

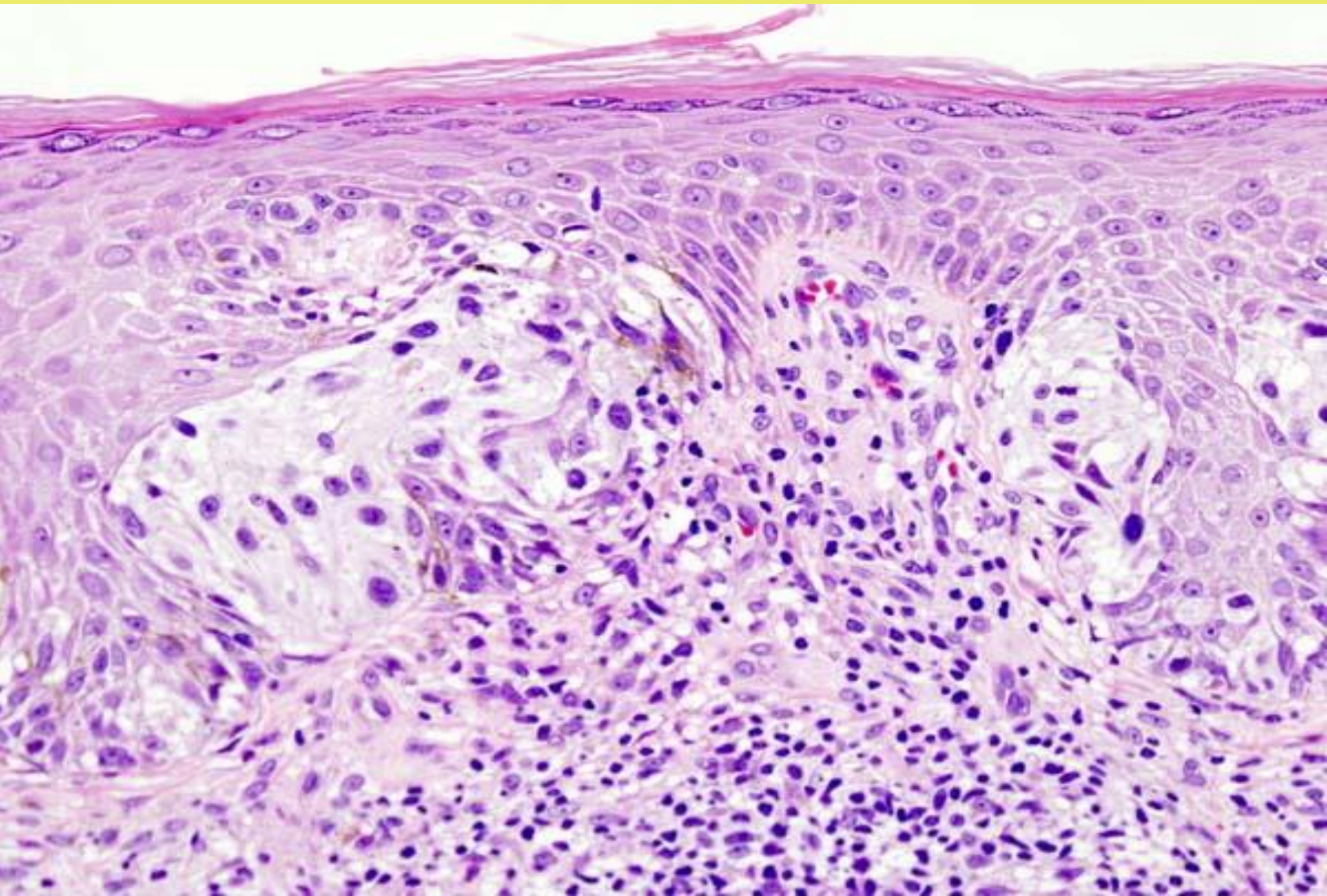
**Discovering Cancer Heterogeneity by
Image Analysis-linked Genomics
using Phenotype-based High-throughput
Laser-aided Isolation and Sequencing (PHLI-seq)**



**Dr. Sunghoon Kwon
Professor, Seoul National University**

July 12, 2018

Subjectivity problem in histopathology



Limitations in conventional histopathological diagnostics

Qualitative Diagnosis based on Tissue Morphology

Malignant

Malignant

Benign

Benign

Malignant

Classifying **ambiguous melanocytic lesions** with FISH and correlation with clinical long-term follow up

Timo Gaiser^{1,2}, Heinz Kutzner³, Gabriele Palmado³, Markus D Siegelin^{1,4}, Thomas Wiesner⁵, Thomas Bruckner⁶, Wolfgang Hartschuh⁷, Alexander H Enk⁷ and Maria R Becker⁷



Human Pathology

Volume 27, Issue 6, June 1996, Pages 528–531



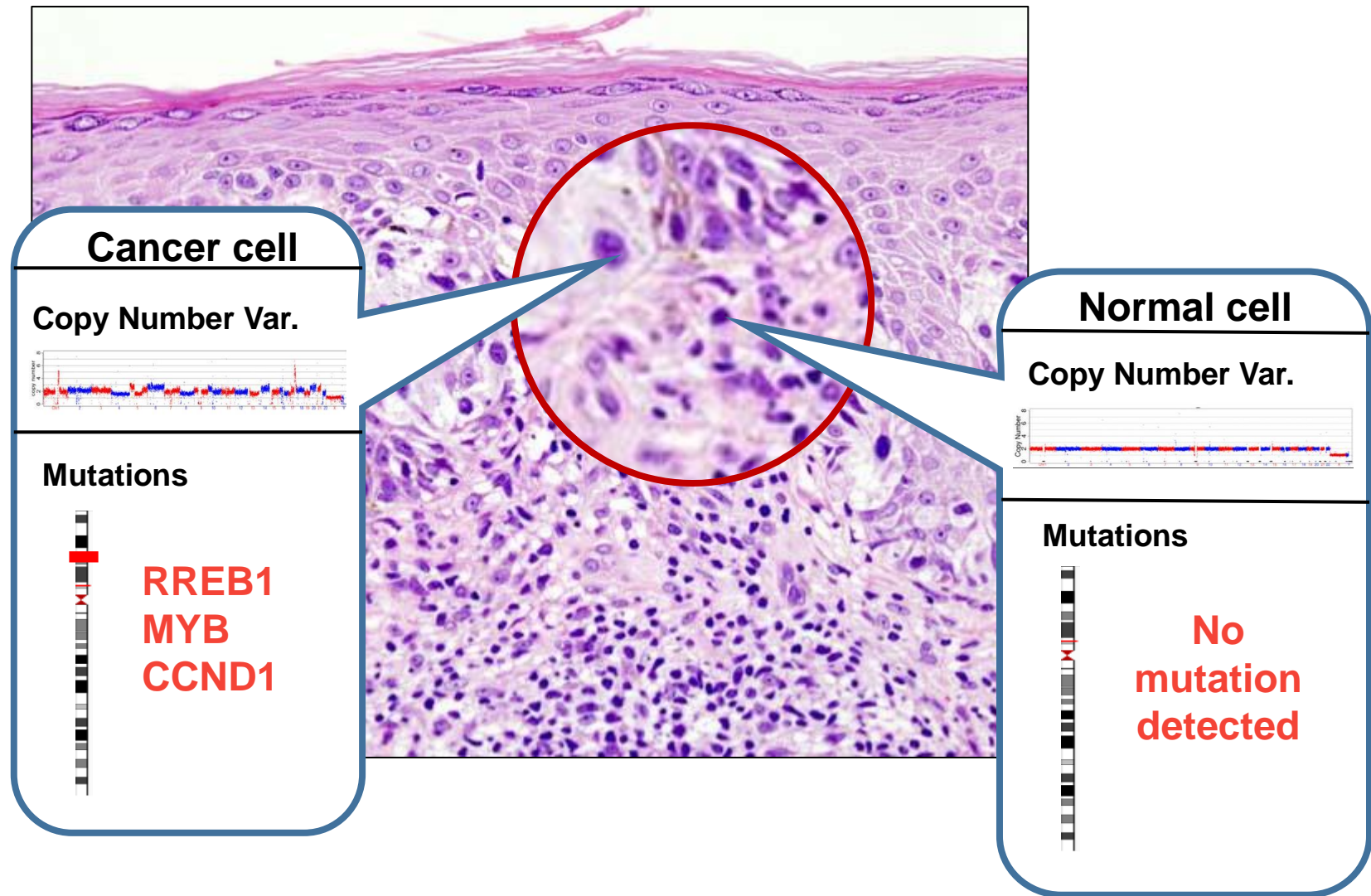
Original contribution

Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi **between expert pathologists**

Evan R Farmer, MD, René Gonin, PhD, Mark P Hanna, MS

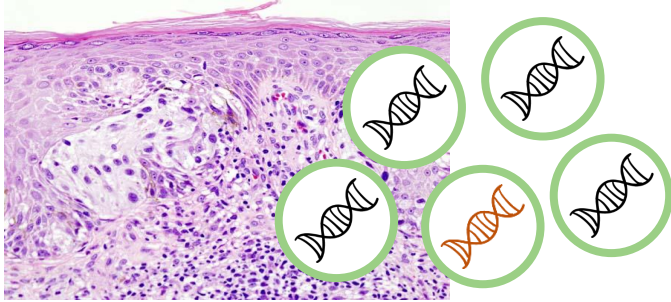
12/22 samples could **not** be diagnosed
→ **Ambiguous histological features**

Vision in cancer genomics through IT-BT Convergence



Quantitative and Qualitative Diagnosis

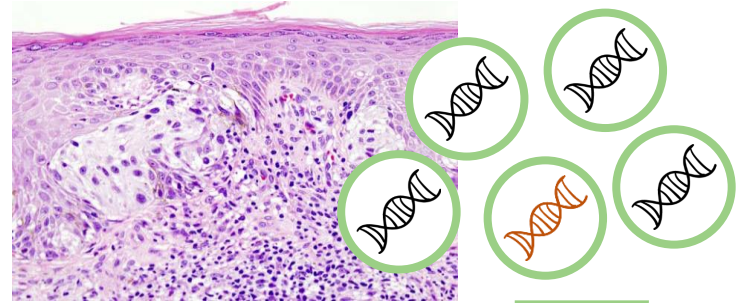
Single cell sequencing for Tumor heterogeneity



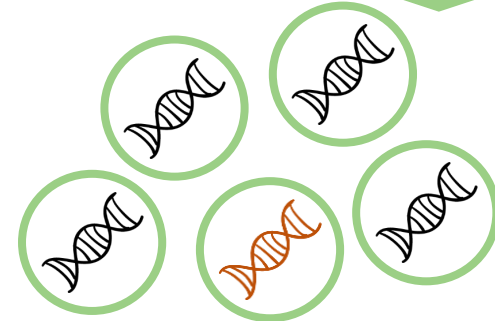
Bulk Sequencing



- Minor mutations are **ignored**
- Inaccurate genetic information, inaccurate diagnosis
- Ignored information can induce **relapse of cancer**



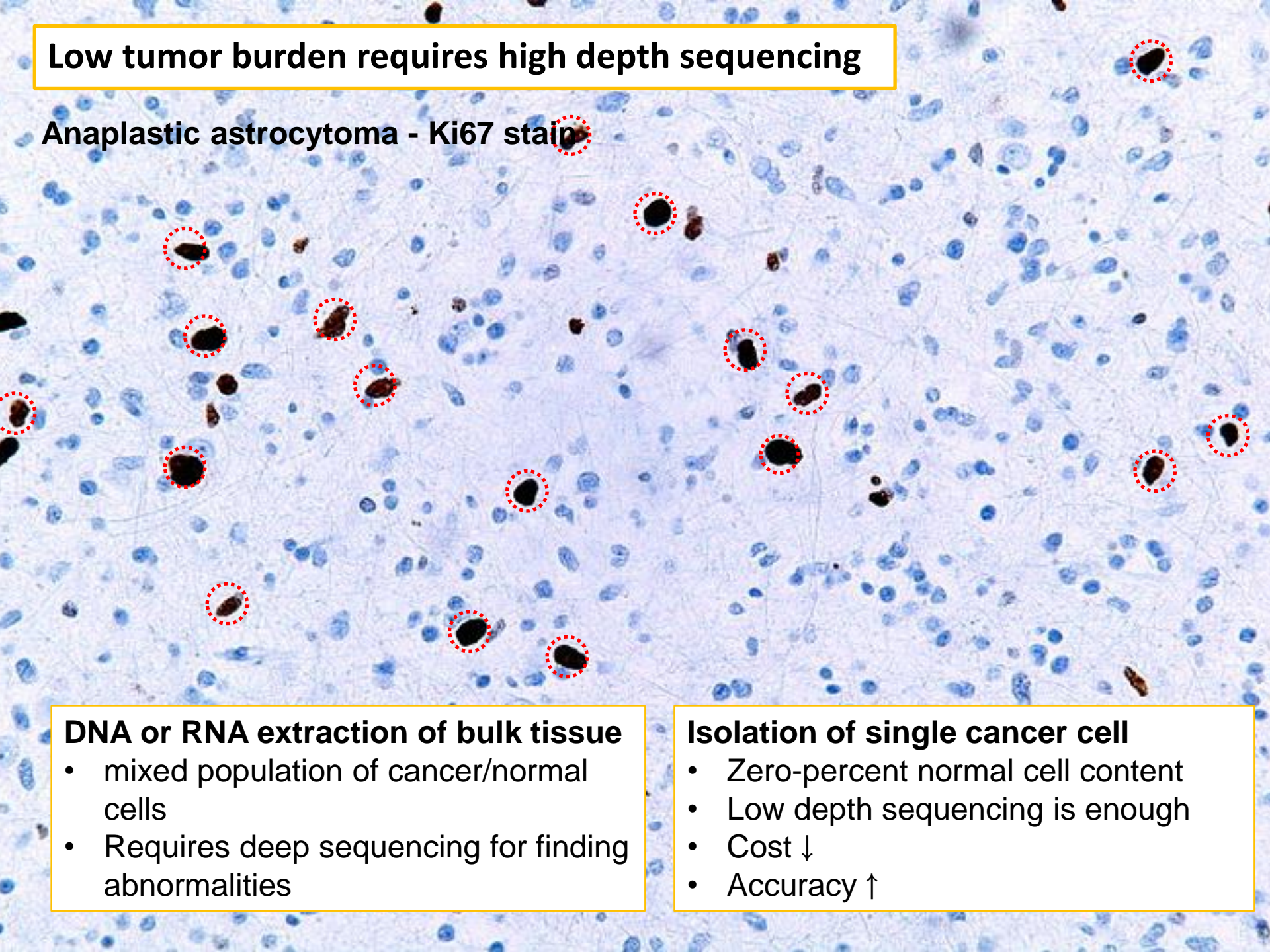
Single Cell Seq



- Minor mutation information **Preserved**
- Accurate genetic information, accurate diagnosis
- Clonal structure of cancer

Low tumor burden requires high depth sequencing

Anaplastic astrocytoma - Ki67 stain



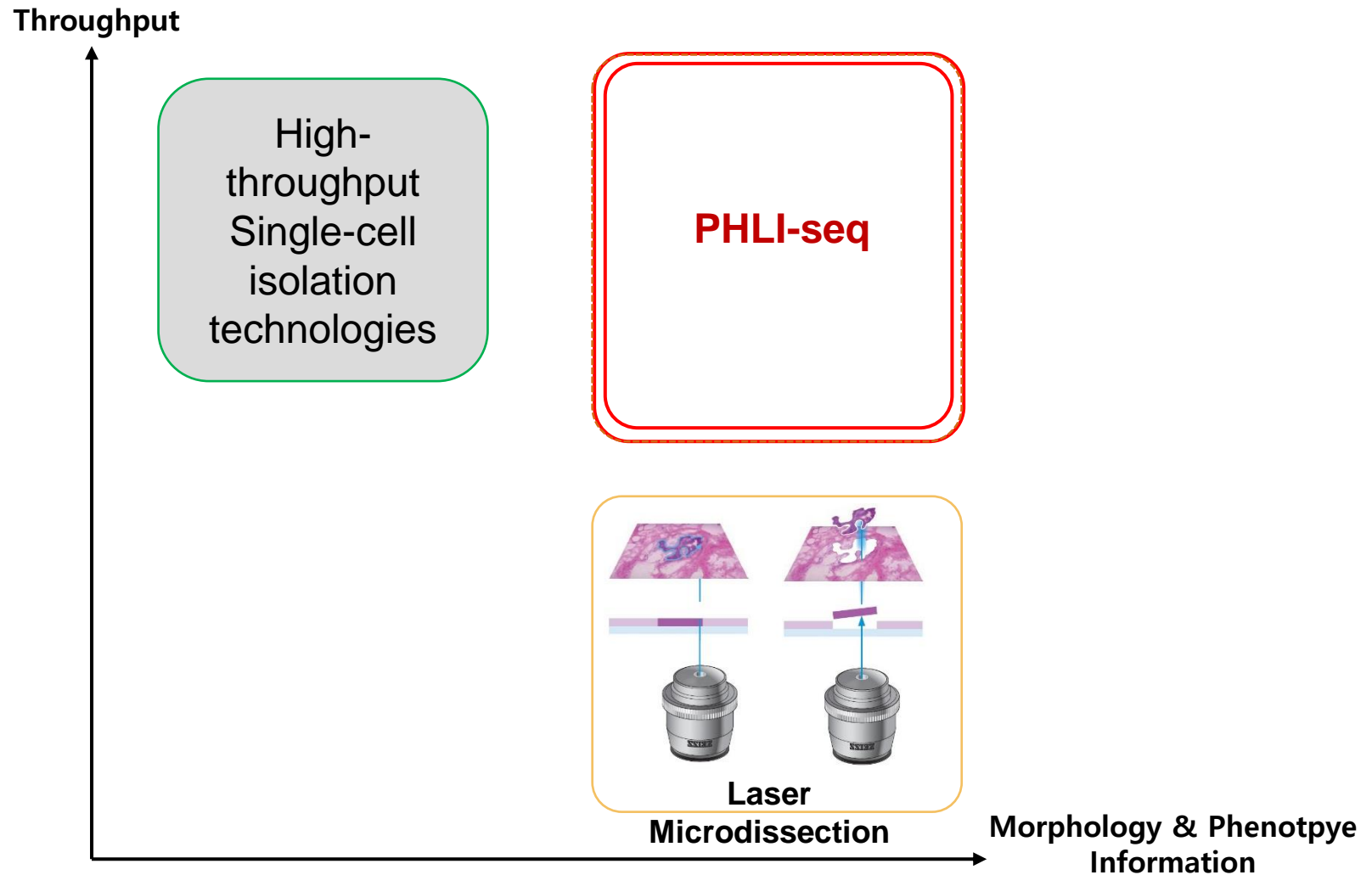
DNA or RNA extraction of bulk tissue

- mixed population of cancer/normal cells
- Requires deep sequencing for finding abnormalities

