The Development and Evaluation of Highly Specific Monoclonal Antibodies against Human LGR5

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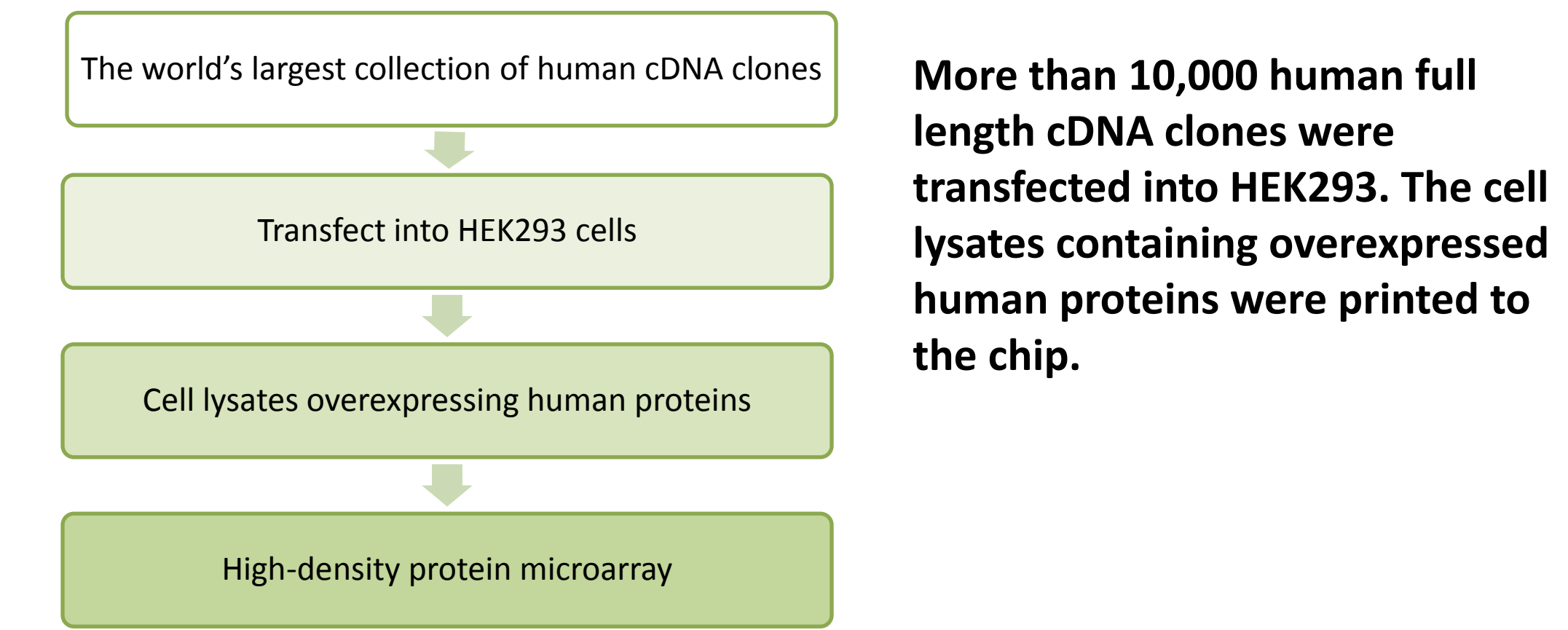
Introduction

The cancer stem cells is a subpopulation of cancer cells which is responsible for cancer initiation, development and metastasis. The identification of cancer stem cells is considered as one of the most important objectives for clinical diagnostic and therapeutic purposes. Research evidences indicated that Leucine-rich repeat containing G-protein-coupled receptor 5 (LGR5) is one of the biomarkers specifically expressed on colon cancer stem cells. LGR5 can be activated by extracellular Wnt signaling molecules and this results in cancer development. Therefore, it is critical to develop a LGR5 antibody with great sensitivity and specificity to detect endogenous LGR5 expression in cancer stem cells.

In this research, several LGR5 mouse monoclonal antibodies were generated for multiple applications. These LGR5 antibodies can be used for flow cytometry application and proved to be highly specific with our proprietary high density protein microarray chip assay. These newly generated LGR5 antibodies could provide new tools for earlier diagnosis and therapeutic guidance on colon cancers.

