

Cas-CLOVER™ Update - Cell Line Development and Engineering

Update to the webinar by Demeetra and Elanco

12.09.2020

Kayla I. Bean

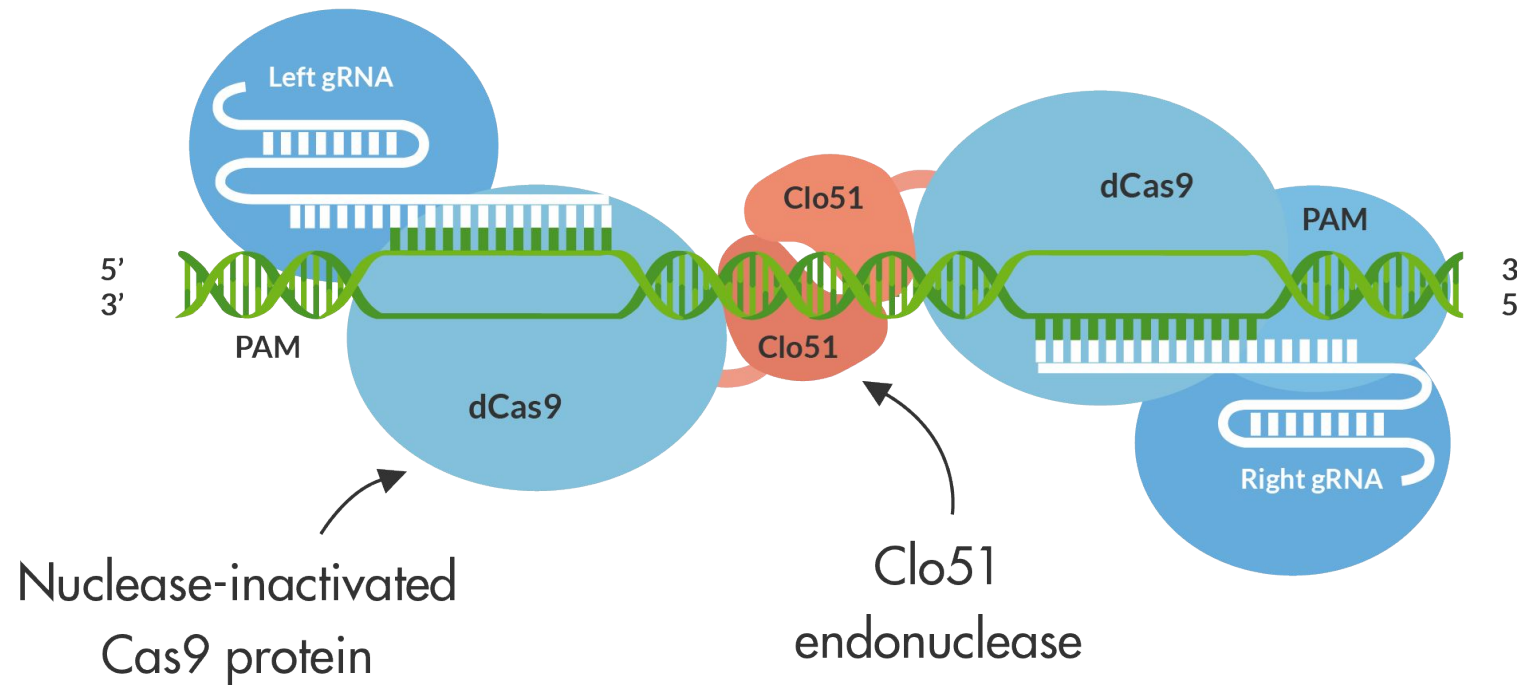
Elanco Animal Health

Discovery Research

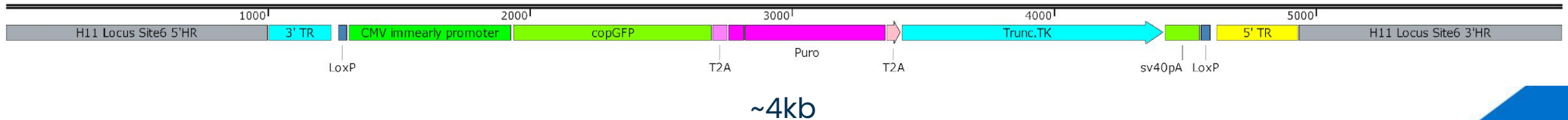
