

# Equipping pooled CRISPR screens with *in situ* tracking of individual sgRNA

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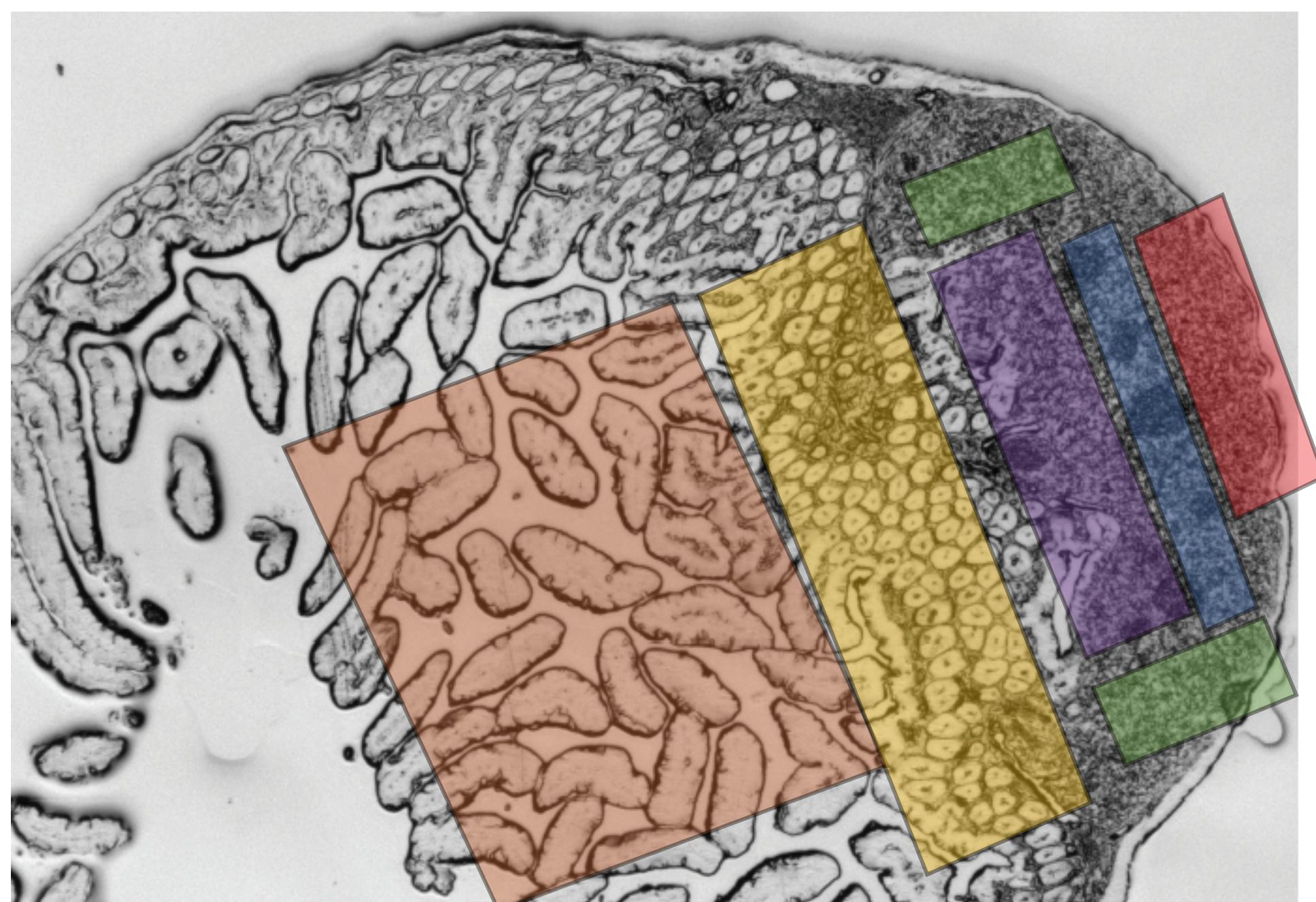
## Introduction