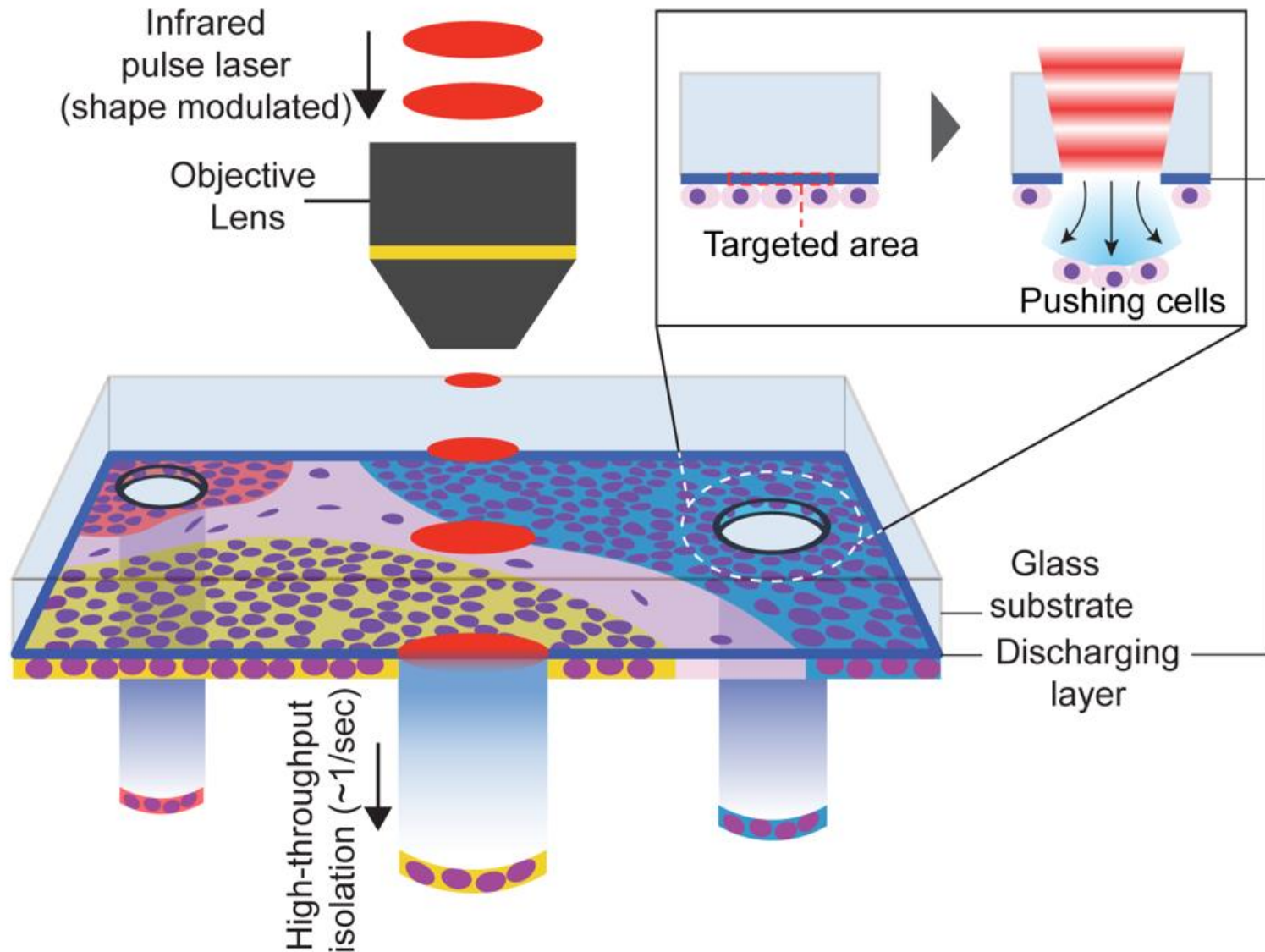
Isolation of single cancer cell

- Zero-percent normal cell content
- Low depth sequencing is enough
- Cost ↓
- Accuracy ↑

Advanced Technologies for Cancer Heterogeneity

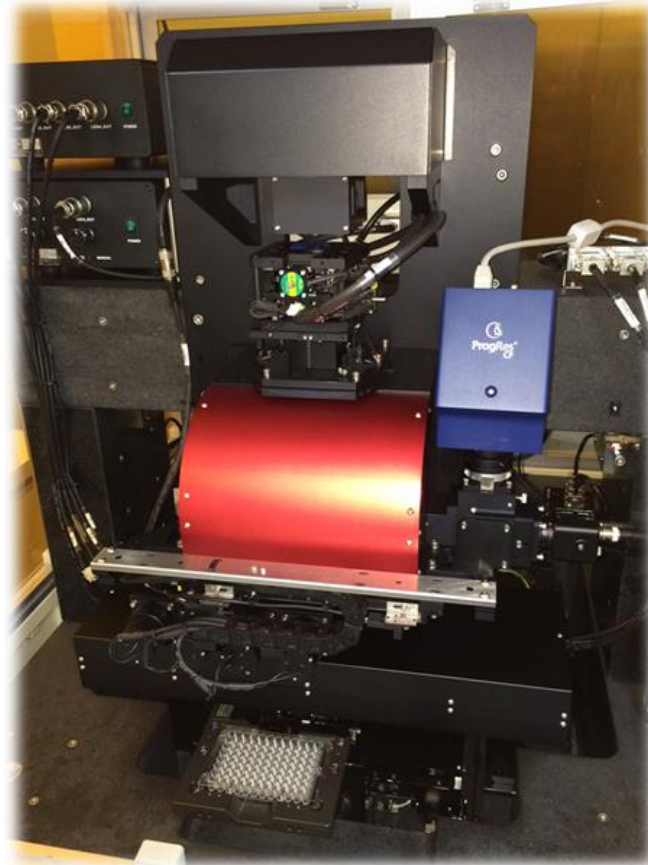
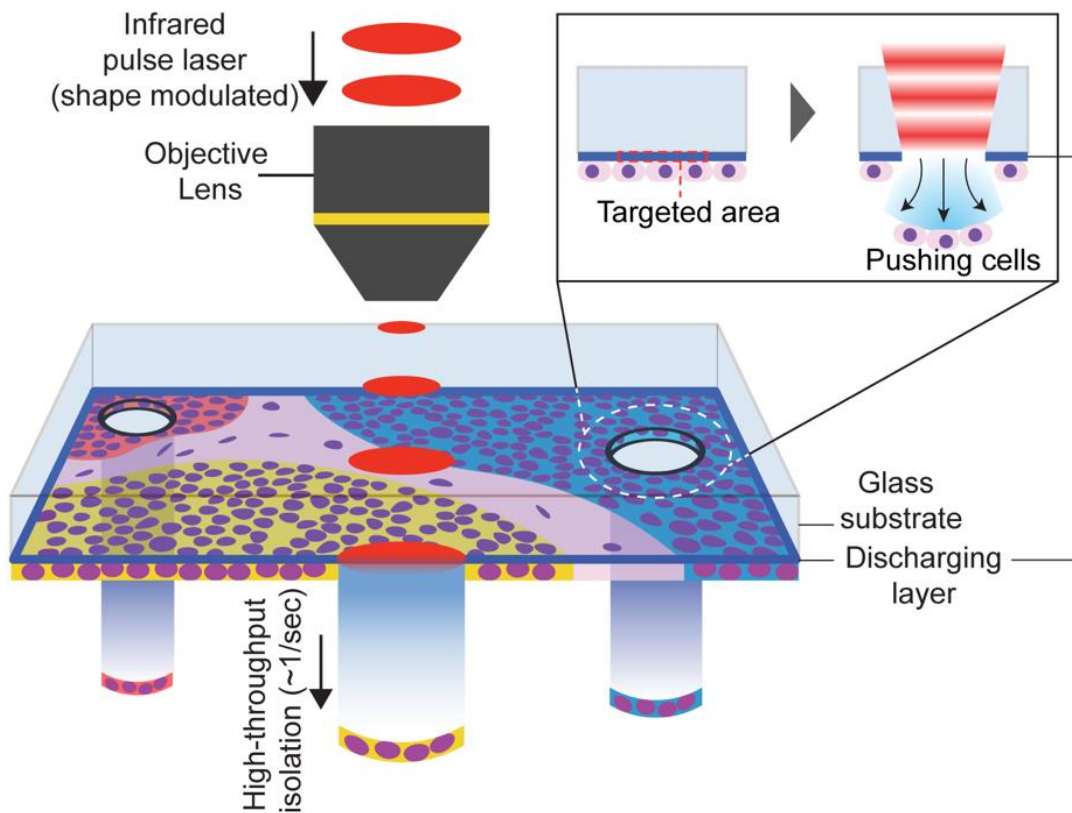


Phenotype-based High-throughput Laser-aided Isolation and Sequencing (PHLI-seq)



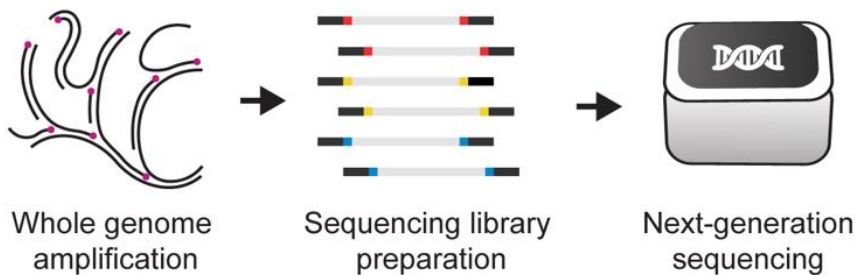
Phenotype-based High-throughput Laser-aided Isolation and Sequencing (PHLI-seq)

cell isolation



(In-house design & Instrumentation)

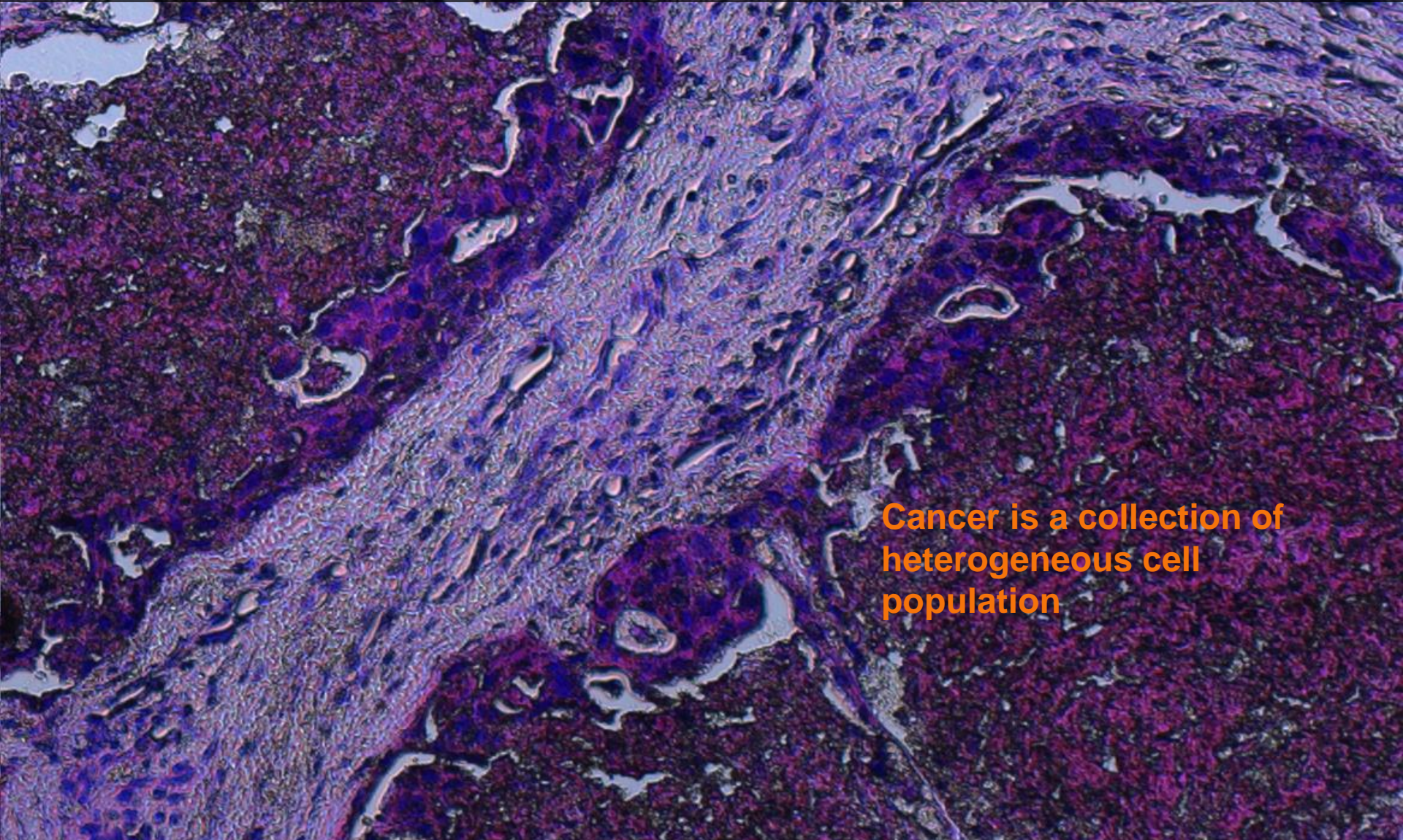
post processing



Single Cell Isolation from Blood Smear



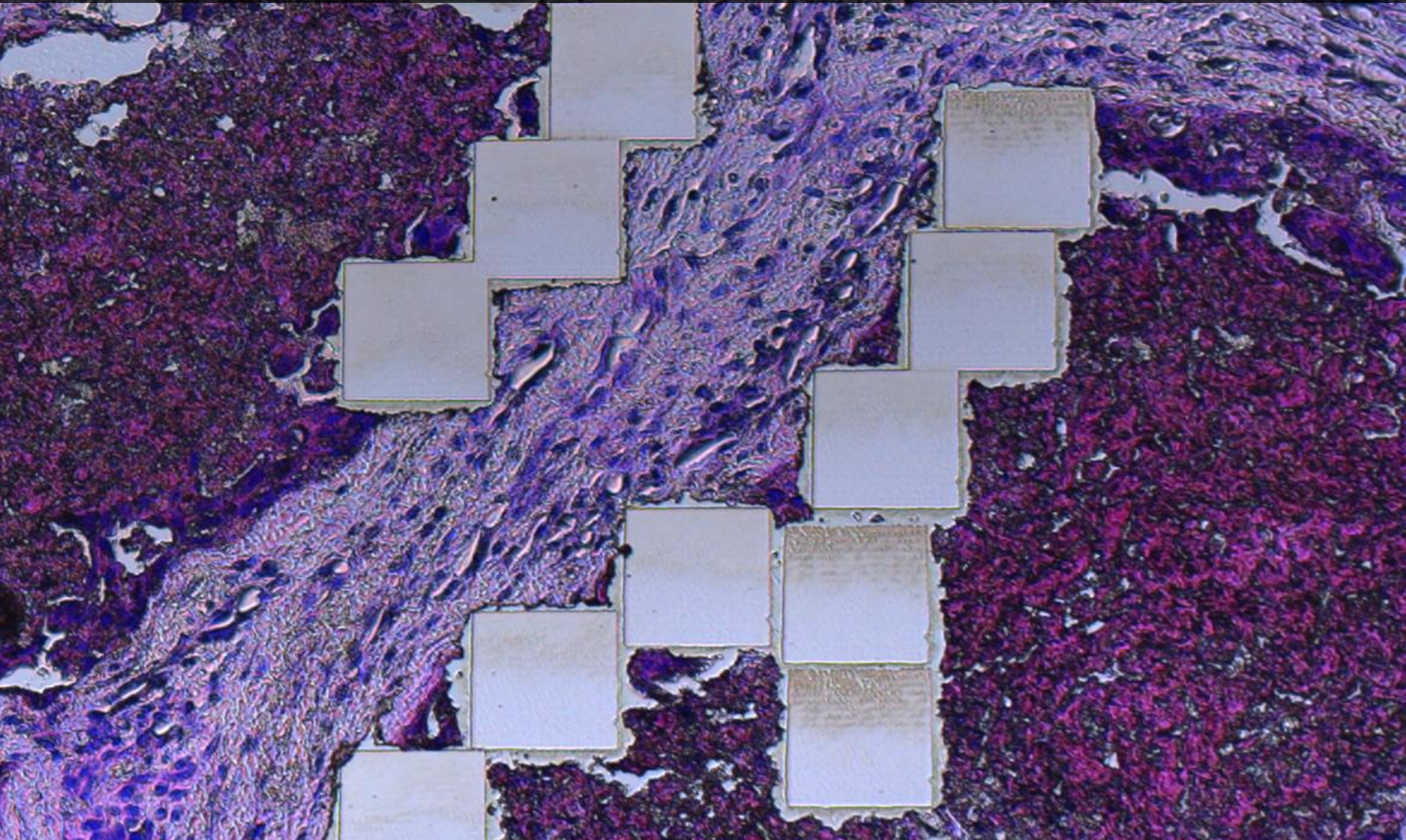
~10-cell Isolation from Breast Cancer Tissue Section



Cancer is a collection of heterogeneous cell population

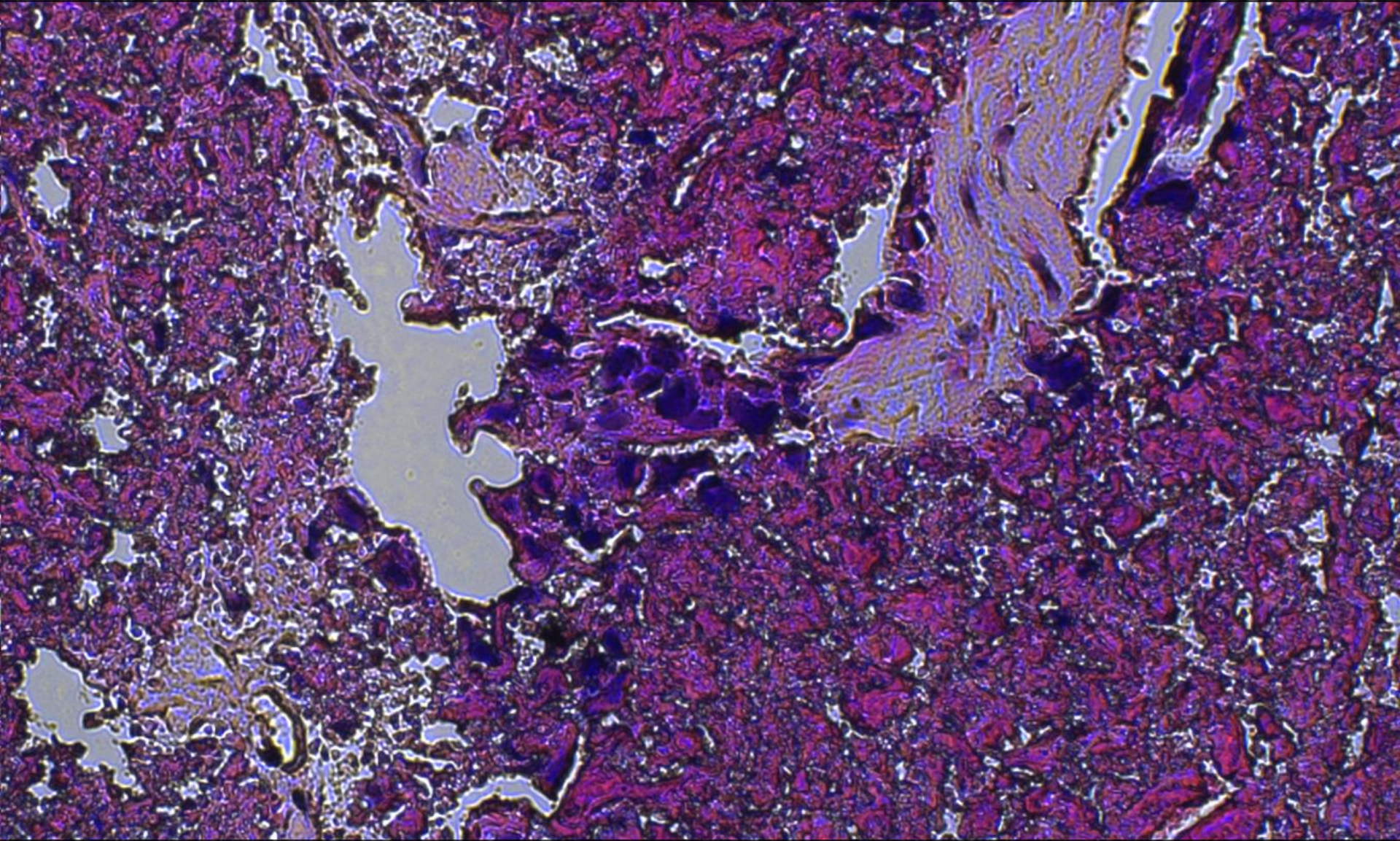
Sample: prof. Won Shik Han (SNUH)

