Probe Architecture

Instrument run

EXAMPLE

mRNA

	Red	Green	Blue	Yellow
	rcR1	rcG1	rcB1	rcY1
	rcR2	**rcG2**	rcB2	rcY2
	rcR3	rcG3	rcB3	rcY3
	rcR4	rcG4	rcB4	rcY4
	rcR5	rcG5	rcB5	rcY5
	rcR6	rcG6	rcB6	rcY6
	rcR7	rcG7	rcB7	**rcY7**
	rcR8	rcG8	rcB8	rcY8
	rcR9	rcG9	rcB9	rcY9
	rcR10	rcG10	rcB10	rcY10
	rcR11	rcG11	rcB11	rcY11
	rcR12	rcG12	rcB12	rcY12
	rcR13	rcG13	rcB13	rcY13
	rcR14	rcG14	rcB14	rcY14
	rcR15	rcG15	rcB15	rcY15
	rcR16	rcG16	rcB16	**rcY16**

Only the matching probes will sequentially bind

Gene #	Barcode
Gene 1	1100001000000001
Gene 2	1100101000000000
Gene 3	0100100000001001
Gene 4	0100100000001001

Photocleavable linkers
Fluorophores (15-60)

Reverse-complement binding sites for landing sites (10-20 nt)

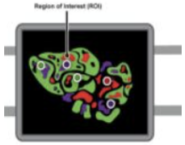| Red	Green	Blue	Yellow
rcR1	rcG1	rcB1	rcY1
rcR2	rcG2	rcB2	rcY2
rcR3	rcG3	rcB3	rcY3
rcR4	rcG4	rcB4	rcY4
rcR5	rcG5	rcB5	rcY5
rcR6	rcG6	rcB6	rcY6
rcR7	rcG7	rcB7	rcY7
rcR8	rcG8	rcB8	rcY8
rcR9	rcG9	rcB9	rcY9
rcR10	rcG10	rcB10	rcY10
rcR11	rcG11	rcB11	rcY11
rcR12	rcG12	rcB12	rcY12
rcR13	rcG13	rcB13	rcY13
rcR14	rcG14	rcB14	rcY14
rcR15	rcG15	rcB15	rcY15
rcR16	rcG16	rcB16	rcY16

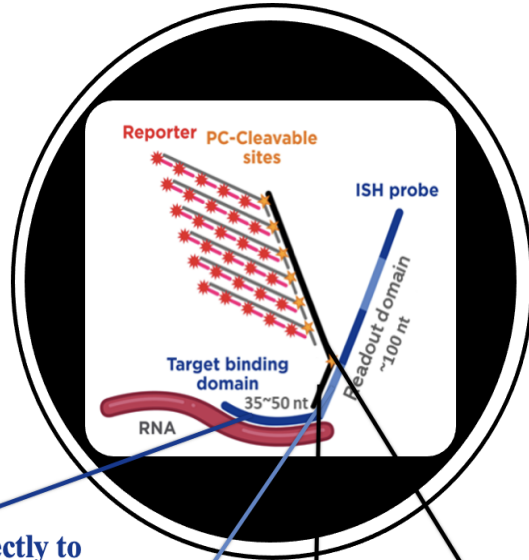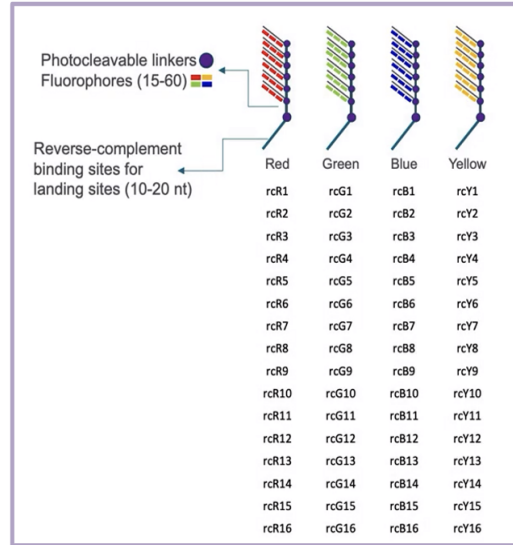**TARGET DOMAIN**
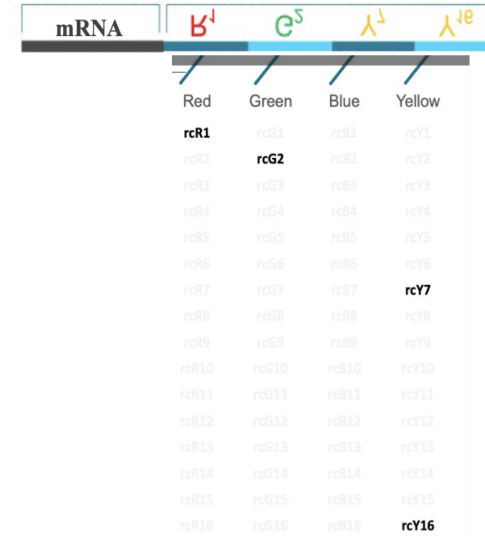Gene specific, binds directly to the target transcript in a specific hybridization domain

