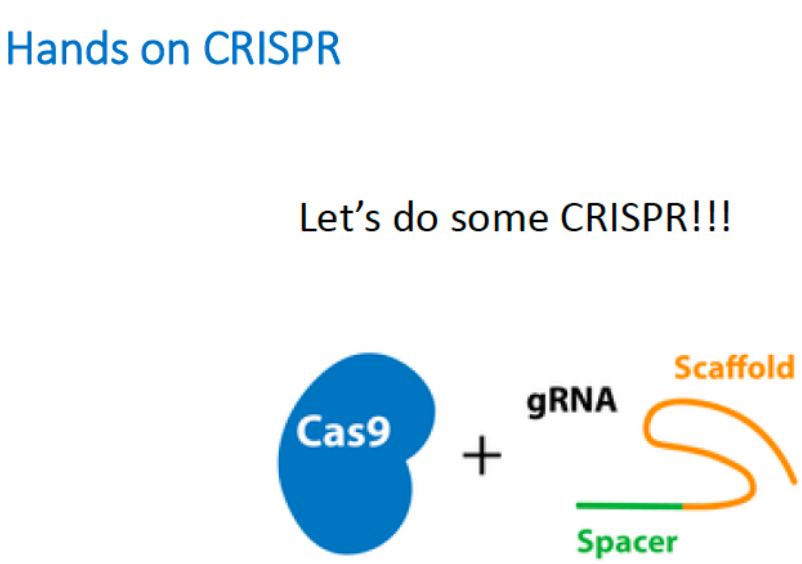
GRANT AGREEMENT NO 2019-1-PT01-KA203-061449
ERASMUS+ PROGRAMME – KA2 – STRATEGIC PARTNERSHIP

HYBRID
LAB NETWORK

Hands on CRISPR

Author: Fábio Ferreira



EnGen sgRNA Synthesis Kit

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Erasmus+ Programme
of the European Union

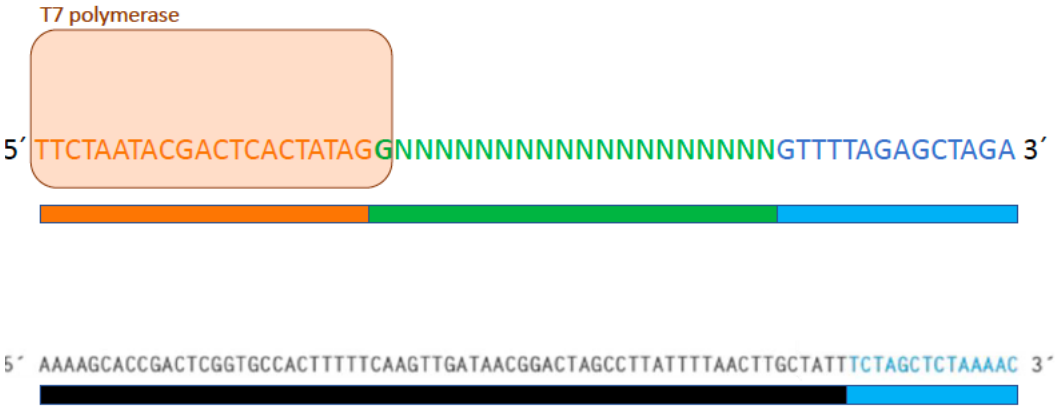
Hands on CRISPR

EnGen sgRNA Synthesis Kit

1. Select 20 nucleotide target sequence (not including the PAM (NGG) sequence). Use of a target DNA selection program is recommended.
2. Check input sequence for presence of "G" at the 5´ end. If there are no "G's" at the 5´ end, add one "G.
3. To the 5´ end; append T7 promoter sequence: TTCTAATACGACTCACTATA
4. To the 3´ end; append 14 nucleotide overlap sequence: GTTTTAGAGCTAGA
5. Check complete oligo sequence:

   5´ TTCTAATACGACTCACTATAG$(N)_{20}$GTTTTAGAGCTAGA 3´

Hands on CRISPR



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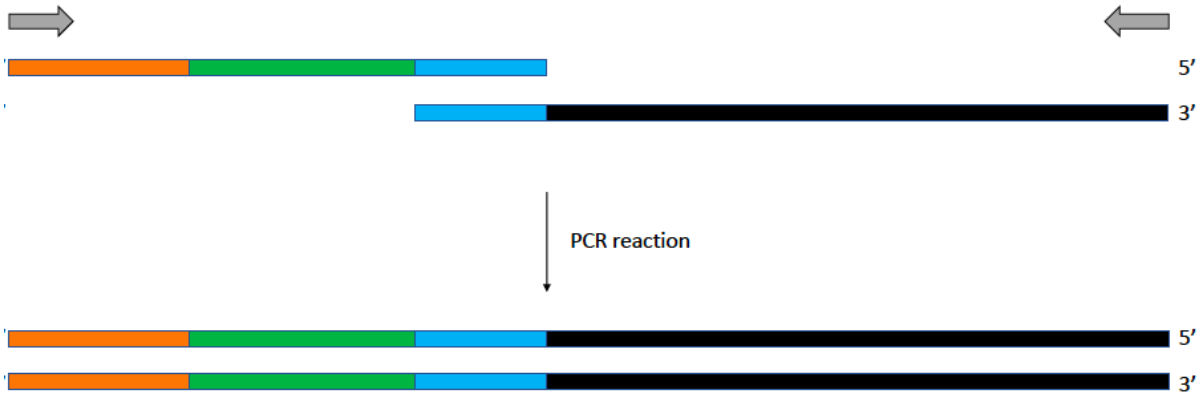
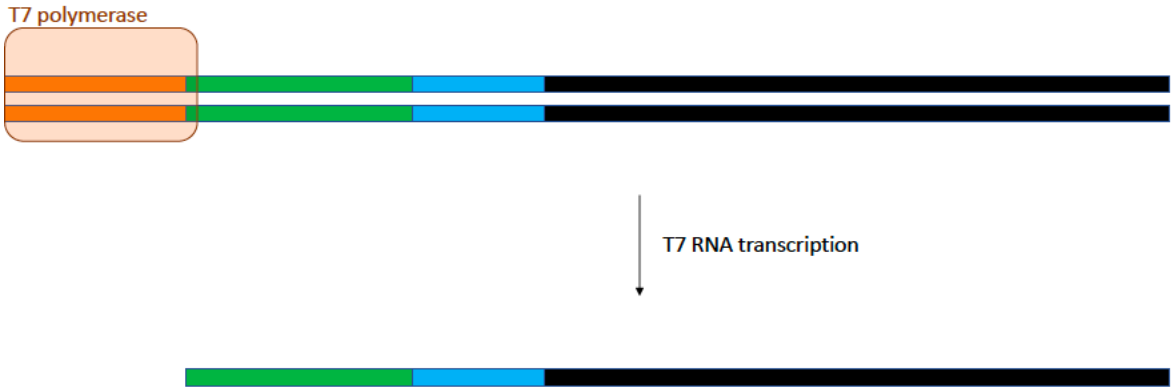
Hands on CRISPR



Hands on CRISPR



Some online tools

- Zhang lab (overview): https://zlab.bio/guide-design-resources
- Benchling: https://www.benchling.com/crispr/ (favorite)
- CRISPick: https://portals.broadinstitute.org/gppx/crispick
- CHOPCHOP: https://chopchop.cbu.uib.no/
- CRISPRscan: https://www.crisprscan.org/ (many species)
- CRISPy: http://staff.biosustain.dtu.dk/laeb/crispy_scoeli/ (S. coelicolor)

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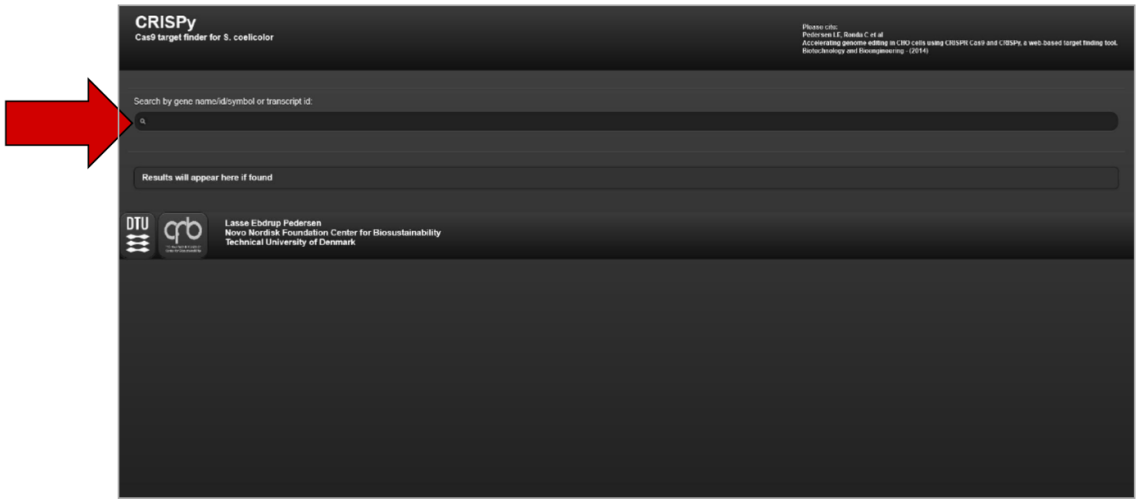
Design sgRNA: Tools and parameters