~10-cell Isolation from Breast Cancer Tissue Section



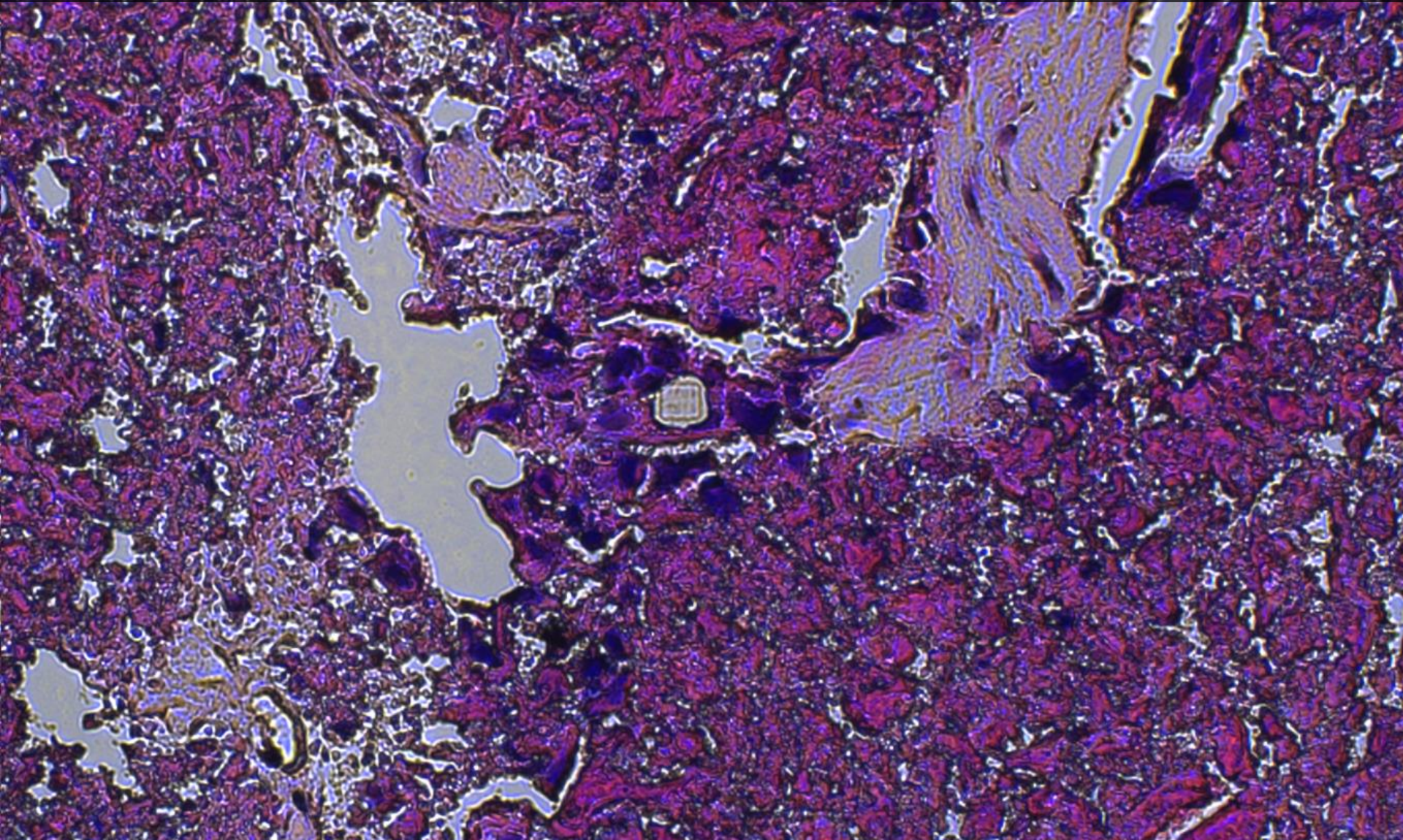
Sample: prof. Won Shik Han (SNUH)

Single-cell Isolation from Breast Cancer Tissue Section



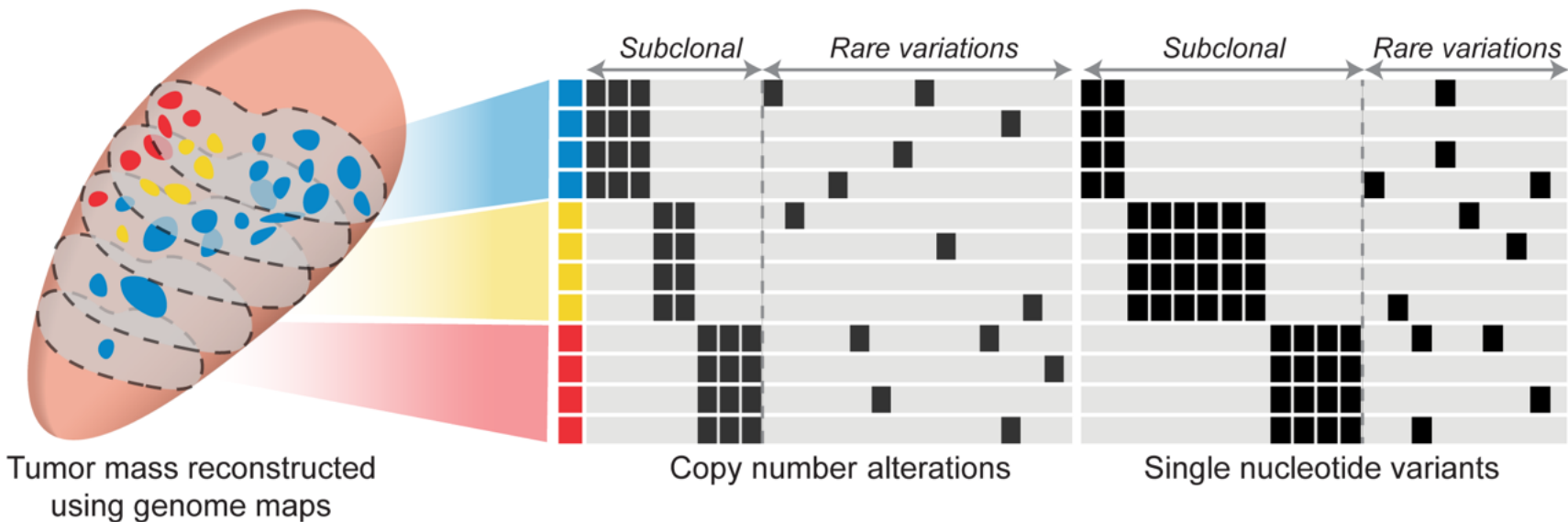
Sample: prof. Won Shik Han (SNUH)

Single-cell Isolation from Breast Cancer Tissue Section

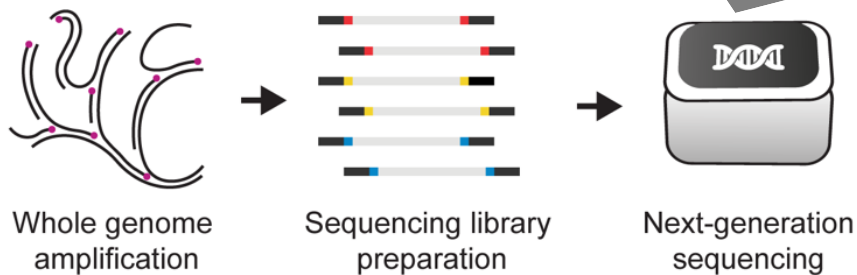


Sample: prof. Won Shik Han (SNUH)

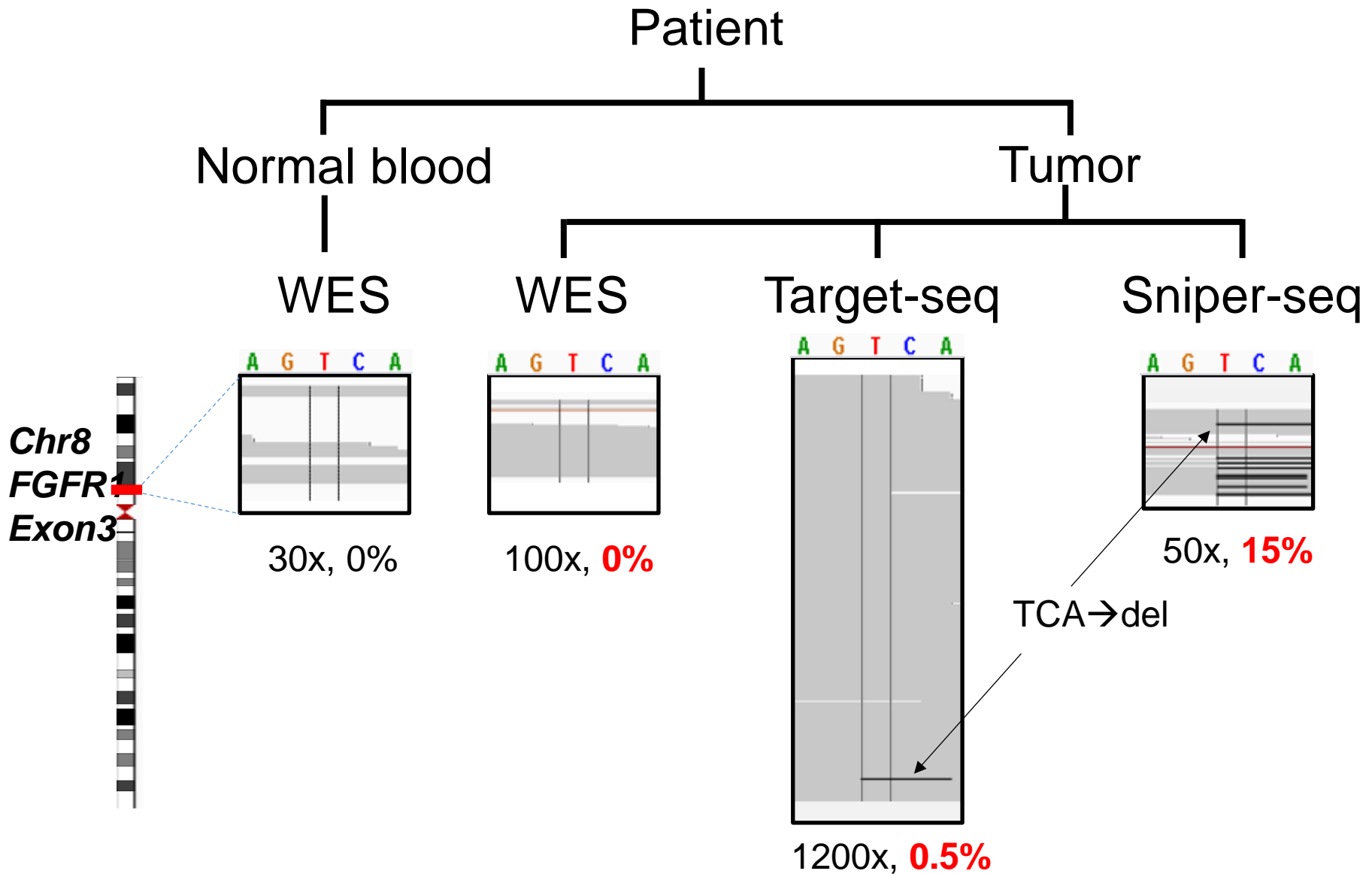
Phenotype-based High-throughput Laser-aided Isolation and Sequencing (PHLI-seq)



post processing

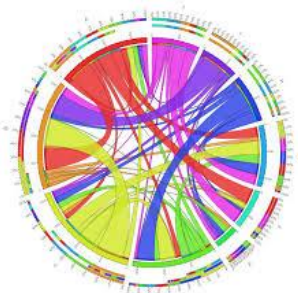
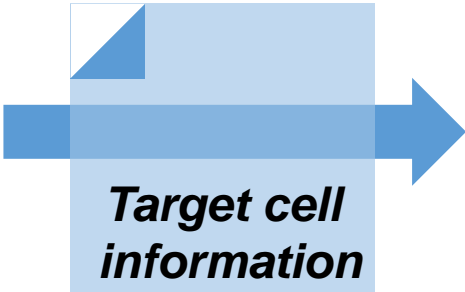
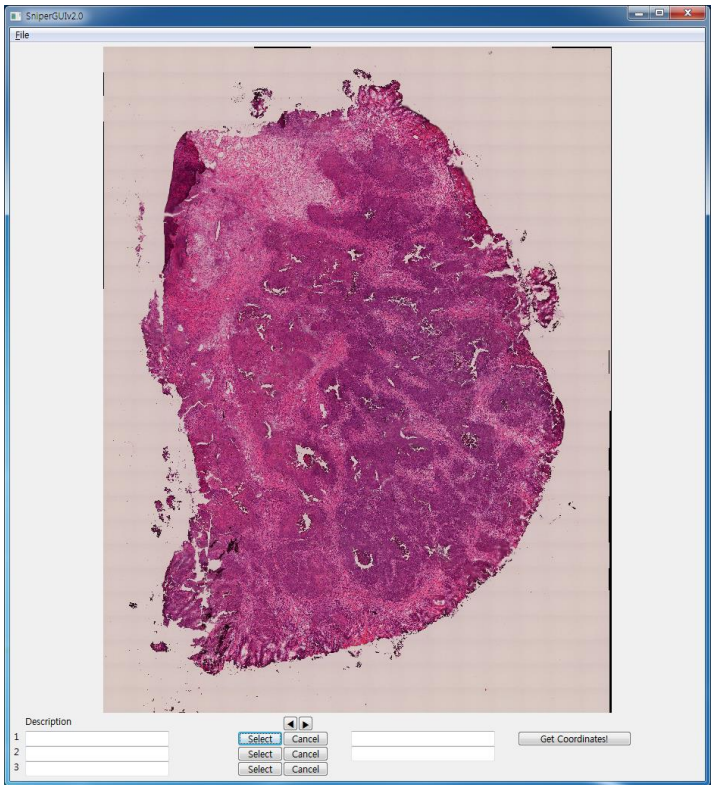


Detection of Rare Variant by Low-depth Sequencing

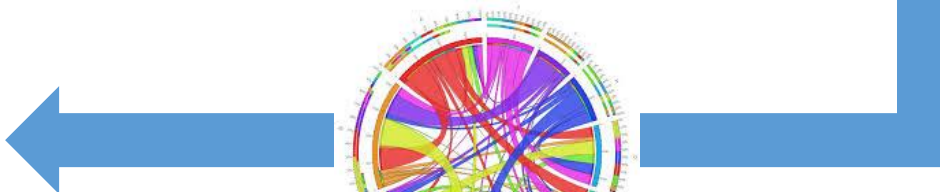


Target sequencing: Prof. Woong-yang Park (SGI)

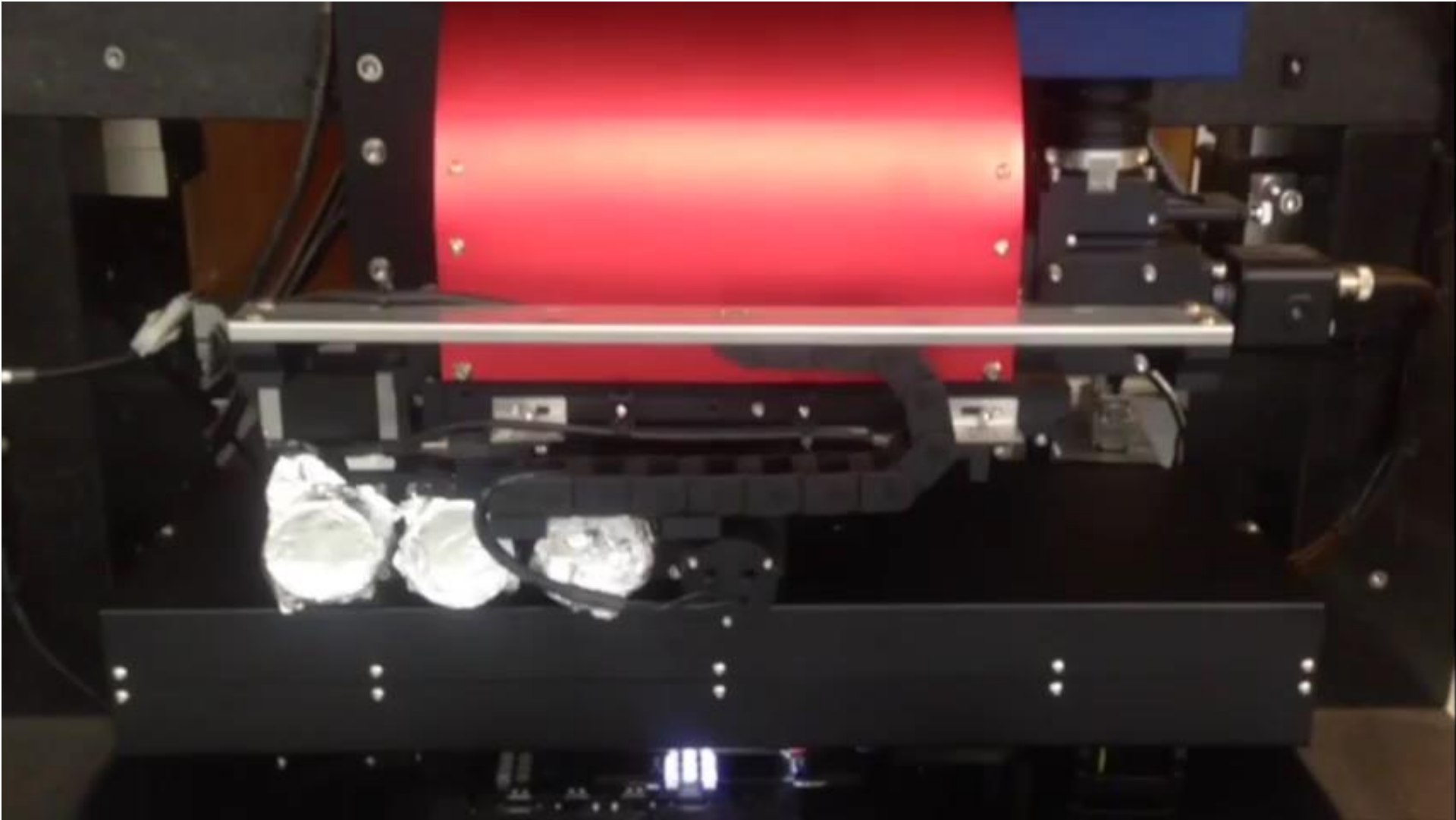
Cooperative Procedure with Hospital



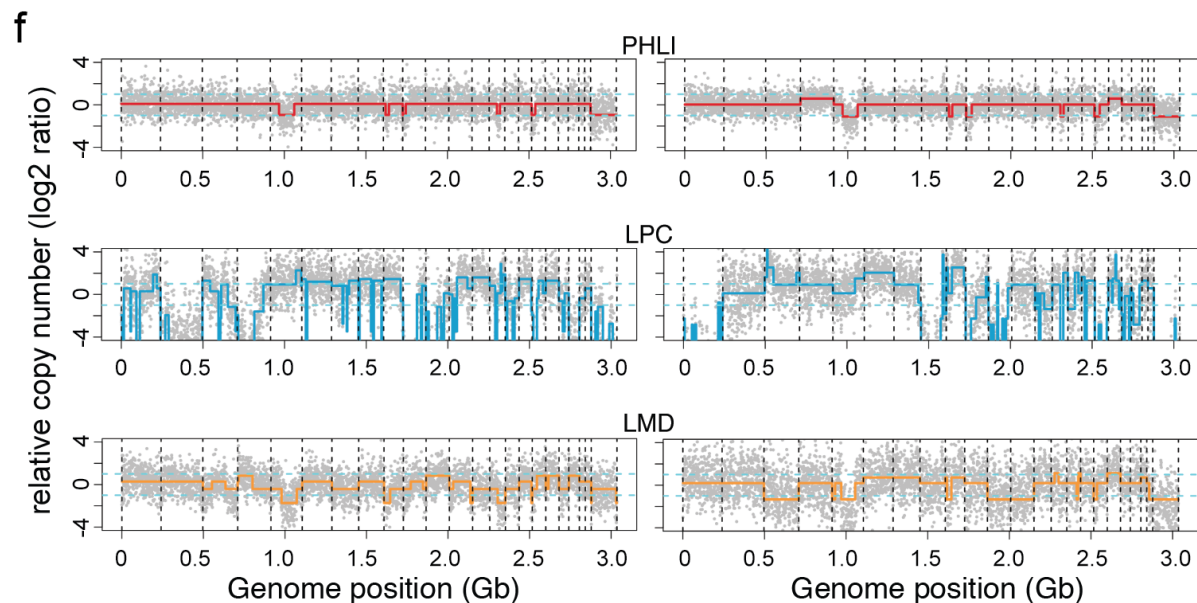
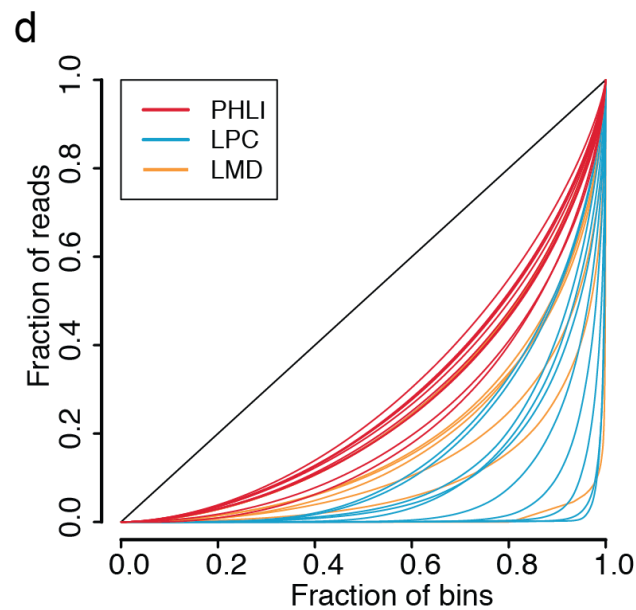
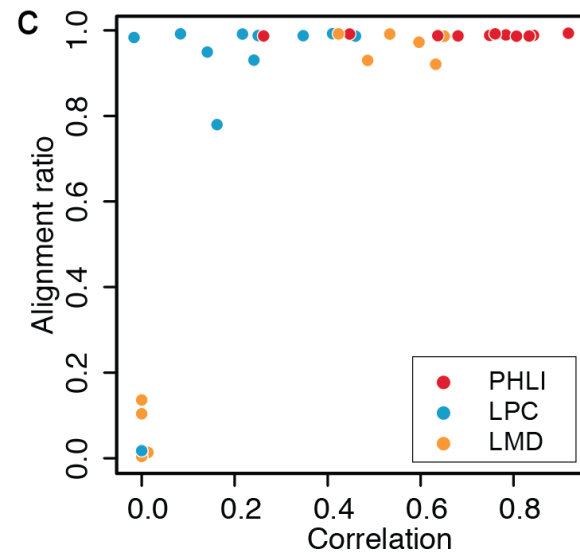
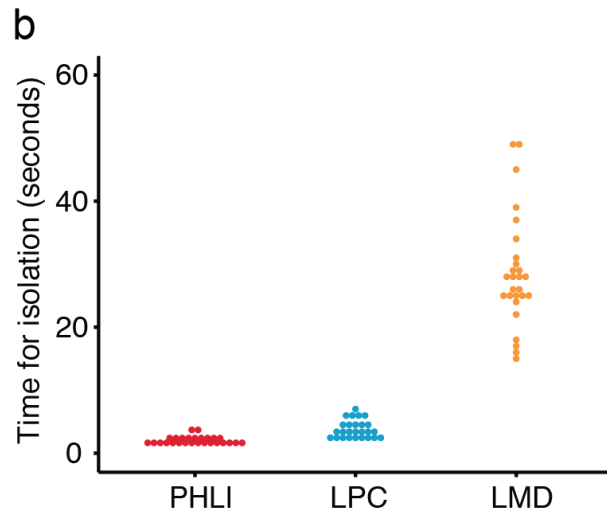
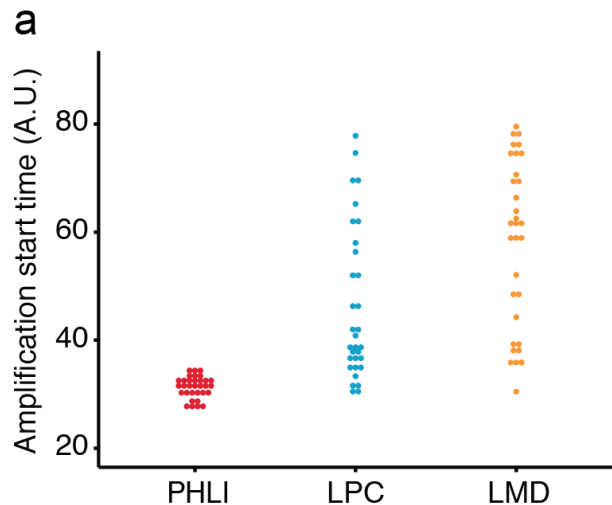
Seqencing data



