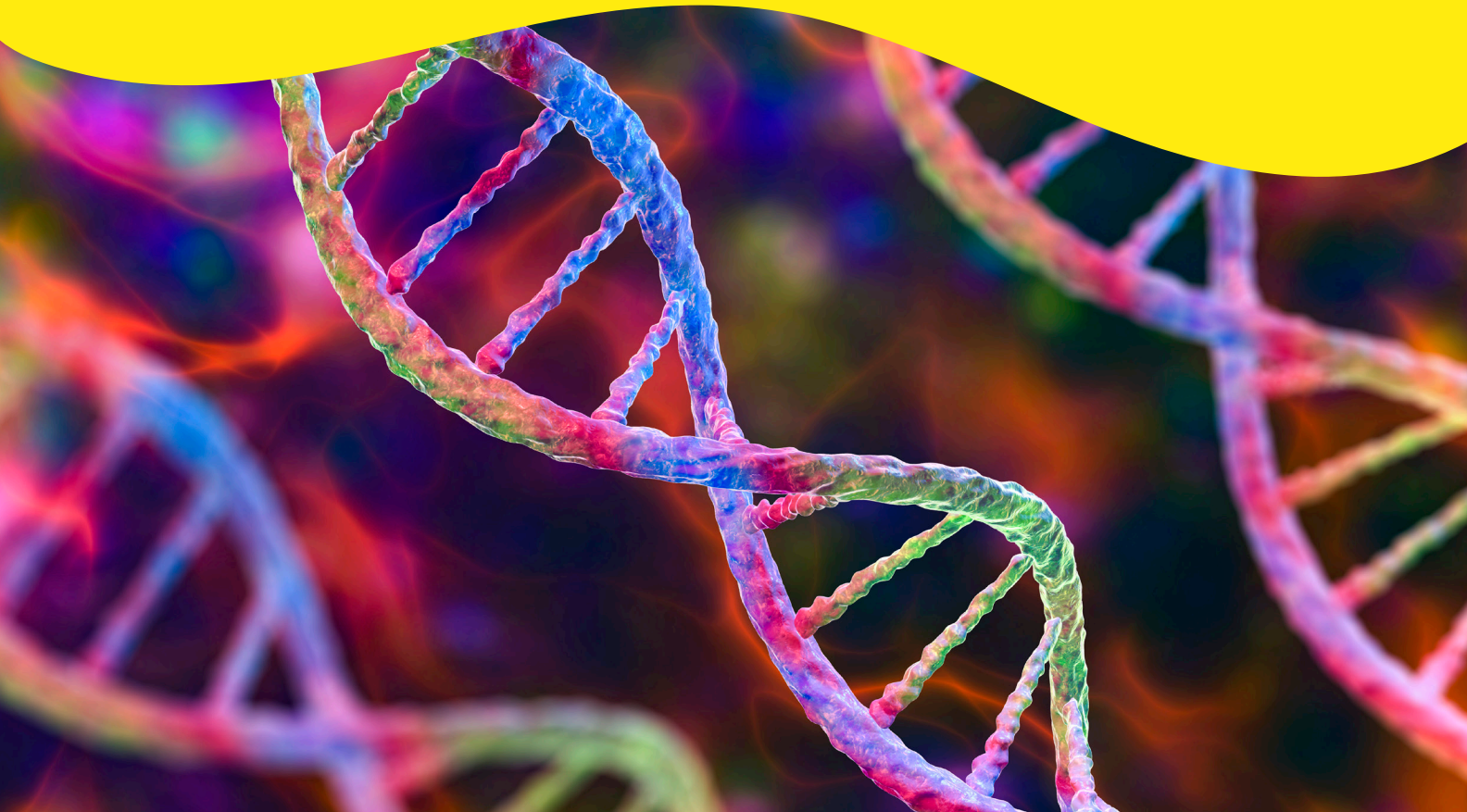
revvity

CRISPR promoter selection guide

A guide for choosing the optimal lentiviral promoter for CRISPR experiments



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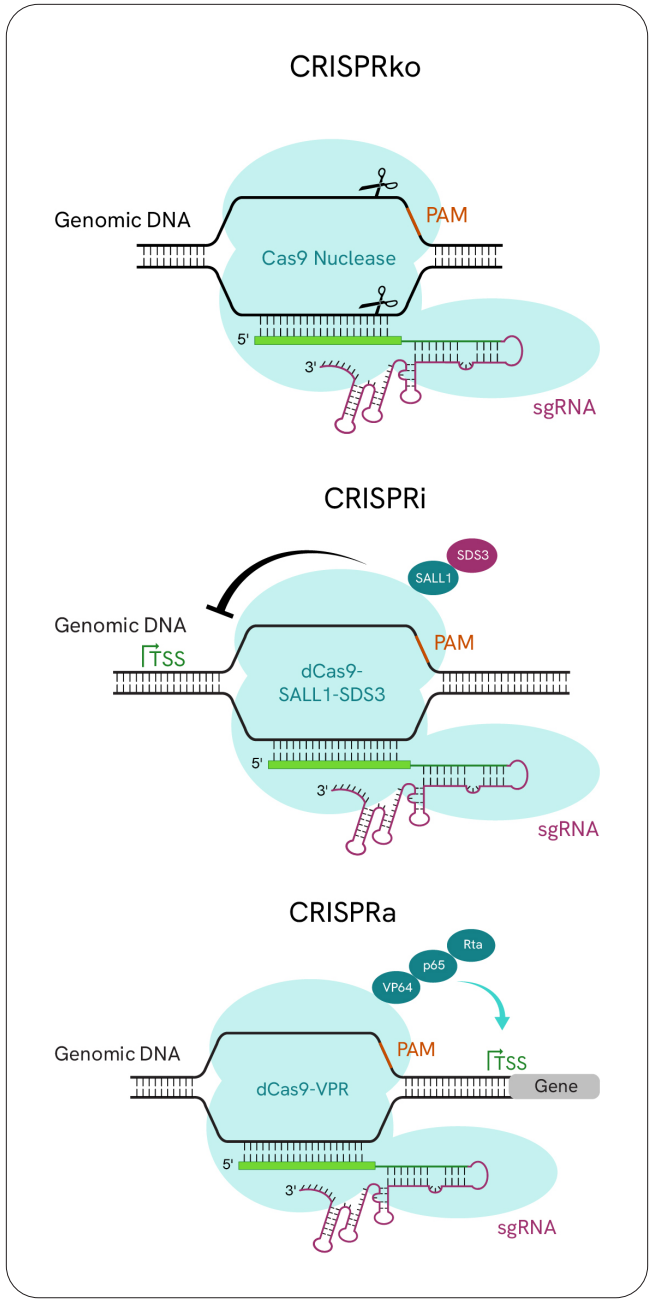
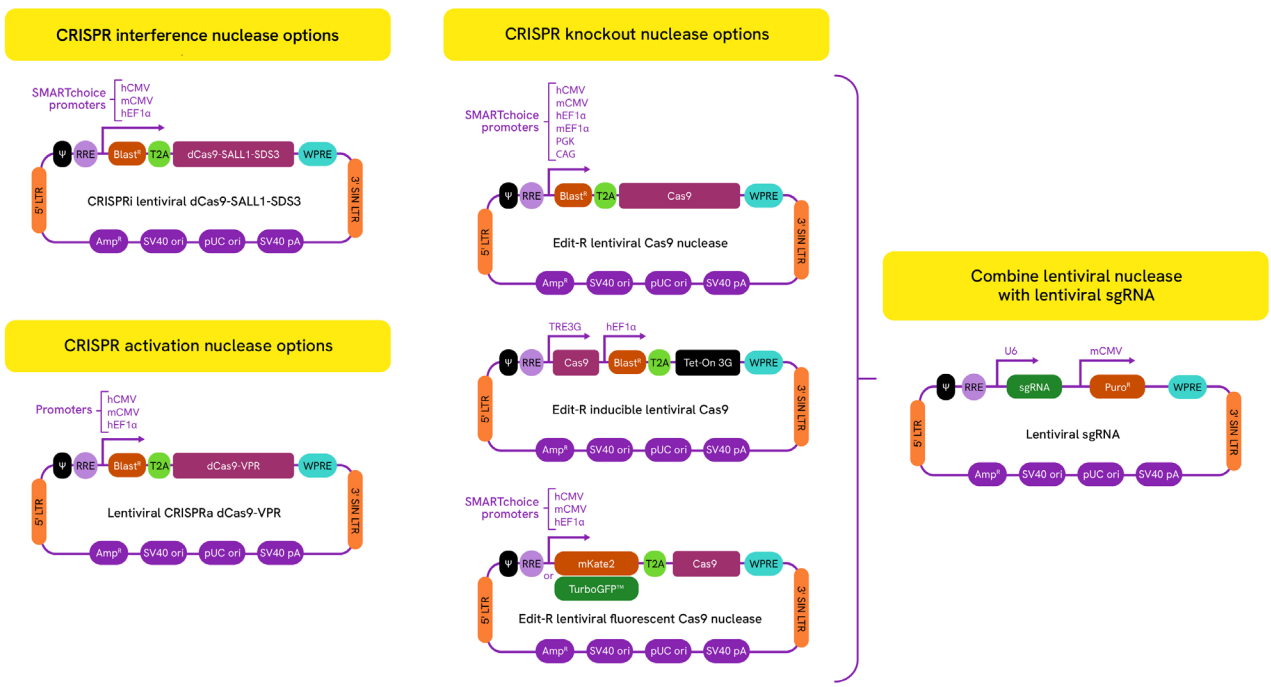
Background information

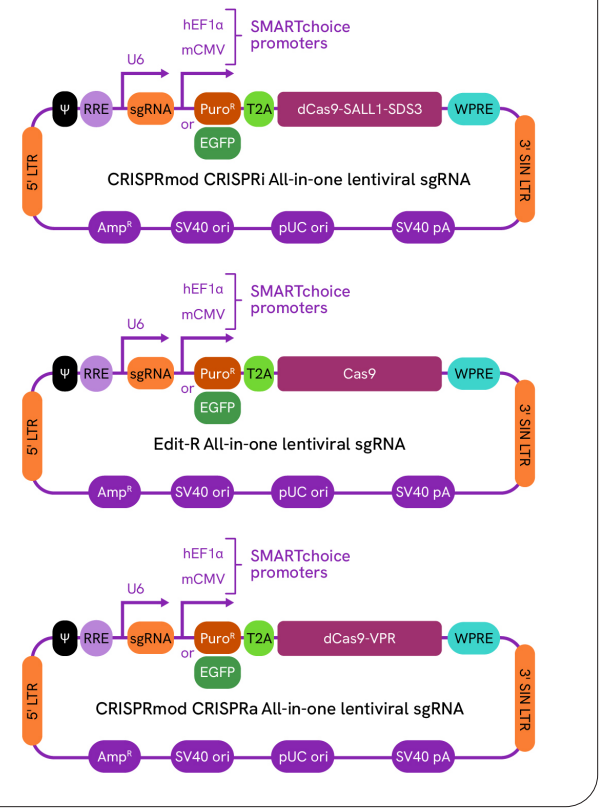
The CRISPR-Cas9 system can be utilized for precise and robust genetic editing in various contexts. Optimizing this system for your experiment can yield groundbreaking results for scientific studies across many fields. Revvity's Dharmacon™ CRISPR products support gene modulation studies with three technologies: **CRISPR knock out (Edit-R™), CRISPR interference (CRISPRmod™ CRISPRi), and CRISPR activation (CRISPRmod™ CRISPRa).** Each of these technologies are offered as synthetic or lentiviral systems. **Different lentiviral options are available,** where the nuclease and guide RNA can be integrated into the cell genome sequentially or simultaneously. Sequential integration will require two transductions, one of nuclease and another of guide RNA. Conversely, simultaneous expression is achieved with the use of the All-in-one system (AIO) for one transduction of nuclease and guide RNA.

