

Identify HIV Host factors with a CRISPR- Based Genome-Wide Functional Screening

Haoquan Wu

Beckman Research Institute, City of Hope

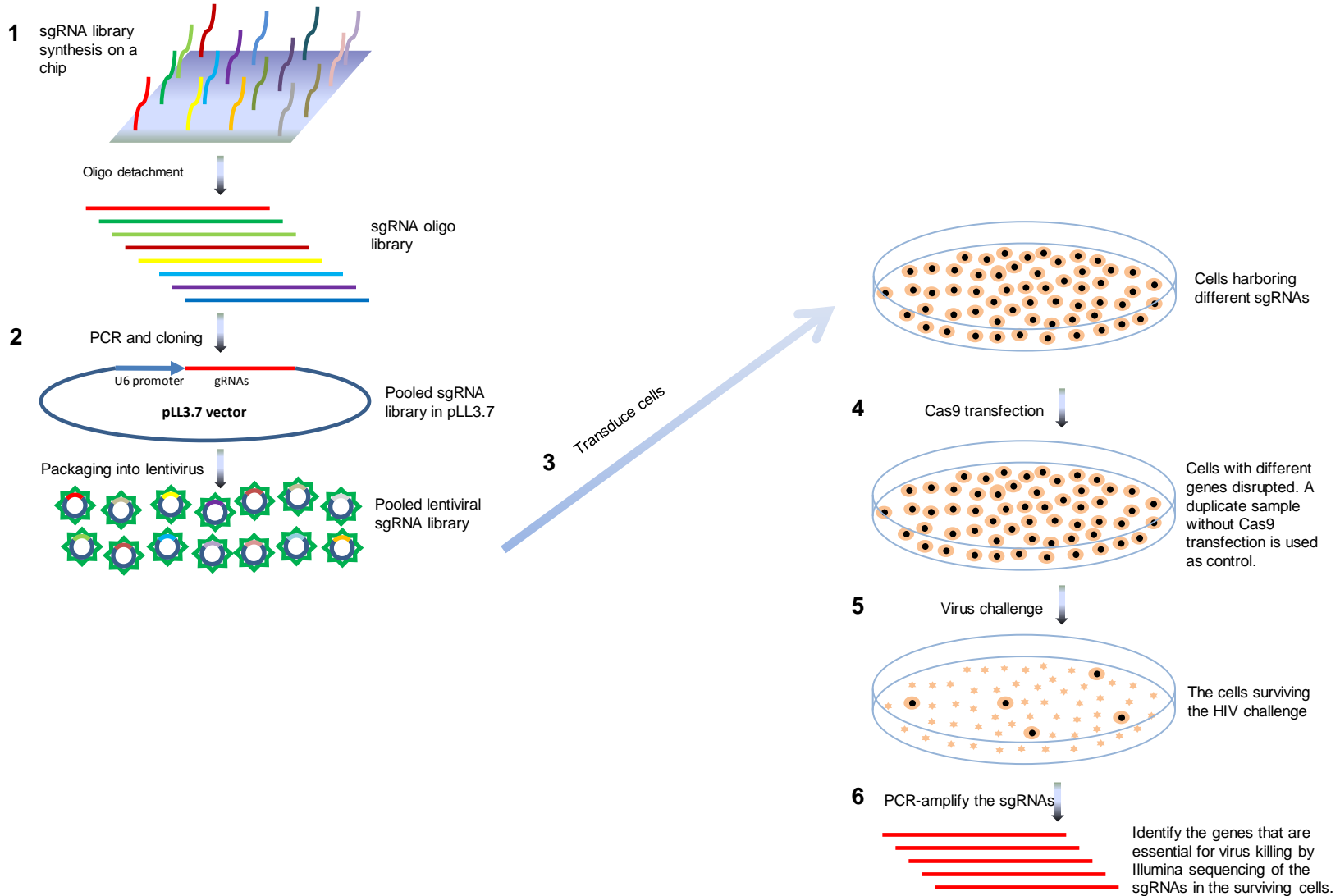
CRISPR-based genome-wide screen

- **CRISPR-Cas9 system**
 - Cas9 + sgRNA**
- **Risk**
 - 1. microRNA lab**
 - 2. very limited resources**

sgRNA library

- 4 sgRNA from each gene
- Total 77,406 unique single-guide RNA (sgRNA) sequences targeting 20,121 genes