EXAMPLE 1- *S. coelicolor*

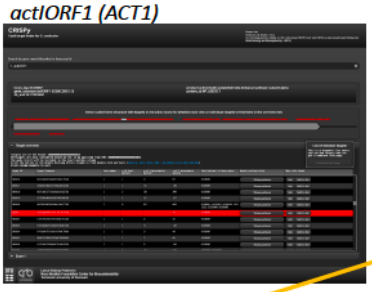
To find *S. coelicolor* target sequence use CRISPy – a specific Cas9 target finder for *S. coelicolor*:
a. actIORF1 (ACT1)
b. actVB (ACT5)

CRISPy: http://staff.biosustain.dtu.dk/laeb/crispy_scoeli/ (S. coelicolor)

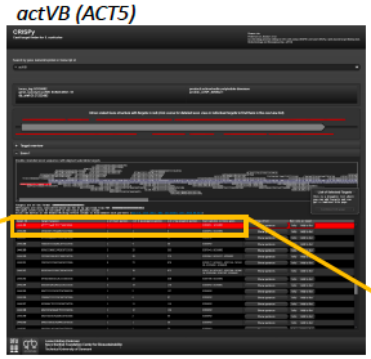


Design sgRNA: Tools and parameters

actIORF1 (ACT1)



actVB (ACT5)



Target ID	Target Sequence	Exon number	# of Exact matches	# of 1 bp mismatch matches	# of 2 bp mismatch matches	Exact matches in these genes
1446187	GTCCCGGAGCATTCCCTGGTCGG	1	1	1	15	SC05092
1446188	TGGCGTCCCGGAGCATTCCCTGG	1	1	4	36	SC05092

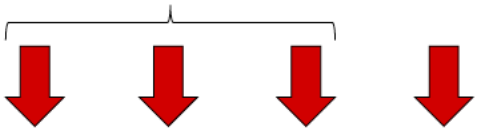
Design sgRNA: Tools and parameters

actVB

Target sequence
GTCCCGGAGCATTCCCTGGTCGG

Guide sequence
GTCCCGGAGCATTCCCTGGT (+ CGG, *PAM*)

Off-targets?



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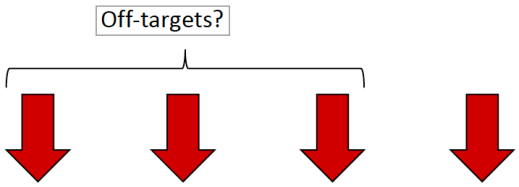

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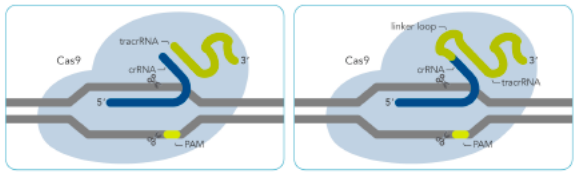

