

MIT CAB/ESCRO Risk Assessment for CRISPR/Cas9

Delivery Methods:	Form	Target	Biosafety Level
Electroporation/transfection/injection	Plasmid Protein <i>in vitro</i> transcribed RNA	Any	Adopt biosafety level appropriate for host
Integrating viral vectors capable of transducing human cells (e.g. amphotropic murine retroviral or lentiviral vectors)	sgRNA and Cas9 encoded on viral genome, constitutive promoter*	Expected oncogenic outcome (e.g. tumor suppressor) or library	BL2+
		Oncogenic outcome not expected	BL2**
	sgRNA and Cas9 encoded on viral genome, inducible promoter*	Expected oncogenic outcome (e.g. tumor suppressor) or library	BL2+
		Oncogenic outcome not expected	BL2
	sgRNA alone	Any	BL2
	Cas9 alone, constitutive promoter	n/a	BL2**
Non-integrating viral vector capable of transducing human cells	Cas9 alone, inducible promoter	n/a	BL2
	sgRNA and Cas9 encoded on viral genome	Expected oncogenic outcome (e.g. tumor suppressor) or library	BL2+
	sgRNA and Cas9 encoded on viral genome	Oncogenic outcome not expected	BL2
	Cas9 alone	n/a	BL2
	sgRNA alone	Any	Adopt biosafety level appropriate for vector

*Delivering Cas9 and the sgRNA using separate viral vectors reduces the chances of an adverse outcome in the case of an exposure.

** The CAB/ESCRO recommends that BL2+ practices be followed, i.e. avoiding sharps, double gloving, decontaminating waste inside the biosafety cabinet before removal. Personnel should be proficient with the viral vectors used before performing experiments with a higher risk level.

Careful consideration should be given to evaluating possible off-target effects of the sgRNAs used.

Catalytically inactivate versions of Cas9 (dCas9) fused to transcriptional modulators or other functional domains, and other variants and homologs of Cas9, will be evaluated based on the expected outcome (oncogenic or not). **Gene drives will be evaluated separately.**