Screen strategy



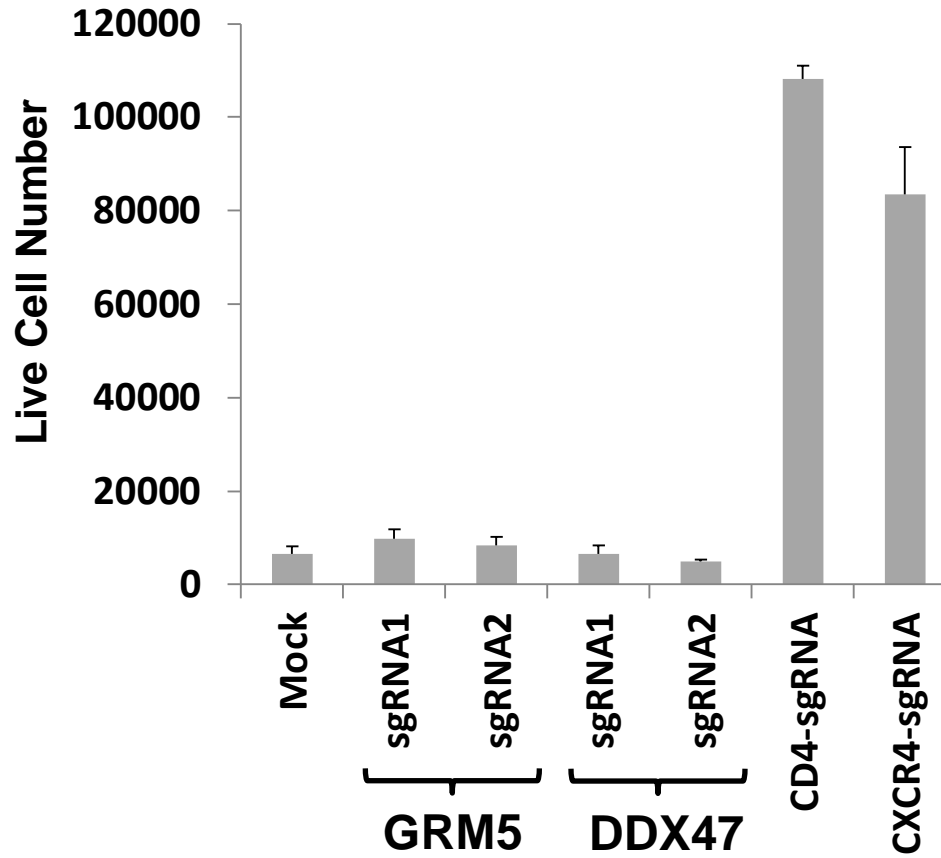
sgRNA reads

		Experiment #1		Experiment #2		Experiment #3	
sgRNA ID	Gene Name	Control	Cas9-treated	Control	Cas9-treated	Control	Cas9-treated
gRNA46855	TMEM60	27	55	363166	266372	55	59
gRNA20434	YIPF5	153035	88186	0	0	0	0
gRNA1107	RXFP2	116103	119191	0	0	0	0
gRNA41328	CXCR4	5	15	36	206538	20	1962
gRNA70805	WDR52	0	0	111167	48570	13	9
gRNA45099	RNF214	99200	57517	0	0	0	0
gRNA66065	BPIFB4	0	0	12	11	25703	79545
gRNA74168	IGFL1	0	0	3564	601	43123	49023
gRNA11409	ALG10B	0	0	7	8	69865	21718
gRNA31487	TOMM5	0	0	7	8	39545	45968
gRNA35493	C18orf25	0	0	7	5	55766	28356
gRNA6880	SLC47A1	0	0	10	8	53262	23444
gRNA17130	ANKRD6	0	0	10	9	40012	35482
gRNA44441	SOBP	0	0	11	5	40654	32311
gRNA56383	SSH1	0	0	7	10	17123	53137
gRNA53685	TCF12	0	0	28271	34952	5	7
gRNA9179	CXCL9	0	0	7	0	19314	38126
gRNA36730	AC008040.1	22	21	36810	13268	0	7
gRNA44750	SERF2	0	0	0	0	44478	5154
gRNA15589	LARP1	0	5	25967	17884	4	4
gRNA42401	VHLL	0	3	19532	17667	5	0
gRNA4442	PHF14	0	0	0	0	8548	28013
gRNA64511	LSM6	4	0	28513	7387	0	0

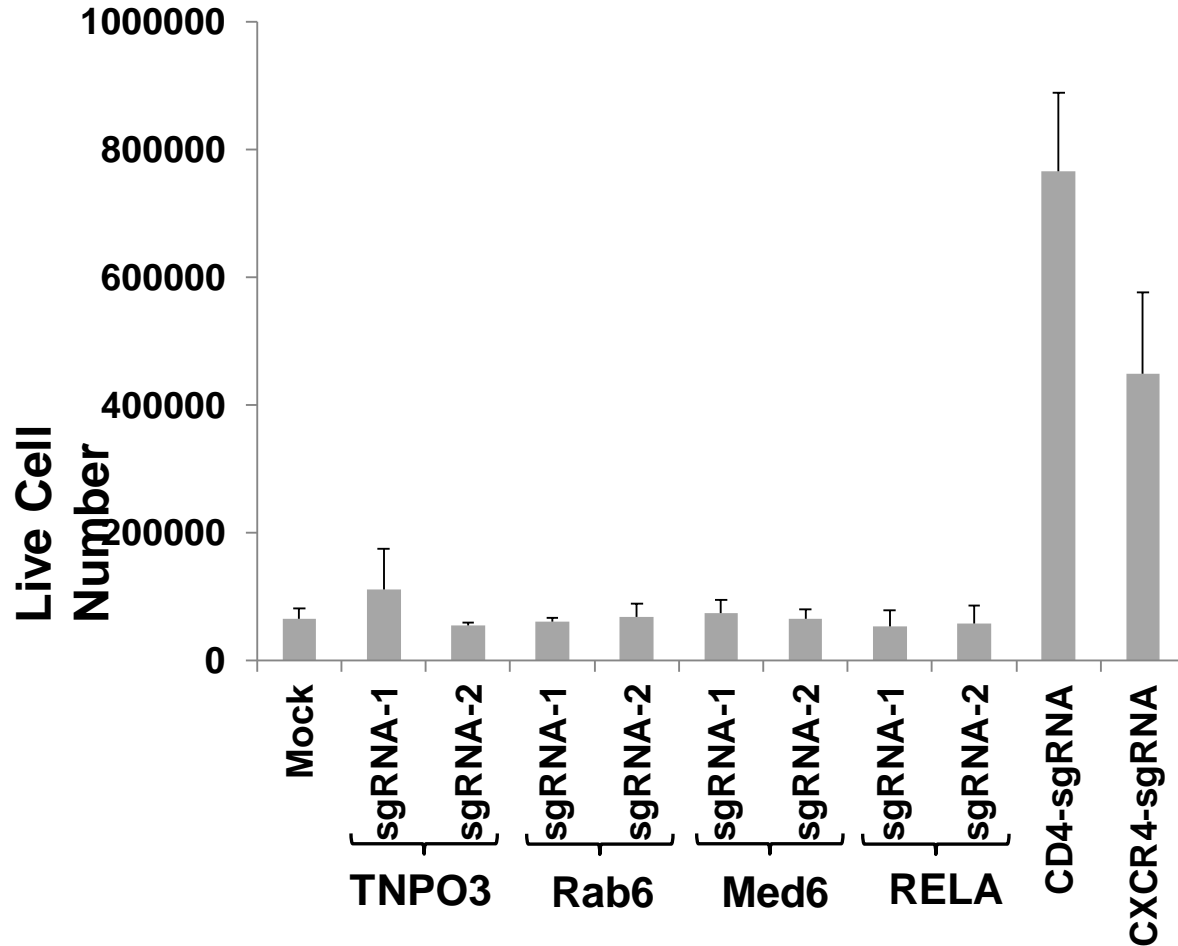
Genes with more than 1 hits

Number	Target Gene	gRNA ID	Target sequence	gRNA Reads			Total Hits
				Exp #1	Exp #2	Exp #3	
1	CXCR4	gRNA41328	<i>AGGGGACTATGACTCCATGA</i>	0	206538	1962	4
		gRNA9820	<i>AGATAACTACACCGAGGAAA</i>	10571	5373	0	
2	CD4	gRNA72942	<i>GCTCCTCCCAGCAGCCACTC</i>	0	0	13768	3
		gRNA13392	<i>AGTGCCTAAAAGGGACTCCC</i>	801	0	2961	
3	GRM5	gRNA7359	<i>AAAGATATGTCAGCGAAGGA</i>	0	238	3524	2
4	DDX47	gRNA31746	<i>ATCATTGGGCTTGCAGAAAC</i>	1032	0	761	2
5	TDRD3	gRNA54359	<i>ATCTGTAATCGCAGCATCCT</i>	0	590	820	2
6	CMBL	gRNA30252	<i>AGTTCAAGTCGAGCACATCA</i>	137	0	0	2
		gRNA15143	<i>ATTGTTCCAGACTTCTTTGT</i>	0	697	0	
7	HLA-DQB1	gRNA66061	<i>AAGAAGGCTTTGCGGATCCC</i>	117	0	0	2
		gRNA32606	<i>AGGTCACAGTTGCTGCCCGA</i>	0	614	0	
8	ANKS1B	gRNA55842	<i>AGCTGCTCGCACTGGAAATG</i>	0	0	265	2
		gRNA17632	<i>GGGGAAGGACCAGGAGCTGC</i>	0	387	0	
9	HRH3	gRNA12248	<i>GGCGAGGCGGCGGCGGCGGG</i>	143	399	0	2
10	PARS2	gRNA38522	<i>ACCACTGTGCCCAAGAAGA</i>	124	0	209	2
11	LINC00493	gRNA41200	<i>AACTATTGGAAATCATGGAC</i>	129	0	0	2
		gRNA37282	<i>ATCGAAATGAGTTCACGGCC</i>	0	0	183	