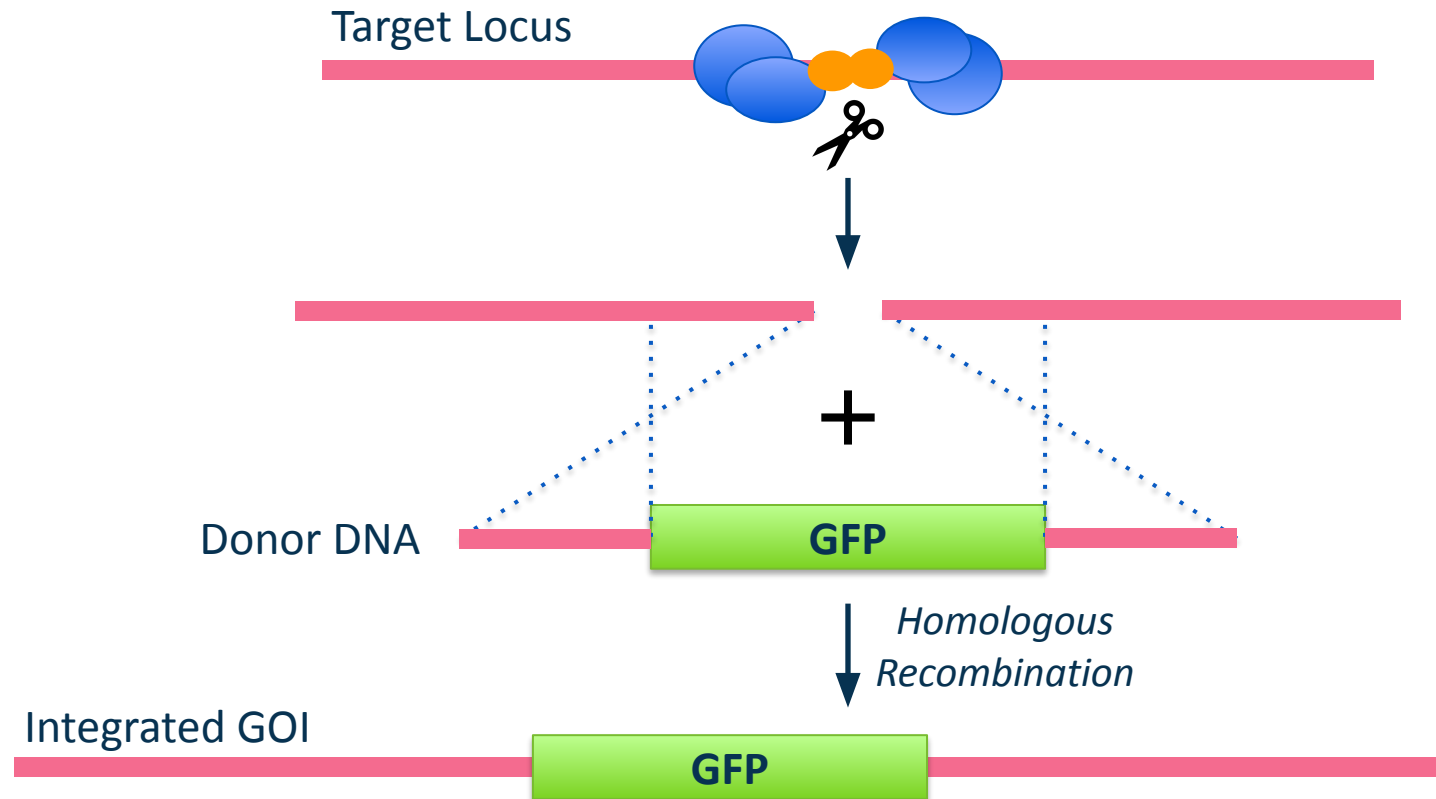


Benefits of Cas-CLOVER System

- Ease of use: flexible double gRNA guided
- High efficiency: dCas9-guided RNA recognition
- High fidelity: cuts only when Clo51 nuclease dimerizes