ISH PROBE LANDING SITE
Binds to the reporter probes. Four sites each chosen from a pool of 64 sequences associated with the 4 possible colors (16 per genes/ fluorophore)
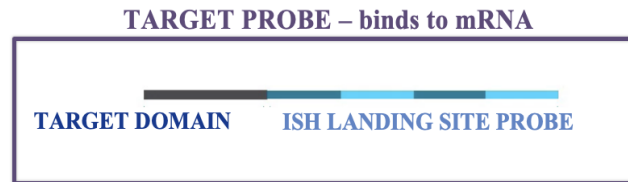
PHOTOCLEAVABLE LINKERS PROBES
Fluorophores

REVERSE COMPLEMENT SEQUANCES BINDING SITE
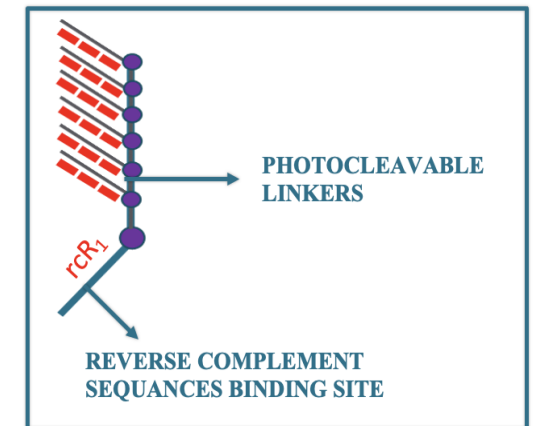Binds to the ISH landing site probe (one of the 4 sites)

TARGET PROBE – binds to mRNA

TARGET DOMAIN   ISH LANDING SITE PROBE

+

REPORTER PROBE – binds to target probe

PHOTOCLEAVABLE LINKERS

REVERSE COMPLEMENT SEQUANCES BINDING SITE

Spatial Transcriptomic_CosMx SMI

Workflow Pre and On Instrument

Experiment Day 1 Pre-Instrument

FFPE tissue on standard slides

Permeabilize, fix — Retrieve targets

Hybridization- RNA specific probes and antibodies bind to target
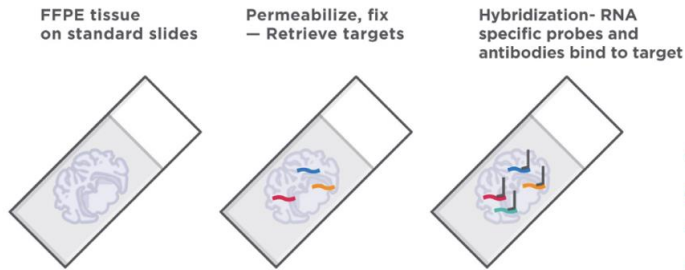
Critical steps:
- **Antigen retrieval**
- **Tissue digestion**
- **Probe Hybridization**