Each of these platforms can be **optimized** for robust results by systematically choosing the best promoter for driving strong nuclease expression. Page 4 shows a schematic representation of Revvity's lentiviral nucleases and AIO expression constructs with the various promoter options available.



Schematics depicting the mechanisms of CRISPR knockout, CRISPR interference, and CRISPR activation.





Schematic overview of Revvity's Dharmacon™ lentiviral CRISPR products. These products are available for three CRISPR technologies: CRISPR interference (CRISPRi), CRISPR knockout (Edit-R), and CRISPR activation (CRISPRa). Promoters can be chosen for each of these technologies for expression of the Cas9/dCas9-effector cassettes with either a fluorescent protein or an antibiotic resistance gene. Guides are expressed by the U6 polymerase III promoter.

Which promoter is best for my experiments?

Revvity's Dharmacon lentiviral CRISPR products contain promoters that recruit either RNA polymerase II or III. In cells, RNA polymerase II is responsible for transcription of all protein coding genes in eukaryotes, while RNA polymerase III transcribes short non-coding RNAs. The promoters that drive transcription of the Cas9/dCas9-effector cassettes are polymerase II promoters, which transcribe the DNA into messenger RNA for further translation into protein. The U6 polymerase III promoter is used for transcription and amplification of the guide RNA to create a complete CRISPR complex for guide RNA binding with the translated Cas9/dCas9 proteins.

Optimizing promoter selection can guide experimental setup, workflow, and outcome. Because polymerase II promoters transcribe messenger RNA, a strong promoter will result in a higher amount of mRNA present in the cell, which typically correlates with higher protein expression. A strong promoter can drive expression of an associated effector protein (nuclease or nuclease fusion), resulting in a more robust and extended gene editing or gene modulation. These features may be important in large screening experiments, or gene activation or inhibition studies.

Conversely, in a knockout experiment where **a single genomic editing event is typically sufficient,** the double-stranded break and DNA repair results in disruption of the coding gene by introducing deletions, inserts, or single nucleotide changes (indels). These changes lead to different possible outcomes of the protein, such as: total protein absence, truncated protein, or protein with loss-of-function (functional knockout). When a homogenous cell-line is needed for further downstream studies and testing, clonal isolation experiments can be conducted to isolate cell clones that do not express the target protein. Because cells with the correct edit are chosen and further expanded, the expression level of the nuclease does not necessarily need to be high to generate indels, but high expression can decrease the clonal screening time because more cells will possess the desired edit.

Lastly, promoters can be constitutive or inducible: constitutive promoters express the gene constantly and are not influenced by regulatory elements in the construct, whereas inducible promoters can be activated in a controlled manner to meet experimental parameters. If controlled nuclease expression is desired, If controlled nuclease expression is desired, Revvity also offers the Dharmacon™ Strict-R™ Inducible Lentiviral Systems with high specificity and efficiency.

Promoter function & experimental optimization

What affects promoter function?

Promoter choice can impact the degree of target protein expression, but cell line and cellular context are also important to consider (reference 2). A high efficiency promoter in one cell line does not always constitute an effective promoter in a different cell line. Many studies, including experiments performed by Revvity R&D, demonstrate that promoters can function differently across cell lines. **See table 1** below for promoter recommendations based on in-house testing in various cell lines, using either Edit-R or CRISPRmod lentiviral products.

Table 1: Overview of cell lines and the optimal promoters tested by Revvity. These cell lines were tested in-house, and depending on the technology used, have shown positive GFP expression, increased mRNA expression or silencing, or editing efficiency via TIDE analysis with using the suggested recommended promoter to drive the nuclease expression (for example data, see pages 8-9 below).