Tissues are highly organized and complex structures that are regulated by their cellular neighborhoods. Pooled CRISPR screening has emerged as a powerful tool for uncovering the key players behind tissue behavior. Pooled CRISPR screens can simultaneously induce several genetic perturbations *in vivo* by leveraging multiple single guide RNAs (sgRNAs) within a single mouse to generate a chimeric organism. However, quantifying the distribution of sgRNAs enriched in a tissue via scRNA-sequencing requires tissue dissociation, consequently destroying the spatial context of those sgRNAs, and losing critical insights with regards to direct cell-to-cell interactions, compartment entry, and regional cellular composition.

Here, we propose an adaptation to pooled CRISPR screening, termed CRISPR-MERFISH that will not only track the sgRNAs expressed within individual cells but will also quantify the transcriptome of those cells, creating a near-comprehensive gene expression map for the entire tissue. (MERFISH: Multiplexed Error Robust Fluorescence *in situ* Hybridization)

To validate this approach, we will target genes involved in B cell affinity maturation within murine Peyer's Patches (lymphatic tissues within the small intestine) and use CRISPR-MERFISH to determine all cell types and sgRNA carried by B and T lymphocytes. We will then use enrichment or de-enrichment of sgRNAs within specific cell populations to test the role of these sgRNA targets in lymphocyte location within Peyer's Patches.

## Tissues have specialized subcompartments

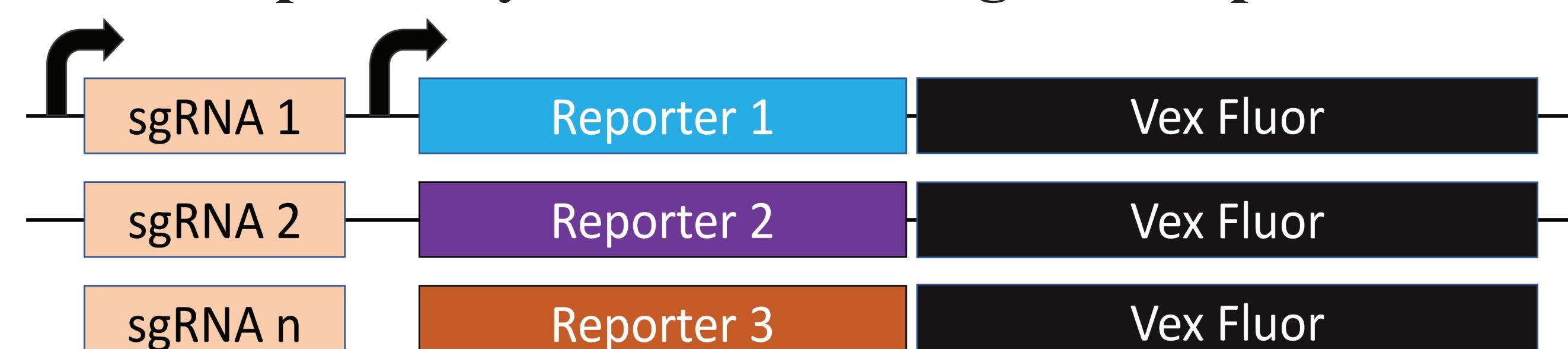


Peyer's Patches contain distinct regions that are differentially regulated

## Methods and Results

MERFISH is a highly scalable approach to single-molecule RNA fluorescence *in situ* hybridization (FISH) capable of tracking thousands of unique RNA targets using fluorescent probes.