PHLI-seq in Action



Performance comparison with Laser Microdissection



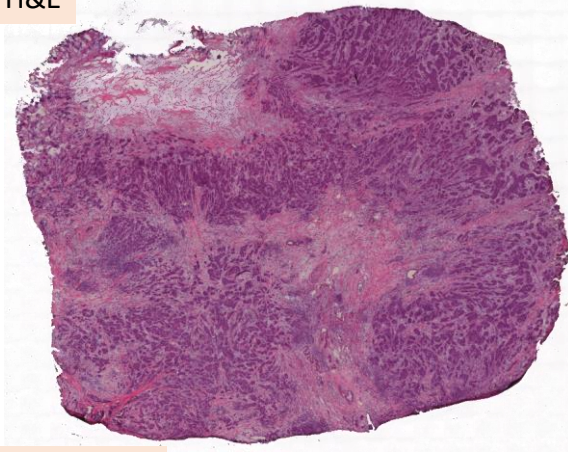
Collaborators

Collaborators \ Procedures		Presentation by Prof. Kwon	1 st meeting for study design	Experiments using pilot samples	Data analysis and 2 nd meeting for discussing main experiments	Experiments using main samples	Data analysis and 3 rd meeting for final discussion	Paper submit
Cancer	한원식 (Dept. of breast surgery 유방내분비외과, SNUH)	[Active]						
	이한별 (서울대학교 병원 유방외과)	[Active]						
	이동순 (Dept of laboratory medicine 진단검사의학과, SNUH)	[Active]						
	송용상 (Dept of Obstetrics and Gynecology 산부인과, SNUH)	[Active]						
	김수지 (서울대학교 병원 산부인과)	[Active]						
	김태유 (Dept of Medical oncology, 중앙내과, SNUH)	[Active]						
	강경훈 (서울대학교 병원 병리과)	[Active]						
	김황필 (Cancer Research Institute, Seoul National University)	[Active]						
	김선영 (충남대학교 병원)	[Active]						
	박용양 (삼성유전체연구소)	[Active]						
	주경민 (삼성유전체연구소)	[Active]						
	박상희 (이대목동병원)	[Active]						
	Mats Nilsson (스톡홀름 대학교)	[Active]						
	김광현 (이대목동병원)	[Active]						
	채영준 (보라매병원)	[Active]						
정연준 (카톨릭 대학교)	[Active]							
이석형 (카톨릭 대학교)	[Active]							
Brain	장미숙 (Dept of Oral Anatomy, 구강해부학과, SNUDH)	[Active]						
	이지연 (Dept of Pediatric Neurosurgery 아동신경외과, SNUH)	[Active]						
	이동수 (Dept. of nuclear medicine 핵의학과, SNUH)	[Active]						
Renal disease	이정표 (Dept of Nephrology 신장내과, SNUH)	[Active]						
Bone disease	김상완 (Dept of Internal medicine 내과, SNUH)	[Active]						
Bacteria	Håkan Jonsson (스웨덴왕립공과대학)	[Active]						
Development	백성희 (서울대학교 생명과학부)	[Active]						

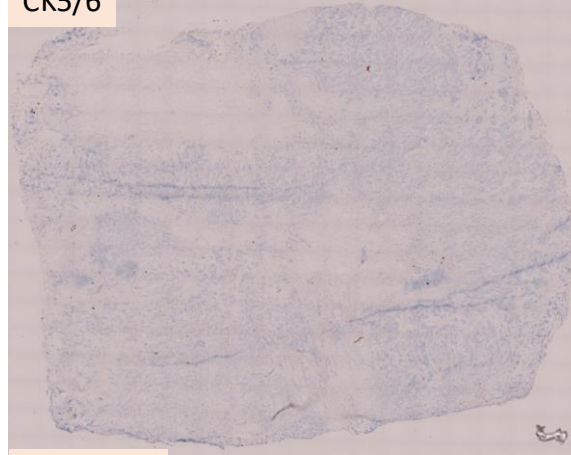
PHLI-seq for Studying Genetic Heterogeneity in Breast Cancer

Application of PHLI-seq to a Her2+ Breast Cancer

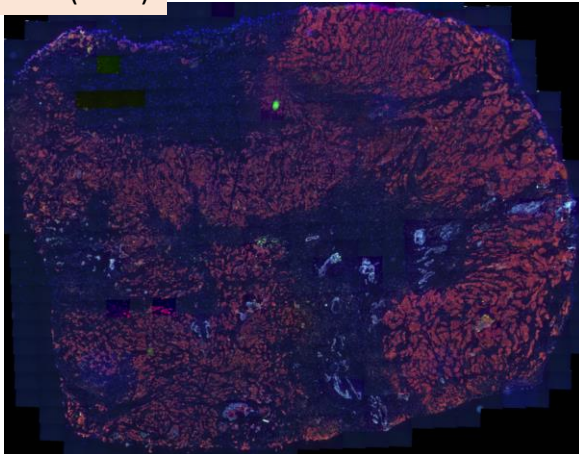
H&E



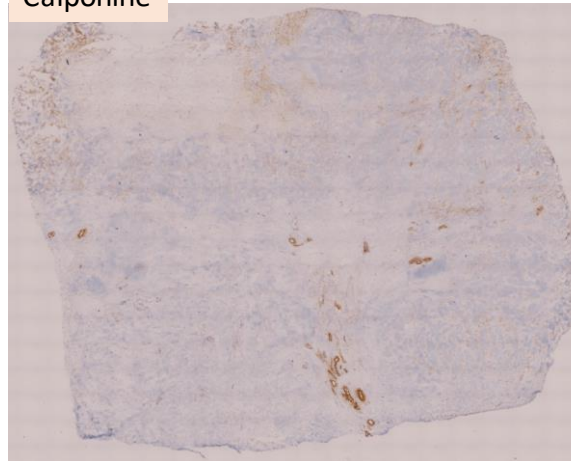
CK5/6



FISH (Her2)

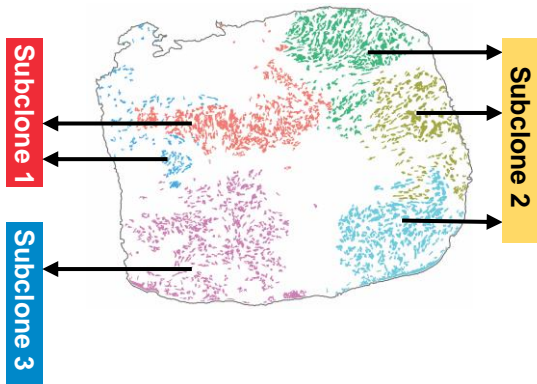


Calponine

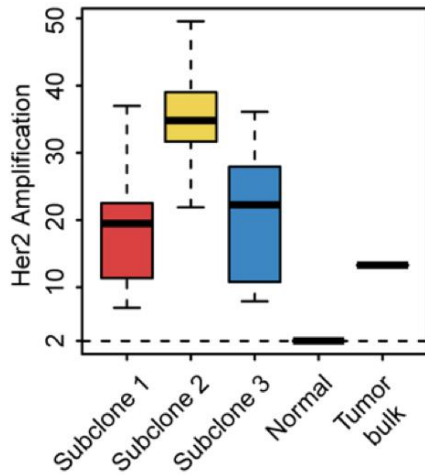
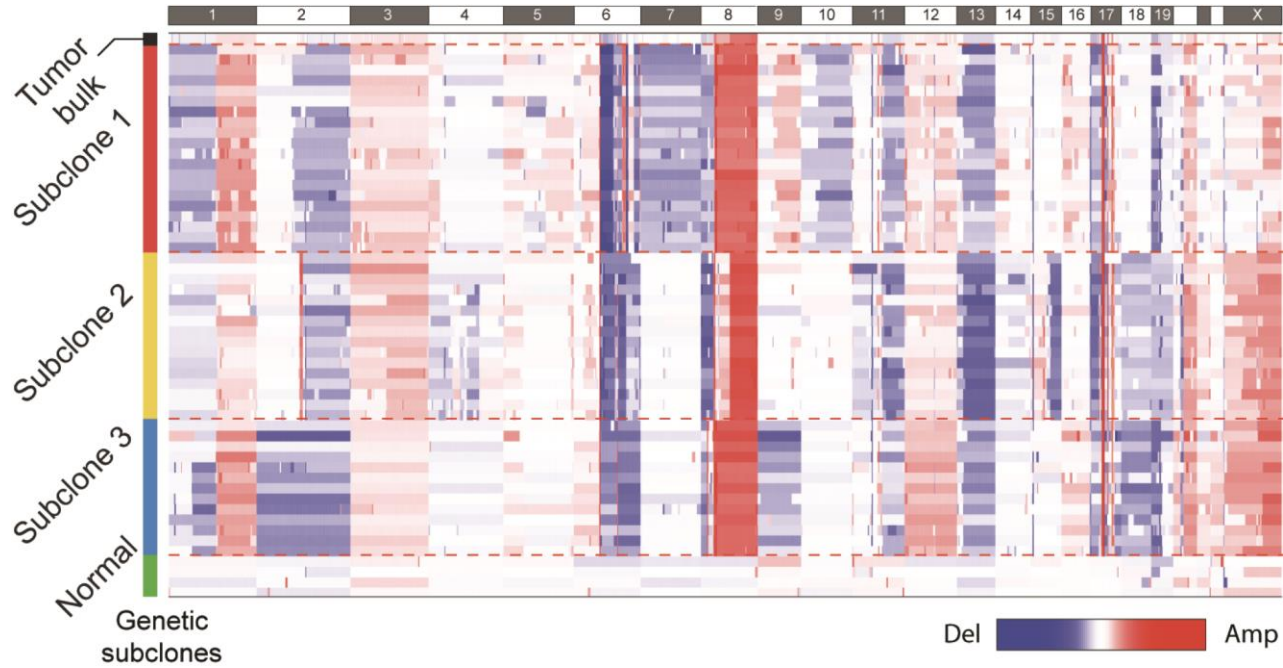


- Cells from 54 regions were isolated by Sniper Cell Sorting
- About 20 cells (similar genetic information) were isolated from each region
- Multiple displacement amplification and NGS (0.03x WGS , 200x targeted sequencing)

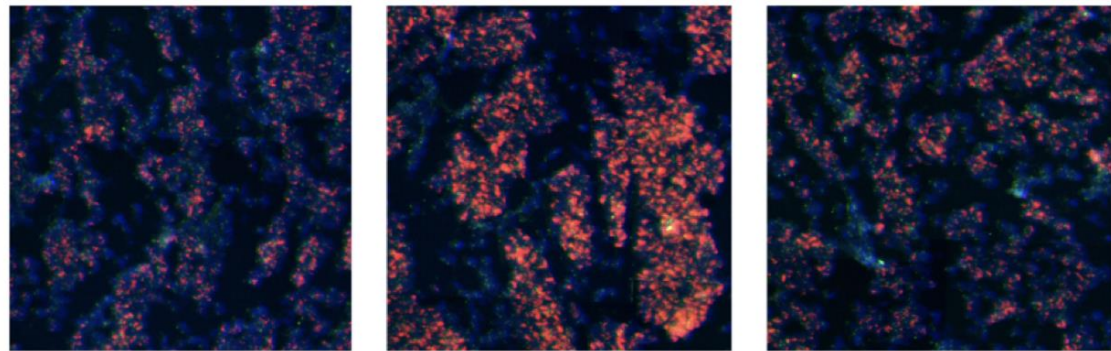
Whole genome sequencing (0.03x) & CNV analysis (N=54)



**Based on copy number variations,
Three subclones were identified**



Her2 FISH



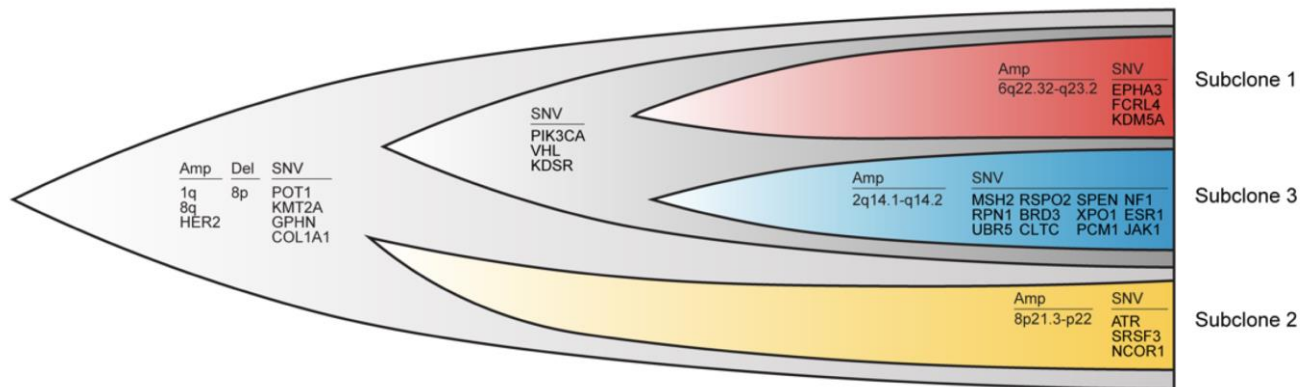
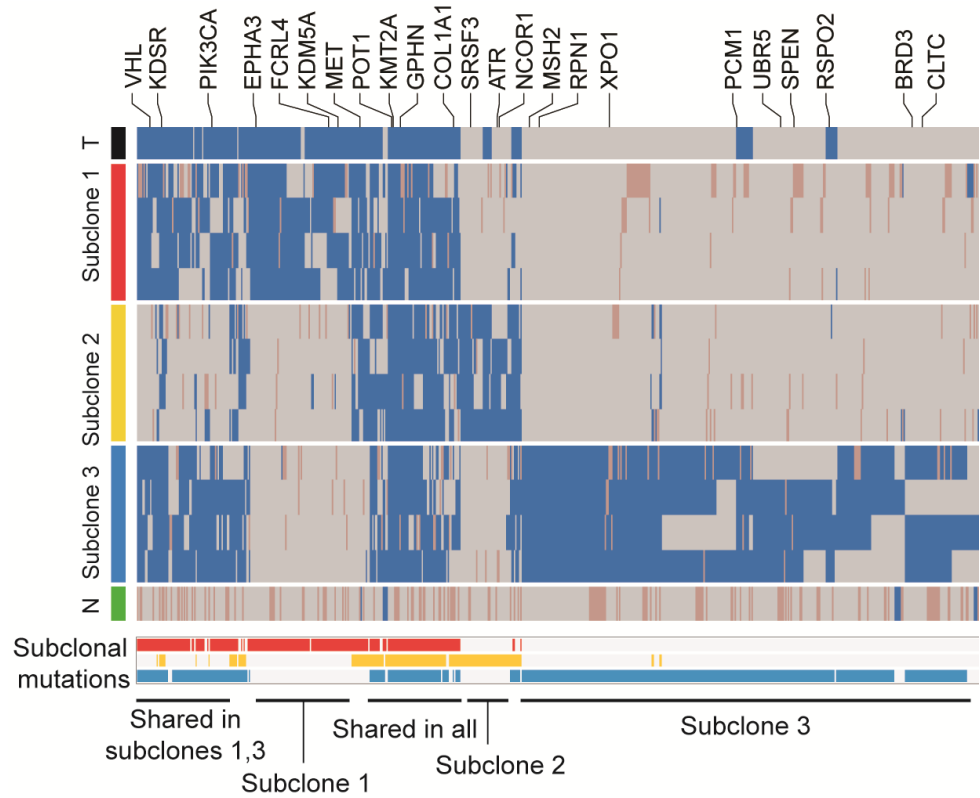
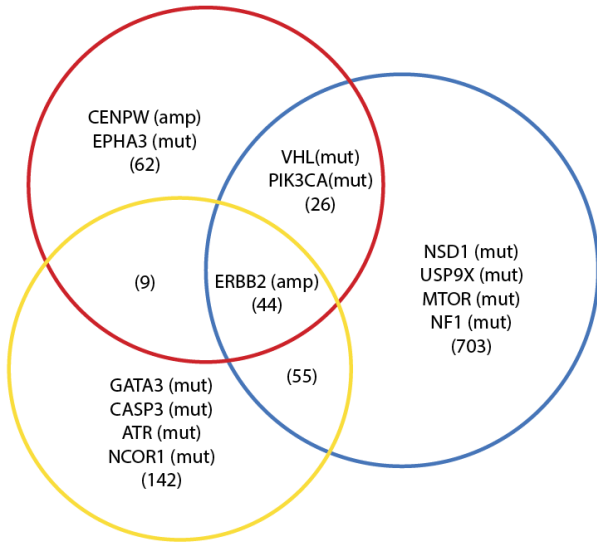
Subclone 1