Validation



Previous “positive” genes



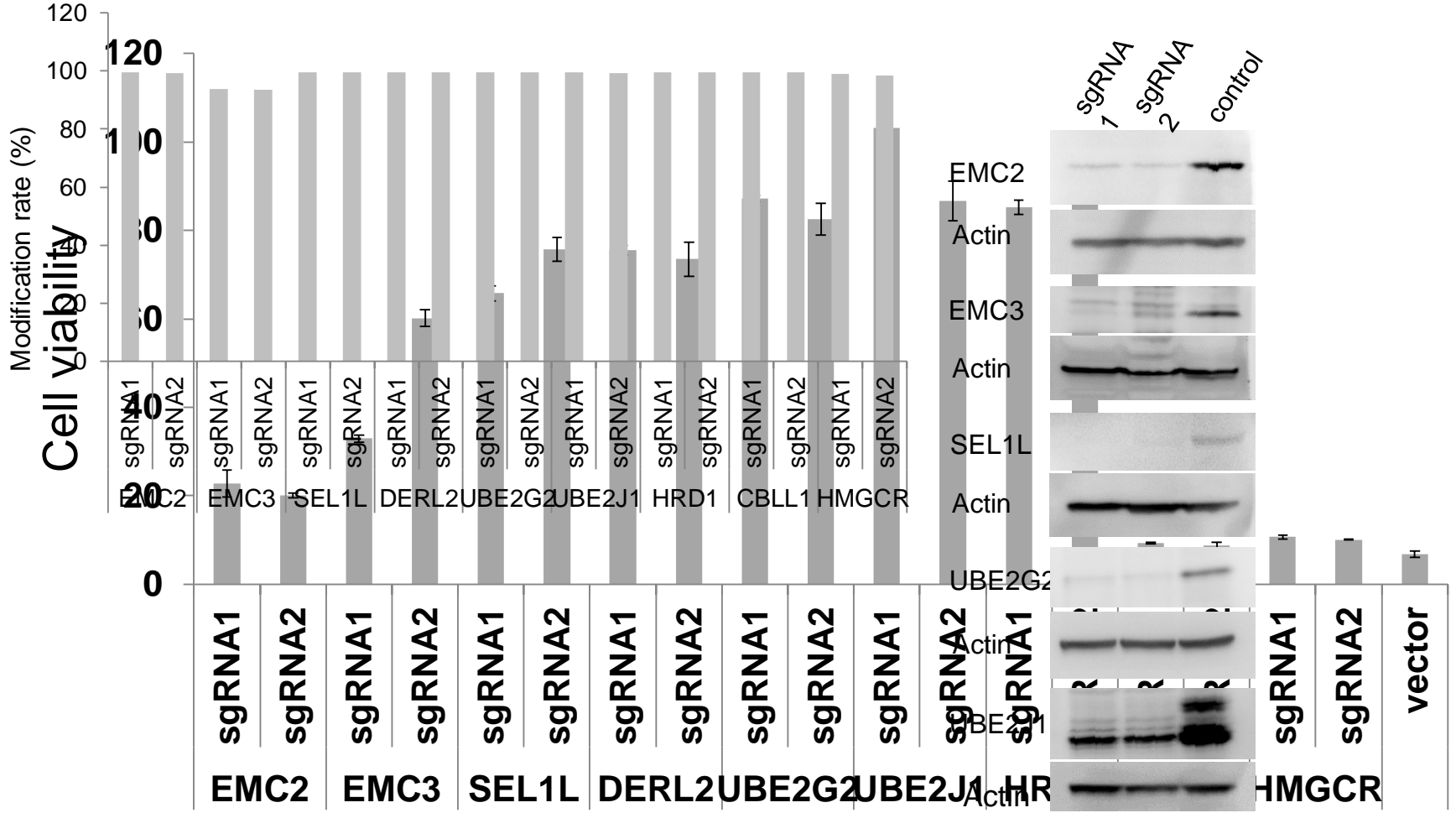
Summary

- CRISPR-mediated genome-wide functional screen can efficiently identify virus host factors.
- After entering into cells, HIV might not require host factors for killing.

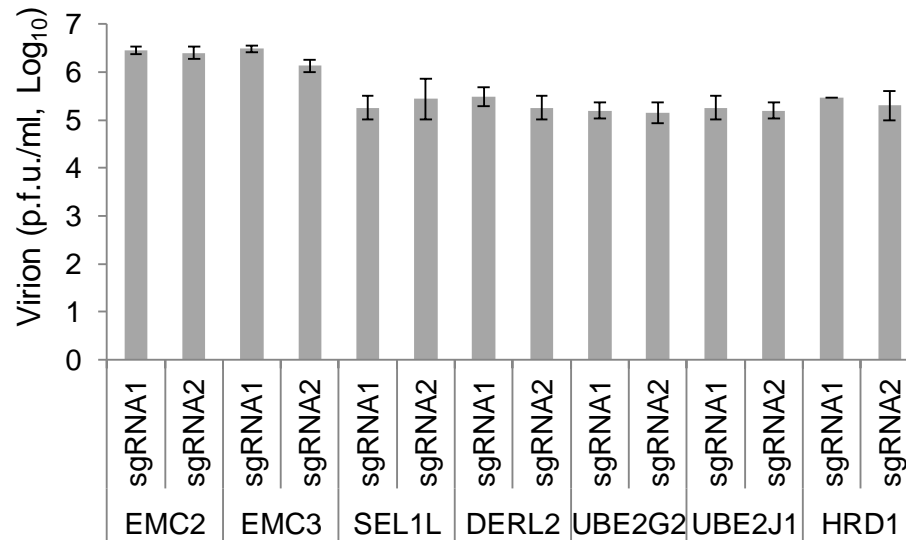
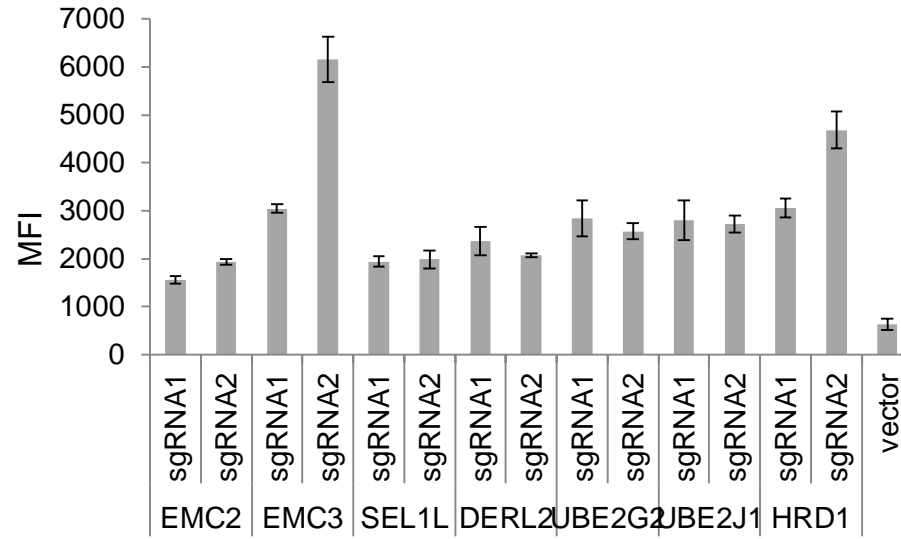
**Human Genes Essential for
West Nile Virus-Induced Cell Death**