## Reporter system will infer sgRNA expression



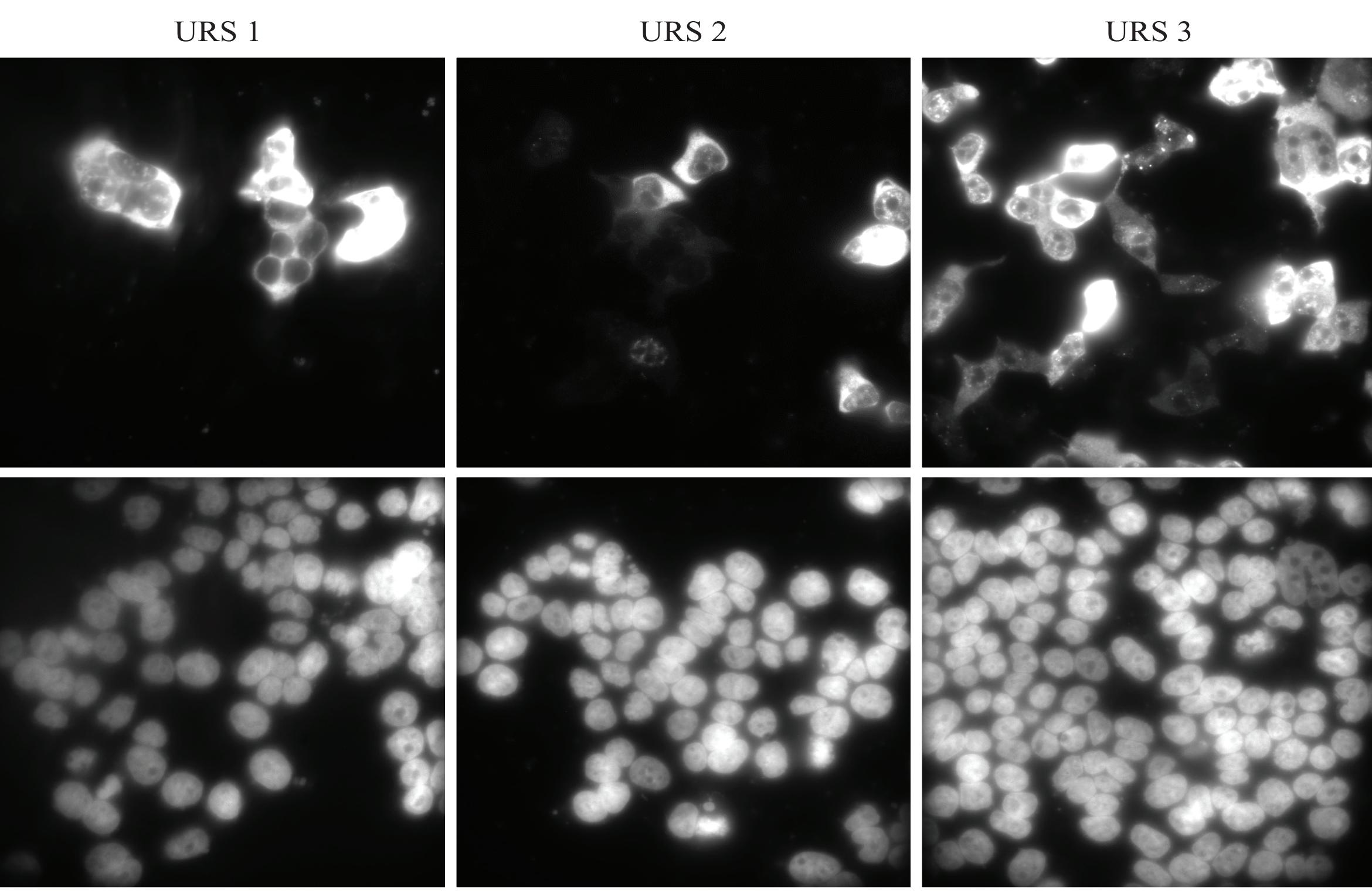
## Methods and Results

### What sgRNAs will validate our approach?

Target Gene	Gene Function	# sgRNAs
Ccr6	SED localization	4
Cxcr5	LZ localization	4
Cxcr4	DZ localization	4
Ebi2	Outer follicle localization	4
S1pr2	GC retention	4
Ccr9	Ileum homing	4
vil1	N/A in immune cells	3
Non-target	N/A	3