Experiment Day 2 Pre-Instrument

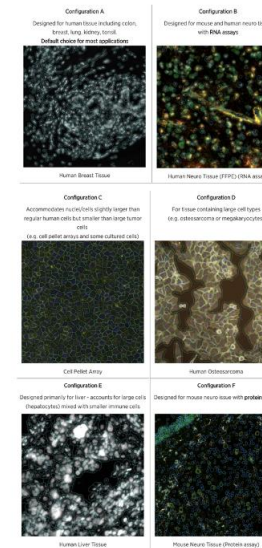**Nuclear and Cell segmentation staining**

Experiment Day 2 Instrument

Instrument loading

LABEL

5 mm
15 mm
5 mm
20 mm — 17 mm

Barcode Scanner
Flow Cell Nest
Buffer 1   Buffer 2
Buffer 3   Buffer 4

Figure 4: CosMx SMI Instrument Interior

Flow Cell setting

Table 6: Pre-bleaching Profile

Tissue Type	RNA	RNA	Protein
	Normal	Malignant	
Brain	Configuration B	Configuration B	Configuration C
Skin	Configuration C	Configuration C	Configuration C
Lung	Configuration C	Configuration C	Configuration C
Breast	Configuration C	Configuration C	Configuration C
Liver	Configuration B	Configuration C	Configuration C
Colorectal	Configuration C	Configuration C	Configuration C
Tonsil	Configuration C	Configuration C	Configuration C
Pancreas	Configuration C	Configuration C	Configuration C
Kidney	Configuration B	Configuration B	Configuration C
Fresh Frozen	Configuration C	Configuration C	Configuration C
CPA	Configuration A	Configuration A	Configuration A

Configuration A: 30 seconds, Configuration B: 90 seconds; Configuration C: 60 seconds

Configuration A
Designed for human tissue including colon, breast, lung, kidney, tonsil.
Default choice for most applications

Human Breast Tissue

Configuration B
Designed for mouse and human neuro tissue with RNA assays

Human Neuro Tissue (FFPE) (RNA assay)

Configuration C
Accommodates nuclei/cells slightly larger than regular human cells but smaller than large tumor cells
(e.g. cell pellet arrays and some cultured cells)

Cell Pellet Array

Configuration D
For tissue containing large cell types
(e.g. osteosarcoma or megakaryocytes)

Human Osteosarcoma

Configuration E
Designed primarily for liver - accounts for large cells (hepatocytes) mixed with smaller immune cells

Human Liver Tissue

Configuration F
Designed for mouse neuro tissue with protein assays

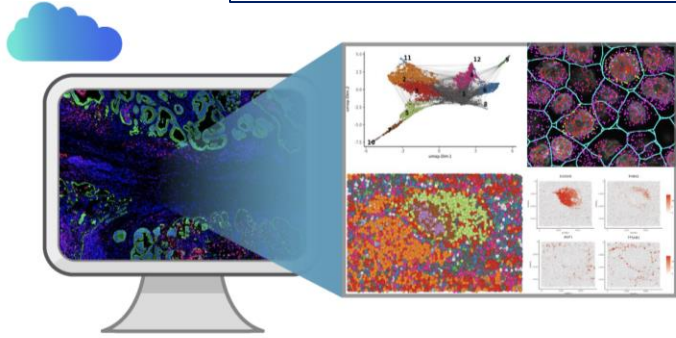Mouse Neuro Tissue (Protein assay)