Subclone 2

Subclone 3

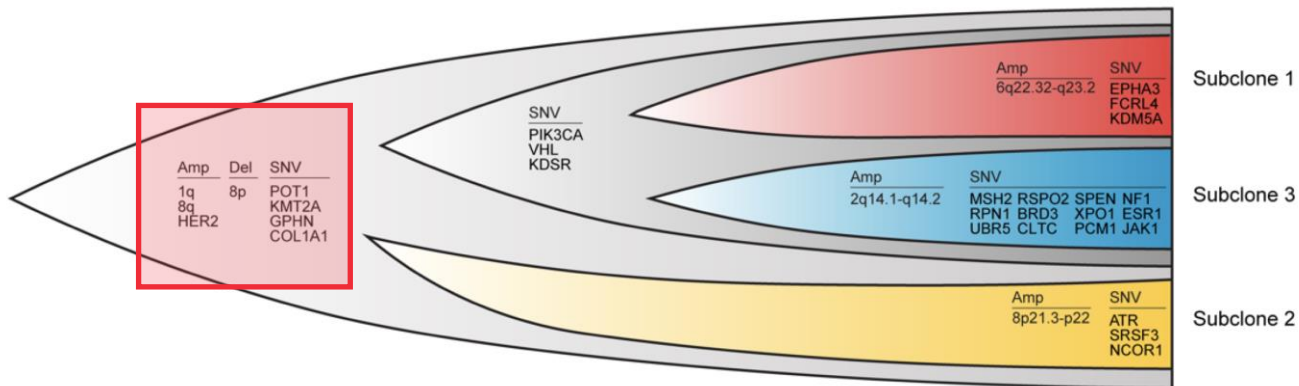
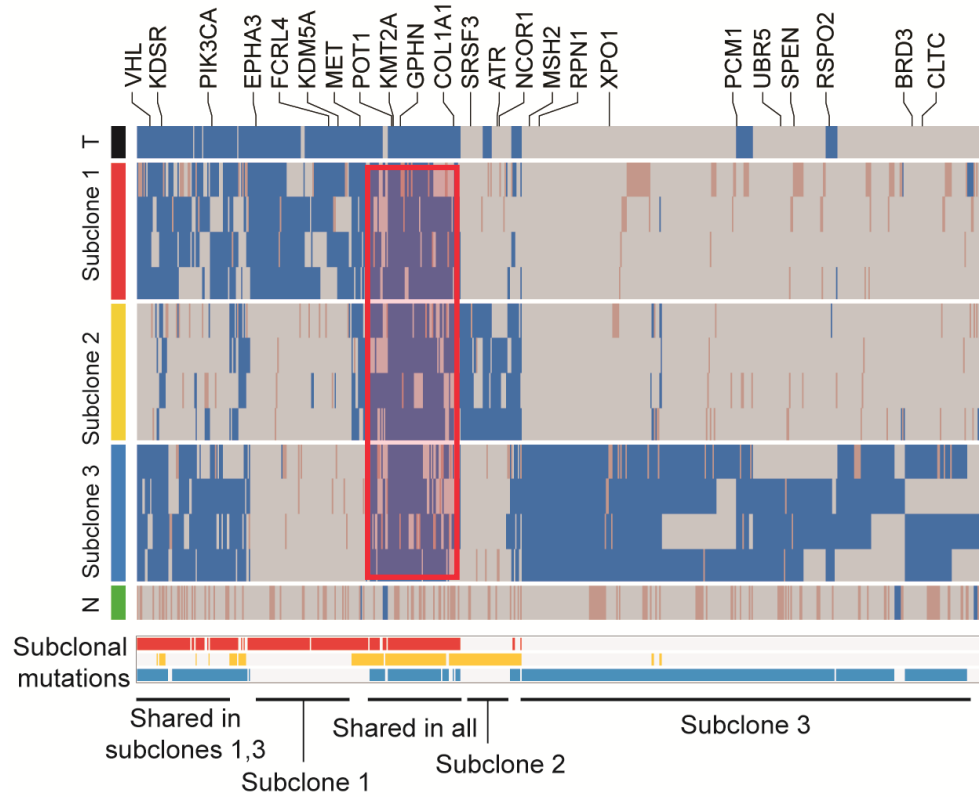
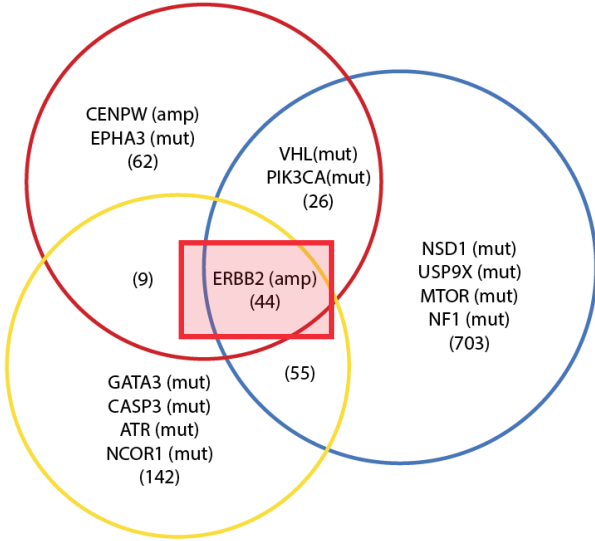
Whole Exome Sequencing Analysis

Potential cancer driving genes in each group



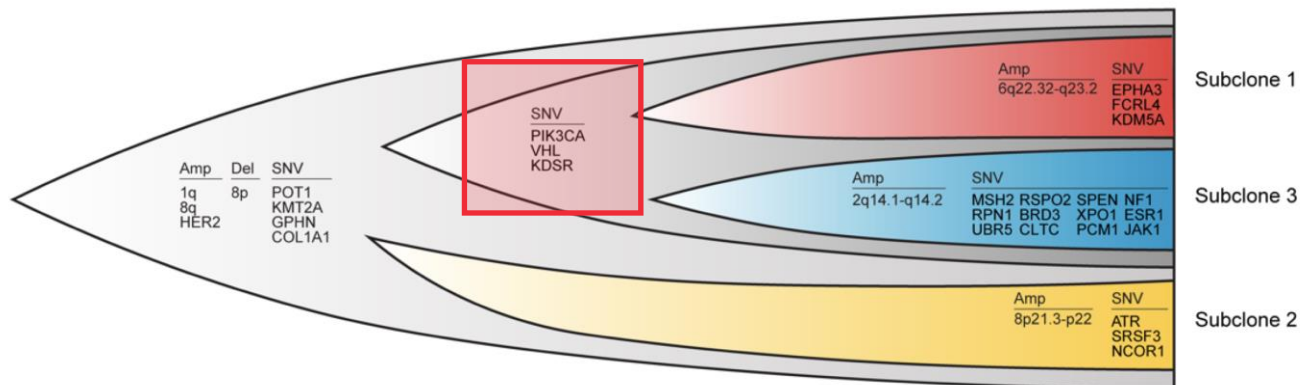
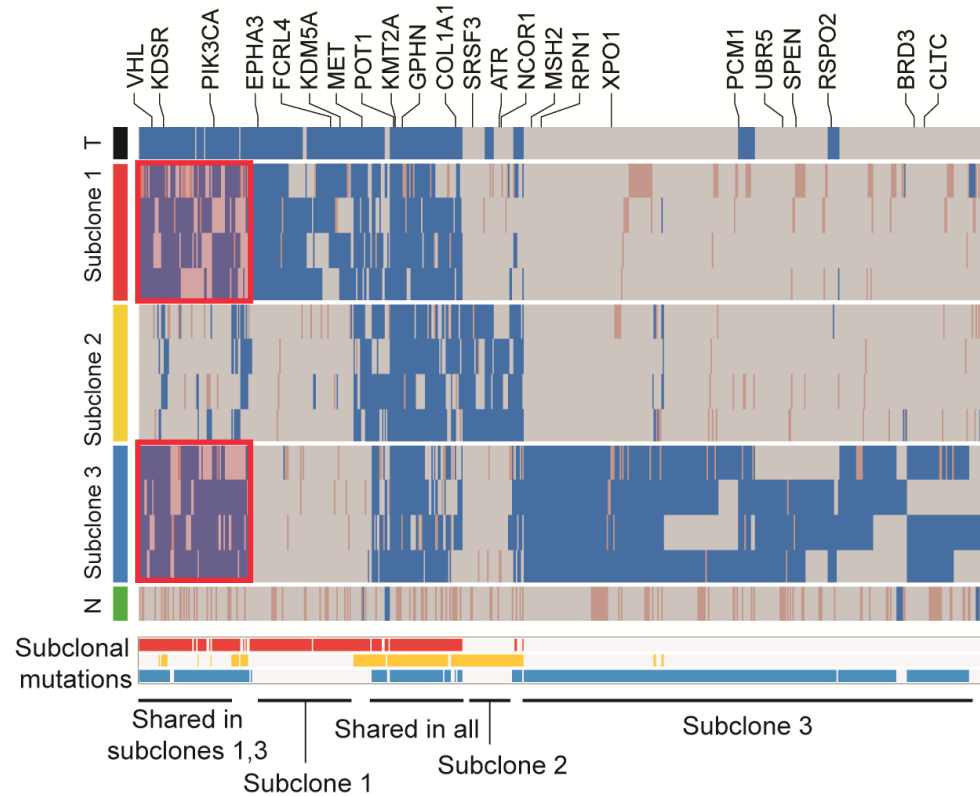
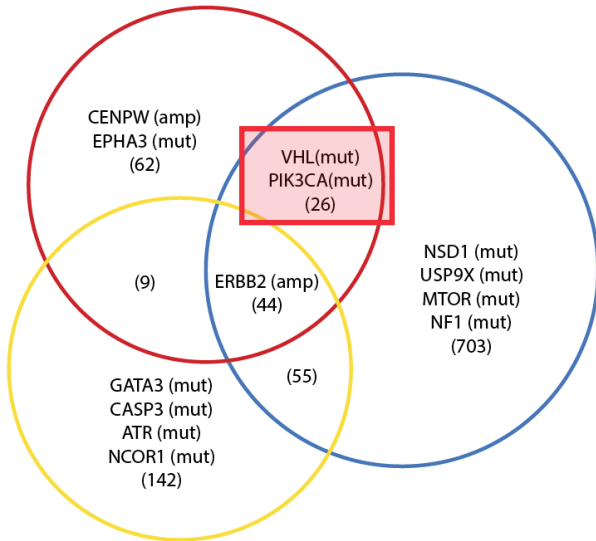
Inferring evolution of the tumor using WES result

Potential cancer driving genes in each group



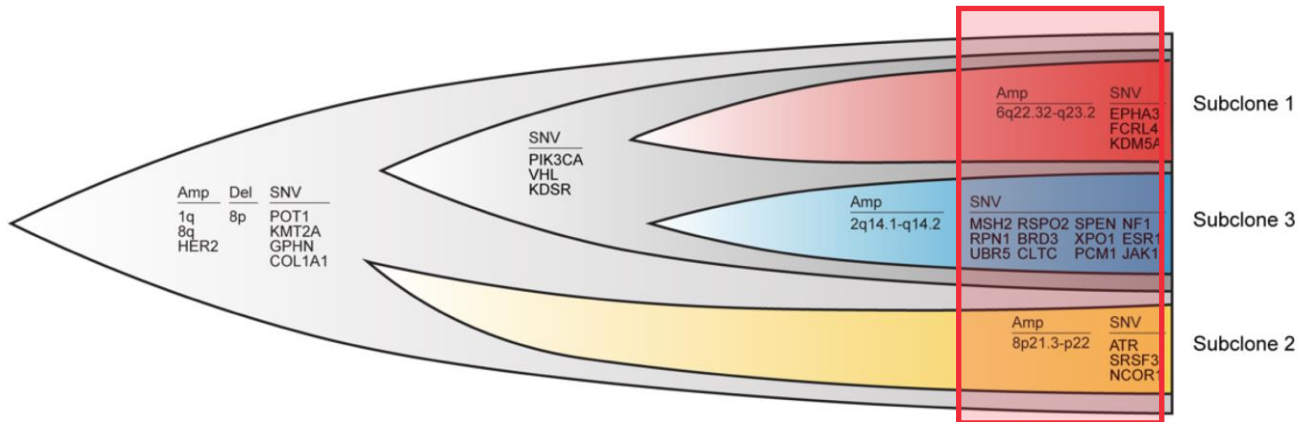
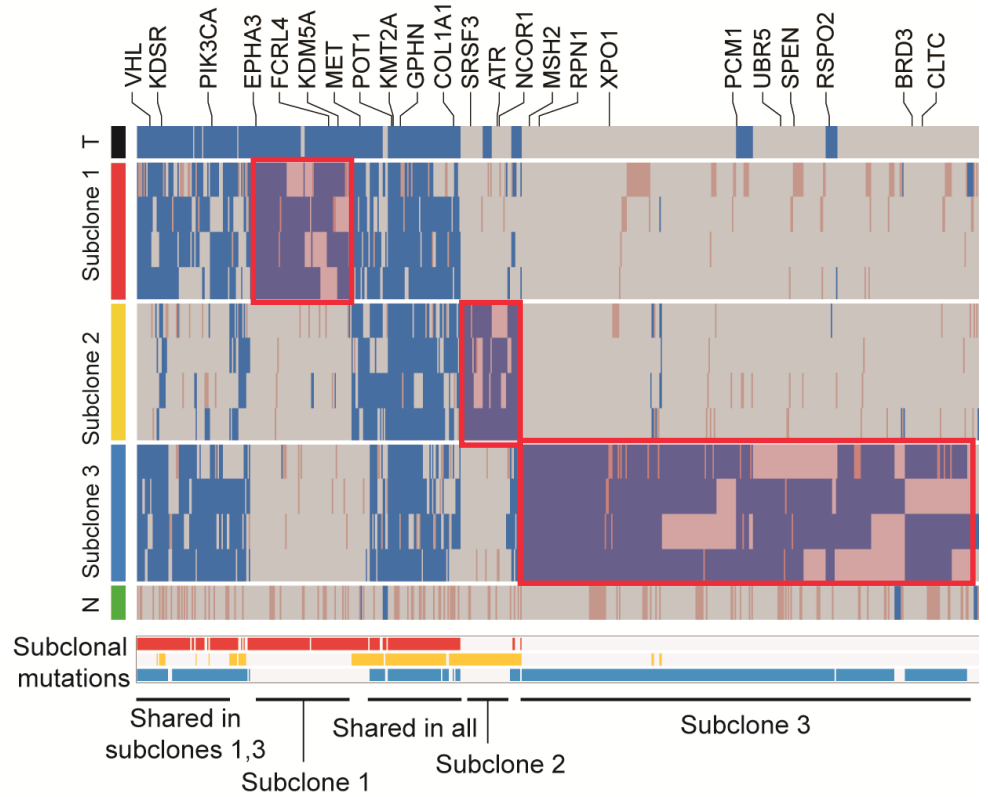
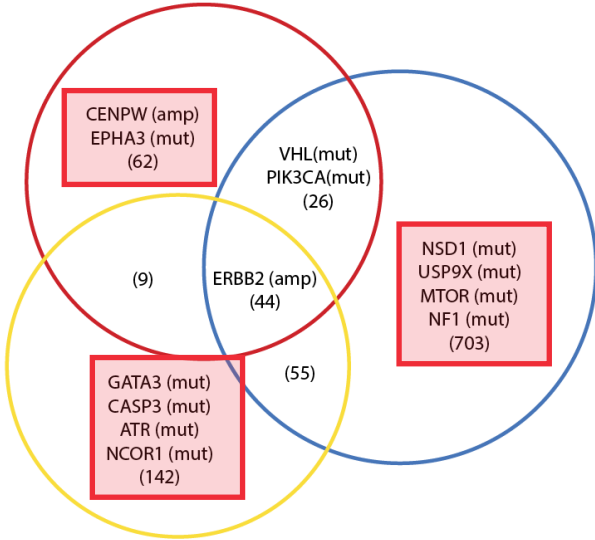
Inferring evolution of the tumor using WES result

Potential cancer driving genes in each group



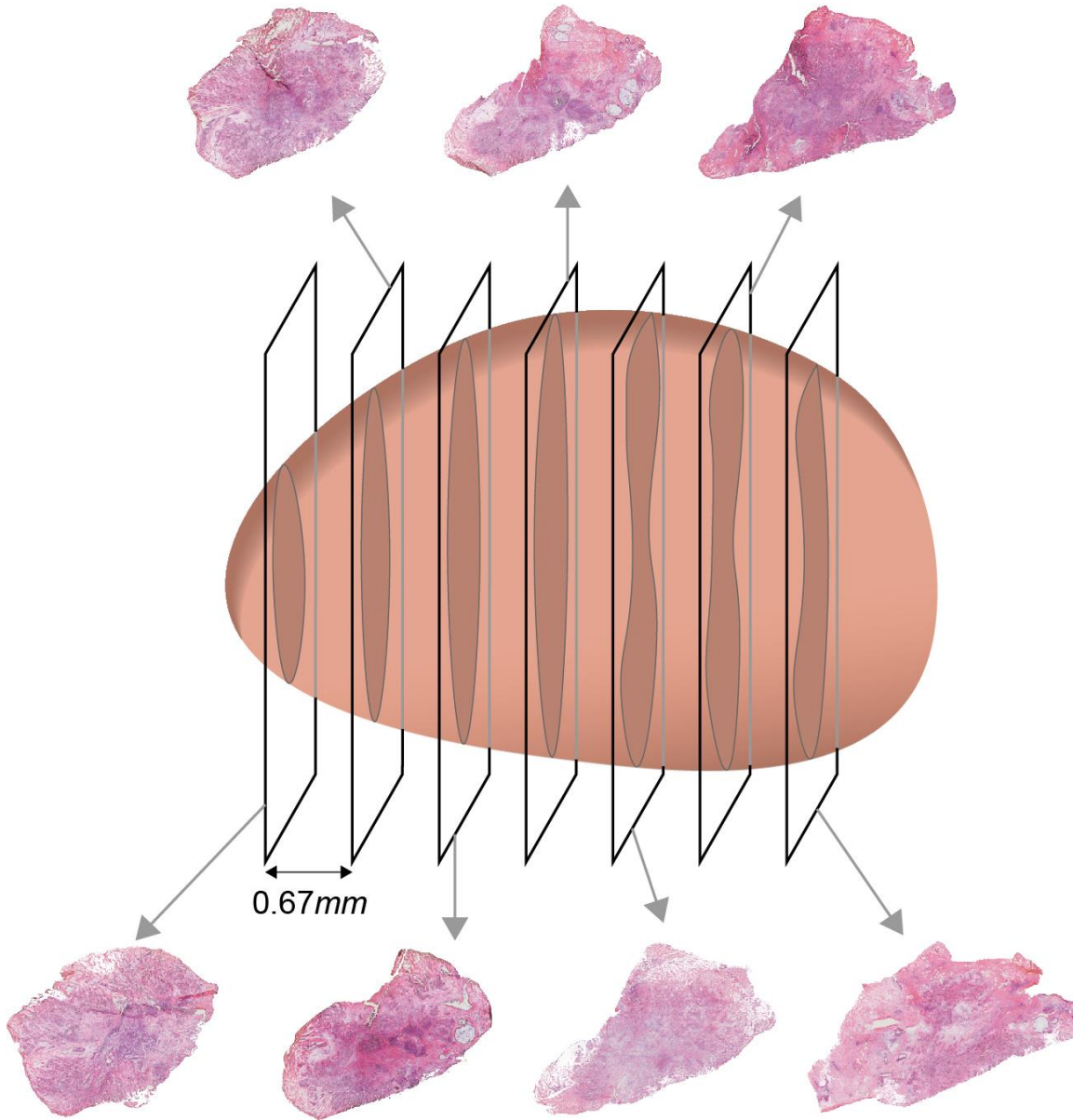
Inferring evolution of the tumor using WES result

Potential cancer driving genes in each group



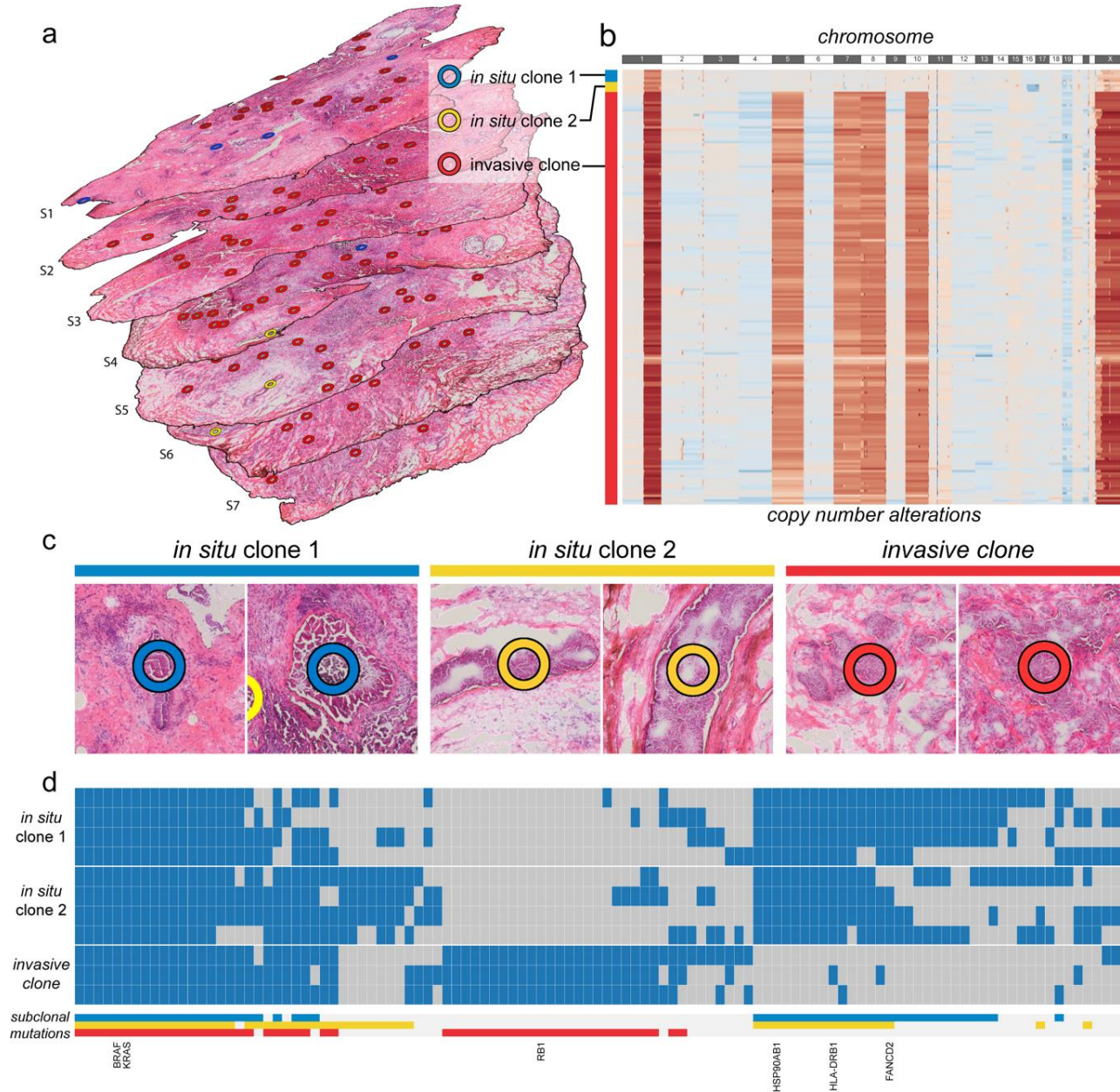
PHLI-seq for Studying Cancer Heterogeneity in 3D Tissue Space

The 3-dimensional tumour mass was investigated using PHLI-seq



- Breast cancer
- Sections were prepared from seven locations
At each location, three H&E section (10um)
- Each location has 0.65 mm interval.