OriGene Protein Microarray Chip for antibody specificity identification
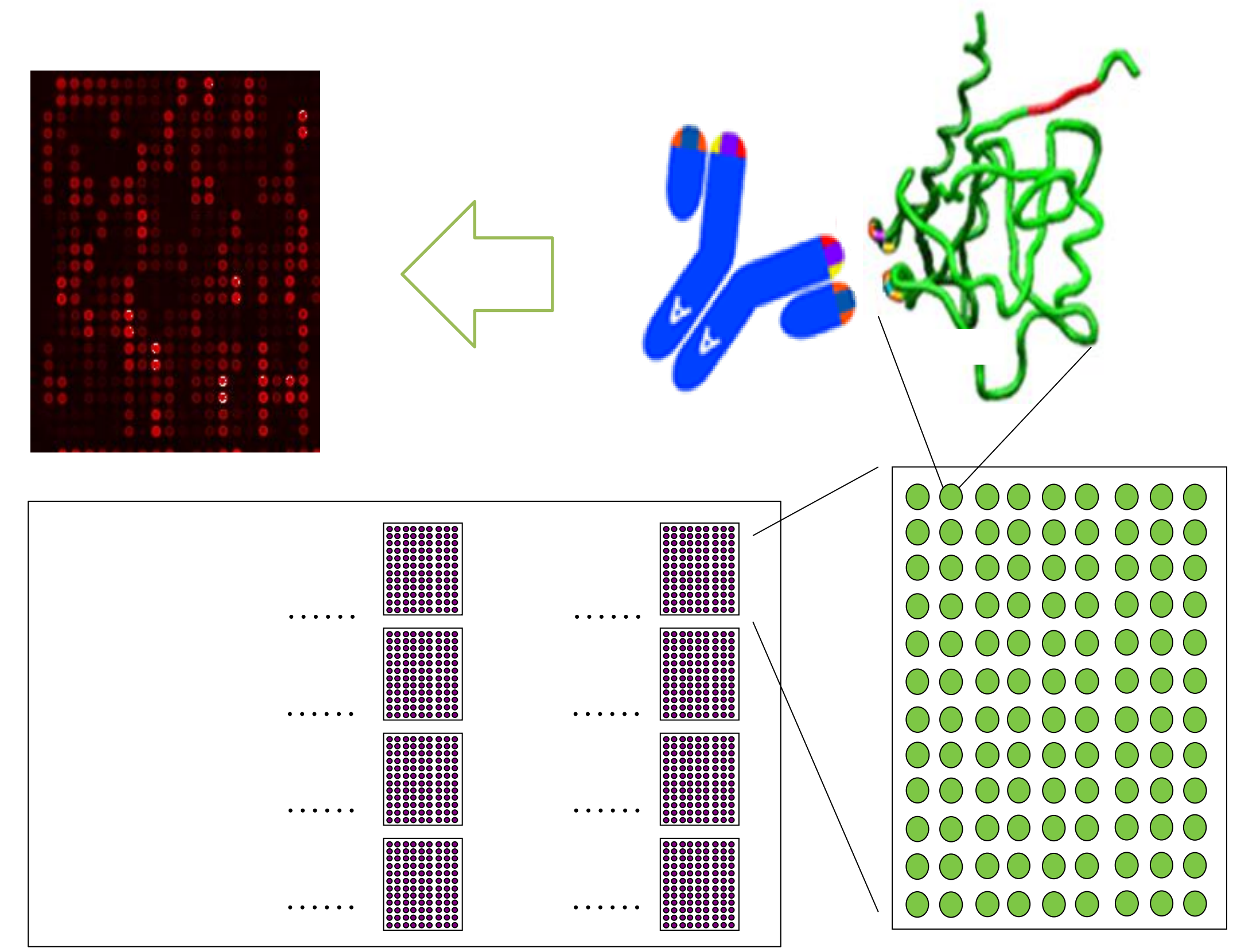
OriGene developed a high-density protein microarray to test antibody-antigen interaction in a high-throughput manner