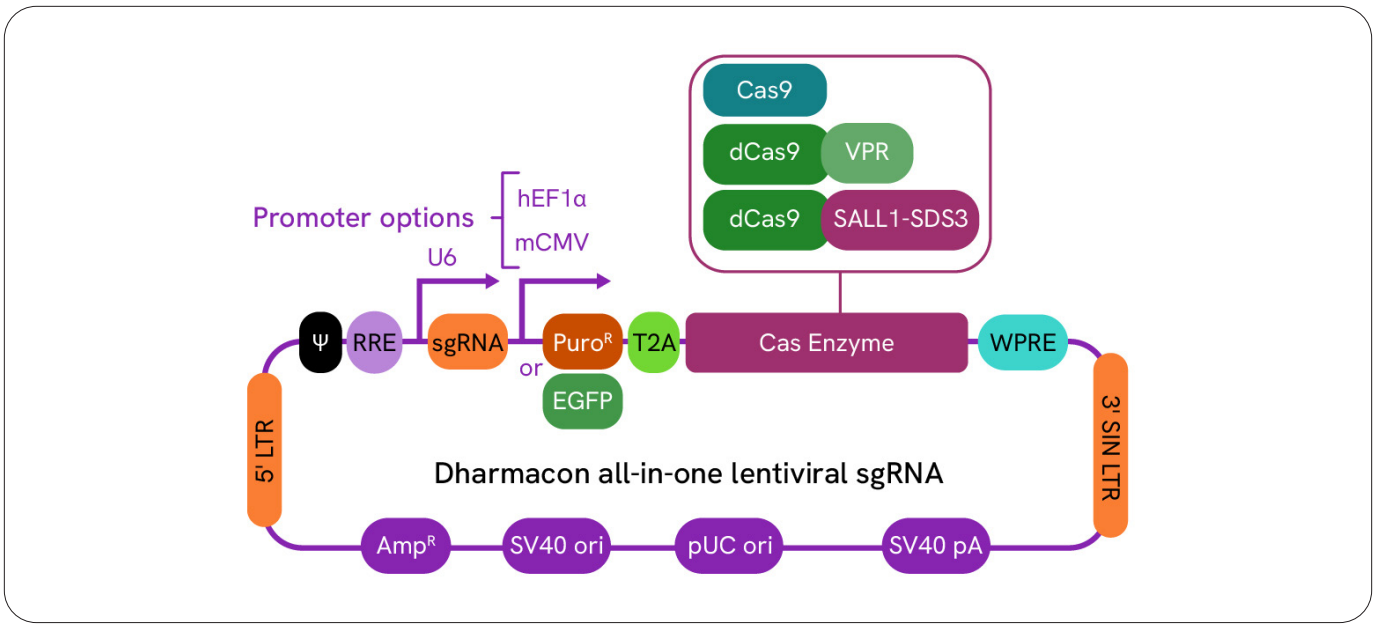
Cell line	Promoter recommendation	Cell line description
A375	mCMV	Human, melanoma
DU 145	mCMV	Human, prostate carcinoma
HT 29	mCMV	Human, colorectal adenocarcinoma
Jurkat	hEF1α	Human, T lymphocyte
Colo 205	hEF1α/mCMV	Human, colorectal adenocarcinoma
K562	mCMV/hEF1α	Human, chronic
PC3	mCMV	Human, prostate adenocarcinoma
HEK 293T	hCMV/ hEF1α	Human, embryonic kidney
HCT 116	mCMV	Human, colorectal carcinoma
MCF7	hCMV/ hEF1α	Human, adenocarcinoma
NIH/3T3	mCMV/ hEF1α	Mouse, embryonic fibroblast
U2OS	hEF1α/mCMV	Human, osteosarcoma
A549	mCMV	Human, lung carcinoma
THP1	hEF1α	Human, acute monocytic leukemia
MCF 10A	mCMV	Human, fibrocystic
hiPSCs	hEF1α	Human, stem cell

Considerations for experimental optimization using expressed nucleases

Our CRISPRmod products have two promoter options, the **human elongation factor 1-α (hEF1α) and the mouse cytomegalovirus (mCMV) promoters**, which are known for strong performance in a wide range of cell lines, demonstrated by Revvity (**see Table 1**) and others in the scientific community (**see promoter recommendations from the literature on pages 10-12**). Therefore, it is recommended that these two promoters be used as a starting point for experimental optimization to meet your specific goals. To initiate experiments involving target gene editing or gene modulation in **a cell line not listed in Table 1,** Revvity suggests comparing activity empirically using these two promoters, as they have been extensively validated and utilized for strong transgene expression in various cell types and contexts (references 3-6).

Simultaneous evaluation of multiple promoters can help optimize nuclease expression, and therefore activation, silencing, or knockout of the target gene. Further, the **downstream assays and experiments must be fully streamlined** in addition to determining the most optimal promoter. Unexpected results may occur even if the promoter is optimized but the downstream experimental conditions are non-ideal. Therefore, these experiments must include robust positive and negative controls to evalidate results.