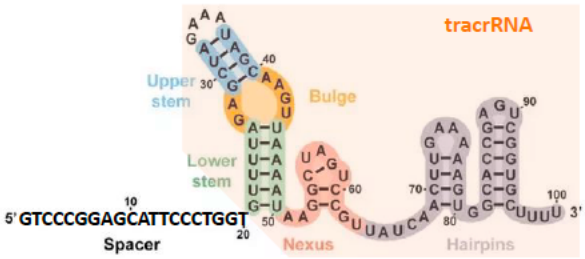

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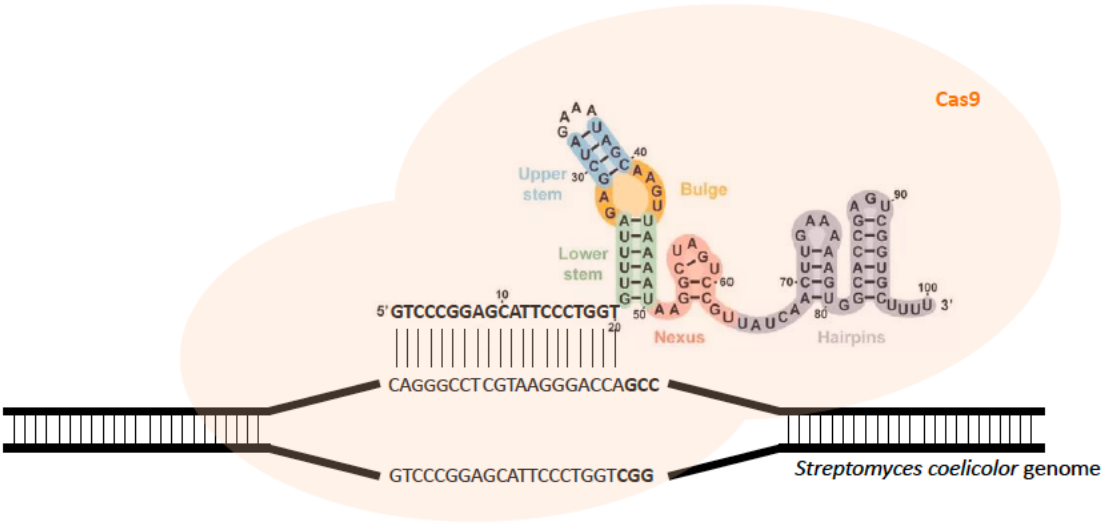

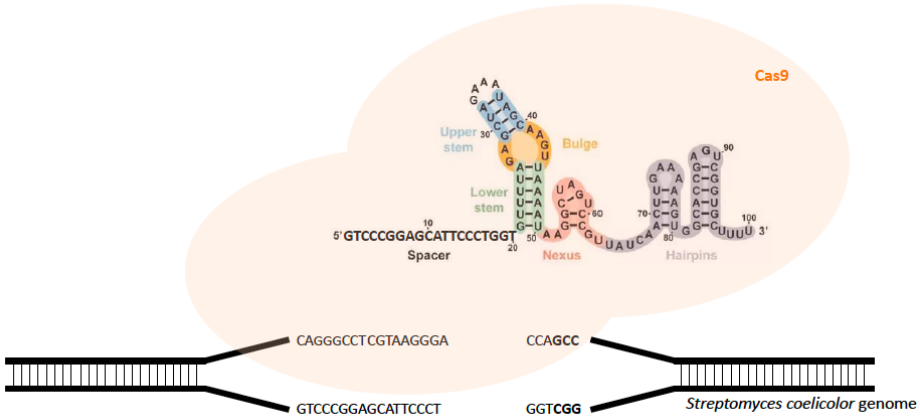

Hands on CRISPR

slide
5´ TTCTAATACGACTCACTATAG**G**NNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGA 3´

sgHL3:
TTCTAATACGACTCACTATA**G**TCCCGGAGCATTCCCTGGTGTTTTAGAGCTAGA

sgHL4:

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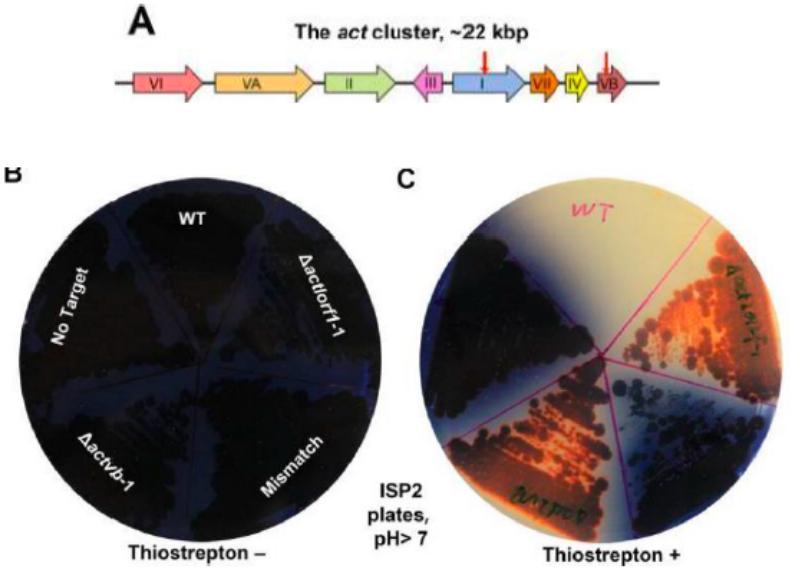
TTCTAATACGACTCACTATA**G**CCAGGCAGACCAGTGCCAGGTTTTAGAGCTAGA