The 3-dimensional tumour mass was investigated using PHLI-seq

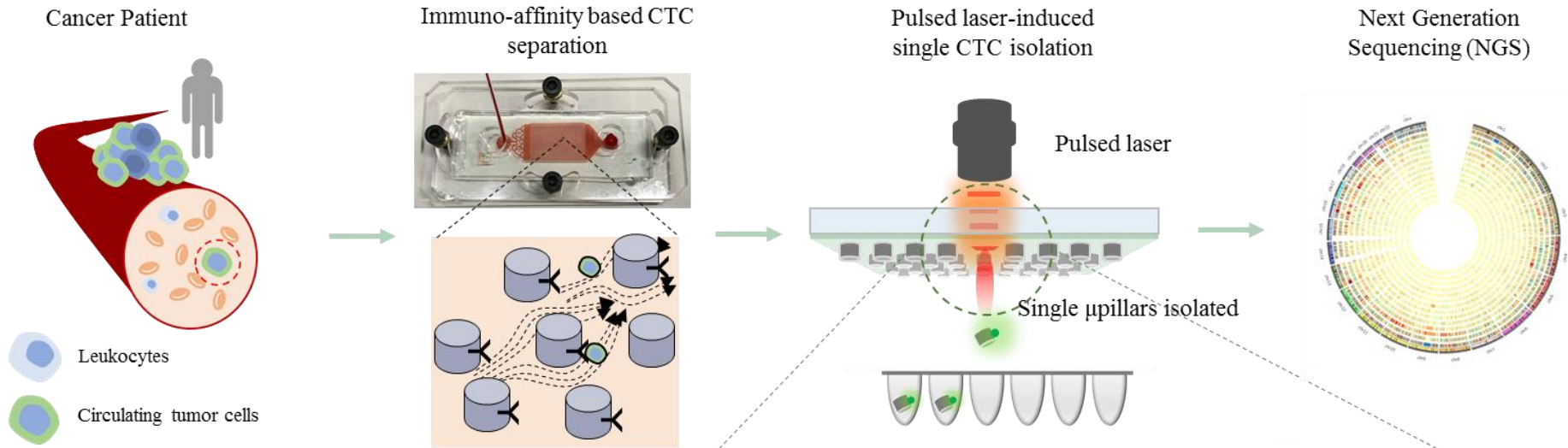




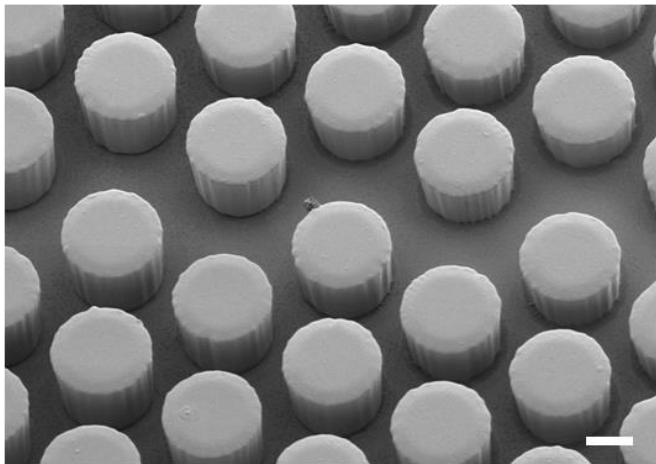
PHLI-seq for Studying Circulating Tumor Cells

Application to Circulating Tumor Cell (CTC) Analysis

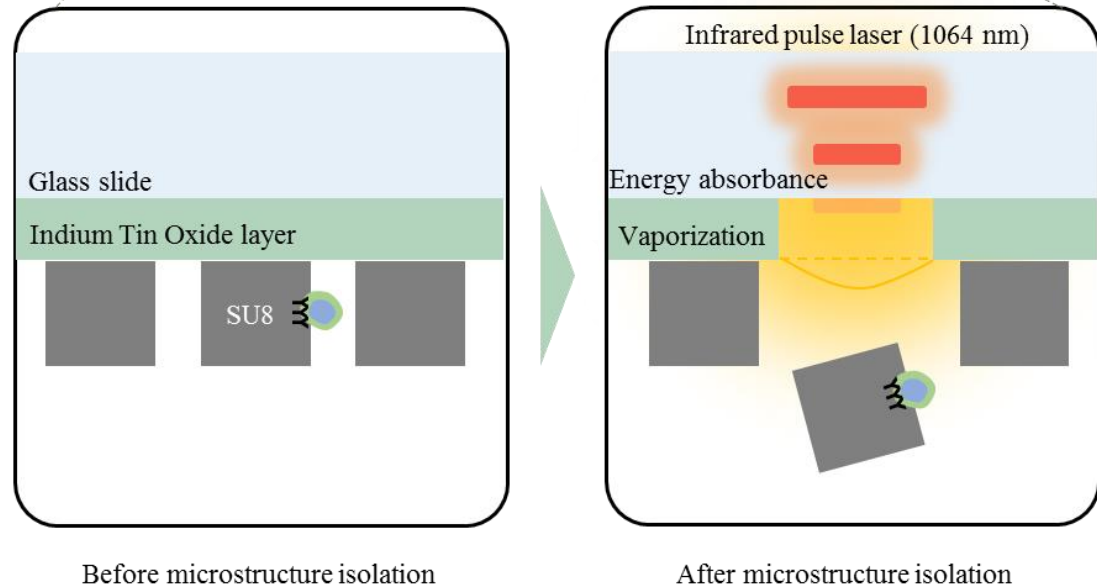
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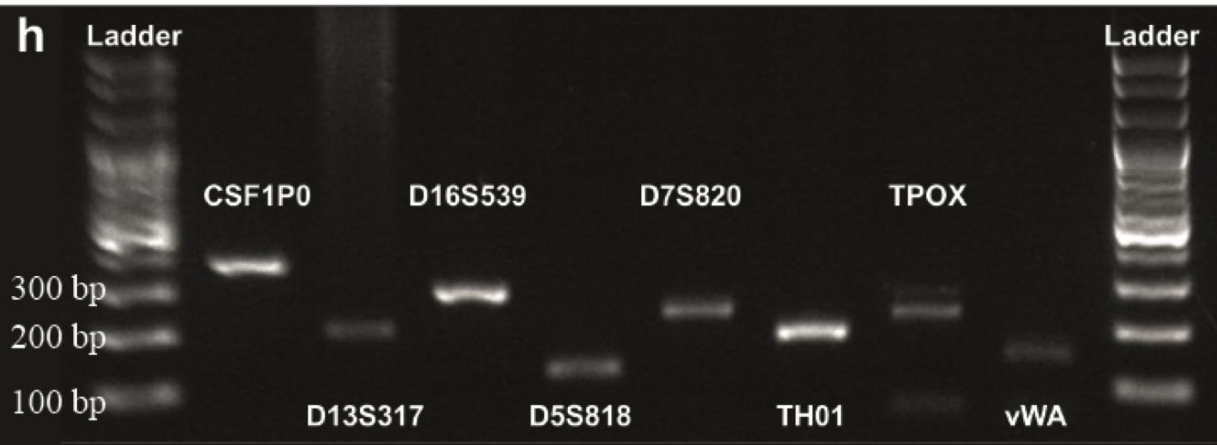
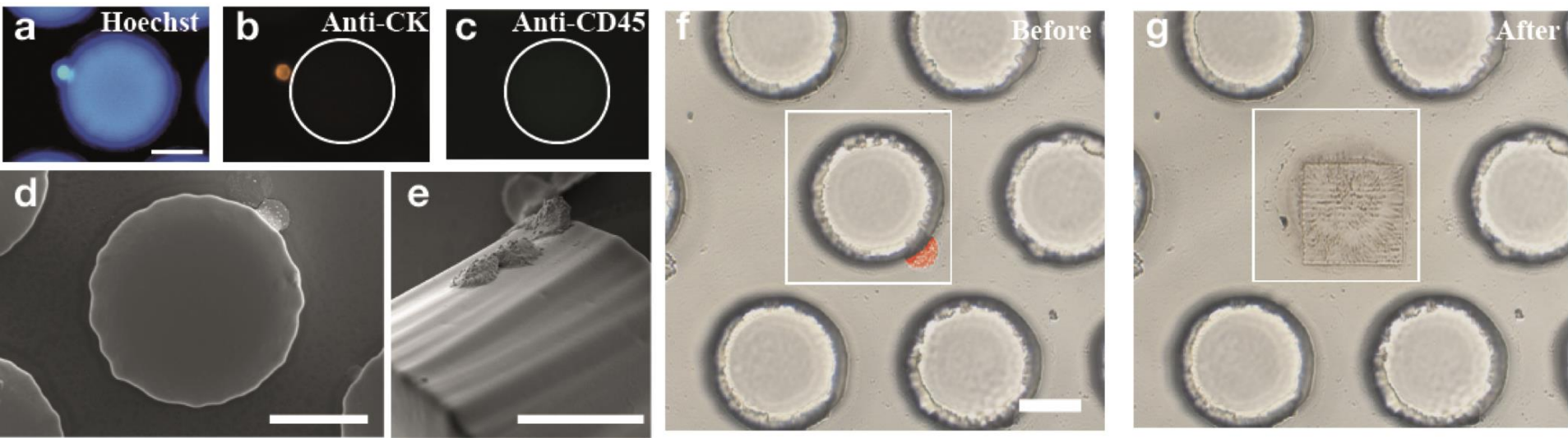
b



c

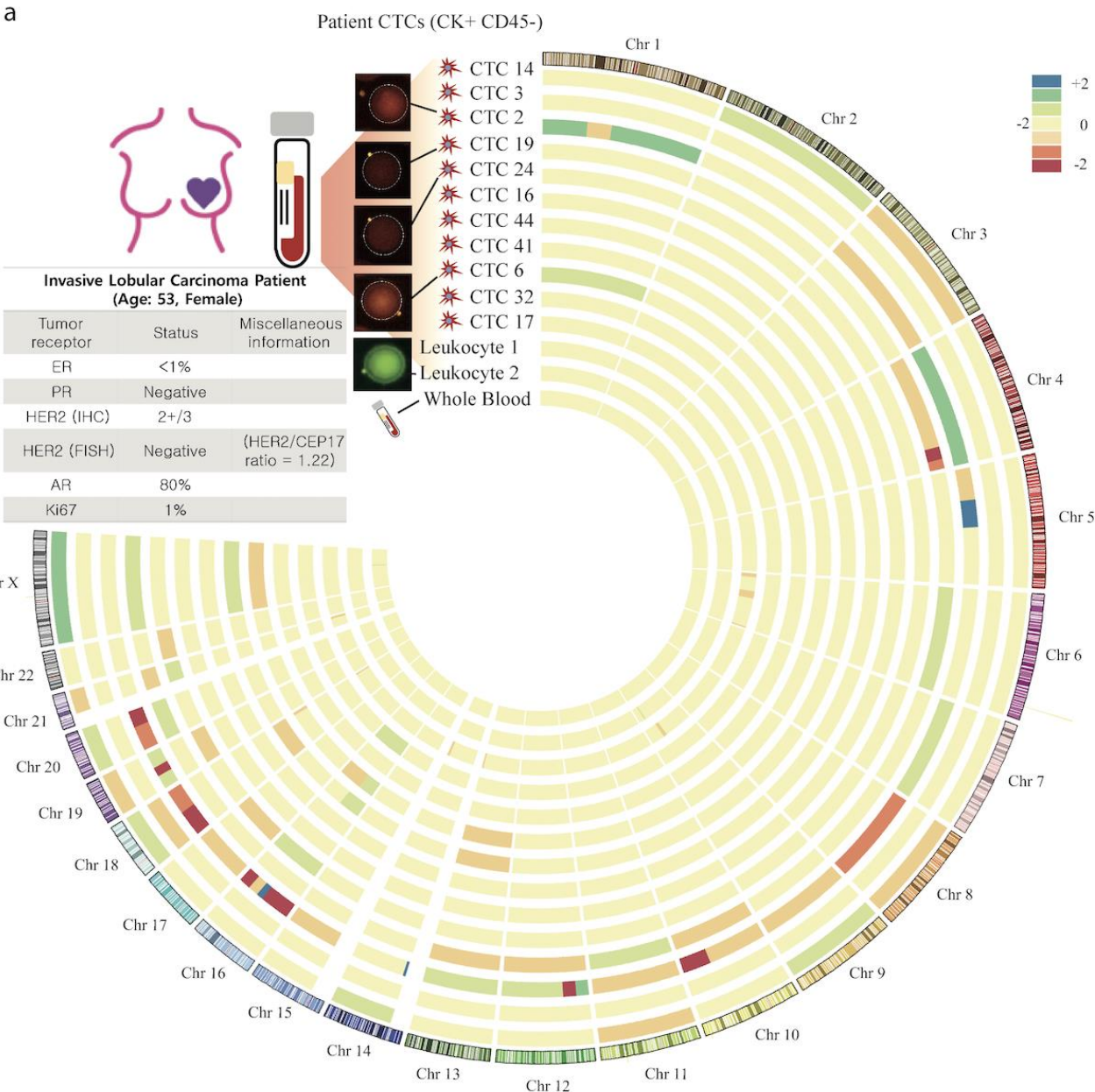


CTC capture and isolation by PHLI-seq method



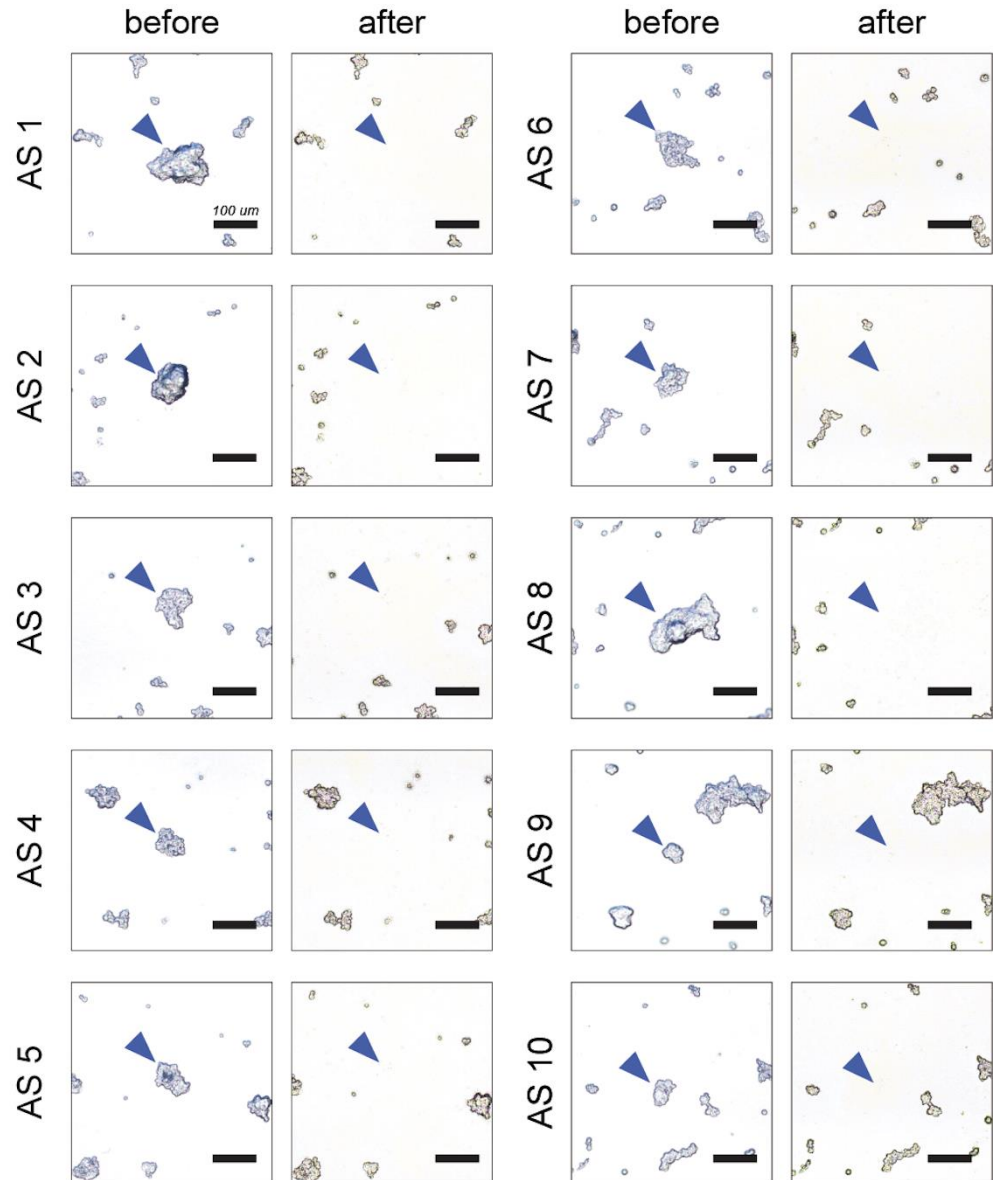
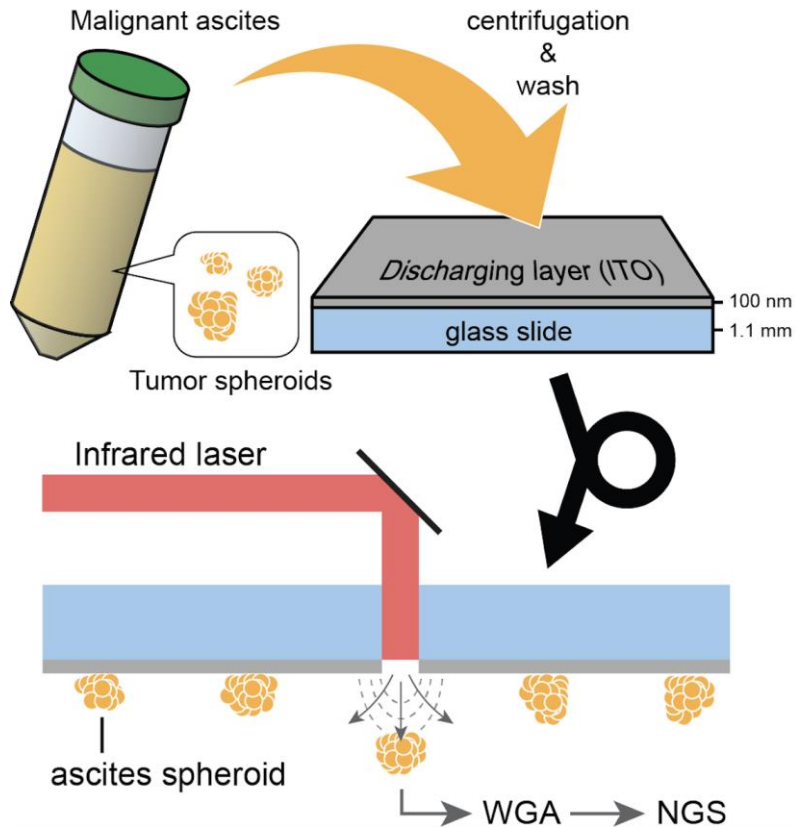
Locus	Position	PCR size (bp)
CSF1P0	Chr5	345
D13S317	Chr13	181,185
D16S539	Chr16	280
D5S818	Chr5	127
D7S820	Chr7	227,239
TH01	Chr11	191,195
TPOX	Chr2	232,244
vWA	Chr12	150