The world's largest collection of human cDNA clones

Transfect into HEK293 cells

Cell lysates overexpressing human proteins
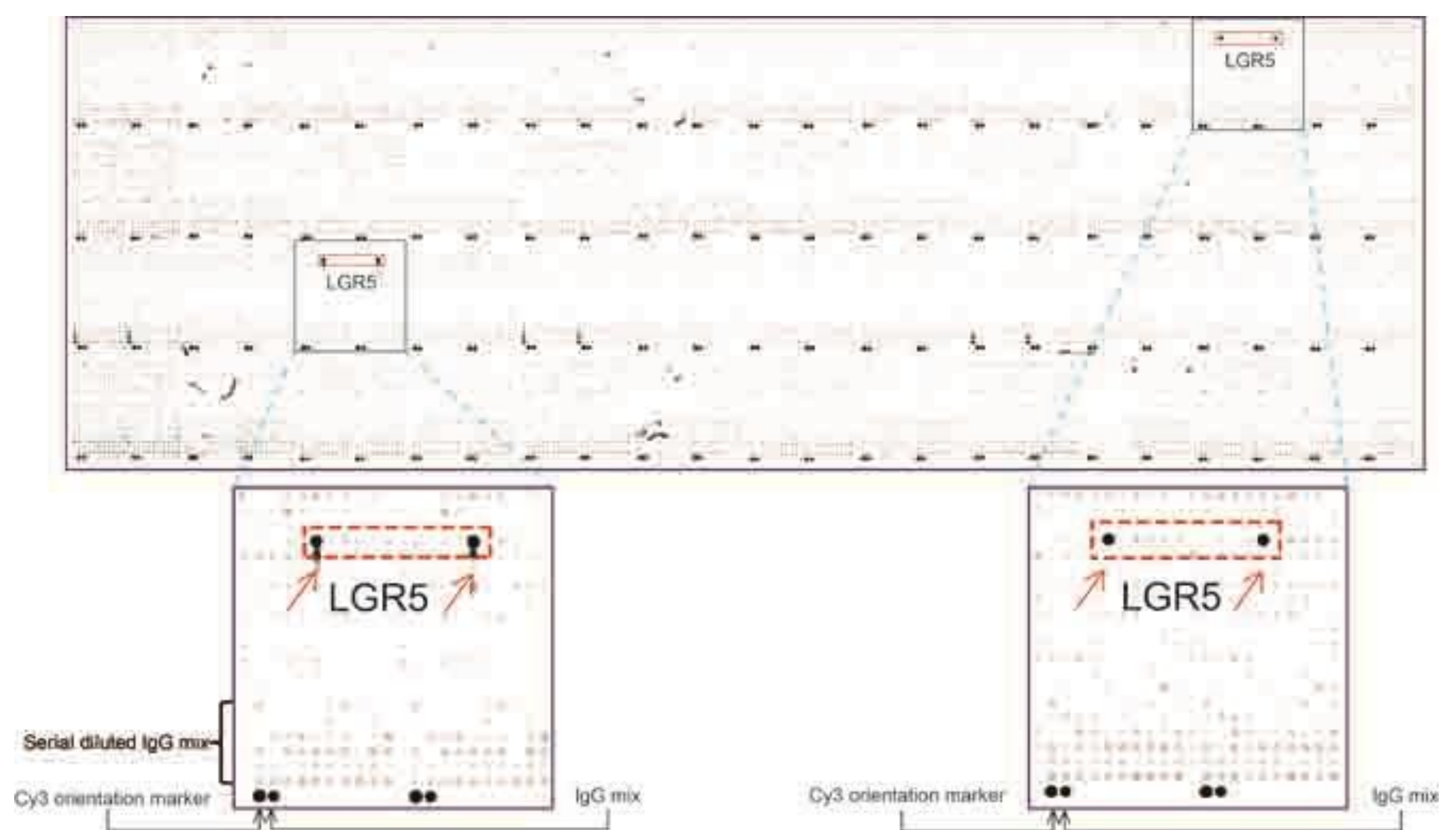
High-density protein microarray

