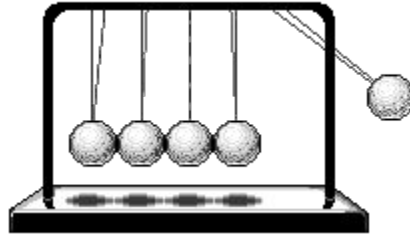


Perturbation assays

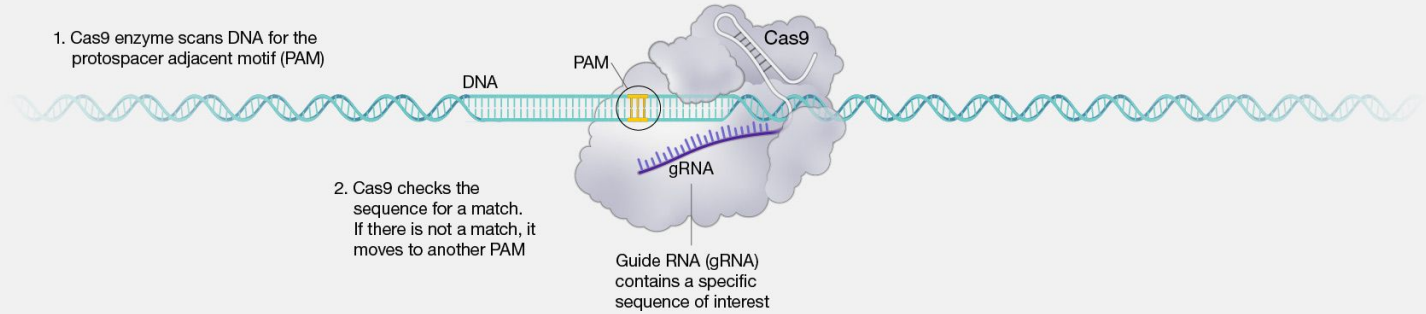


Michael Love

Differential expression:
case vs control,
drug vs control

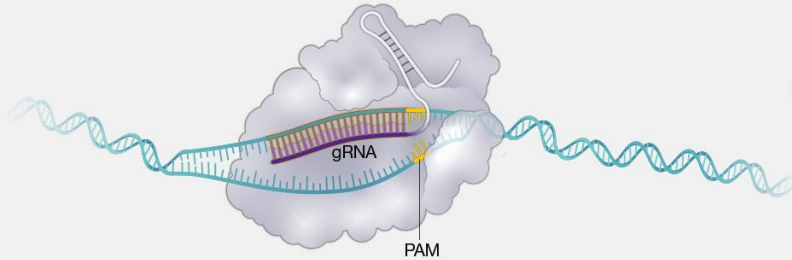
CRISPR-Cas9

1. Cas9 enzyme scans DNA for the protospacer adjacent motif (PAM)

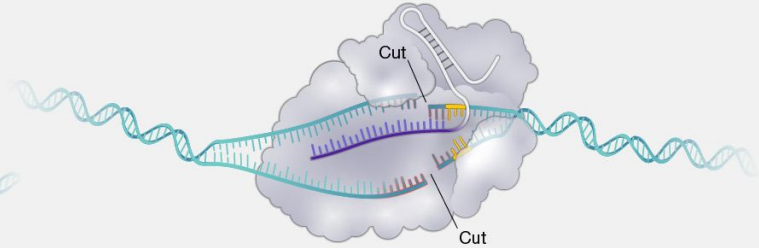


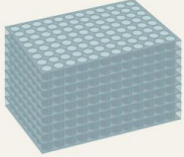
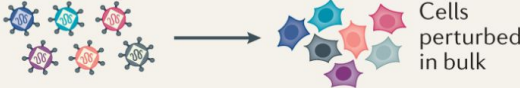
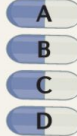
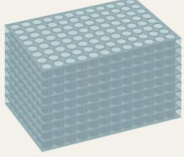
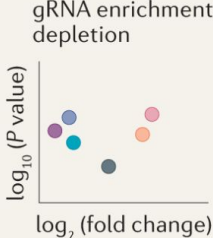
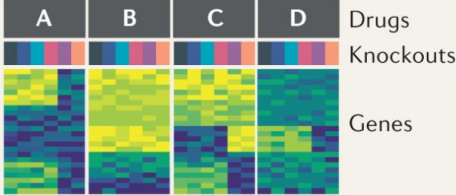
2. Cas9 checks the sequence for a match. If there is not a match, it moves to another PAM

