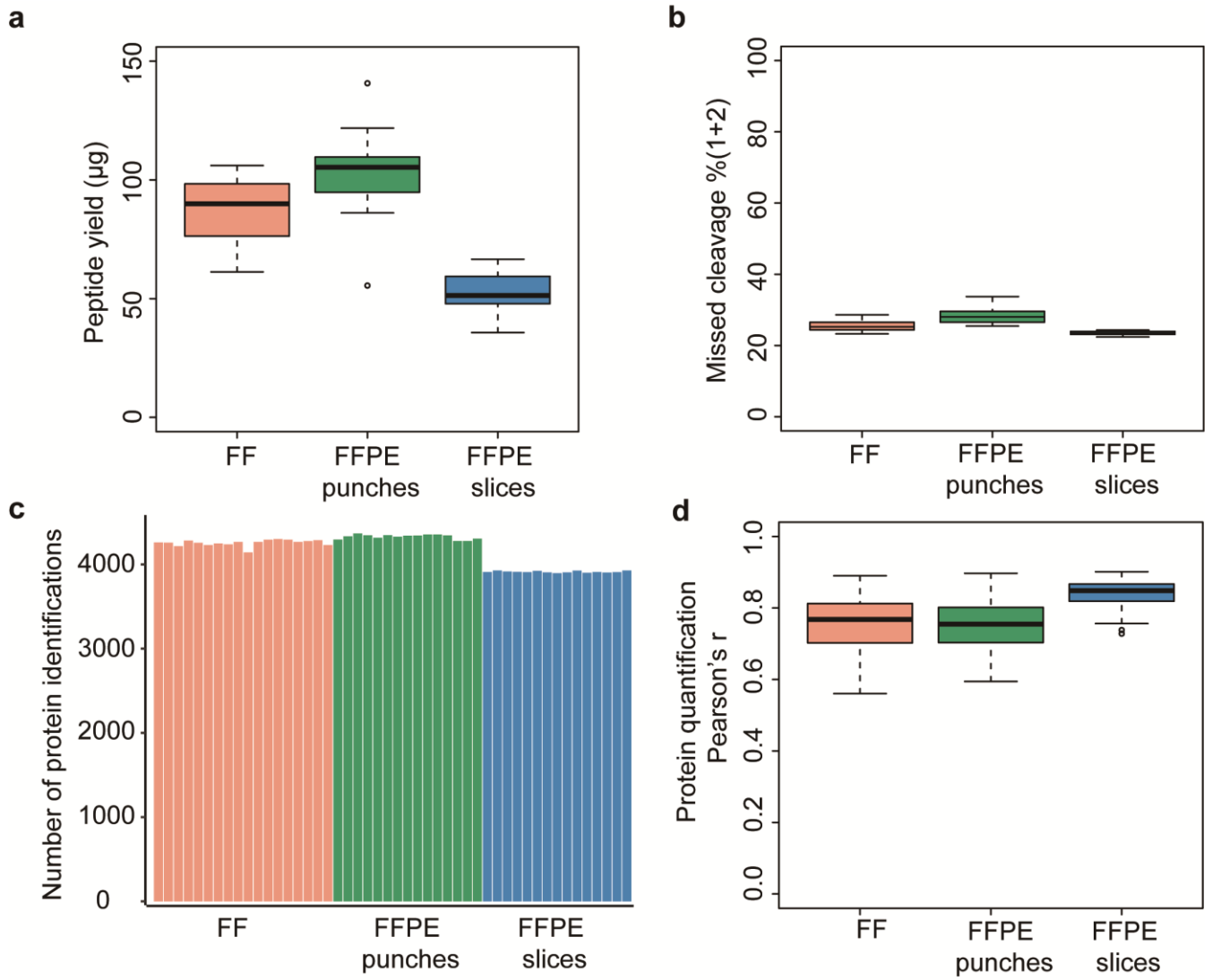