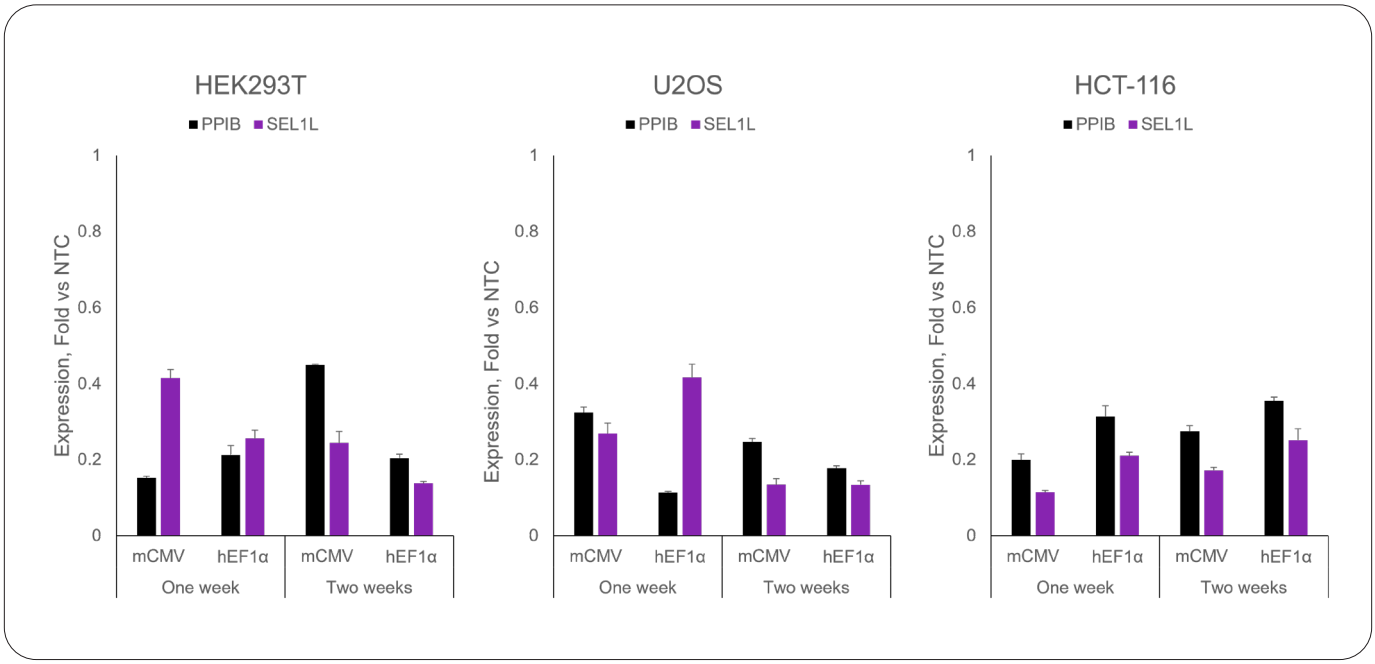
Additionally, the Multiplicity of Infection (MOI) and delivery method in a transduction experiment should be evaluated and optimized to ensure proper integration of the constructs. MOI is defined as the ratio of transducing units of lentiviral particles to target cells (reference 7). Ideally, **multiple viral integration events per cell should be avoided,** as they can lead to cell toxicity and aberrant results. Statistically, an **MOI of ≤0.3** provides the best chance that individual cells in a bulk transduction experiment will harbor a single integration event. To generate cells with single integration events, optimization should include testing a range of viral MOIs as well as downstream assessments of gene expression such as RT-qPCR or fluorescence microscopy. For more information about experimental optimization for transductions and MOI, please refer to our product pages or technical manuals.



The optimal promoter for gene knockout, activation or repression depends on the experimental cell line.

Impact of promotor choice using CRISPR interference

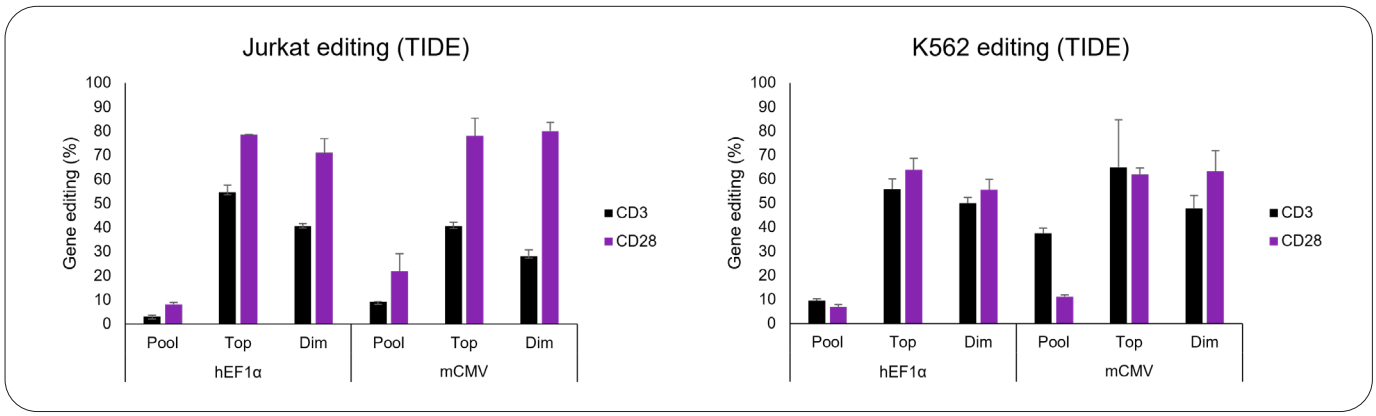
CRISPRmod CRISPRi AIO products targeting the SEL1L and PPIB genes were used to examine the impact of promotor choice in different cell lines. The hEF1α promoter is optimal in HEK293T cells, achieving ~80-85% silencing of PPIB and SEL1L after two weeks; however, the mCMV promoter is best in U2OS and HCT-116 cells, achieving ~75-90% silencing of PPIB and SEL1L at one- and two-weeks post-transduction.



Experimental setup: HEK293T, U2OS, and HCT-116 cells were transduced with CRISPRmod CRISPRi AIO lentivirus containing sgRNAs targeting PPIB, and SEL1L, while the nuclease expression was driven by either the hEF1α or mCMV promoter.