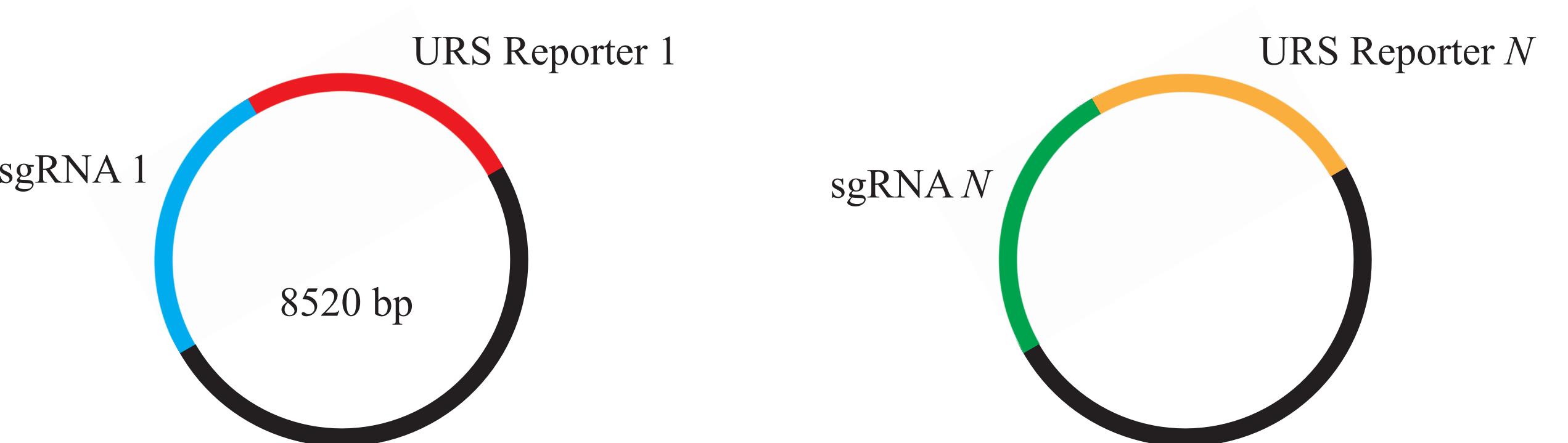
### What will we use as reporters?