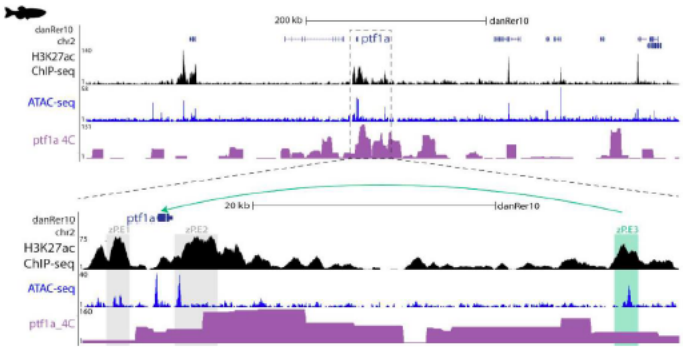
IMAGENS A B C – Tong et al, 2015

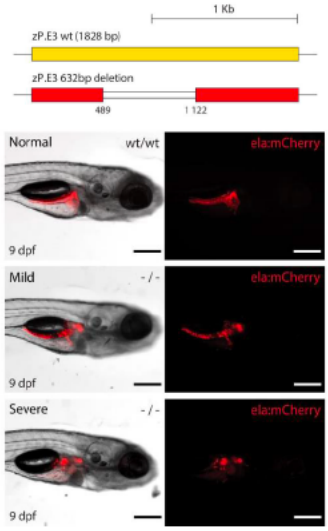


Tong et al., 2015

Zebrafish example

A non-coding genomic region from Zebrafish (*Danio rerio*)





Bordeira-Carriço, Teixeira, Duque... Bessa, ***BioRxiv*** (2020)

Design sgRNAs

CRISPRscan (many species):
https://www.crisprscan.org/

Design sgRNAs

CRISPRscan:
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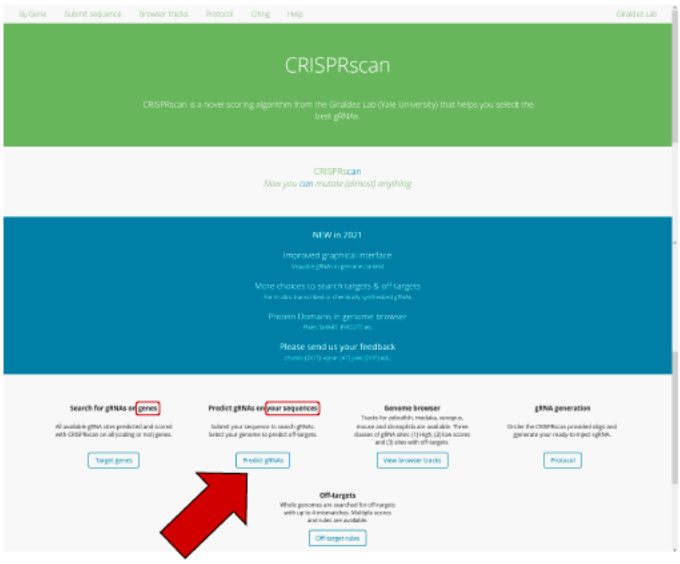