Impact of promotor choice in CRISPR knockout

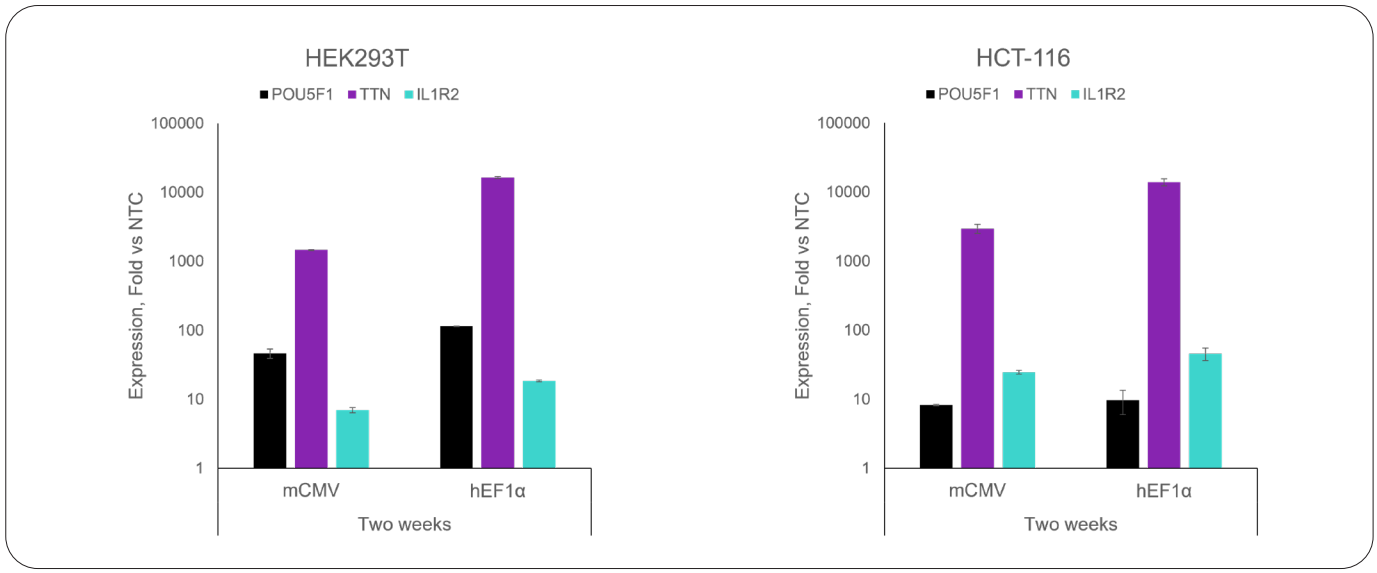
Using the Edit-R AIO EGFP lentivirus to generate CD3 or CD28 knockout, the hEF1α promoter resulted in higher editing efficiency in Jurkat cells, showing ~40-60% knockdown of CD3 as compared to ~30-40% knockdown of CD3 with the mCMV promoter. On the other hand, in K562 cells the Edit-R AIO lentivirus with the nuclease driven by the mCMV promoter yielded higher gene editing for CD3: f ~50-70% knockdown compared to the hEF1α promoter driven Cas9, which achieved 50-60% knockdown.



Experimental setup: Jurkat and K562 cells were transduced with Edit-R AIO EGFP lentivirus containing sgRNAs targeting CD3 or CD28, while the nuclease expression was driven by either the hEF1α or mCMV promoter. The cells were then sorted after expansion to enrich for the brightest (top) or dimmest (dim) EGFP expressing cells. Unsorted cell population is labelled as "pool". The populations were then subjected to TIDE analysis to determine the percentage of gene editing.

Impact of promotor choice using CRISPR activation

The CRISPRmod CRISPRa AIO lentivirus showed stronger activation of the target genes POU5F1, TTN and Il1R2 with the hEF1α promoter in both the HEK293T and HCT-116 cells with compared to the CRISPRmod CRISPRa AIO with the mCMV promoter.



Experimental setup: HEK293T and HCT-116 cells were transduced with CRISPRmod CRISPRa AIO lentivirus containing sgRNAs targeting POU5F1, TTN, and IL1R2 with either the mCMV or the hEF1α promoter driving the nuclease expression. Cells were harvested two weeks post transduction and the level of activation of each gene was determined using RT-qPCR.

Examples of promoters used in corresponding cell lines from peer-reviewed articles using Dharmacon products

CRISPR knockout using Edit-R All-in-one products

CRISPR knockout all-in-one Product				
Cell line	**Functional data**	**Recommendation**	**Cell line description**	**Reference**
A549	CRISPR KO AIO	mCMV/hEF1α	Human, lung adenocarcinoma	11
EKVX	CRISPR KO AIO	mCMV	Human, lung adenocarcinoma	
SW-620	CRISPR KO AIO	mCMV	Human, colorectal cancer	
HCT116	CRISPR KO AIO	mCMV	Human, colorectal carcinoma	
U251	CRISPR KO AIO	mCMV	Human, brain	
MCF7	CRISPR KO AIO	mCMV	Human, breast cancer	
BxPC3	CRISPR KO AIO	mCMV	Human, pancreas adenocarcinoma	
PSN1	CRISPR KO AIO	mCMV	Human, pancreas adenocarcinoma	
U87 MG	CRISPR KO AIO	mCMV	Human, glioblastoma	
HPDE6c7	CRISPR KO AIO	mCMV	Human, pancreatic ductal epithelium	
MCF-10A	CRISPR KO AIO	mCMV	Human, breast cells	
MDA-MB-231	CRISPR KO AIO	mCMV/hEF1α	Human, breast cancer	
CaoV3	CRISPR KO AIO	hEF1α	Human, ovarian cancer	12
OVCAR3	CRISPR KO AIO	hEF1α	Human, ovarian adenocarcinoma	
OVCAR4	CRISPR KO AIO	hEF1α	Human, ovarian carcinoma	
OVCAR5	CRISPR KO AIO	hEF1α	Human, ovarian tumor	
A2780	CRISPR KO AIO	hEF1α	Human, ovarian cancer	
A2780/CP70	CRISPR KO AIO	hEF1α	Human, ovarian adenocarcinoma	
HeyA8	CRISPR KO AIO	hEF1α	Human, ovarian adenocarcinoma	
TykNu	CRISPR KO AIO	hEF1α	Human, ovarian adenocarcinoma	
SKOV3	CRISPR KO AIO	hEF1α	Human, ovarian adenocarcinoma	
ID8	CRISPR KO AIO	hEF1α	Mouse, ovarian cancer	
MDA-MB-231.BM1	CRISPR KO AIO	hEF1α	Human, breast cancer	
MCF-7	CRISPR KO AIO	hEF1α	Human, breast cancer	
M6C	CRISPR KO AIO	hEF1α	Human, mammary carcinoma	
E0771	CRISPR KO AIO	hEF1α	Human, mammary carcinoma	
E0771.LMB	CRISPR KO AIO	hEF1α	Human, mammary carcinoma	
RKO	CRISPR KO AIO	hEF1α	Human, colon carcinoma	
T98G	CRISPR KO AIO	hEF1α	Human, brain glioblastoma	
U-2OS	CRISPR KO AIO	hEF1α	Human, bone osteosarcoma	