More than 10,000 human full length cDNA clones were transfected into HEK293. The cell lysates containing overexpressed human proteins were printed to the chip.
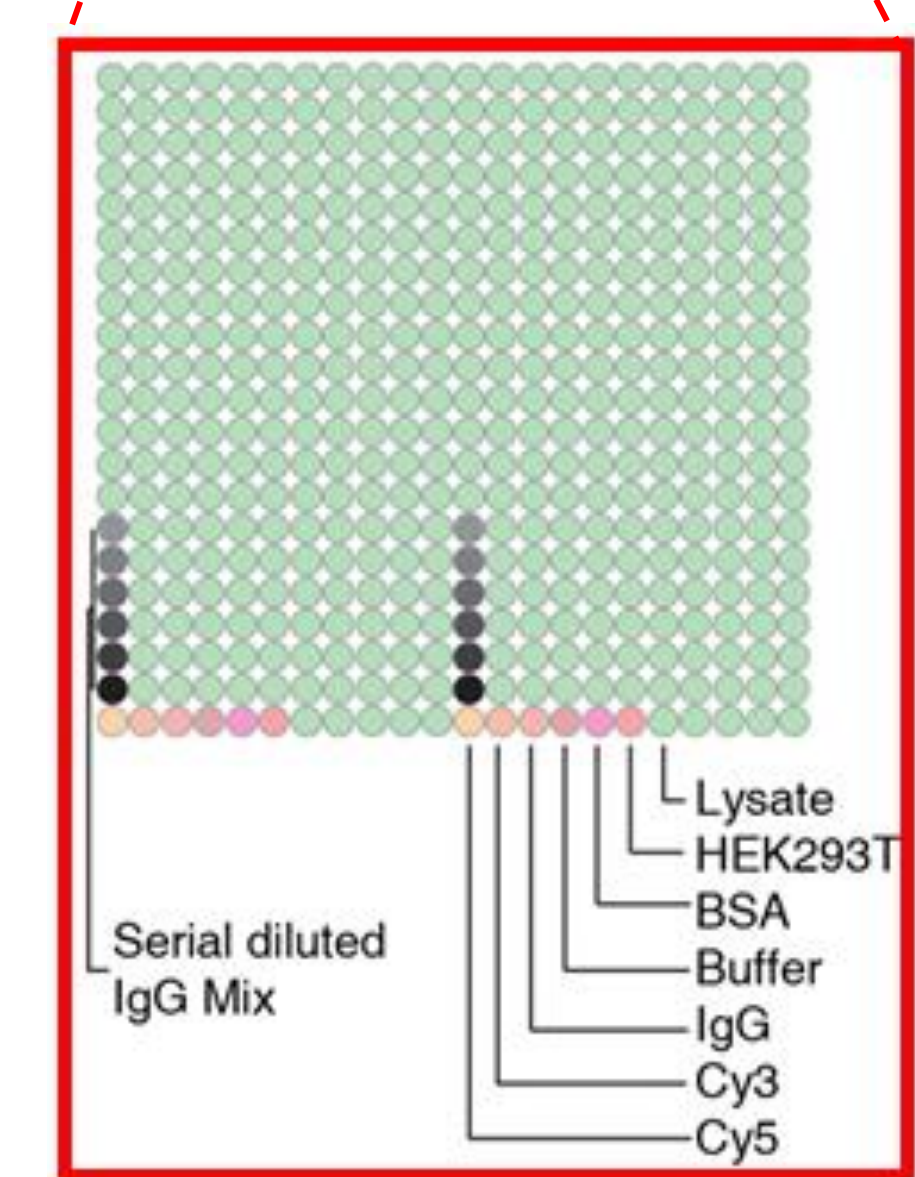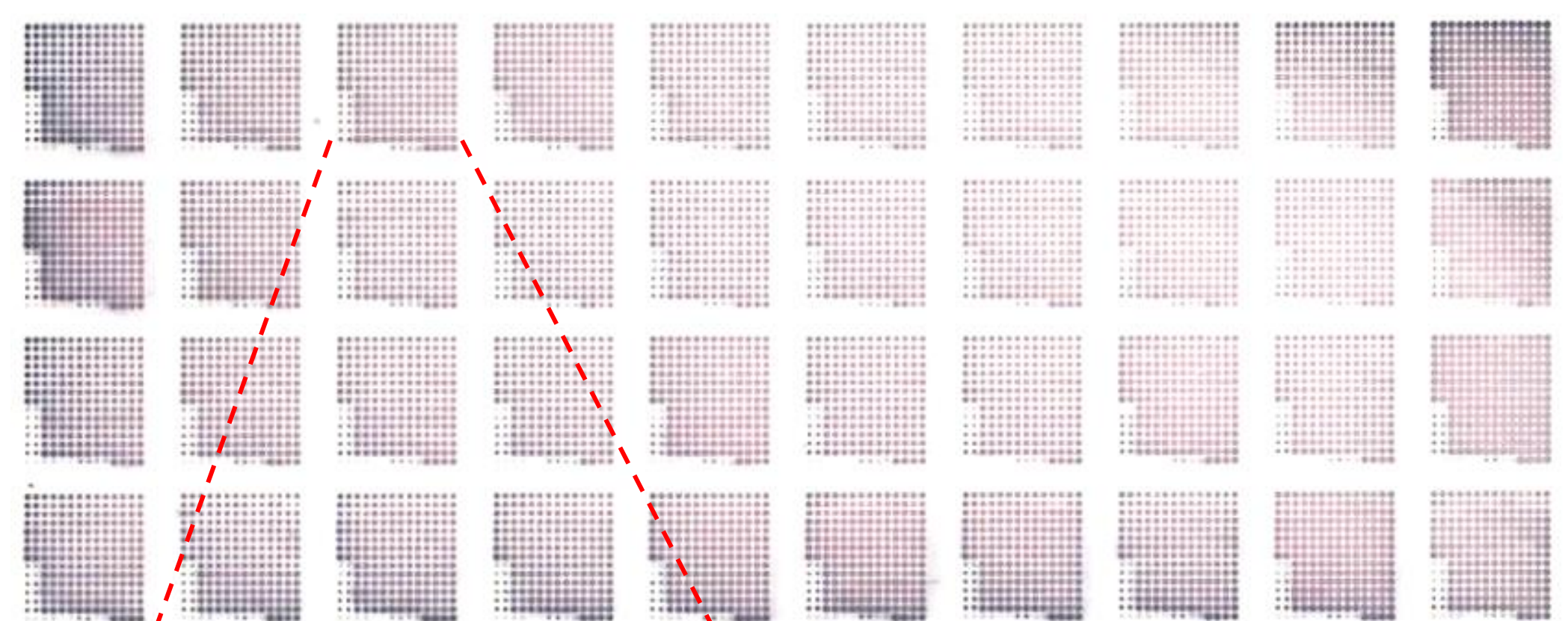
Multiple kinds of controls are also printed on each sub-array of the microarray chip. These controls help eliminate the false negative results during data analysis and normalize the expression levels of different proteins.