FOV (Field Of View) selection
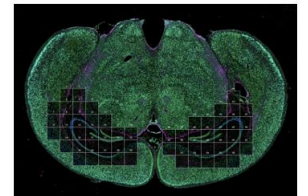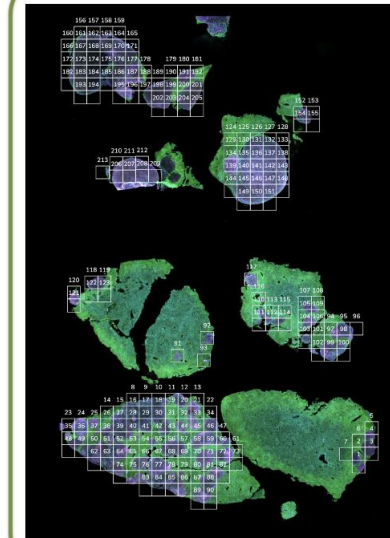
FOV are the areas that will be "sequenced".
FOV are fixed 0.5 x 0.5 mm, based on total number of FOV the instrument will run X days
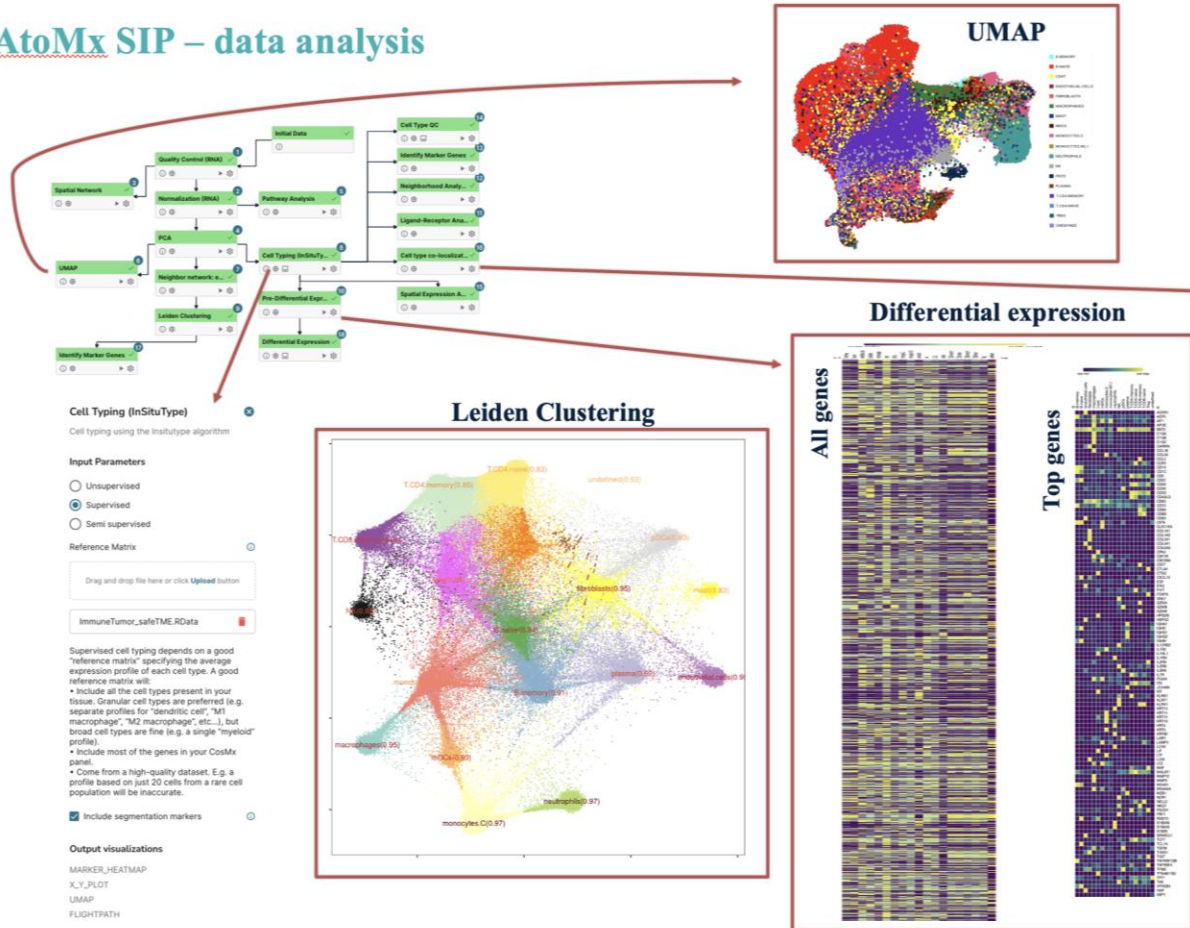
CRITICAL STEP!

AtoMx™ Spatial Informatics Platform (SIP) is a cloud-based, fully-integrated informatics platform for spatial biology.

No coding experience is required to use AtoMx SIP. Researchers can analyze and visualize spatial multiomics data with ease while utilizing NanoString-configured analysis modules and pipelines.

Read more about AtoMx here

AtoMx SIP – data analysis