西尼罗河病毒的宿主因子筛选

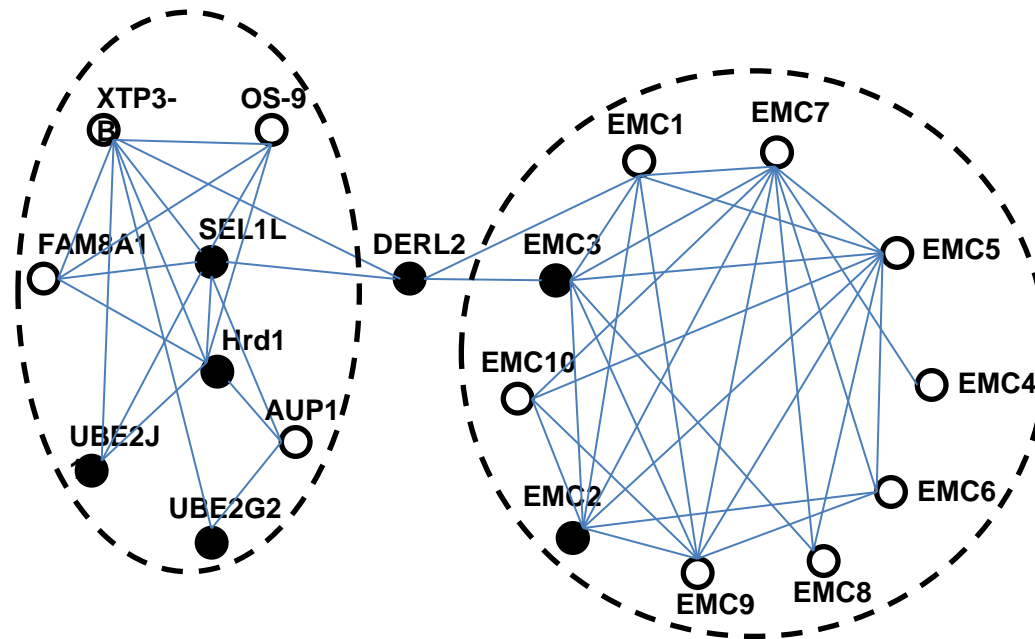
Top genes with strong phenotype



Knockout of the genes did not block WNV replication



ERAD in WNV-induced cell death



Missing connection/缺失的一环

WNV replication



ERAD?



Cell death

**Optimizing sgRNA structure to
improve knockout efficiency**

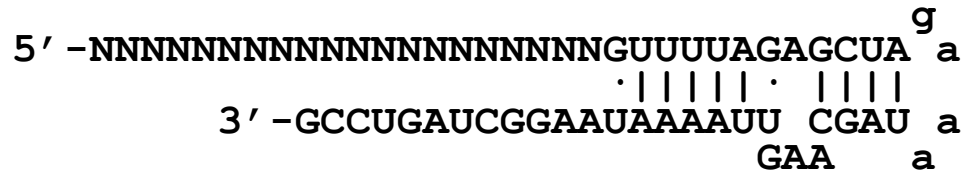
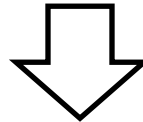
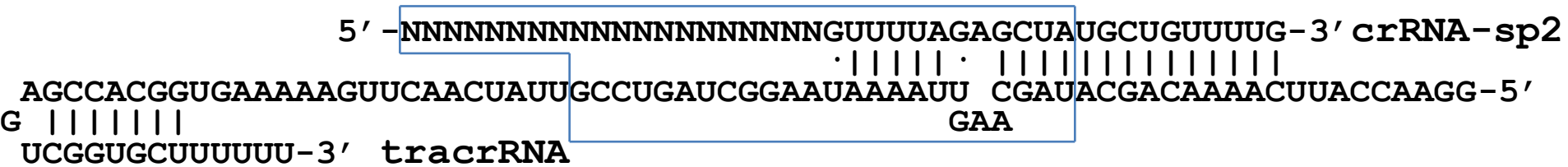
优化sgRNA结构提高筛选效率

Optimizing sgRNA structure to
improve knockout efficiency

Inconsistent Knockout Efficiency

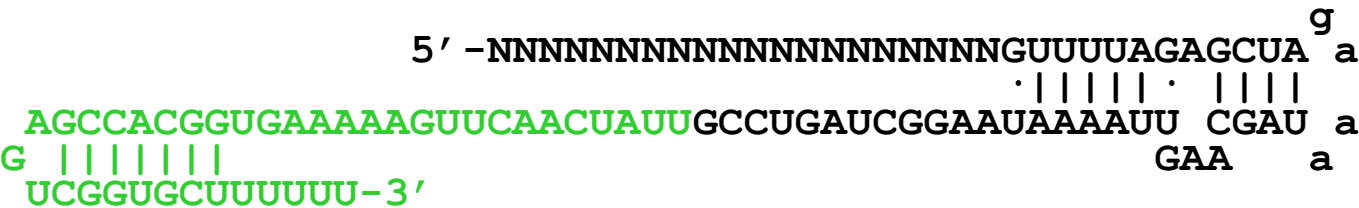
SEL1L	grnas3343	GAGCTTGGCCTCGGCGTCCT	2911	926	0
	grnas3348	GTCTCCAAATCCAGAAAACA	5424	24743	3042
	grnas3345	ACTGCAGGCAGAGTAGTTGC	3029	9036	19356
	grnas3346	AGAATCCTCTATTCAAGAAG	6480	1060	2745
	grnas3344	GACATCAGATGAGTCAGTAA	16207	12492	18521
	grnas3349	ATGAAGAGCCAAAGAAAGTA	2659	5648	10678
	grnas3342	GCTGACGCTGCTGCTGTGTG	30940	66404	77112
	grnas3347	AGACAGCCTCAAGAGCCAAG	19	8112	22346
UBE2G2	grnas3884	AGTGGGAAACTCAGGATGGC	981	0	0
	grnas3887	GCTACGAGAGCAGCGCGGAG	4983	5139	55068
	grnas3886	ATTTCCATCCTCCACGCGCC	0	32	0
	grnas3885	ACAGGTAAATCTCATCTTTG	84	1892	0
	grnas3888	GGAGAAGATCCTGCTGTCGG	9740	6671	5047
	grnas3889	ACGTGGATGCGTCCAAAATG	468	0	1963
	grnas3883	ATGAAGAGAACTTTTTTTGAA	0	164	0
	grnas3882	GACCGCGCTCAAGAGGCTGA	337	3402	6134