For more information, please check OriGene protein microarray video at Youtube https://www.youtube.com/watch?feature=player_embedded&v=e4-xn9x2pck

The newly developed LGR5 monoclonal antibody (UMAB212) is highly specific

UltraMAB anti-LGR5 mouse monoclonal antibody (UMAB212) on protein microarray chip. The positive reactive proteins are highlighted with two red arrows in the enlarged sub-arrays.

UMAB212 recognizes both human and mouse LGR5, but does not cross-react with LGR4 and LGR6
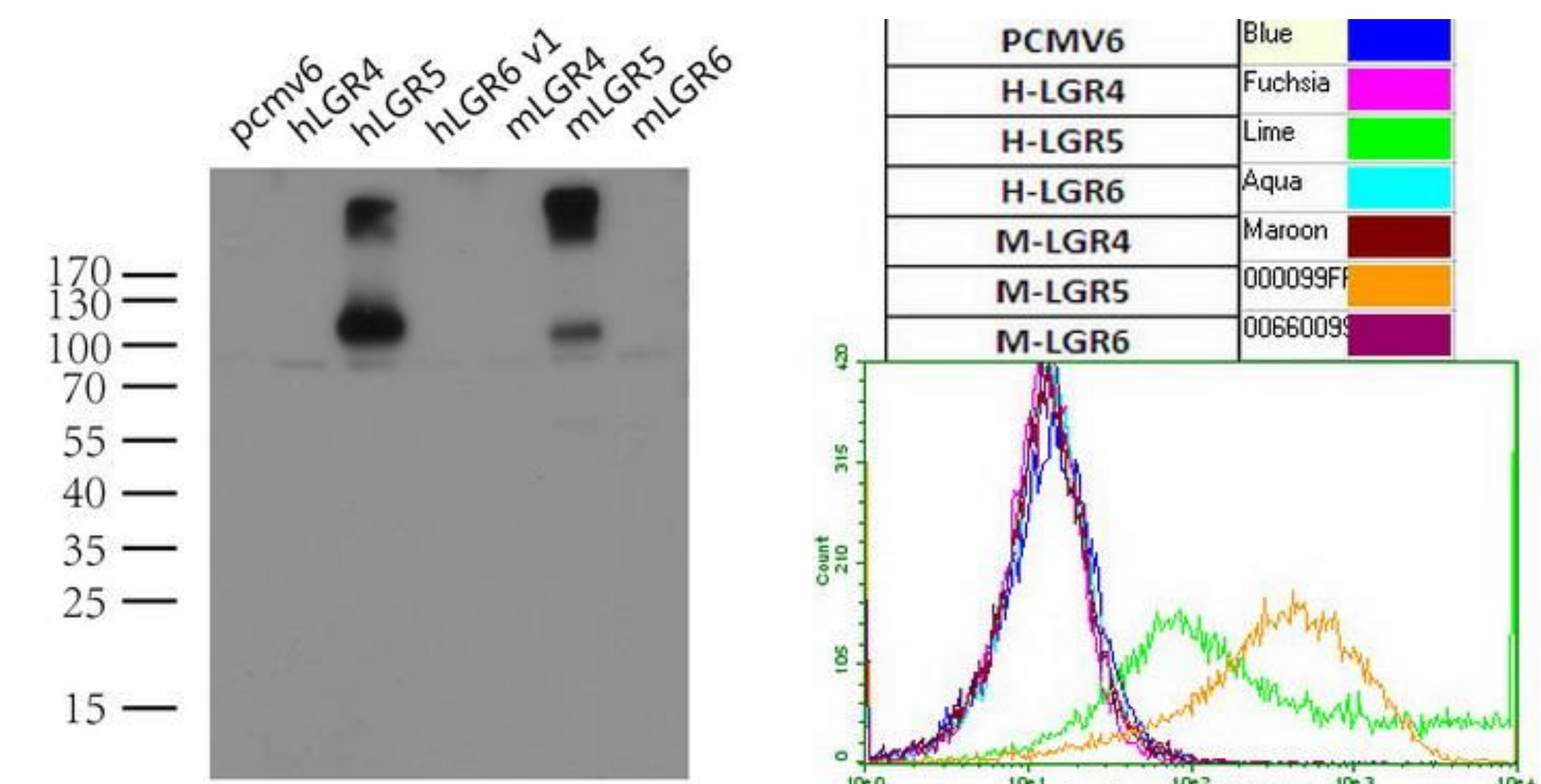
Western blot analysis of extracts from seven different cDNA transiently transfected HEK293T cell lysates by using UMAB212

Flow cytometric Analysis of HEK293T cells transiently transfected with human or mouse LGR4, human or mouse LGR5, human or mouse LGR6, or control vector pCMV6-Entry using UMAB212

