

# A proposal to extend standardized organ mapping antibody panels (OMAPs) to integrate protein and RNA analysis in spatial biology

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## Abstract

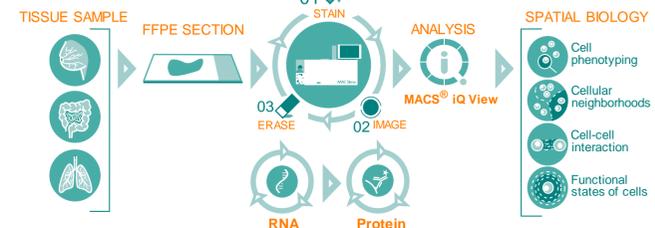
The Human BioMolecular Atlas Program (HuBMAP) consortium has developed a new standard to report on normal histological samples using multicolor immunofluorescence imaging. An Organ Mapping Antibody Panel (OMAP) currently describes in a table the antibodies used, the cycle number assigned to a given reagent, and provides the rationale for using a particular antibody to better understand features of the tissue. This table is accompanied by antibody validation templates that show example images from the tissue described for the OMAP table. In addition, links to existing databases, gene symbols, the antibody features and colors are included. For each tissue, a further table called "Anatomical Structures, Cell Types, plus Biomarkers" (ASCT+B), which contains structures of the tissue, cell types in a given tissue structure, key RNA transcripts for a given cell type, and antibody stains as used in the OMAP table, is created. So far, this process has not been established for spatial multiomic datasets including protein and RNA detection on the same tissue section.

We have extended spatial biology methodology to combine cyclic RNA transcript detection with cyclic antibody-based protein detection using the MACSima™ Imaging Cyclic Staining (MICS) technology as well as H&E-staining on the same tissue section. For such datasets, the current OMAP data table needs to be extended by adding information on the RNA detection, cycle IDs and RNA validation templates, demonstrating the correct performance of a given RNA probe detection.

This poster will demonstrate a proposal to extend the OMAP table structure on an extended version of the "tonsil OMAP (OMAP-10)", which was published on the Zenodo data repository page (<https://zenodo.org/records/7875938>). In addition, we will apply this extended OMAP to a colorectal cancer sample, to highlight the applicability and relevance of the OMAP structure for tumor tissue.



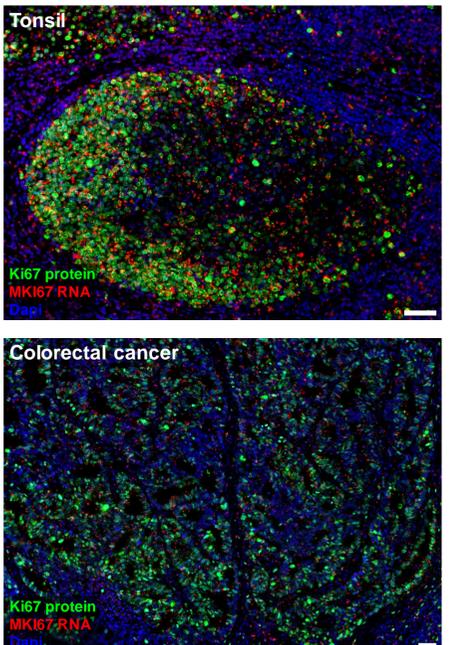
MACSima Imaging Cyclic Staining (MICS)



## 2. Concept of the "RNA Probe Validation Report"

In addition to the extended "OMAP+" table structure, we are suggesting a validation report for RNA probes, similar to the existing Antibody Validation Reports currently generated for HuBMAP. A concept of the "RNA Probe Validation Report (RVR)" for a targeted padlock probe detecting MKI67 transcripts is shown below. Like the Antibody Validation Report, the "RVR" includes example images from the tissue described for the "OMAP+" table. To the right a combined visualization of RNA and protein detection for Ki67 via targeted padlock probe and antibody is shown. Images show a germinal center within a palatine tonsil, and the tumor area of a colorectal cancer tissue sample. Scale bars = 50 µm.

Antibody Validation Report				RNA Probe Validation Report			
<b>I. Target Information</b>				<b>I. Target Information</b>			
Target name	MG67	HGNC ID	HGNC:7107	Target name	MG67	HGNC ID	HGNC:7107
UniProt accession number	P46013	UniProt accession number	P46013	UniProt accession number	P46013	UniProt accession number	P46013
<b>II. Antibody Information</b>				<b>II. RNA Probe Information</b>			
RRID	AB_2905309	RRID		RRID		RRID	
Host	human	Probe type	Padlock probe	Probe type	Padlock probe	Probe number	
Isotype	IgG1	Probe number		Mutation specific	No	Vendor	Miltenyi Biotec
Clonality	monoclonal	Vendor	Miltenyi Biotec	Catalog number	pending	Organ or tissue used for validation	Palatine tonsil
Vendor	Miltenyi Biotec	Recombinant (Y/N)	yes	Tissue preservation method	FFPE	Organ UBERon ID	UBERON:0002373
Catalog number	130-127-837	Tissue preservation method	FFPE	Organ or tissue used for validation	Palatine tonsil	Organ UBERon ID	UBERON:0002373
Antibody-based imaging method	MICS (MACSima)	Organ UBERon ID	UBERON:0002373	Antibody-based imaging method	MICS (MACSima)	Conjugate	PE
Conjugate		Antibody-based imaging method	MICS (MACSima)	Conjugate		Author ORCID	0000-0002-1297-9725
Author ORCID	0000-0002-1297-9725	Conjugate		Author ORCID	0000-0002-1297-9725	Vendor Affiliation	www.miltenyibiotec.com
Vendor Affiliation	www.miltenyibiotec.com	Author ORCID	0000-0002-1297-9725	Vendor Affiliation	www.miltenyibiotec.com		