Two different versions of sgRNA



Short version

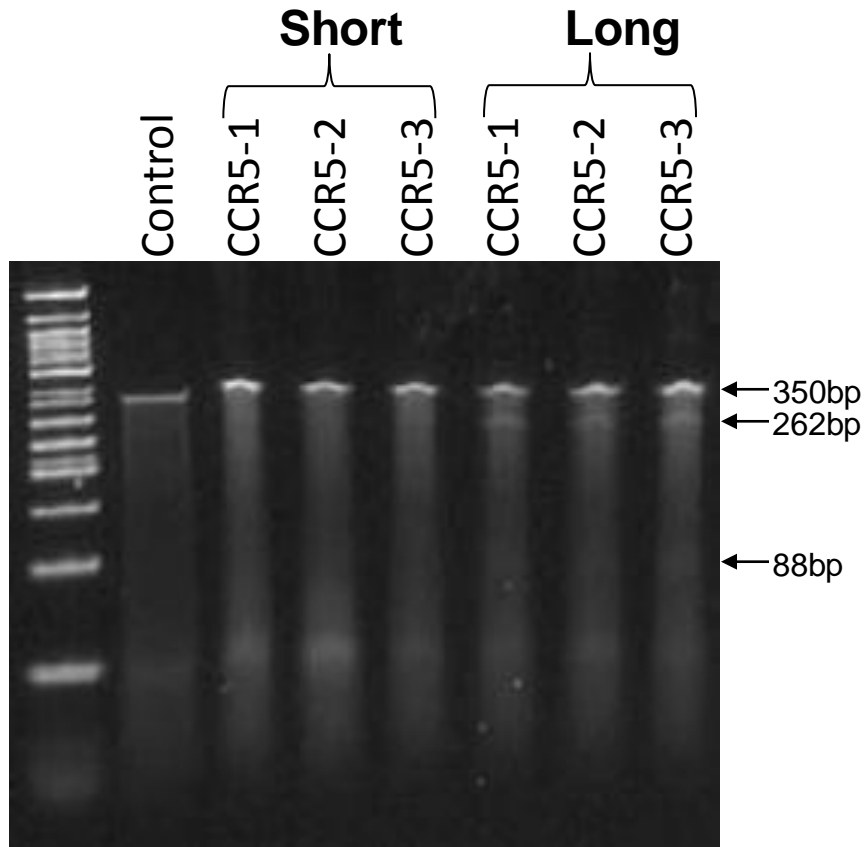
Jinek et al. 2012



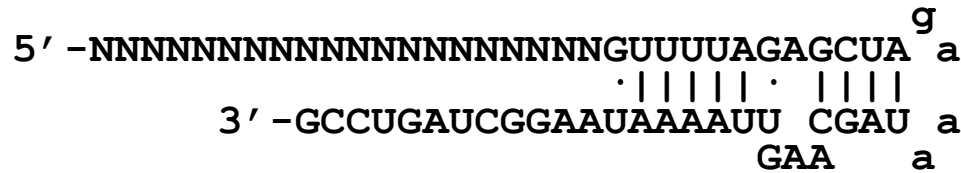
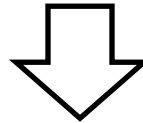
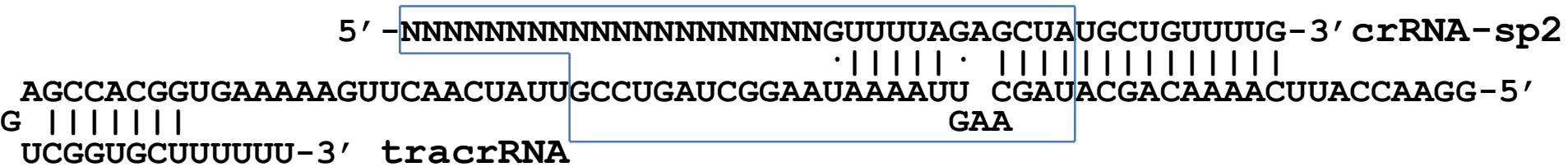
Long version