CRISPR Knockout using Edit-R All-in-one products *continued*

CRISPR knockout all-in-one Product				
Cell line	**Functional data**	**Recommendation**	**Cell line description**	**Reference**
SAOS-2	CRISPR KO AIO	hEF1α	Human, bone osteosarcoma	12
HepG2	CRISPR KO AIO	hEF1α	Human, liver carcinoma	
NCI-H552	CRISPR KO AIO	hEF1α	Human, lung adenocarcinoma	
LLC1	CRISPR KO AIO	hEF1α	Human, lung carcinoma	
B16F10	CRISPR KO AIO	hEF1α	Human, melanoma	
MEL888	CRISPR KO AIO	hEF1α	Human, melanoma	
MEL1106	CRISPR KO AIO	hEF1α	Human, melanoma	
SK-MEL-28	CRISPR KO AIO	hEF1α	Human, melanoma	
PANC1	CRISPR KO AIO	hEF1α	Human, pancreatic carcinoma	
K562	CRISPR KO AIO	hEF1α	Human, myelogenous leukemia	

CRISPR activation using CRISPRmod Cas9-VPR Products

CRISPR activation using dCas9-VPR products				
Cell line	**Functional data**	**Recommendation**	**Cell line description**	**Reference**
U2OS	CRISPRa dCas9	hEF1α/mCMV	Human, bone osteosarcoma	13
HEK293T	CRISPRa dCas9	hEF1α/mCMV	Human, embryonic kidney	
MCF-10A	CRISPRa dCas9	hEF1α/mCMV	Human, fibrocystic breast	
NIH/3T3	CRISPRa dCas9	hEF1α/mCMV	Human, embryonic fibroblast	
K-562	CRISPRa dCas9	hEF1α/mCMV	Human, myelogenous leukemia	
HCT116	CRISPRa dCas9	hEF1α	Human, colorectal carcinoma	14
Panc1	CRISPRa dCas9	hEF1α	Human, epithelioid carcinoma	
MCF-7	CRISPRa dCas9	hEF1α	Human, breast carcinoma	15
PSN1	CRISPRa dCas9	hCMV	Human, pancreas adenocarcinoma	16
HCC1806	CRISPRa dCas9	hCMV	Human, mammary carcinoma	
Panc1	CRISPRa dCas9	hCMV	Human, pancreas adenocarcinoma	
SW1990	CRISPRa dCas9	hCMV	Human, pancreas adenocarcinoma	
HS766T	CRISPRa dCas9	hCMV	Human, pancreas carcinoma	
PANC0504	CRISPRa dCas9	hCMV	Human, pancreas adenocarcinoma	
NCIH1792	CRISPRa dCas9	hCMV	Human, lung adenocarcinoma	
NCIH1651	CRISPRa dCas9	hCMV	Human, lung adenocarcinoma	
NCIH23	CRISPRa dCas9	hCMV	Human, lung adenocarcinoma	
MDA-MB-436	CRISPRa dCas9	hCMV	human, breast adenocarcinoma	
CHP-134	CRISPRa dCas9	hCMV	Human, brain neuroblastoma	17
In vivo mice tail injection	CRISPRa dCas9-VPR plasmid	mCMV	*In vivo*	18

Gene knockout using Edit-R CRISPR nuclease products *continued*

CRISPR-Cas9 knockout				
Cell line	**Functional data**	**Recommendation**	**Cell line description**	**Reference**
βTC3	CRISPR-Cas9	mCMV	Human, pancreas insulinoma	19
SW480	CRISPR-Cas9	hEF1α	Human, colon adenocarcinoma	20
HCT116	CRISPR-Cas9	hEF1α	Human, colorectal carcinoma	21
UM-UC-3	CRISPR-Cas9	hEF1α	Human, urinary bladder	22
HK-2	CRISPR-Cas9	hEF1α	Human, kidney papilloma	23
THP-1	CRISPR-Cas9	hEF1α	Human, monocytic leukemia	24
Panc-1	CRISPR-Cas9	hEF1α	Human, pancreas adenocarcinoma	25
MCF7	CRISPR-Cas9	hEF1α	Human, breast carcinoma	26
MB-157	CRISPR-Cas9	hEF1α	Human, breast carcinoma	27
NCI-H1703	CRISPR-Cas9	hEF1α	Human, lung cancer	28
MDA-MB-157	CRISPR-Cas9	hEF1α	Human, breast carcinoma	
SK-BR-3	CRISPR-Cas9	hEF1α	Human, breast adenocarcinoma	
MDA-MB-468	CRISPR-Cas9	hEF1α	Human, breast adenocarcinoma	
MCF7	CRISPR-Cas9	hEF1α	Human, breast carcinoma	
HCC1954	CRISPR-Cas9	hEF1α	Human, breast duct carcinoma	
HaCaT	CRISPR-Cas9	hCMV	Human, immortalized keratinocyte	29
SCC13	CRISPR-Cas9	hCMV	Human, squamous cell carcinoma	
A2058	CRISPR-Cas9	hCMV	Human, melanoma	30
NCI-H1703	CRISPR-Cas9	hCMV	Hunman, non-small cell lung cancer	31
OVCAR-8	CRISPR-Cas9	hCMV	Human, ovarian adenocarcinoma	
G1S-CCPM (U2OS)	CRISPR-Cas9	CAG	Human, bone osteosarcoma	32
NCI-H1373	CRISPR-Cas9	CAG	Human, adenocarcinoma	33
inDCs	CRISPR-Cas9	CAG	Immortalized immature dendritic cells	34