Untranslated Random Sequences (URS): 500bp, 45-55% GC, no ATG

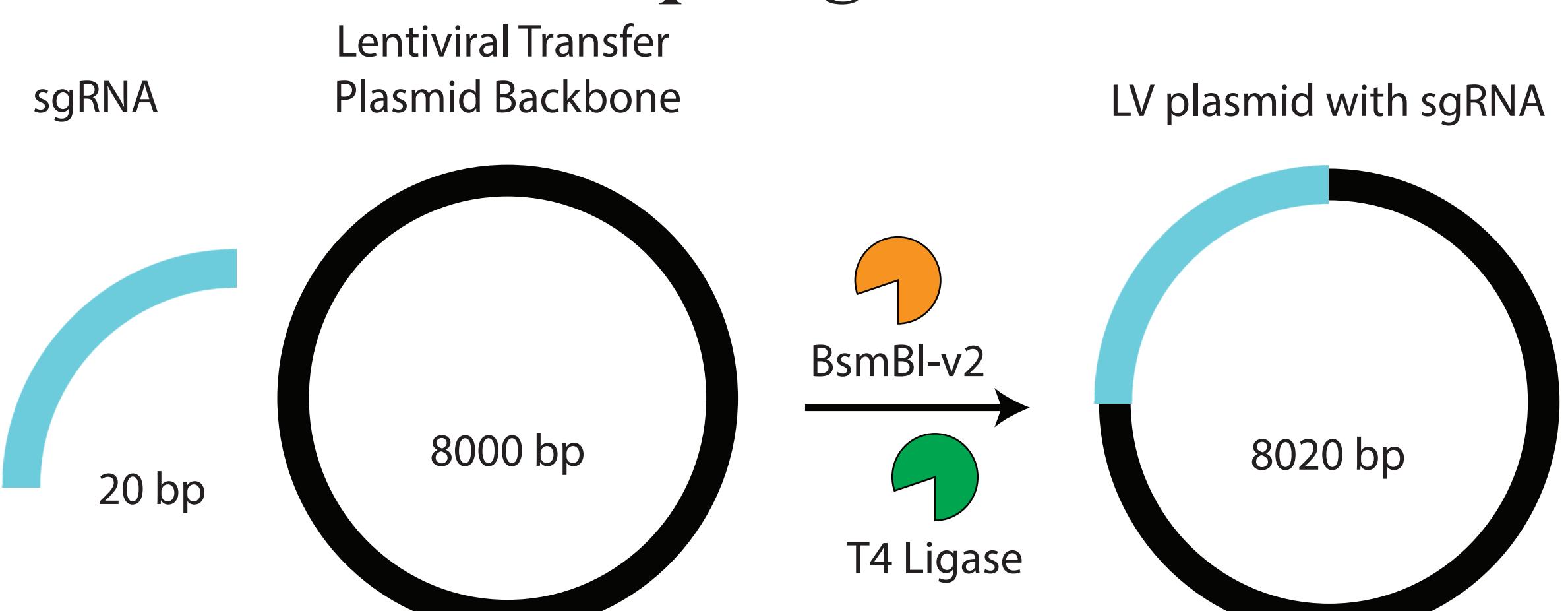


Transfection shows expression and detection of URS via MERFISH in HEK293 cells

## Goal 1: Design the lentiviral transfer plasmids

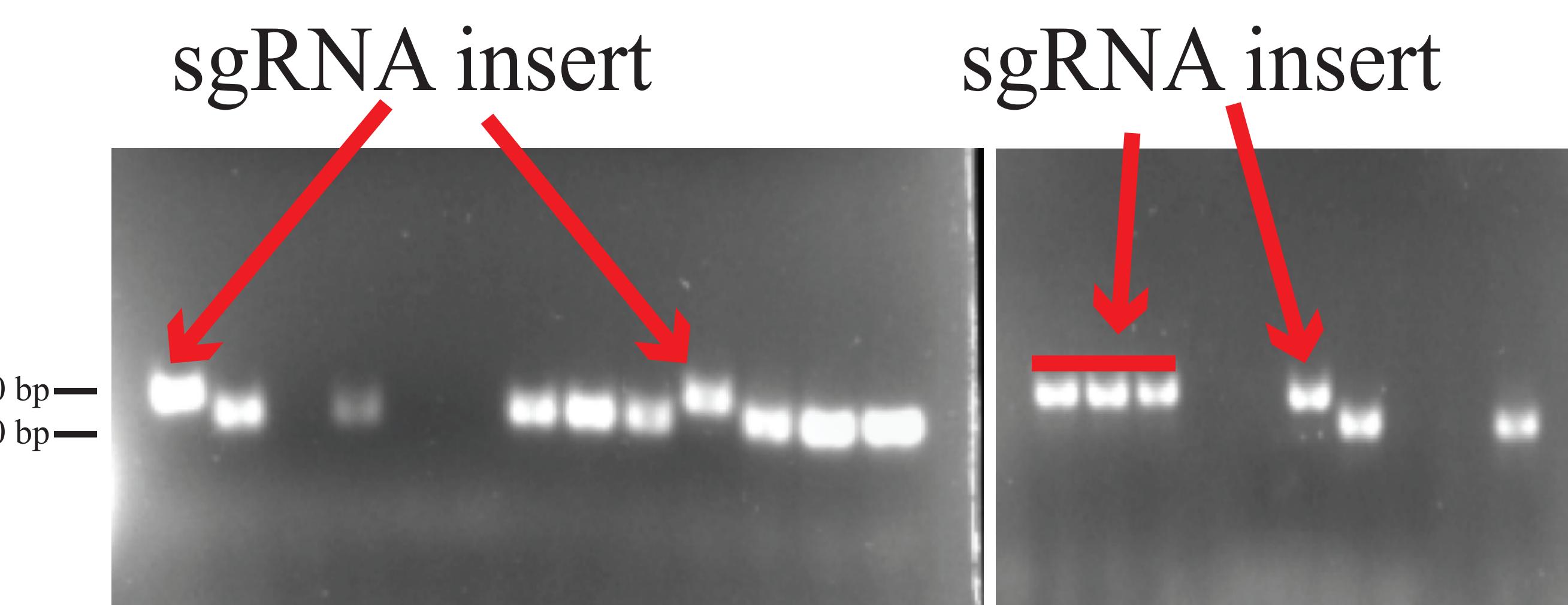


## Goal 2: Insert 30 unique sgRNAs

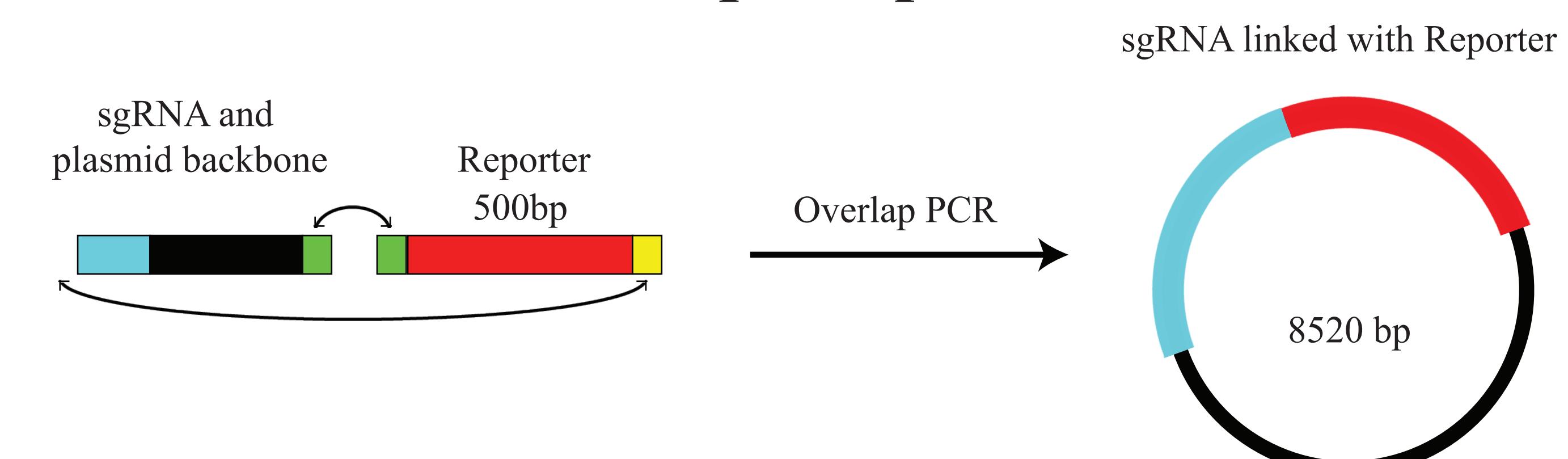