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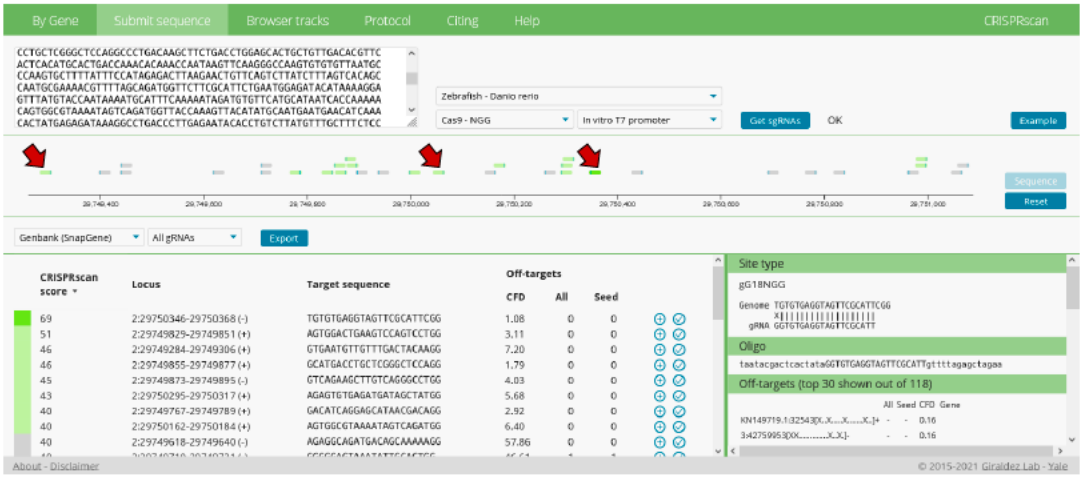
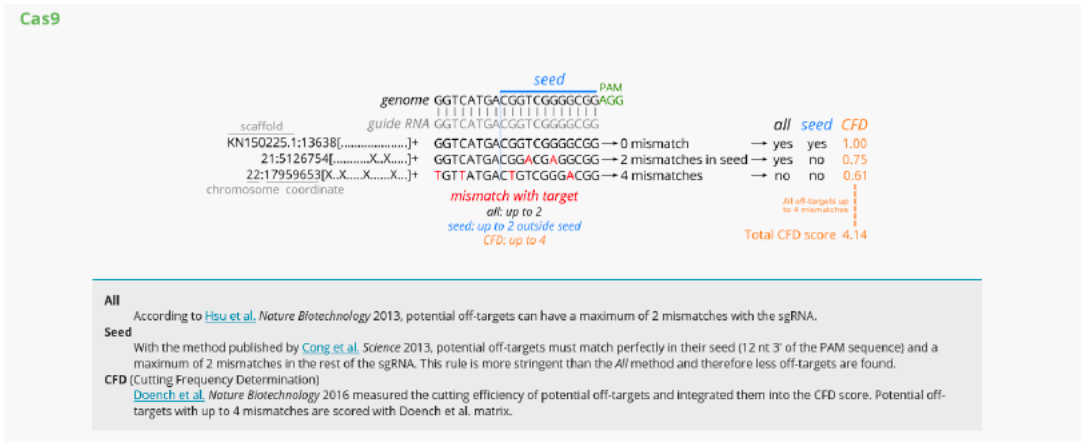
Design sgRNAs



Design sgRNAs



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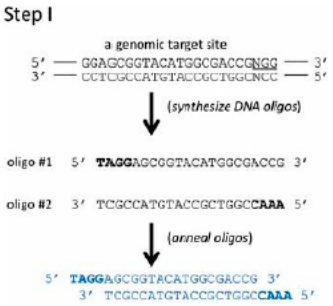
Design sgRNAs

Clone sgRNAs



sgRNA name	Sequence (with overhangs)
Ptf1aE3_Sg1_F	TAGGGAATGTTGTTTGACTACA
Ptf1aE3_Sg1_R	AAACTGTAGTCAAACAACATTCCC
Ptf1aE3_Sg3_F	TAGGCGAAAACGTTTTAGCAGA
Ptf1aE3_Sg3_R	AAACTCTGCTAAAACGTTTTCGCC
Ptf1aE3_Sg4_F	TAGGTGTGAGGTAGTTCGCATT
Ptf1aE3_Sg4_R	AAACAATGCGAACTACCTCACACC