Mali et al. 2013

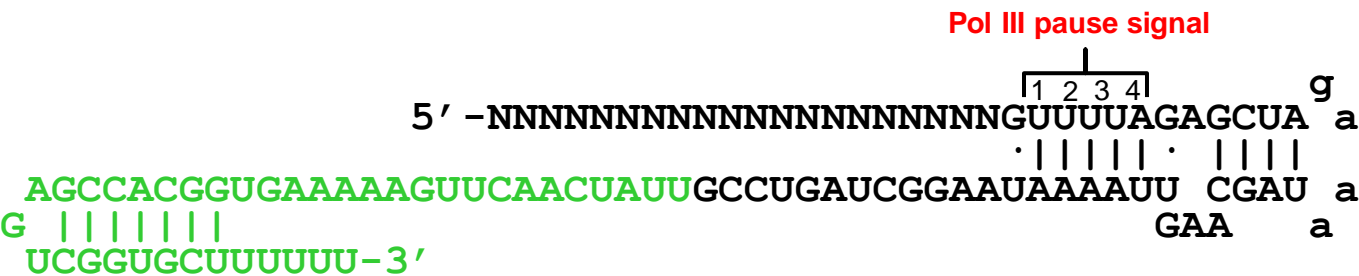
Two different versions of sgRNA



Two different versions of sgRNA

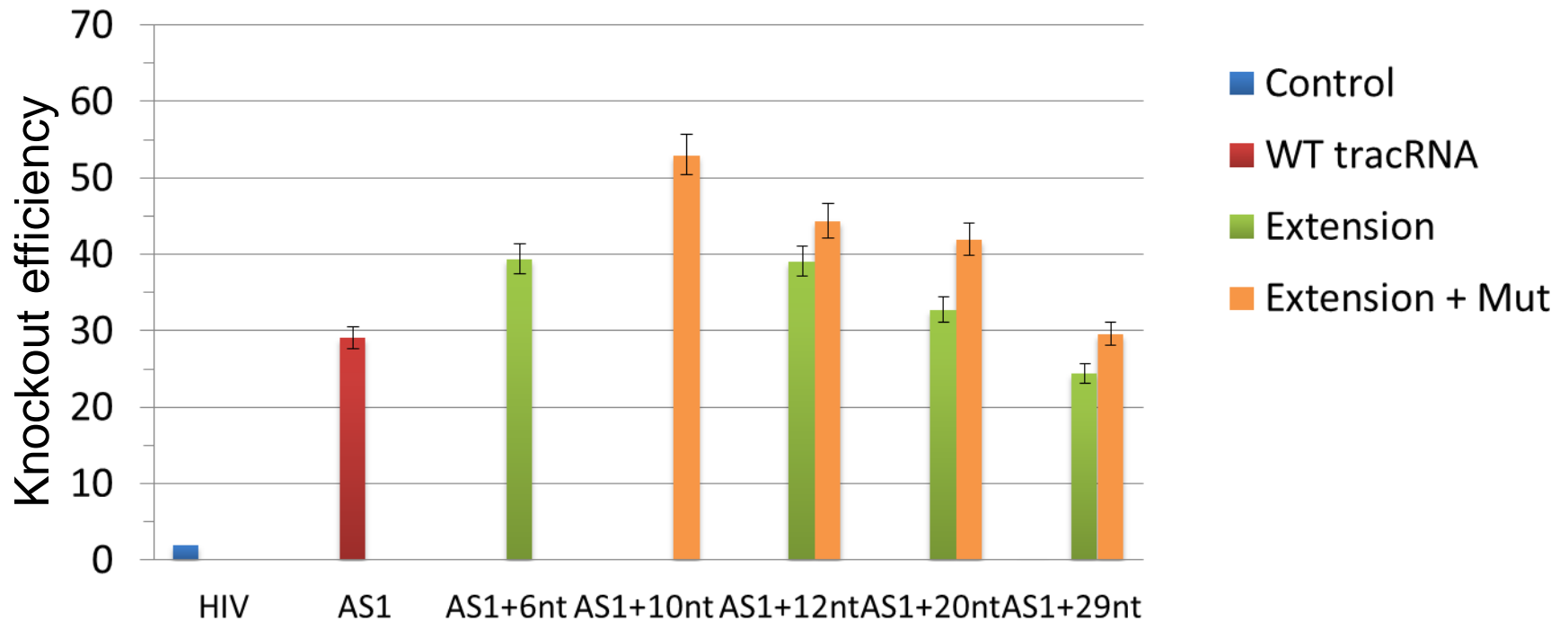


Short version

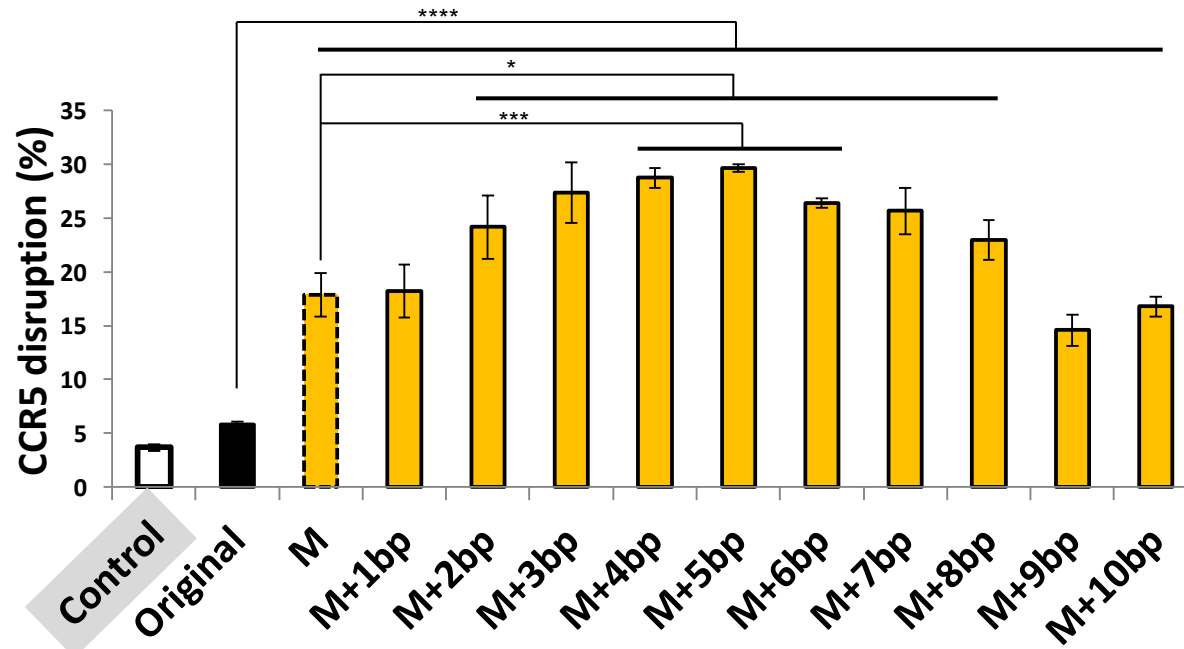
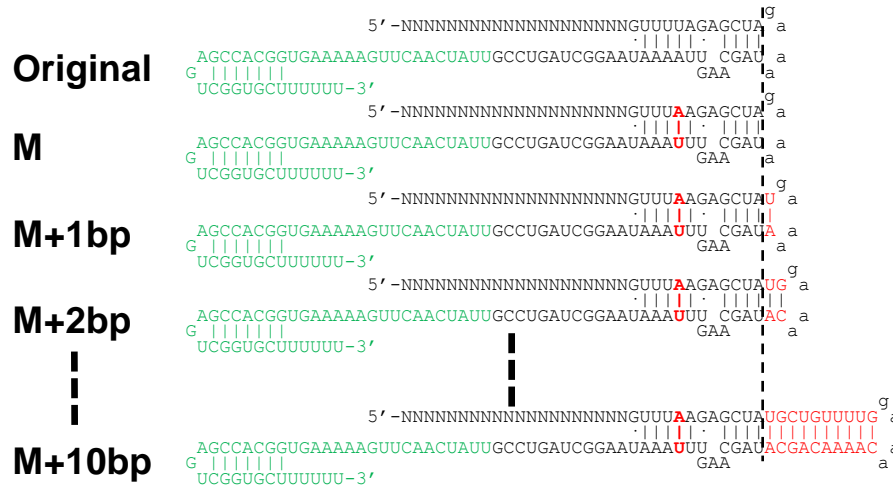


Long version

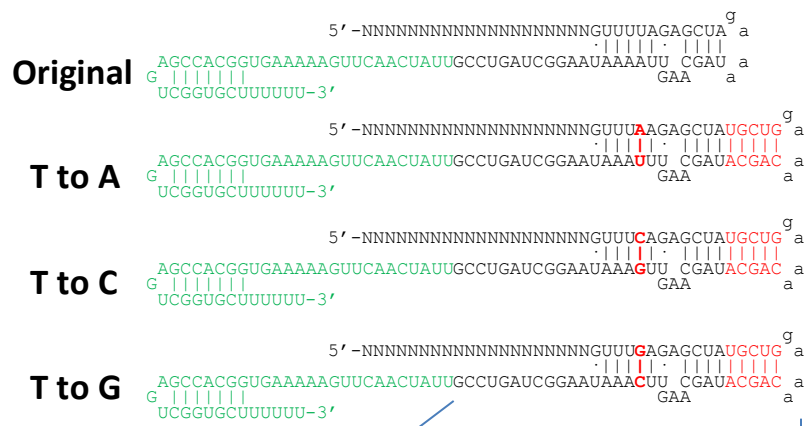
Extend the duplex and mutating continuous Ts increase knockout efficiency



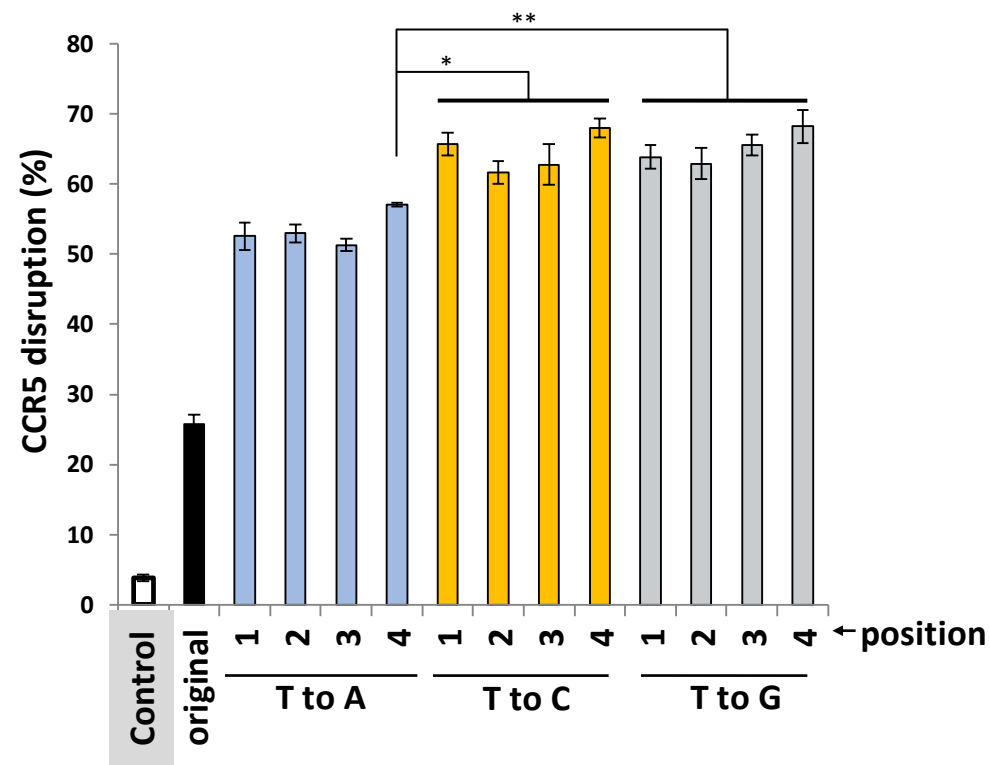
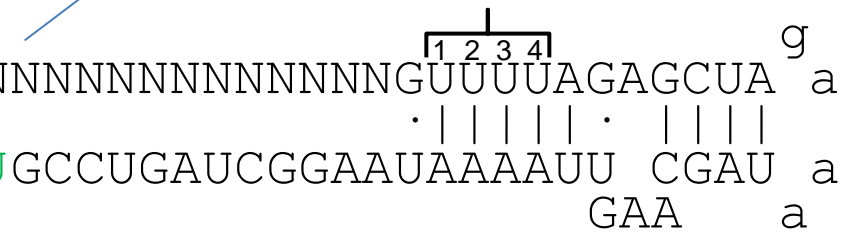
Extending the duplex