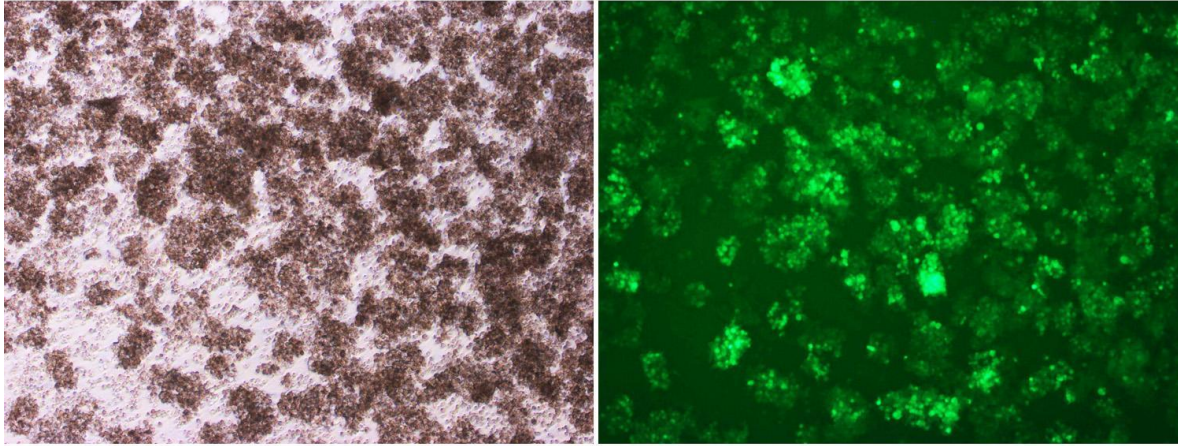


Knock-In Strategy Using Cas-CLOVER

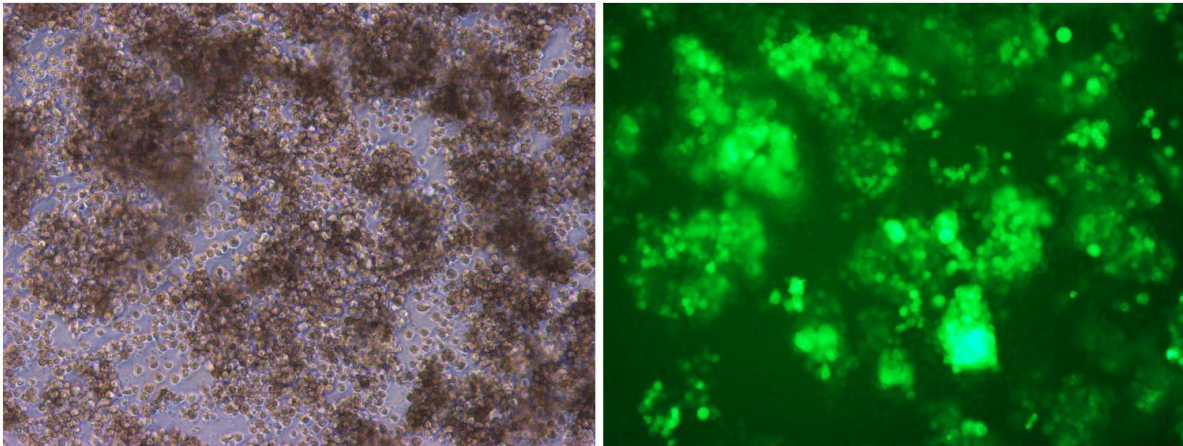


Cas-CLOVER high efficiency gene of interest (GOI) targeted knock-in

5x objective



20x objective



- Estimated 80-90% targeting efficiency of the GOI at the H11 safe-harbor locus
- Projects take **10-17 days**
- Quickly knock-in 1 or more copies of the GOI for consistent and high protein expression



Sequence confirmation of GOI targeted

Genomic to 5' Hom Arm

- Sequence Confirmed

5' Flanking Genomic Sequence

5' Homology Arm



CTCAGGAGAAGGGTCTTTGAATGCAAGAATGCACCTCA																TTGGGCATGGTGGATTATATAATGCTTCTAATCTCTGCATTAGCGGGTAGATGCAGGAGGATCTCTCTTAAGTACGAGGCCAGCAAGAGTTACATAAAATCTTGCCCGTCTGTCTGTCTGTCTCTCTCT																																																			
ctcaggagaagggctctttgaatgcaagaatgcacctca																ttgggcattgggtgattatataatgcttctaatctctgcatttagcgggtagatgcaggaggatctctcttaagtacgaggccagcaagagttacataaaatcttgcccgctgtctgtctgtctgtctctctct																																																			
CTCAGGAGAAGGGTCTTTGAATGCAAGAATGCACCTCA																TTGGGCATGGTGGATTATATAATGCTTCTAATCTCTGCATTAGCGGGTAGATGCAGGAGGATCTCTCTTAAGTACGAGGCCAGCAAGAGTTACATAAAATCTTGCCCGTCTGTCTGTCTGTCTCTCTCTCT																																																			
5160				5170				5180				5190				5200				5210				5220				5230				5240				5250				5260				5270				5280				5290				5300				5310				5320			