## Methods and Results

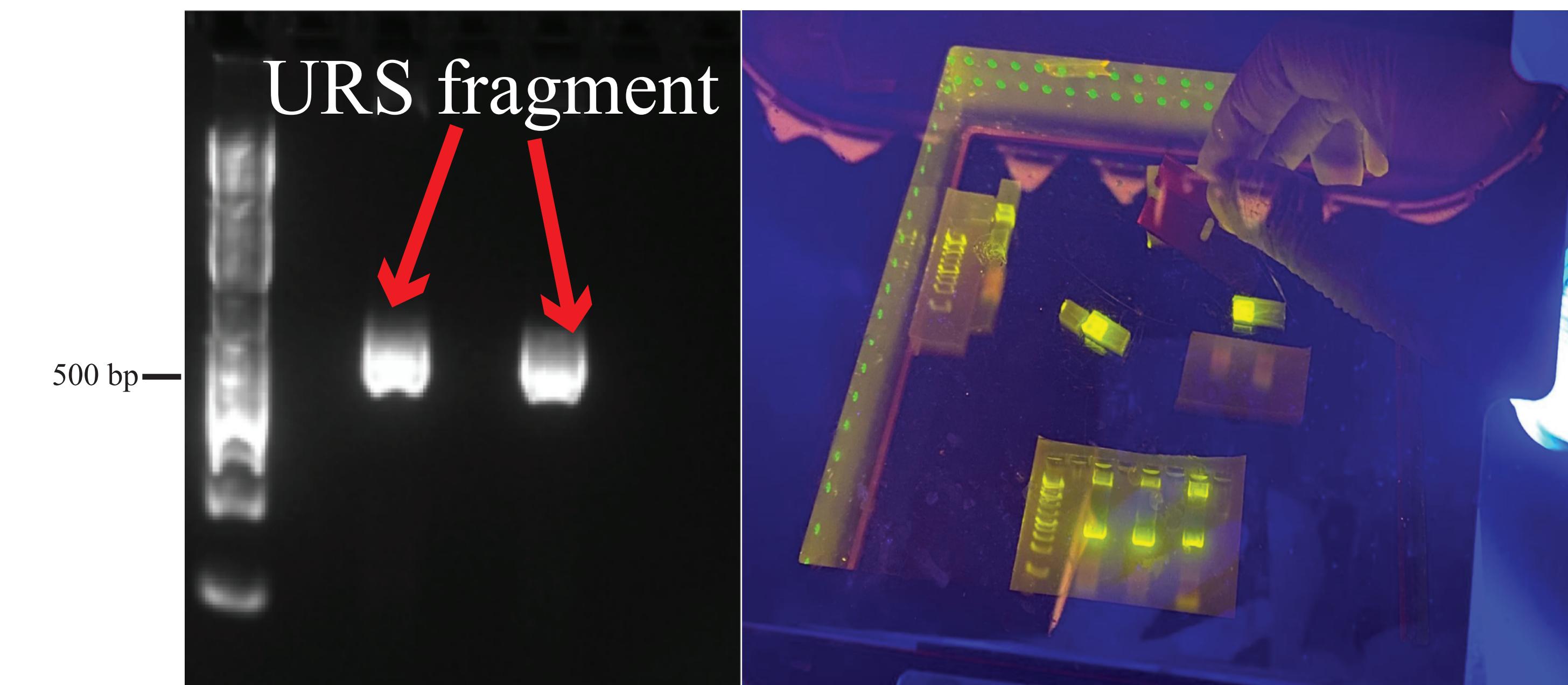
### Colony PCRs indicate sgRNA insertion



## Goal 3: Insert 30 unique reporters



## PCR amplification of URS fragments for assembly



## Conclusion

Development of CRISPR-MERFISH has wide reaching applications for all biological fields and will greatly strengthen the hypothesis generated from traditional pooled CRISPR screens. CRISPR-MERFISH has the potential to inform us about the genes involved in B cell maturation inside the Peyer's patch and better understand the relationship between immune cells and gut microbiome in a spatial context.

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