Whole genome sequencing results of isolated CTCs



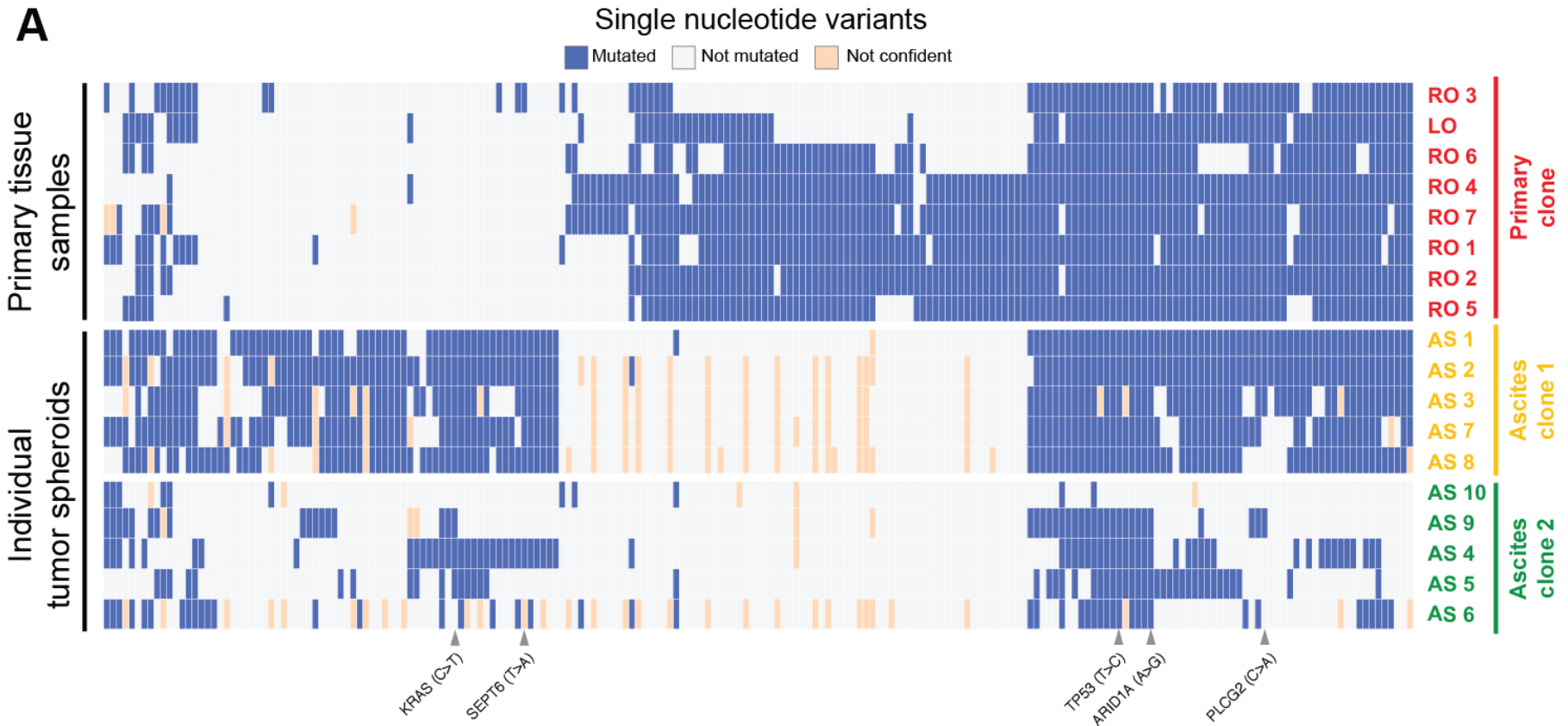
PHLI-seq for Studying
Individual Tumor Spheroids in a
Malignant Ascites of Ovarian Cancer

Isolating Ascites Spheroids



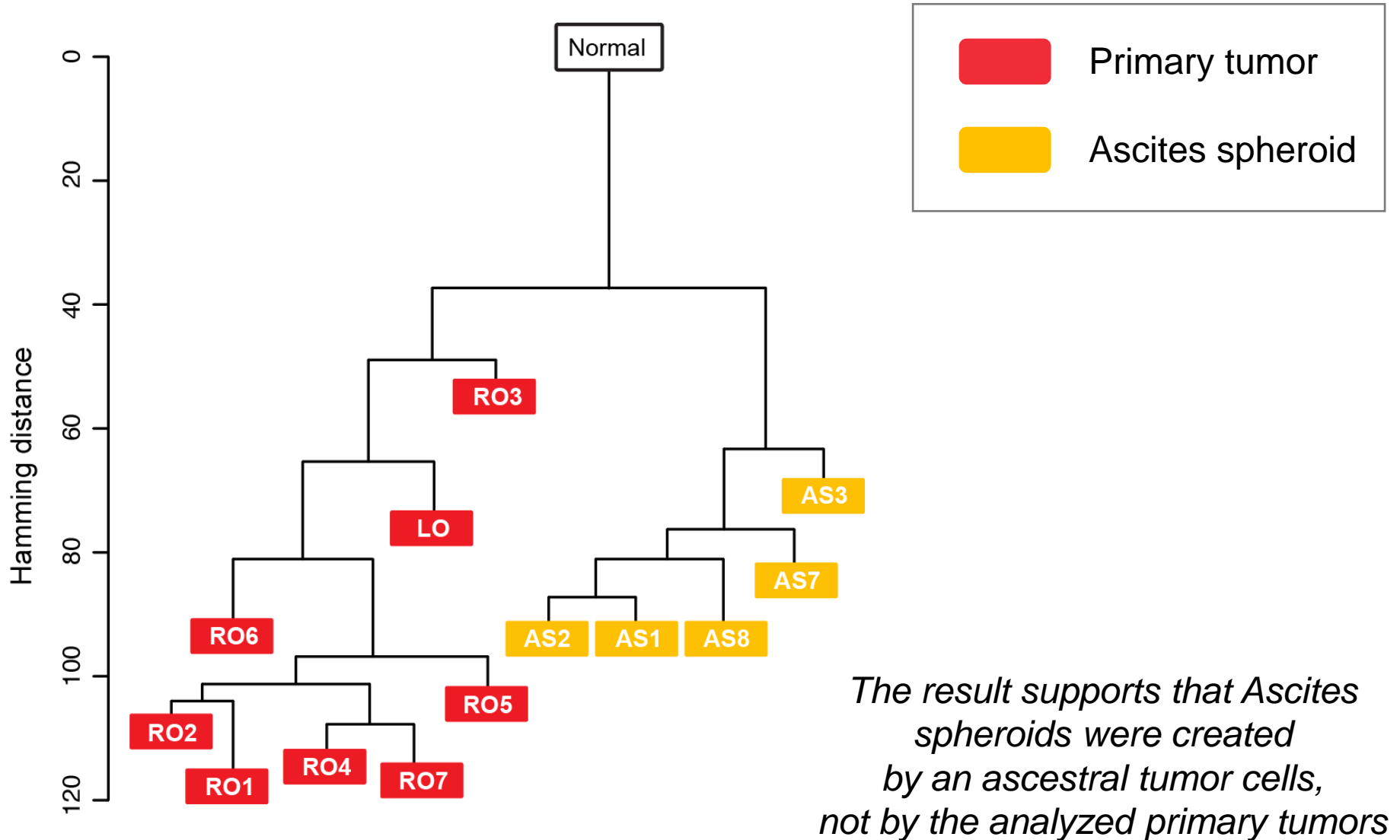
*10 Ascites spheroids
from a ovarian cancer patient
were analyzed*

WES Analysis of Primary tumors and Ascites Spheroids

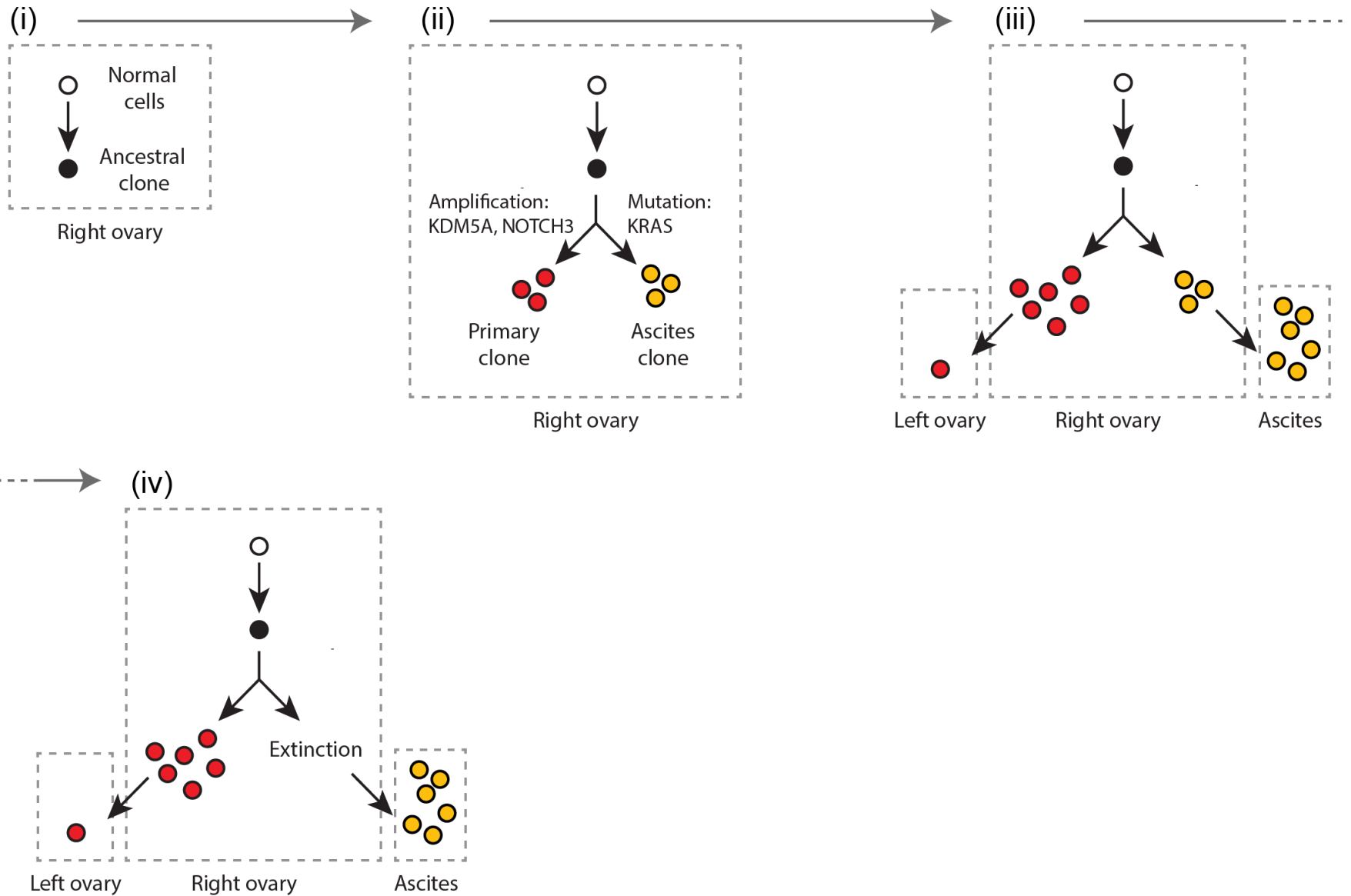


- Primary tumors and ascites spheroids have distinct variant profiles
- Analysis of variant allele frequency support that tumor spheroids included in **Ascites clone 2** had large portion of normal cells, which can explain the lower variant detection rate than **Ascites clone 1**

Phylogenetic tree analysis based on WES data

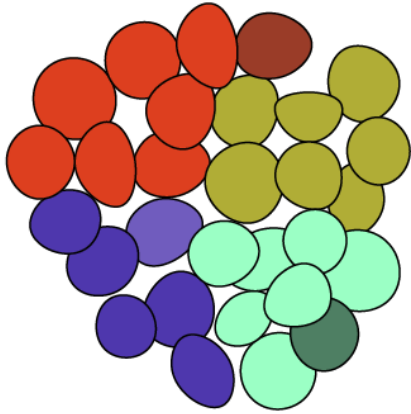


Inferring the tumor evolution process

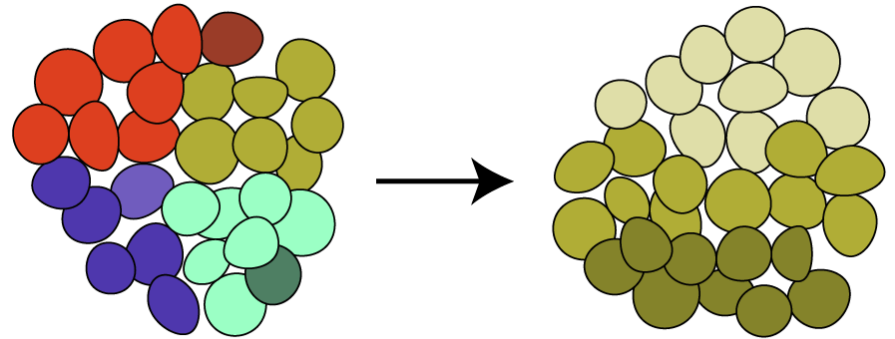


Potential Research Topics of Sniper cell sorting

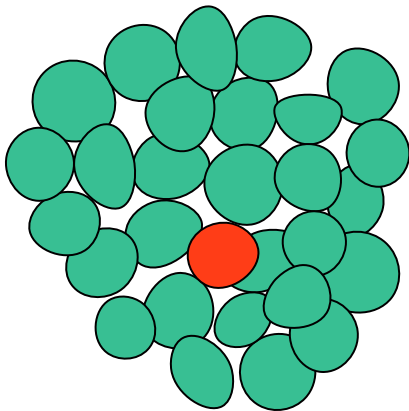
Cancer heterogeneity



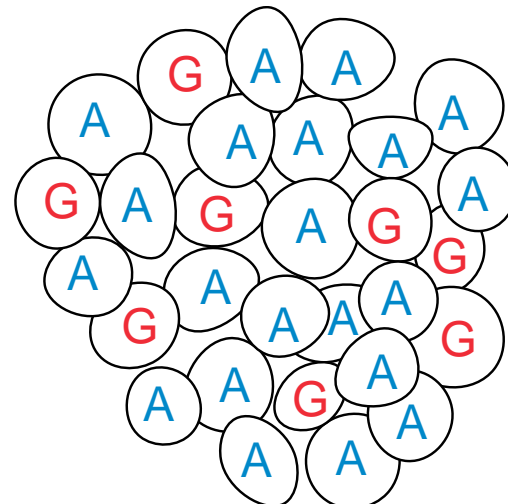
Cancer metastasis



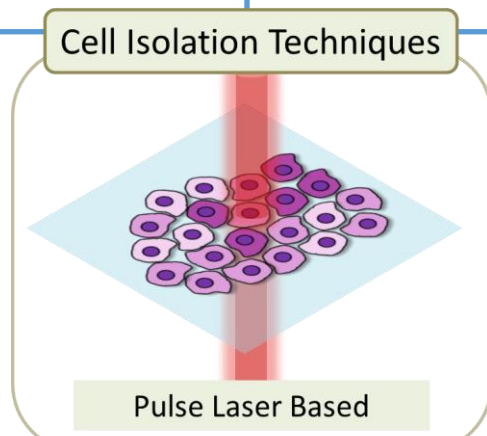
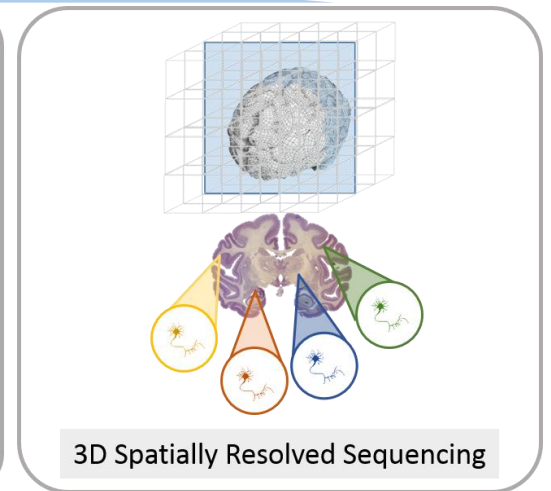
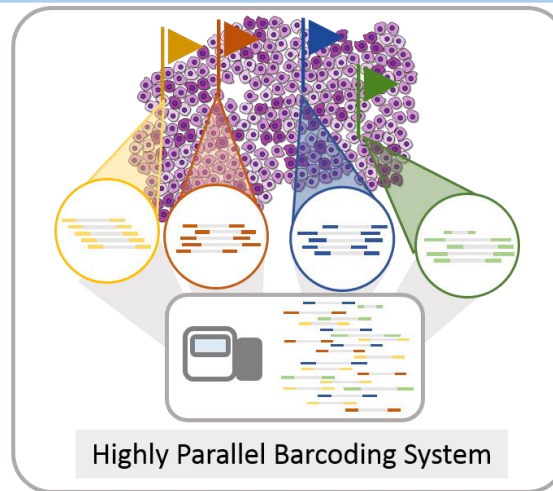
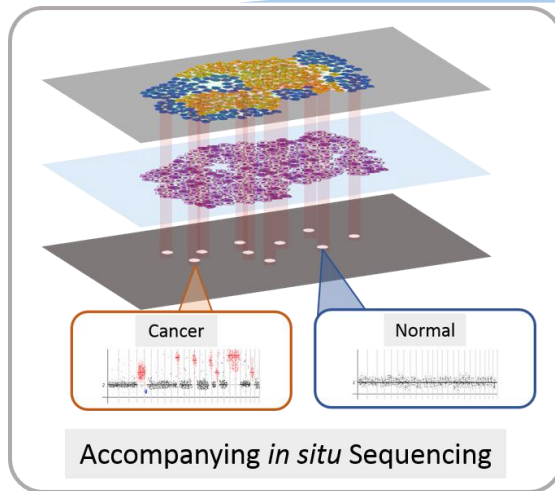
Rare cell sequencing
(cancer stem cells)



Single-cell genotyping



Long Term Plan of Spatially Resolved Sequencing



Conclusion

- Heterogeneous genetic information can be explored by analyzing single-cells or relatively homogeneous small number of cells
- Technological advance, such as PHLI-seq, will enable researchers to find out new biological phenomena in heterogeneous cell population
- High-throughput and high-resolution genetic analysis of histological specimen will provide detail story of cancer development

Acknowledgements

Prof. Won Shik Han
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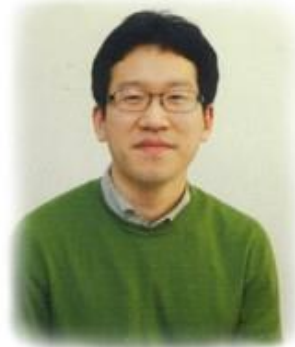
Hyoki Kim
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Yonsei University



Prof. Dongsoon Lee
Seoul National University Hosp



Acknowledgements

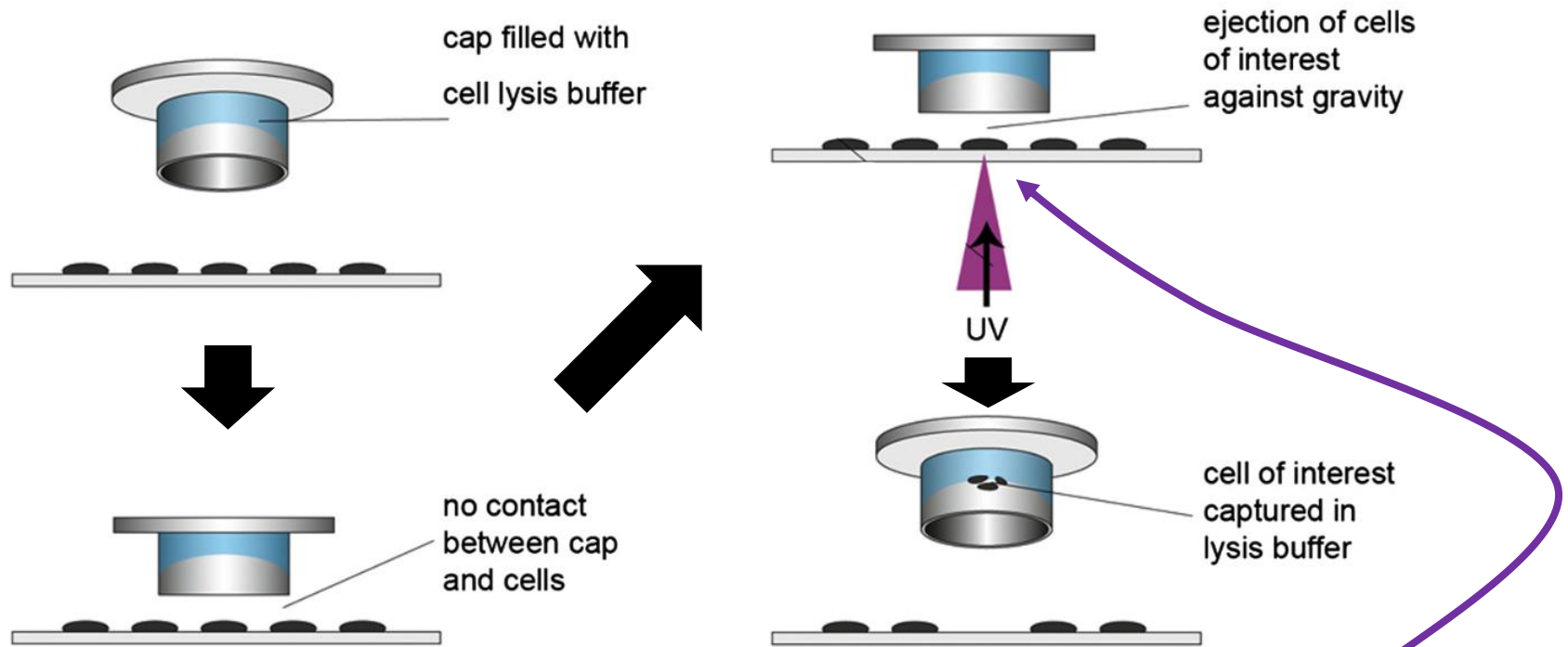
BiNEL Members
@ Mt. Kwanak



Thank you

Competing Technology: Ultraviolet Laser Capture Microdissection (LCM)

LCM from Zeiss



Disadvantage

- UV laser generates damages to cells
- Sometimes, isolation failure occurs

UV damages cells



All other microdissection systems use only a UV laser for microdissection, without the advantage of the gentle IR laser. The UV cutting technique uses a higher power to burn through connective tissue during dissection. It has been proven through independent studies and internal research that UV dissection can damage DNA, RNA, and proteins in dissected cells smaller than 30 μm in diameter, making UV cutting a better tool for isolating larger structures or whole tumors from tissue sections, but not individual cells.