## 1. Concept of the extended OMAP table structure

The table below shows the concept of the "OMAP+" table structure, an extended version of the OMAP table, containing additional categories which allow adding relevant information for spatial multiomics methods combining RNA transcript detection and antibody-based protein detection, such as the MACSima Imaging Cyclic Staining (MICS) technology. The blue highlighted section shows information on RNA detection, the yellow highlighted section is presenting information on antibody-based protein detection. Columns are narrowed for visibility. The shown conceptual "OMAP+" table was generated for a data set acquired on a palatine tonsil sample.

omap_id	reagent_type	probe_type	probe_numbers	probe_sequence	mutation_specific	uniprot_accession_number	epitope	hgnc_id	hgnc_symbol	host	isotype	clonality	clone_id	vendor	catalog_number	lot_number	recombinant	concentration_value	dilution_factor	conjugate	rrid	name	instrument	tissue_preservation	cycle_number	fluorescent_reporter	protocol_doi	manuscript_doi	author_orcid	vendor_affiliation	core_panel	rationale	organ	organ_uberon	cell_line	cell_line_ontology_id	cell_line_reporter				
	RNA probe	padlock probe	P29070					HGNC:9544	PSMB4	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	1	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	PSMB4 contributes to the complete assembly of the 20S proteasome, which is palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P01730					HGNC:1678	CD4	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-114-702		Yes	50	FTIC	AB_2728763	MCS	MACSima	FFPE	2	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	CD4 is expressed by subsets of T cells. This marker is also expressed on a subset palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P11336					HGNC:1787	CDNA2	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-125-975		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	1	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	CDNA2 encodes for two proteins, p16 and p14ARF, both of which regulate palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P11336					HGNC:7315	MSA1	human	IgG1	Monoclonal	REAL1142	Miltenyi Biotec	130-128-467		Yes	50	FTIC	AB_2905078	MCS	MACSima	FFPE	2	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	MSA1 is expressed by epithelial cells and used to distinguish the epithelium palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P42771	CDN226				HGNC:1387	CDN226	human	IgG1	Monoclonal	REAL1142	Miltenyi Biotec	130-128-467		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	2	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	CDN226 is expressed on the surface of all B cells, beginning as the pro-B stage, palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P04226					HGNC:6770	SMAD4	human	IgG1	Monoclonal	REAL1142	Miltenyi Biotec	130-128-467		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	2	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	SMAD4 is a transcription factor and marker of the TGF-beta signaling pathway palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P04226					HGNC:3430	ERBB2	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	3	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	ERBB2 (Her2) is a receptor tyrosine kinase, and functions as an oncogene in palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P04226					HGNC:12980	VEGFA	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	3	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	VEGFA is a growth factor involved in vascular permeability and angiogenesis, palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P04234					HGNC:1673	CD33	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	3	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	CD33 is an essential marker of T cells. This marker represents a core panel palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P01136					HGNC:7353	MIC	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	3	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	MIC is a transcription factor, which regulates cell cycle progression and adopt palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P19622					HGNC:11529	EPICAM	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	4	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	EPICAM is expressed by epithelial cells and used to distinguish the epithelium palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P08575					HGNC:9666	CD45	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	4	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	CD45 is expressed by all hematopoietic cells. This marker represents a preprint palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P46013					HGNC:7107	MKI67	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-128-467		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	4	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	Ki67 is essential for assessing cell division and proliferation. This marker negates palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P04537					HGNC:11998	TP53	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	4	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	p53 is a regulatory protein, which is often mutated in many human cancer type palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P12004					HGNC:8729	PCNA	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	5	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	PCNA is a cofactor of DNA polymerase delta and found in the cell nucleus. It palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P22771					HGNC:4172	GATA3	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	5	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	GATA3 is a transcription factor and controls a variety of biological processes, palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P06484					HGNC:9588	PTEN	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	5	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	PTEN is a phosphatase in the Akt signaling pathway, which plays a role in cell palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P01857					HGNC:5525	IGHG1	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	5	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	IGHG1 is expressed by plasma cells and, together with IGHK, is used to detect palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P03033					HGNC:3258	EGFR	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	5	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	EGFR is a transmembrane receptor. Mutations can lead to overexpression in palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P01116					HGNC:6407	KRAS	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	6	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	Kras is a protein involved in cell proliferation and differentiation. It is frequently palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P10415					HGNC:390	Bcl2	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	6	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	BCL2 is an anti-apoptotic protein highly expressed in mantle zone B cells and palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P01334					HGNC:5716	Ki6C	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	6	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	Ki6C is expressed by plasma cells and, together with Ki67, is used to detect palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	P34810					HGNC:1693	CD88	human	IgG1	Monoclonal	REAL1024	Miltenyi Biotec	130-117-200		Yes	50	FTIC	AB_2857414	MCS	MACSima	FFPE	7	https://doi.org/10.1101/2023.10.27.564191	0000-0002-1297-9725	yes	Y	CD88 is expressed by many subsets of myeloid cells (weakly body macrophage palatine tonsil	UBERON:0002373											
	RNA probe	padlock probe	OR024C					HGNC:14582	LAMP3	human	IgG1																														