UMAB212 for multiple applications (WB, Flow cytometry, and IF)
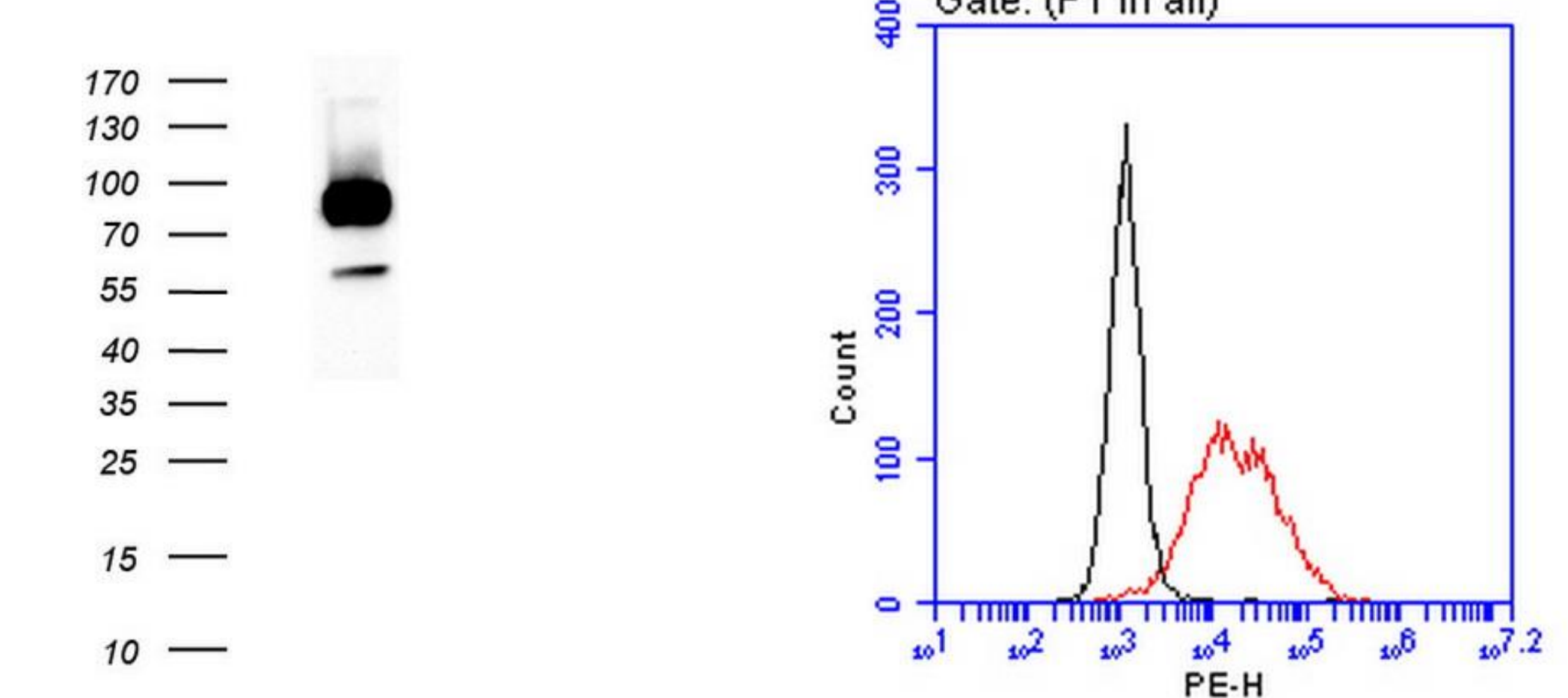
NIH-3T3 (Left lane) or stable expressed LGR5-3T3 cell lysate (right lane) were immunoblotted with anti-LGR5 UMAB212