(<https://www.thermofisher.com>)

Journal of Pathology

J Pathol 2017; **241**: 208–218

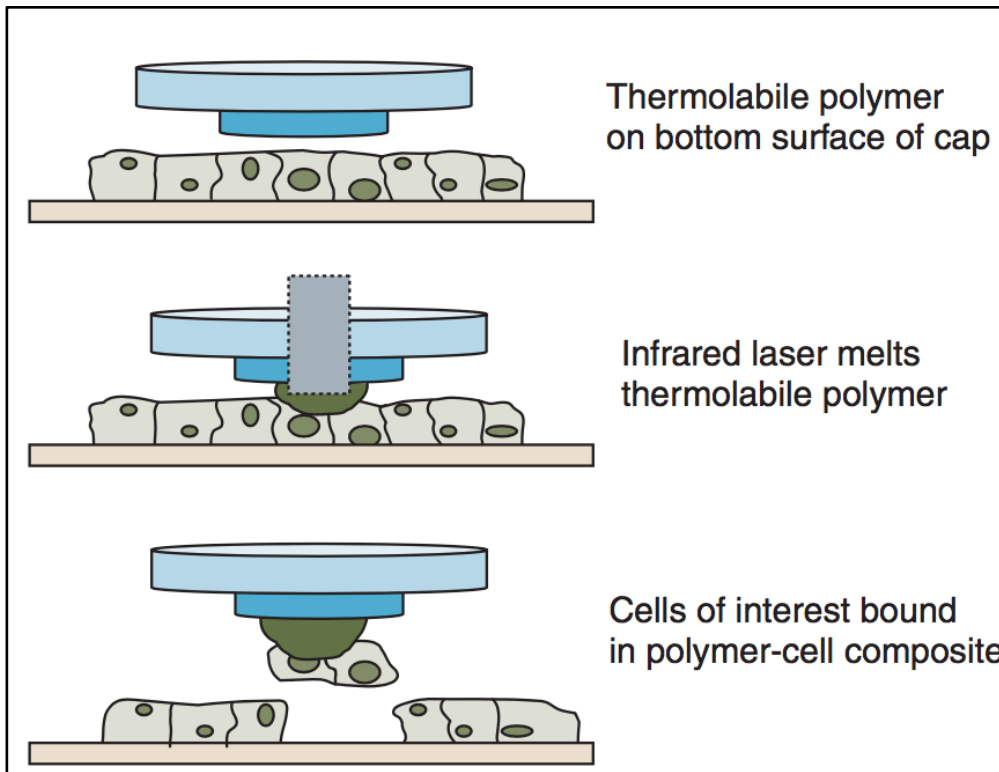
Published online 27 November 2016 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/path.4840

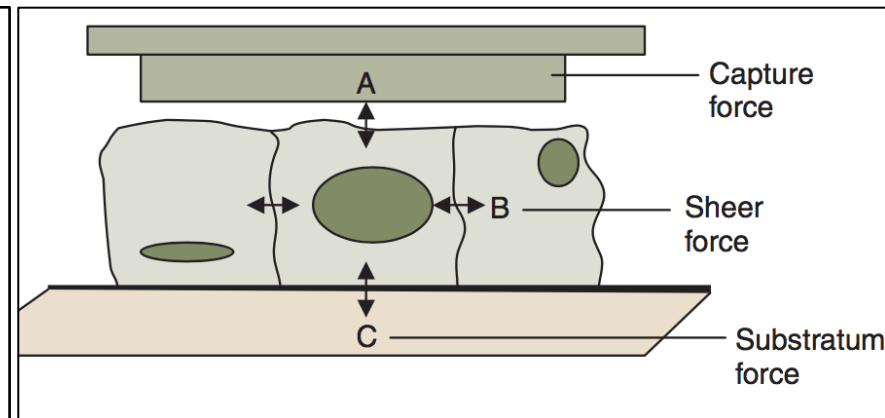
in combination with SCS methods. Technical issues such as UV lasers damaging DNA and RNA prior to amplification or cells being cut in half during tissue

Infrared Laser Capture Microdissection Has Limited Applicability Depending on the Condition of Samples

LCM from Thermo Fisher Scientific



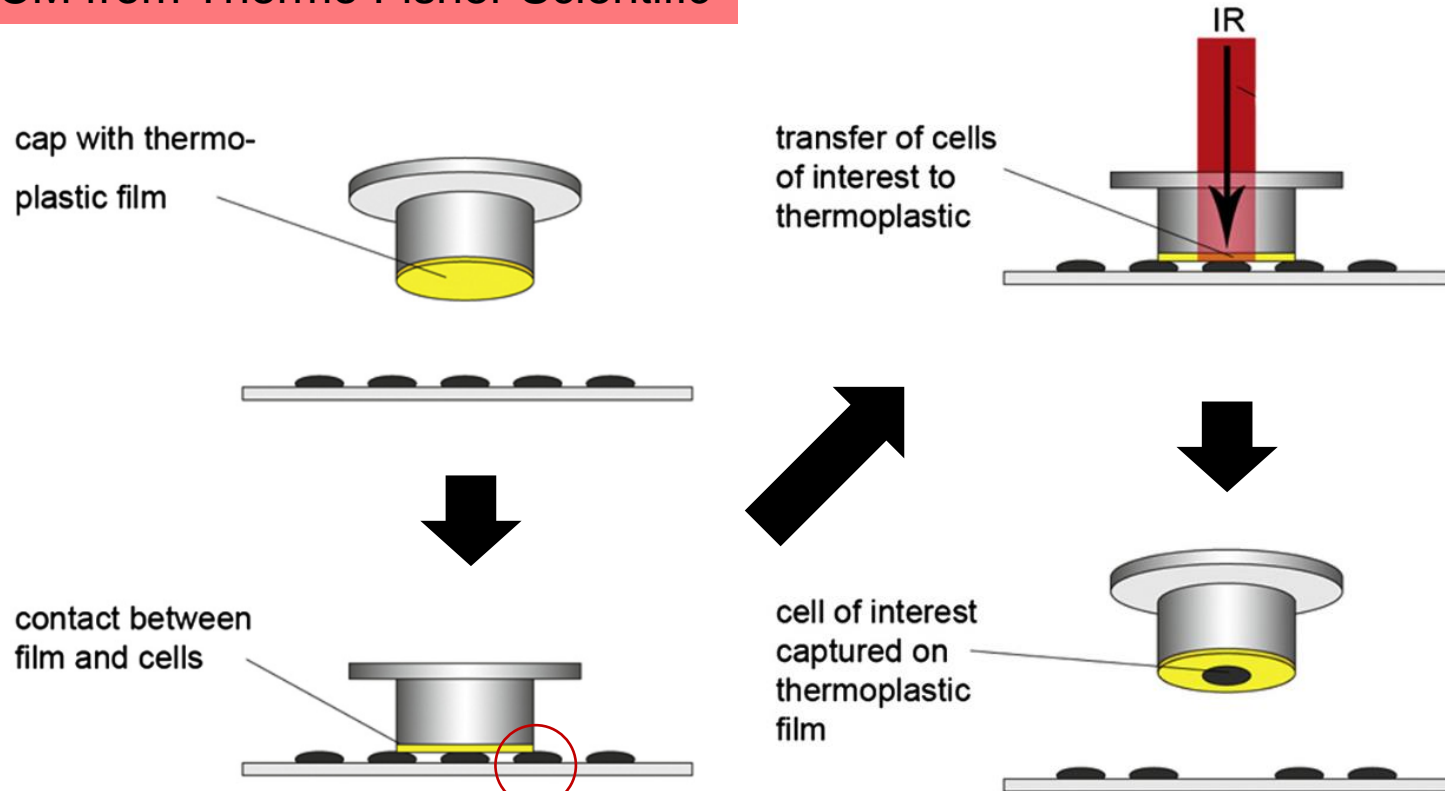
Cell capture mechanism



Physical forces involved in LCM

Competing Technology: Infrared Laser Capture Microdissection (LCM)

LCM from Thermo Fisher Scientific



Disadvantage:

- limited throughput and scalability
- debris or non-selected cell adhesion
- applicability depending on the condition of samples (next page)

Laser Microdissection Companies and Markets



strength

Instrument/
microscope

Instrument/
microscope

Sample preparation/
Post LCM biochemistry

Market
portion

40%

40%

15%

Market
size

USD 124.97 Million by 2020 from USD 72.45 Million in 2015
at a CAGR of 11.52% between 2015 and 2020.

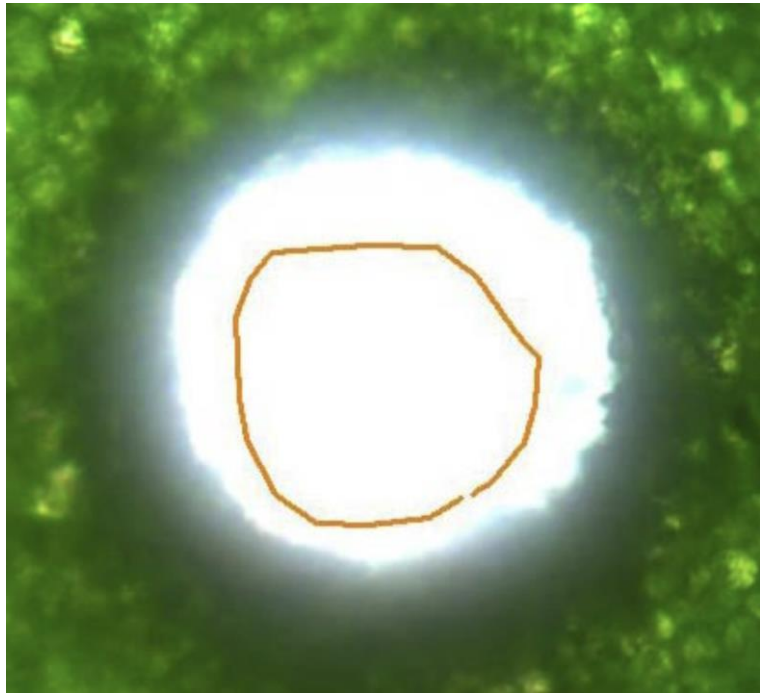
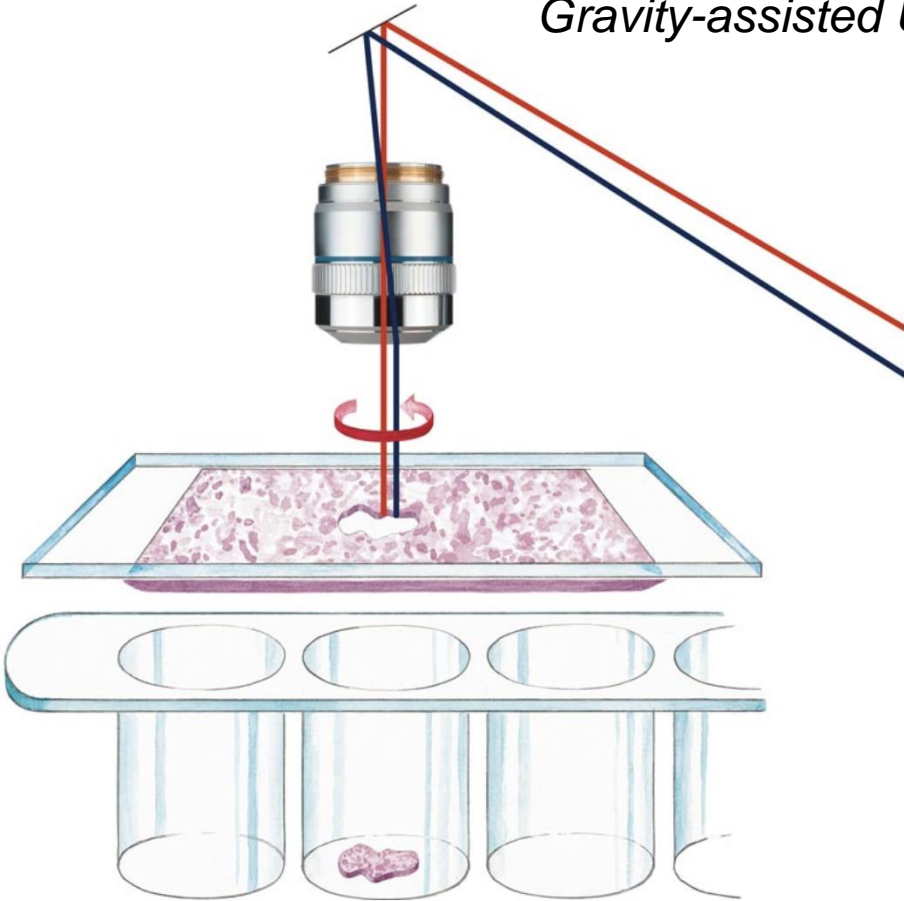
Majority of the market is instruments.

→ Globally 500 대 판매/년

→ Genomics 분야의 성장과 함께 동반 성장하는 시장

MarketsandMarkets Research

Gravity-assisted UV cutting



Leica (movie)

Leica Laser Microdissection - [Live]

File View Camera Image Laser AutoShape Options Help

Save Save Live Freeze MicCtrl Specimen Collector Focus Unload Unload Laser Edit New

Select
Line
Ellipse
Rect
Reuse
Detect
ABC
Text
Length

Zoom Factor: 1.0 FullScreen

Draw Shape(s)
 Close Line(s)
 Single Shape Multiple Shapes

Cut Shape(s)
 Draw + Cut Draw + Scan
 Move + Cut

Start Cut Stop Cut

X-Y-Z Precision:
 Fine Coarse

Database:
 Connect to Database
Specimen ID: <Unknown>
Specimen Data

Nr.	Area (μm²)	Mag.	Col.	Objects
1	422	40x	1	1
2	482	40x	1	1
3	280	40x	1	1
4	289	40x	1	1
5	774	40x	1	1

Total selected area (μm²): 2441

Erase Erase All Summary

Collector Device: Tube Caps
A B No Cap C D

Slide Holder

Stage Position Memory
Erase Memory
Add Erase All

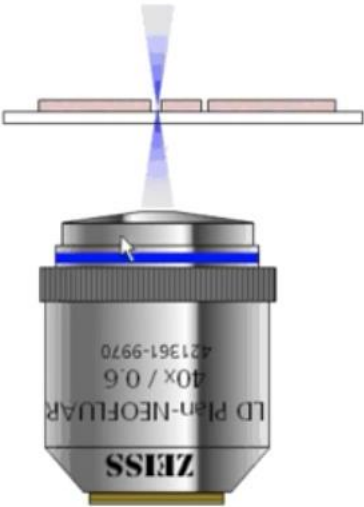
Specimen Overview...
Create Fast Overview

LEICA LMD7000

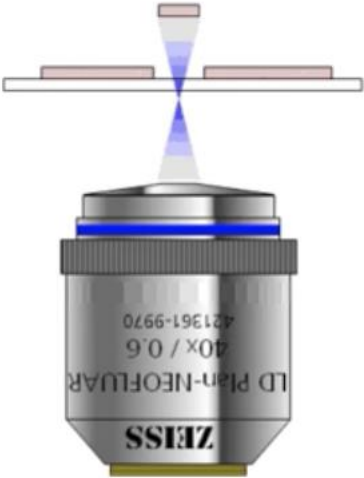
Ready ThreeSlides Magnification: 40x



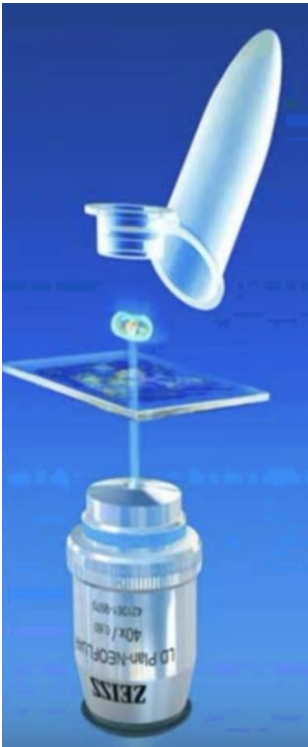
Cutting and lift-off transfer by UV laser



Cutting



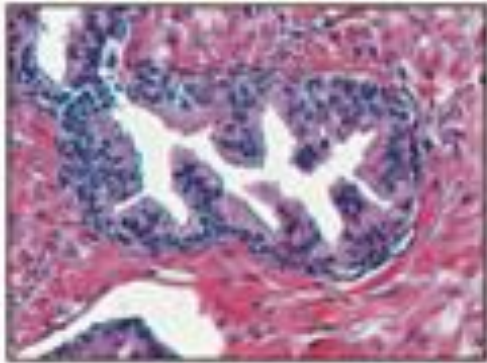
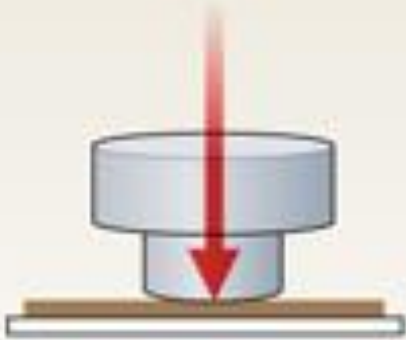
Transfer



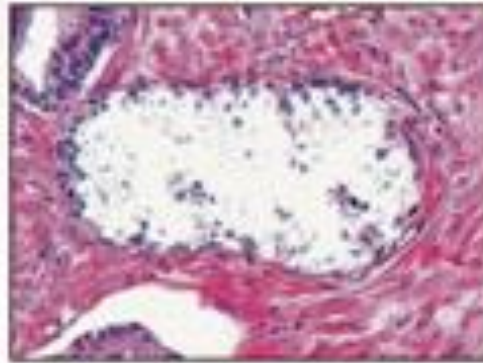
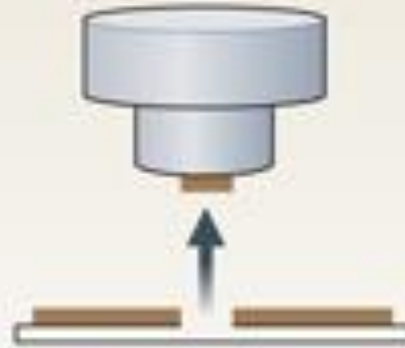
Thermo Fisher Scientific

ThermoFisher
S C I E N T I F I C

Infrared laser capture



Lift cap from slide



Cap with captured cells



Thermo Fisher Scientific (movie)