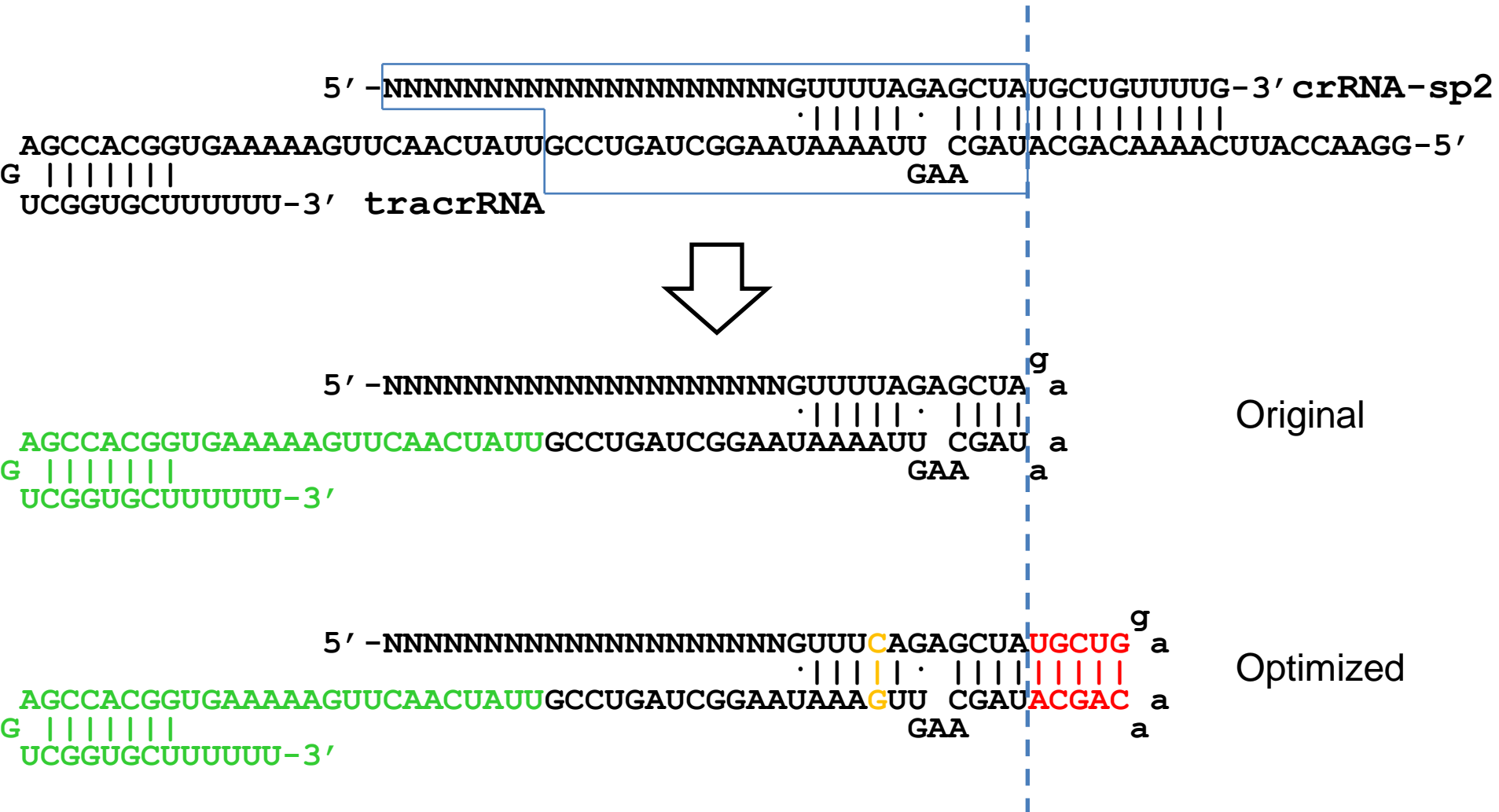
Mutation to C or G in the fourth U



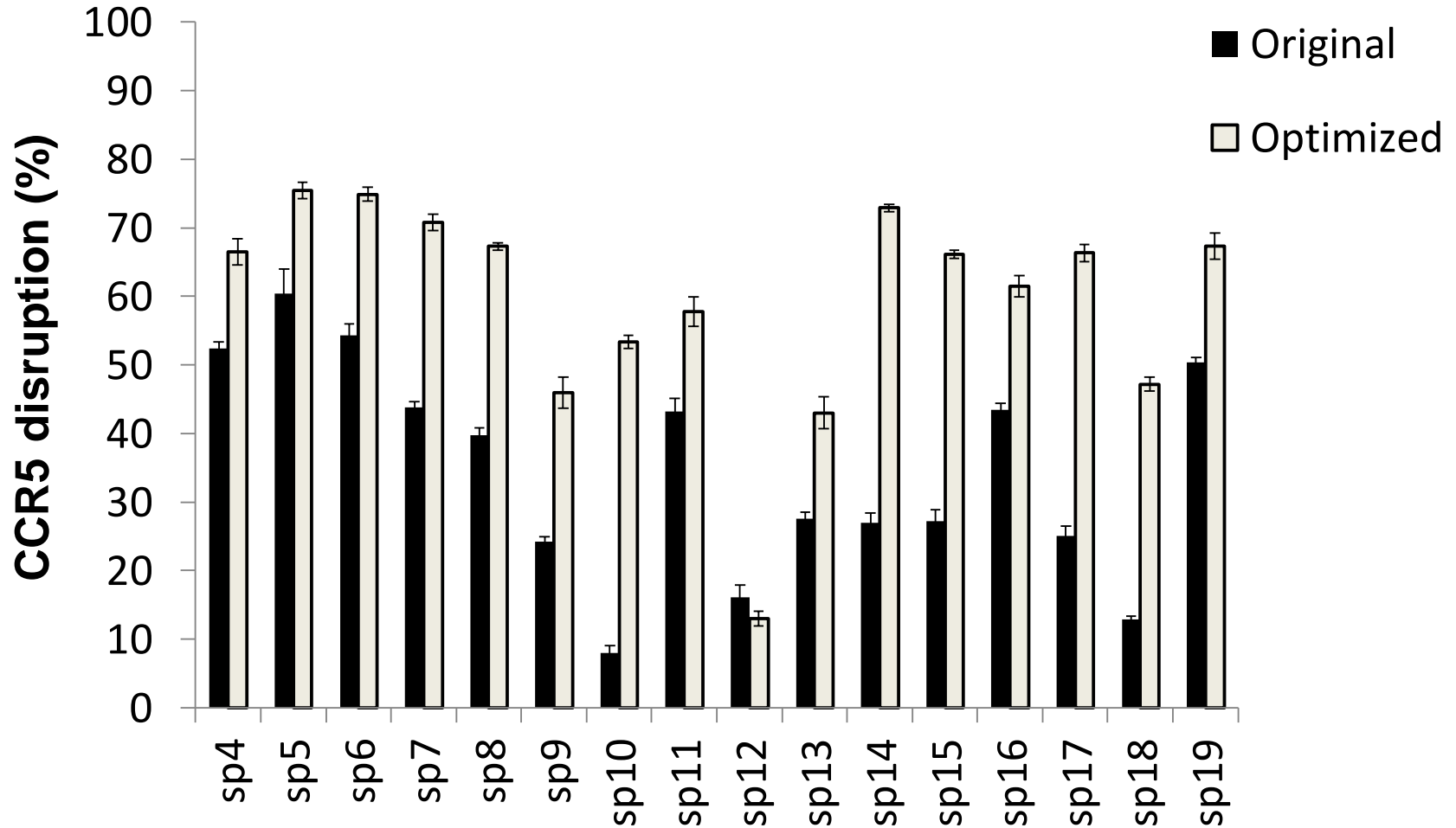
Pol III pause signal



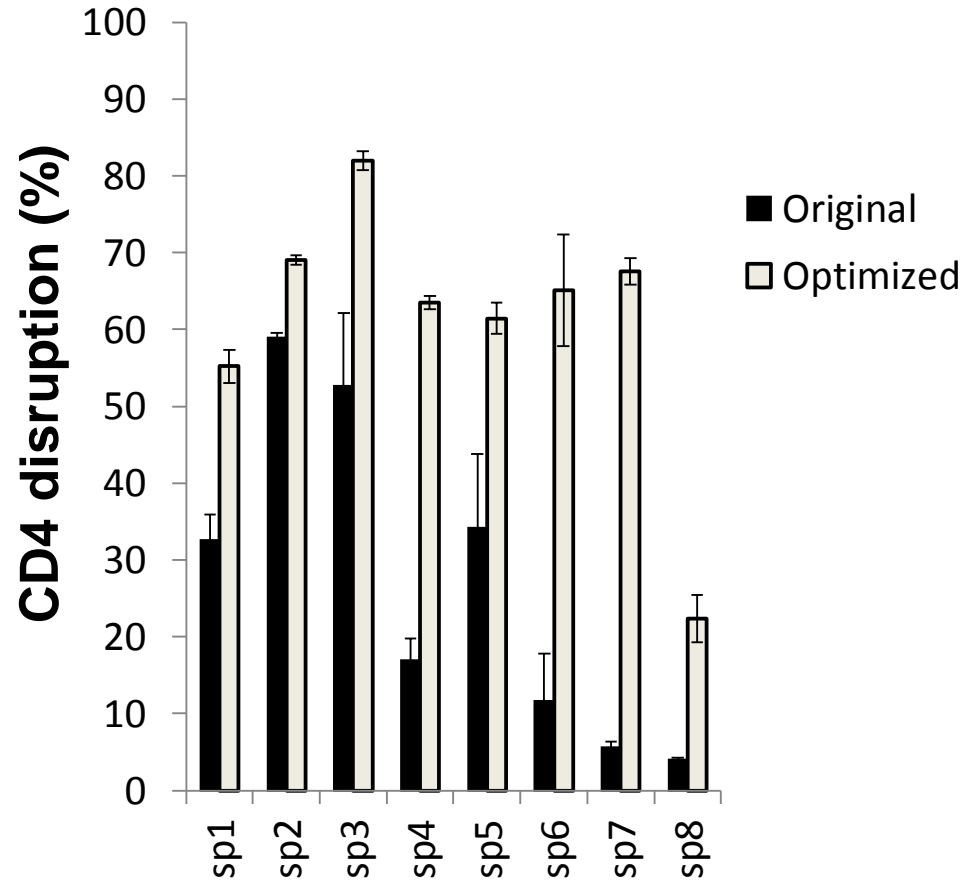
Optimized sgRNA



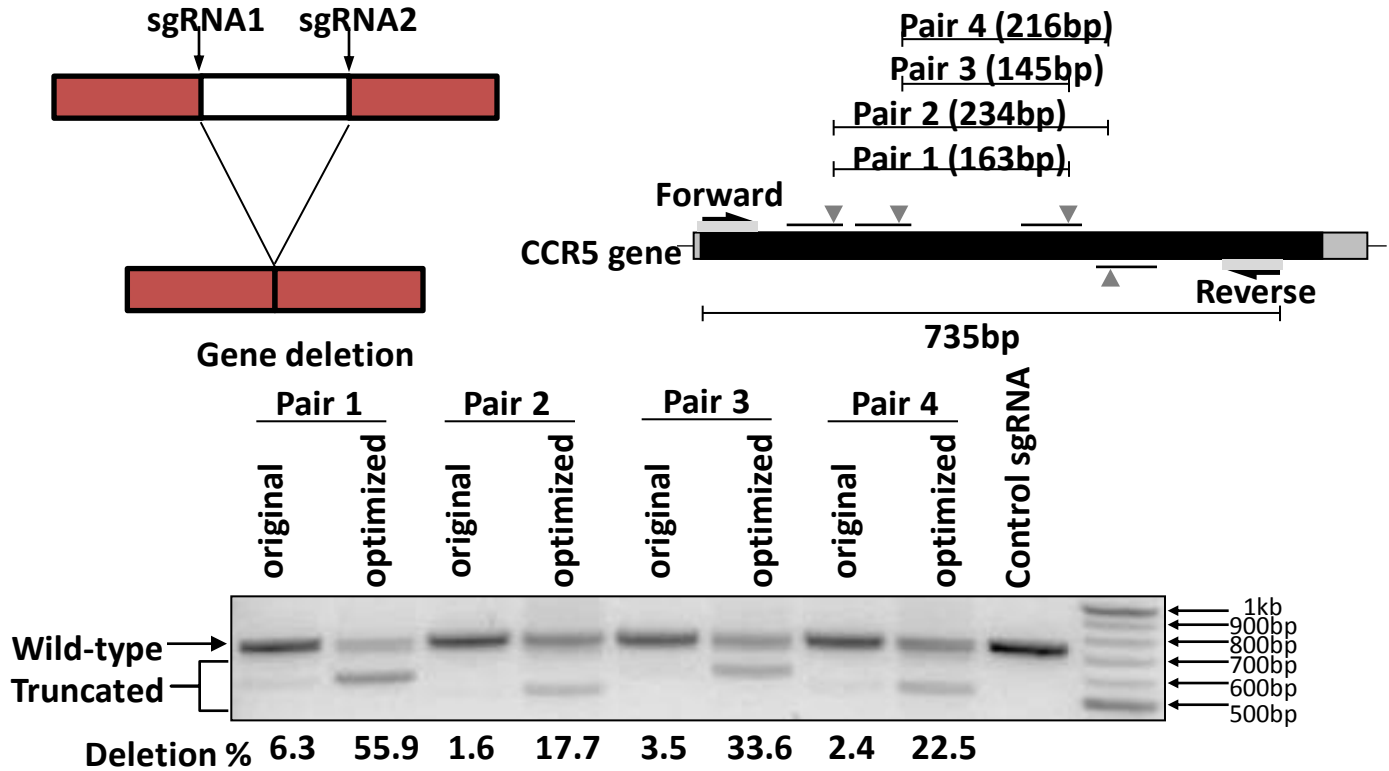
Optimized sgRNA is good!



Optimized sgRNA is good!



Gene deletion efficiency



A new sgRNA library

- Based on optimized sgRNA
- Should have better performance

Acknowledgement

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