Flow cytometric analysis of the live stable expression of LGR5 in NIH3T3 cells using anti-LGR5 antibody (UMAB212) (Red) vs negative CTL (Black)
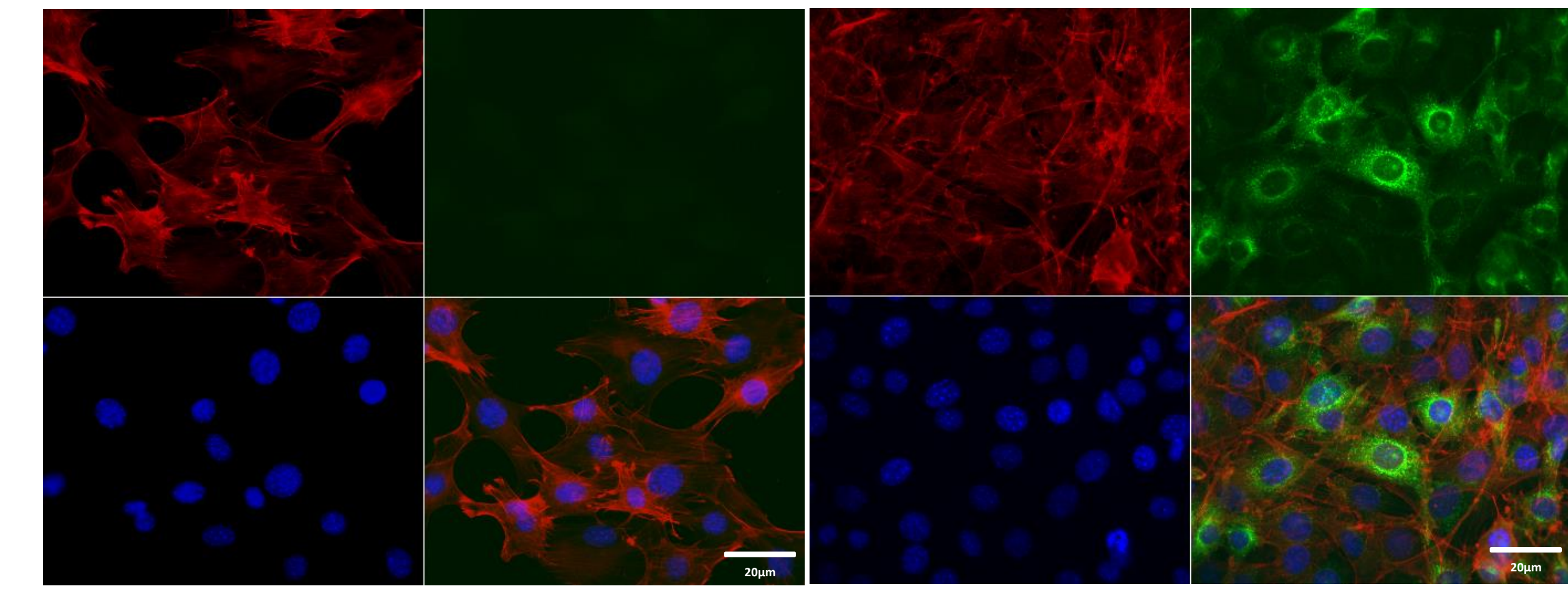
Immunofluorescence staining of 3T3 cells (left panel) or LGR5 stable expression 3T3 cells (right panel) using LGR5 antibody (UMAB212, green), Phalloidin (red) and DAPI (blue)

Conclusions

1. We developed a highly specific LGR5 UltraMAB with our high density protein microarray chip technology.
2. UMAB212 does not cross-react with other LGR family members such as LGR4 or LGR6.
3. UMAB212 recognizes mouse LGR5.
4. UMAB212 can be used for multiple immunoassays (Western blot, Flow and IF).

Current UltraMAB availability (http://www.origene.com/UltraMAB/)

ABAT	ADIPOQ	AFP	ALDH1L1	ALX4	AMACR	BBOX1	BID
BMP4	BTLA	BUB1B	C14orf166	CA12	CD19	CD1C	CD2
CD20	CD3E	CD4	CD40	CD44	CD5	CD68	CD80
CDH1	CDH2	CHGA	CRABP2	CTAG1B	CYP1A2	DDX56	DEF6
DES	DOCK2	EGFR	EPCAM	ERCC1	ERG	FCER2	FCGR1A
FCGR2A	FGF21	FOLH1	FOXP1	FTCD	GBA	GEMIN8	GFAP
GLI1	HADH	HAO1	HE4	HEMGN	HER2	HMBS	HP
IDO1	IRF6	JUN	KI67	KLK8	KRT18	KRT19	KRT20
KRT7	KRT8	L1CAM	LGALS3	LGR5	MADCAM1	MCAM	MEF2C
MET	MGMT	MLF1	MLH1	MMP7	MRPS34	MRPS7	MSI1
MSMB	MUC1	NBN	NCAM1	NKX2-1	NKX3-1	NME1	P53
PARN	PBX1	PDCD1	PDSS2	PECAM1	PCR	PROCR	PRPH
PSMA6	RAPGEF1	RRM1	S100A9	S100P	SDCBP	SERPINB4	SLC7A8
SOX5	SQSTM1	ß-Catenin	SYP	SYT4	TNFRSF18	TNNC1	TNNT2
TOP2A	TP53	TYMP	VBP1	VIM	VSNL1	WIBG	XPF
XRCC1	ZSCAN18						

References

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