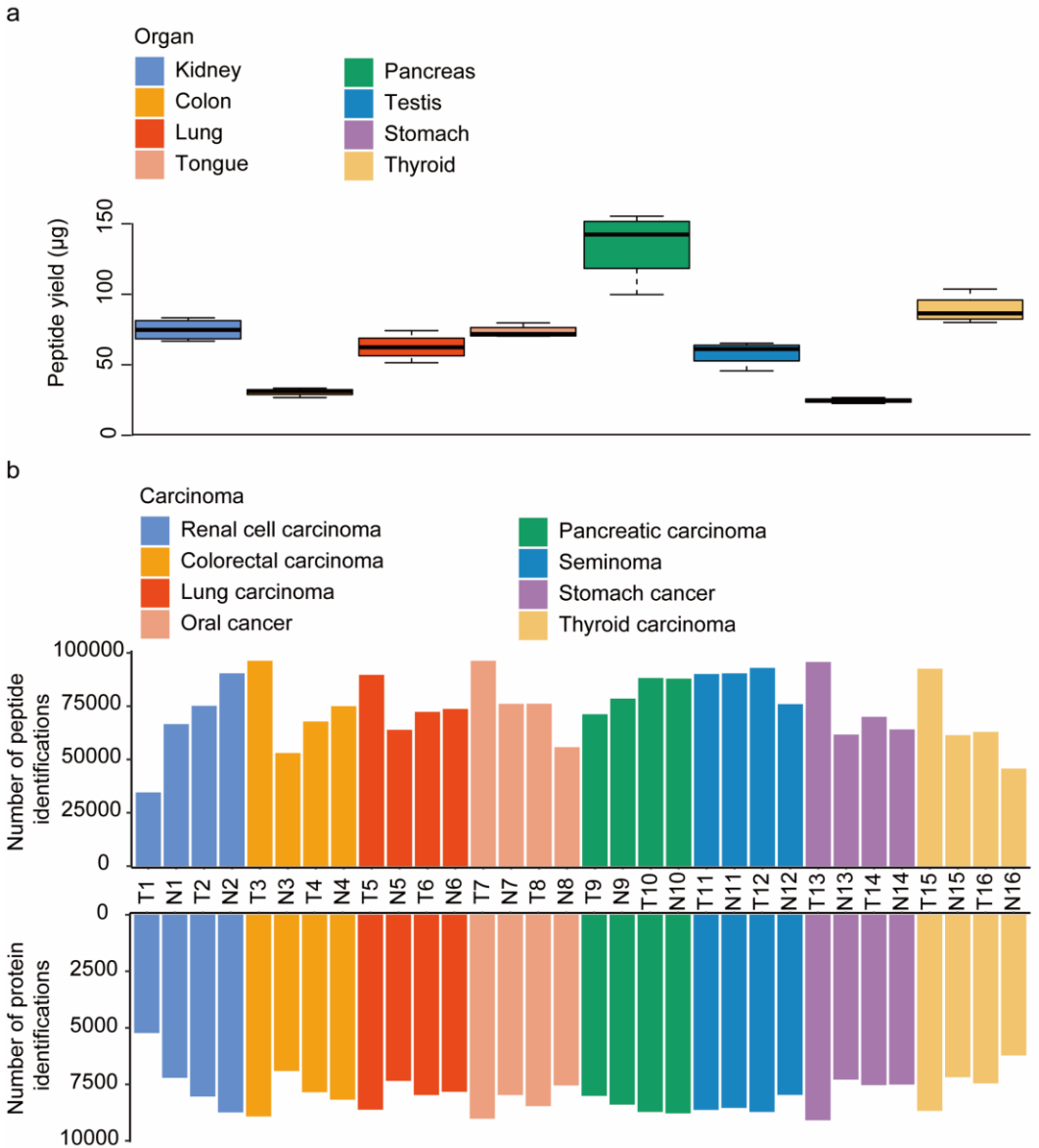
Data presentation