5' Hom Arm to ITR

- Sequence Confirmed

TCTCAAAACAATACAAAAC		CAGAGAGAAC	CCCAAAC																		TCCATT	CGGTAG	CCCAAC	CCCTCCT	TTTTTA	AATTAAC	CCCTAG	AAAGATA	AATCAT	ATTGTG	ACGTAC	GTTAAAG	AATCAT	CGCTAAA	ATTGAC	GCATGT	GTTTTAT	CGGT	CTGT	TATAT	CGAGG	TTTATT
tctcaaaacaatacaaaac		cagagagAAC	cccaaac																		tccatt	cggtag	cccaac	ccctcct	ttttta	aatTAAC	ccctag	aaagata	aatCAT	attGTG	acgtac	gTTAAAG	aatCAT	cgctaaa	attgac	gcattGT	gTTTTAT	cggt	ctgt	tatat	cgagg	tttatt
TCTCAAAACAATACAAAAC		CAGAGAGAAC	CCCAAAC																		TCCATT	CGGTAG	CCCAAC	CCCTCCT	TTTTTA	AATTAAC	CCCTAG	AAAGATA	AATCAT	ATTGTG	ACGTAC	GTTAAAG	AATCAT	CGCTAAA	ATTGAC	GCATGT	GTTTTAT	CGGT	CTGT	TATAT	CGAGG	TTTATT
6170	6180	6190	6200	6210	6220	6230	6240	6250	6260	6270	6280	6290	6300	6310	6320	6330																										

ITR to 3' Hom Arm

- Sequence Confirmed

TCGCGCTATTTAGAAAAGAGAGCAATAT										TTCAAGAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAAAGACCTTGAGACCCTAGGCTGTAGACGTGGGTCTCTCAGTATTCCCACTCACTCAGCCTGCTGTCAACCTTTGACAGTGTATCCCTCCATA																							
TCGCGCTATTTAGAAAAGAGAGCAATAT										TTCAAGAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGT										TAAAAGACCTTGAGACCCTAGGCTGTAGACGTGGGTCTCTCAGTATTCCCACTCACTCAGCCTGCTGTCAACCTTTGACAGTGTATCCCTCCATA													
TCGCGCTATTTAGAAAAGAGAGCAATAT										TTCAAGAATGCATGCGTCAATTTTACGCAGACTATCTTTCTAGGGTTAAAAGACCTTGAGACCCTAGGCTGTAGACGTGGGTCTCTCAGTATTCCCACTCACTCAGCCTGCTGTCAACCTTTGACAGTGTATCCCTCCATA										TAAAAGACCTTGAGACCCTAGGCTGTAGACGTGGGTCTCTCAGTATTCCCACTCACTCAGCCTGCTGTCAACCTTTGACAGTGTATCCCTCCATA													
10100		10110		10120		10130		10140		10150		10160		10170		10180		10190		10200		10210		10220		10230		10240		10250		10260	

3' Hom Arm to Genomic

- Sequence Confirmed

3' Homology Arm

3' Flanking Genomic Sequence



CCTGTAGATCACATTAAAGGTGAGTTAGCATGTTTAGACTTCTCTGT					TACCTATGGGCCCACATTCTCACTAAGCCATGATGTGATCGCTGCCAACCATCTTCAGATAATCAGACTGCCAAGTGCTTTCTCCTGTAGTCTTAATGACCGTCCCTCCCACTCCTAGCAATG											
cctgtagatcacattaaagggtgagttagcatgtttagacttctctgt					tacctatgggcccacattctcactaagccatgatgtgatcgctgccaaccatcttcagataatcagactgccaaagtgcctttctcctgtagtcttaatagaccgctccctcccaactcctagcaatg											
CCTGTAGATCACATTAAAGGTGAGTTAGCATGTTTAGACTTCTCTGT					TACCTATGGGCCCACATTCTCACTAAGCCATGATGTGATCGCTGCCAACCATCTTCAGATAATCAGACTGCCAAGTGCTTTCTCCTGTAGTCTTAATGACCGTCCCTCCCACTCCTAGCAATG											
11090	11100	11110	11120	11130	11140	11150	11160	11170	11180	11190	11200	11210	11220	11230	11240	11250





Special Cas-CLOVER bioprocessing evaluation offer

Exclusive rights to gene editing technologies **Cas-CLOVER** for pharmaceutical bioprocessing

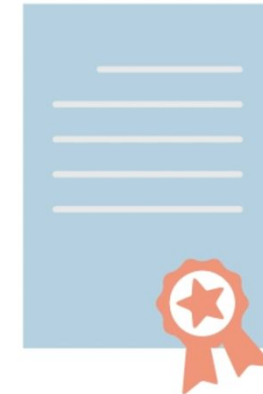
Full **freedom to operate in a single license** for commercial use

With a Cas-CLOVER Evaluation for Cell Line Development you get:

- Access to technology being used by Big Pharma and large CDMO's
- Validated Cas-CLOVER gRNA pairs at the GS locus (knockouts) and H11 safe-harbor site (knock-ins)
- Supply of Cas-CLOVER mRNA
- Optimized protocols for suspension CHO cells for both transfection and nucleofection

Following successful evaluation, we offer highly accessible (a fraction of CRISPR/Cas9 with no royalties) commercial licenses

Optional services include custom gRNA pairs and custom cell line development services. If you would like us to edit your platform cells, we can do it!



**Pharmaceutical
Bioprocessing**