Summary and conclusions

When choosing a promoter for your experimental needs, **consider:**

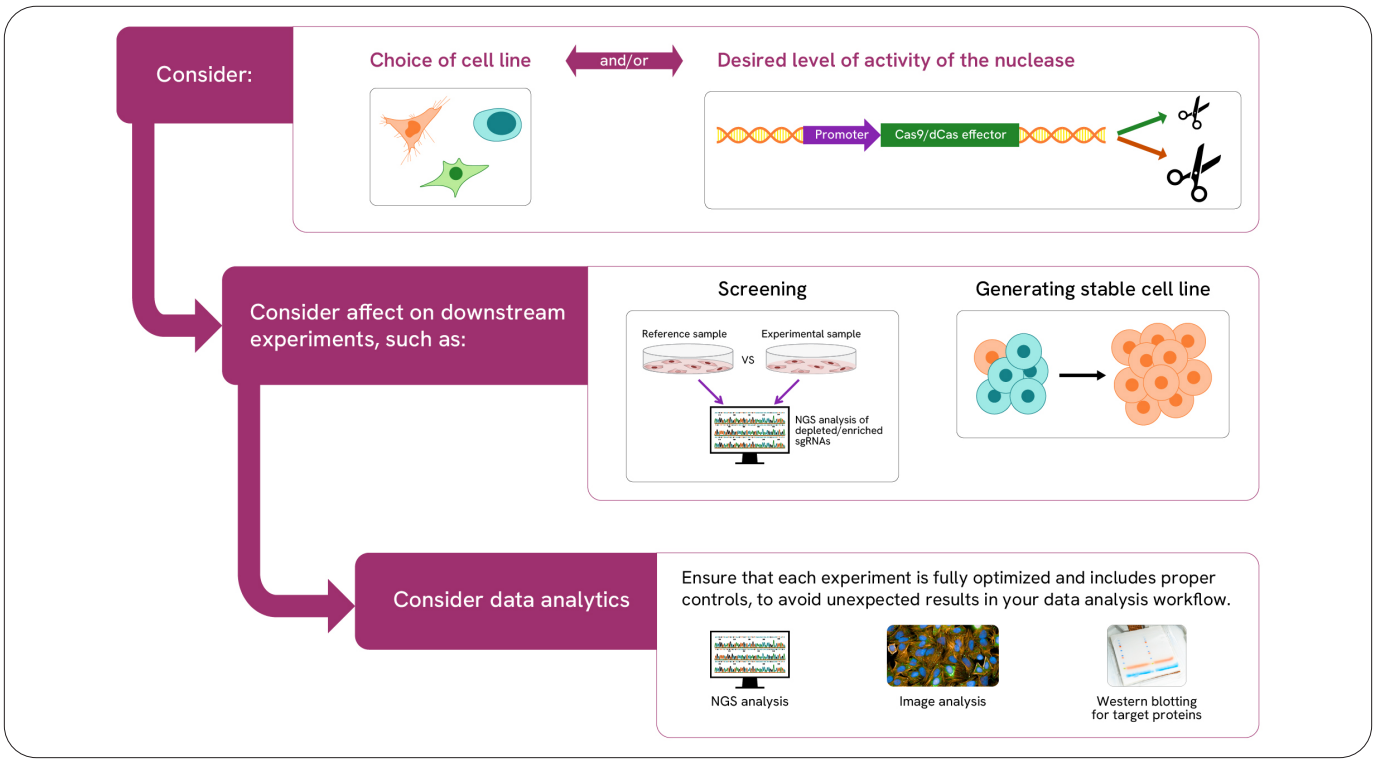
1. Level of nuclease activity required by experiment
2. Experimental cell line
3. Downstream experiments

The experimental results shown on pages 8-9 of this guide highlight the **importance of systematic experimental optimization;** target parameters and experimental goals may benefit from testing different promoters to achieve optimal results.

This guide should be used as a starting point for promoter selection; however, it is recommended to **try at least two promoters to determine optimal nuclease expression in your study.** As shown, the same promoter can perform differently depending on the cell line. Positive and negative controls should also be included when evaluating experimental targets and determining optimal promoter expression. For optimization in a cell line not listed, consider our AIO lentiviral delivery controls expressing EGFP and the nuclease cassette driven by either the hEF1α or the mCMV promoters found on the website to optimize the nuclease expression in your cell line of interest.

See our technical manuals and controls found on our website for quick and efficient experimental optimization and use our **delivery controls** to optimize the promoter selection and delivery conditions. These lentiviral products can be used **to determine optimal titering units to achieve desired MOI** by evaluating the level of EGFP fluorescence using flow cytometry or microscopy as compared to positive and negative controls in the experiment.

For additional questions and advice about promoter selection, experimental optimization, or getting started with your CRISPR experiment, please contact our Technical Support Team (technical@horizondiscovery.com)



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