

<b>Class of Intervention</b>	<b>Existing Technology</b>	<b>Additional Challenges</b>
<b>Optimize Cutting</b>	Target multiple sites	Eliminate guide RNA repetitiveness
<b>Minimize Fitness Cost</b>	Use paired nickases Use truncated guide RNAs Use Cas9-FokI fusion	Need twice as many guide RNAs Express truncated guide RNAs Need twice as many guide RNAs
<b>Avoid Resistant Alleles</b>	Target important genes Target multiple sites	Ensure recoded version is viable Eliminate guide RNA repetitiveness
<b>Eliminate Repetitiveness</b>	Use alternative promoters Use guide RNA variants Multiple guides per promoter Invert and separate repeats	Confirm activity in each species Determine most divergent set of variants None known Could cause problems for subsequent drives
<b>Optimize HR</b>	Generate optimal overhang Cut during optimal cell stage Activate HR genes Repress NHEJ genes Tether HR proteins near cut	Determine optimal overhang Identify optimal stage, promoter for species Optimize timing and verify effectiveness Optimize timing and verify effectiveness Determine whether this increases HR