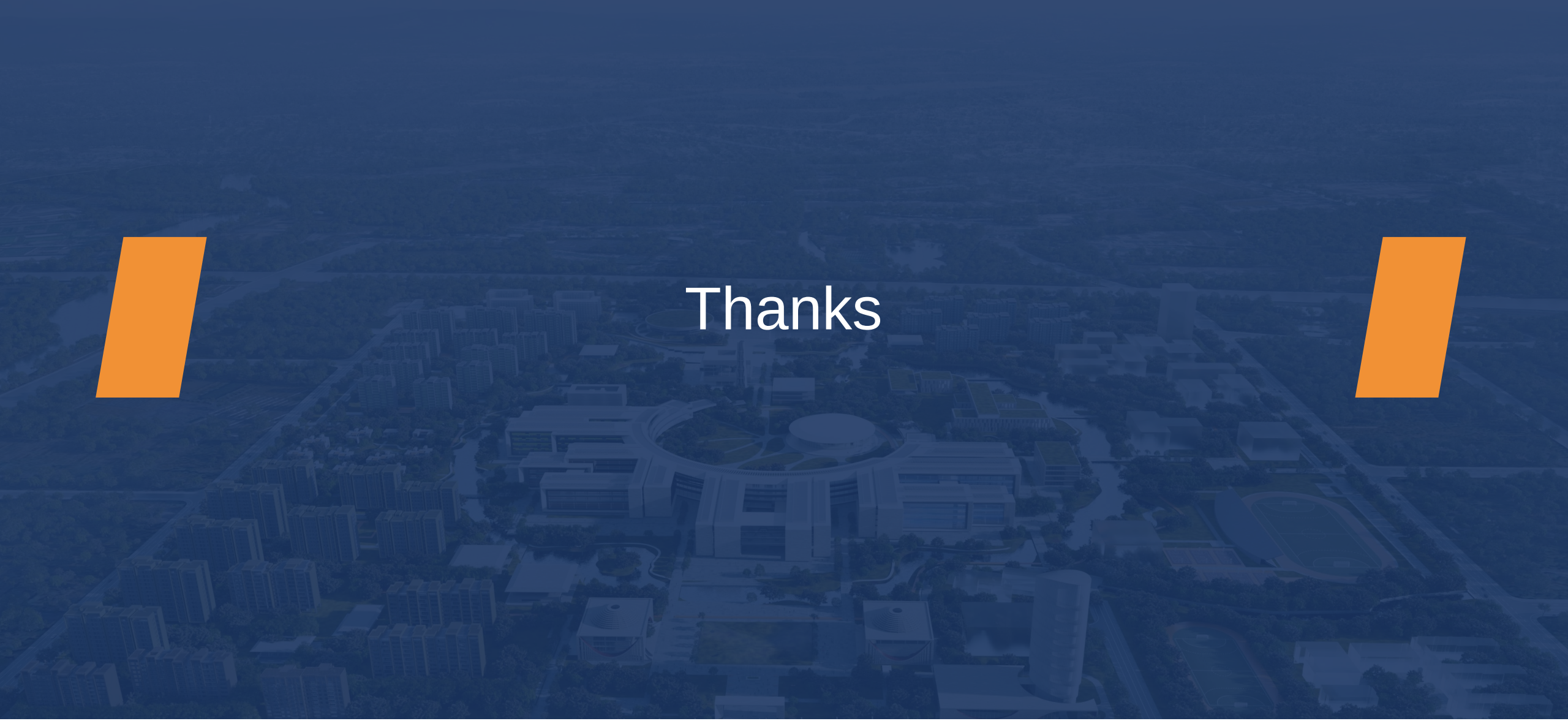


- Mouse kidney
- 1 mg FF, 1 mg FFPE punches, 20 µm FFPE slices
- 60 min DDA
- QE-HF

Data presentation



- 1 mg FFPE punches
- 16 cancer patients, paired samples
- 60 min *2 part, PulseDIA PASEF
- TimsTOF pro



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