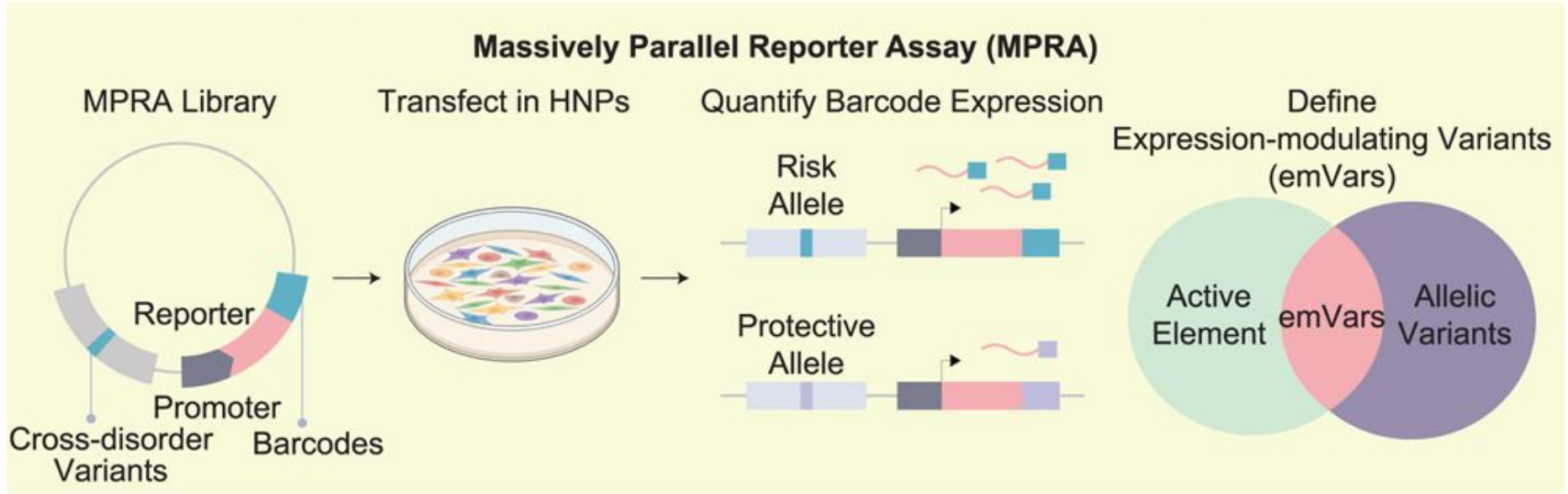
3. Cas9 gRNA hybridizes to matching DNA once identified