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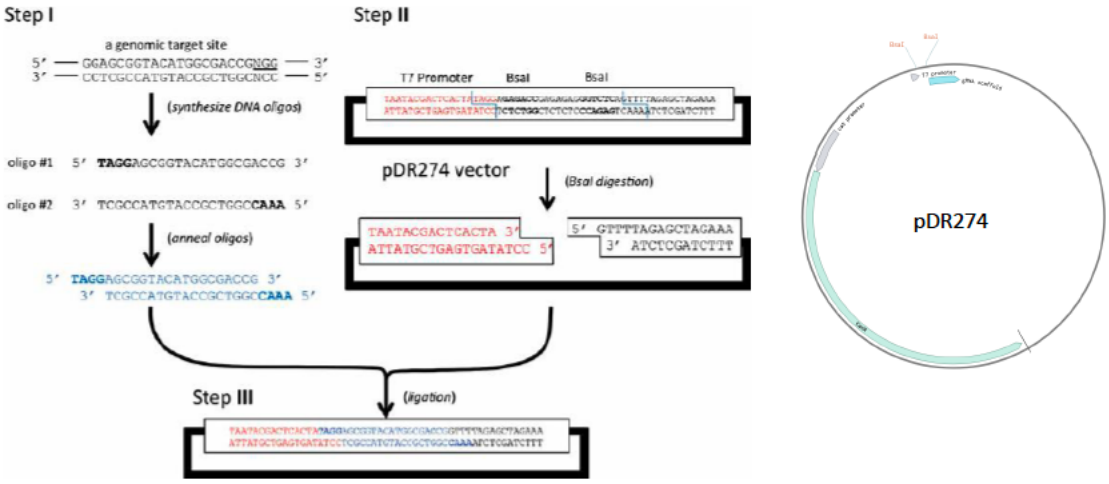
Clone sgRNAs



Clone sgRNAs

Cleave DNA in vitro

DNA Template (zebrafish ptf1a enhancer) – PCR product (~1800bp)

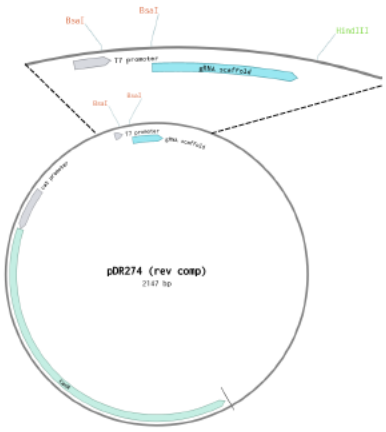
+

sgRNA (synthetized with T7 DNA polimerase)

- includes spacer and tracrRNA

+

Cas9 protein



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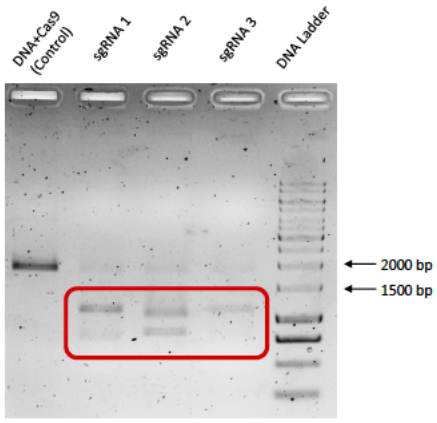
Cleave DNA in vitro

Check cleavage efficiency in an agarose gel:



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In silico activity Protocol

Design the target for the sgRNA - Target-specific oligo design

This kit contains the S. pyogenes Cas9 Scaffold Oligo within the EnGen 2X sgRNA Reaction Mix, S. pyogenes. Target-specific oligos are designed by the user as follows:

1. Select 20 nucleotide target sequence (not including the PAM (NGG) sequence). Use of a target DNA selection program is recommended. We recommend ChopChop.
2. Check input sequence for presence of "G" at the 5´ end. If there are no "G's" at the 5´ end, add one "G" (making it a total of at least one G at the 5´ end).
3. To the 5´ end; append T7 promoter sequence: TTCTAATACGACTCACTATA
4. To the 3´ end; append 14 nucleotide overlap sequence: GTTTTAGAGCTAGA
5. Check complete oligo sequence: 5´ TTCTAATACGACTCACTATAG(N)20GTTTTAGAGCTAGA

Design the respective sequences to synthetize one sgRNA to target the "red" sequence of the partial (Bull's) sequence **- Template for oligo design and sequence to mutate**

The challenge!

Design the Genomic target region that should have the following configuration:
Target(20N) NGG

Design the sequence of the oligo to order, required for the sgRNA transcription, having the following configuration:
T7Promoter (G) Target(20N) Linker

Design the full template for the sgRNA sequence transcription, that must have the following configuration:
T7Promoter (G) Target(20N) Scaffold

In silico Components:

T7 promoter
TTCTAATACGACTCACTATA**(G)**

Linker
GTTTTAGAGCTAGA

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Scafold (5>3)

GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC
CGAGTCGGTGCTTTT

Sequence to mutate:

TCGGTCCCGTGTATCCATGGCCCAGCCGGCACTTGTCAGACTGTCACAGATCGGGGGTGAGATA
TTTCTCCTGTTCCAACACGTGATGATTTCGCTTGCAATGGCTCGTGGCCAACATAGCGAGTATGG
ACTCCAGGACGTCCTGATCCCGGATGCCTGGGGTTTTTACCAGTTTCGTCAGAGTCCTCAGGATA
CTACGCCCCACTTCTATCGCCAAGTTTCATGAGAAACACTCGCCTTTGGATTAATCTCAGCGACC
CAGGGCACGCAAGAGGGGTGCGTCGTCGAAGCCAGGTCACCACTTACAACTTGGGGGGGCCTA
GTGGACGGGGTATTCGATCCTTCCGTAGCTCAAACTTTATAGAGTCACAGACACCTCCCACTGAC
CTTTGTAGTTGGTGCCGGTCCACTACAGGCTACAAGGTCTTGAGTAGTGGCGGGTGGGGTACTC
GAGAACACGTTAGTCAGCTCCGGCGTTCCACTTCAGCCTAGCCTAAGACAAATAACCCTTGCAG
TACCACTAACAAGGAGCGCCGGGGCGGGATATTTTAAATTAAAAAAACAACTTACCCCTACGAA
CGATGTGAACGAGCGTAAAATTGCATGGTGACCCGGGCGCCAAGTCTCTCTTGACTGCCCGCGA
GCCTTGGGCGTTCGTCTTTACTGAGTCTCGGGTTACGTCCTGCCTTCTATTGTCCCAAATTAGAAC
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CAATTGATGAGAGCAGGGCTGTAGATGCAAGCTCGTTCAGCATGTCCCTCACGTACCTCCTTGG
CGTATGGCTGGTCACCTAGGTACGGTGGCTGATGAAAGGGAGGCTTAGCTGGGTGCAGAGGTA
TATAAGGGTCGTCGGTGATCCTTTGTTTCAGCGGAAGCAACGCGGACAGGCATCAGACTCATTG
CGGTAGGGAAGCCCGCATCAAGAATTATATGTCCTGTGTCGCCGAGCTCTCTGAGCGTGTTCGG
AGCCTGTGGTTCTCGGTGTCATCCGTGGACACCTAGAAGAGCGGTGGTAGGTGCGGTAGGATG
GCTTTAGTACACGTGAGAGACTGAAAGATCTATAAGCTCGGGCTTTAAACGAAACCGTCAACCAA
GTCGCTCGAGCGTAAAATATAGATTCCCATCGGGCGCCAAGTTTGTTTCAGCGGTAGCTACGGG
GAGGAAGGTTTAAAGCTAGCATTTTCTCAGTCTCGTAGGTACGTTTTCAACTCGCTTCACGCTGTG
TTGGTTTGGAAGGCATGCAGTATGTATGTATGATCAACTAAATGCTCGATAAAAGGAGATGTTATG
AGGCTTTCGCTGATGCCTGTTAGGTTGGATGGATGGATCCGGCAGACAGCAGCGATTCATGACTT
ACTCCACATCTGACTCTACGCCTAGCTTCACATGCATGCTATTAGAGCTAGGCCACTGGCTGGGA
TACGCCGTCCCAATGGTACATAGTGGCCAGTAAGATCATCGTTCGTATTCTATGCATGGTGACCT
CTCTCAGGTTAGTTCGTATTCTACCTGCCAAATTTGGTTGGGCTGTTGATCGTGAGTCCACCGCAT
CCCCCCCAAAACAATCGGGTTTAGCTTCTAAACTAGAGCTTCATCAGGAACCGTCGGGATGGTTA
CGTAATCGAGTGTCAGTGCGTGTCTCTGTCGCGCTCCCACCCTGATAAGTGCGTCTTCGAGCCG
GCGATTCGTCCGTCGCGTCGAGGGAGCCCAGTGATAGAAGTGTAACGTACCCCTGGGTAAGTTG
TCACTTGGTTCCGCGTTTTTTTAAGCTGTATTTAAAGGTGTGAGTGTCGAATCCTCCCAGATTAGCT
AGCATTTTCTGACTGGAGGCTCTACCGTACGCCCAAGAGTTACGTTACGGTTCGCTAGAAATGGG
TCACCCGTGT

TTCTAATACGACTCACTATAGTGCCTGTTAGGTTGGATGGATGG GTTTTAGAGCTAGA

Please order mine: TTCTAATACGACTCACTATAGTGCCTGTTAGGTTGGATGGATGG
GTTTTAGAGCTAGA

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