4. Cas9 cuts DNA

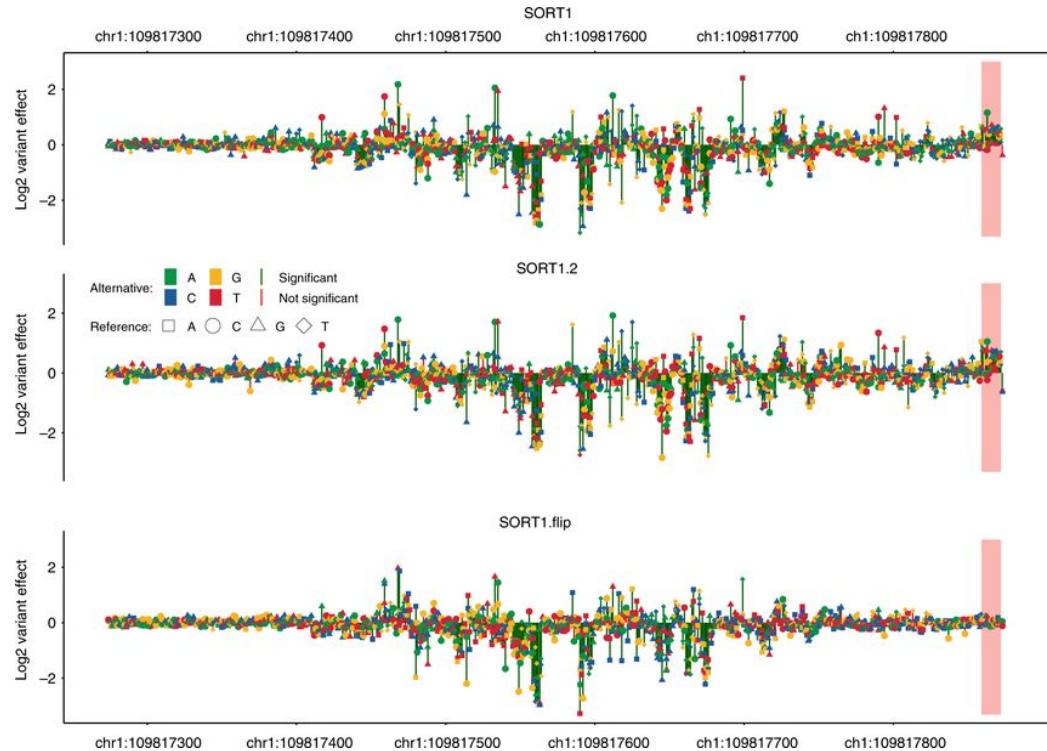


	Pooled CRISPR screen	Arrayed CRISPR screen
Model Cell lines, primary cells, organoids, model organisms (in vivo screens)	Cas9-expressing cells in bulk 	Cas9-expressing cells in separate wells 
Perturbation CRISPR knockout, interference, activation, base editing, prime editing	CRISPR gRNA library 	Cells perturbed in separate wells 
Challenge Cell survival and proliferation, drug treatment and resistance, virus/pathogen infection, metabolic challenges	 Cells challenged in bulk (for example with a drug) 	Cells challenged in separate wells (for example with a drug)  
Read-out Sequencing-based counting of gRNA frequencies, single-cell RNA sequencing, multi-omics profiling, imaging	 gRNA sequencing 	Molecular phenotyping 



“Saturation mutagenesis”

Wild type	M	W	I	K	I	P	E	D	I	A	*
I5A	M	W	I	K	A	P	E	D	I	A	*
I5C	M	W	I	K	C	P	E	D	I	A	*
I5D	M	W	I	K	D	P	E	D	I	A	*
I5E	M	W	I	K	E	P	E	D	I	A	*
I5F	M	W	I	K	F	P	E	D	I	A	*
I5G	M	W	I	K	G	P	E	D	I	A	*
I5H	M	W	I	K	H	P	E	D	I	A	*
I5K	M	W	I	K	K	P	E	D	I	A	*
I5L	M	W	I	K	L	P	E	D	I	A	*
I5M	M	W	I	K	M	P	E	D	I	A	*
I5N	M	W	I	K	N	P	E	D	I	A	*
I5P	M	W	I	K	P	P	E	D	I	A	*
I5Q	M	W	I	K	Q	P	E	D	I	A	*
I5R	M	W	I	K	R	P	E	D	I	A	*
I5S	M	W	I	K	S	P	E	D	I	A	*
I5T	M	W	I	K	T	P	E	D	I	A	*
I5V	M	W	I	K	V	P	E	D	I	A	*
I5W	M	W	I	K	W	P	E	D	I	A	*
I5Y	M	W	I	K	Y	P	E	D	I	A	*



Kircher, M., Xiong, C., Martin, B. *et al.* Saturation mutagenesis of twenty disease-associated regulatory elements at single base-pair resolution. *Nat Commun* **10**, 3583 (2019). <https://doi.org/10.1038/s41467-019-11526-w>