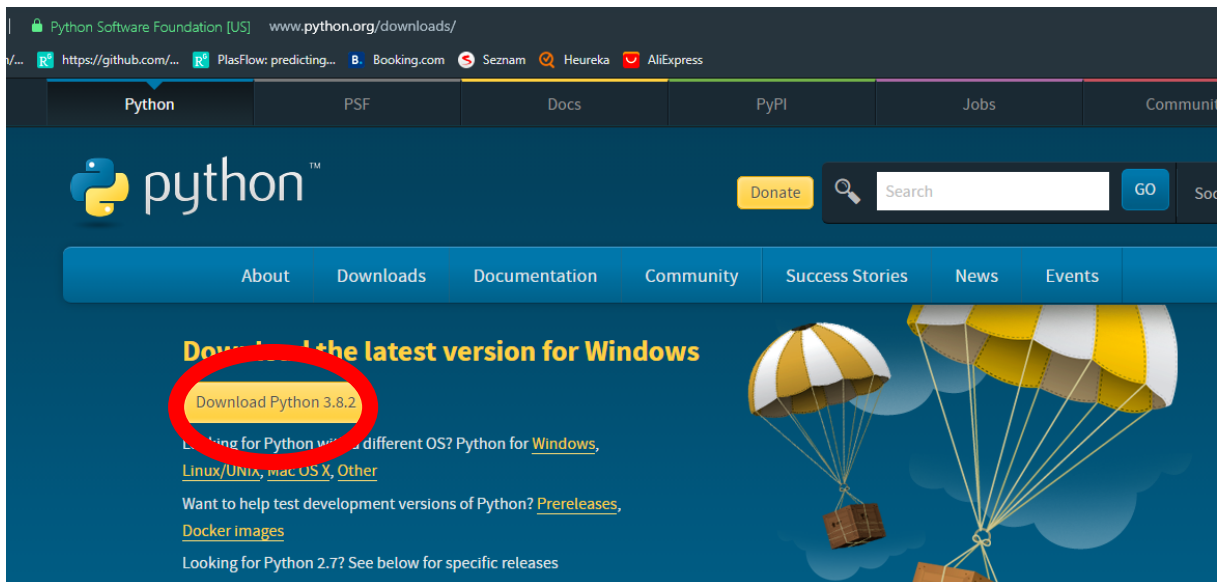


## Návrh protospaceru pomocí skriptu

### 1. Instalace Pythonu

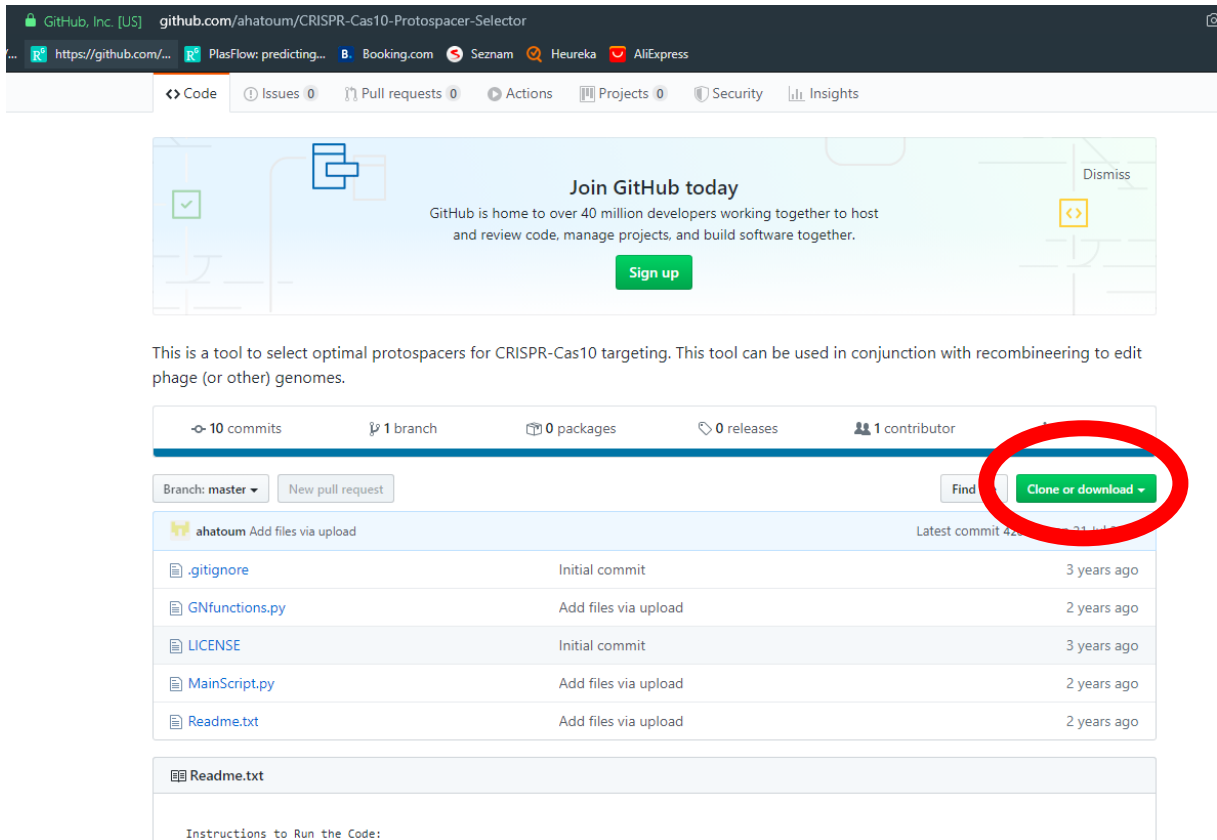
Stáhněte si a nainstalujte verzi Pythonu vhodnou pro váš operační systém ze stránky:

<https://www.python.org/downloads/>



### 2. Stažení skriptu

Ze stránky <https://github.com/ahatoum/CRISPR-Cas10-Protospacer-Selector> si stáhněte skripty MainScript.py, GNfunctions.py. Oba skripty si uložte ve stejné složce.



### 3. Stažení sekvence Major Capsid proteinu (MCP)

Na stránkách Genbank si pod přístupovým kódem MH844528 najdete sekvenci Major capsid proteinu. Uložte si kódující sekvenci proteinu spolu s 500 bp upstream a downstream ve Fasta formátu.

>MH844528\_phi812\_MCP\_500upanddown

```
AATTTCTGAAATCCAAGCTGAAGATGTTTCTAAATCAGTAGACACTGATGAAGAAGCTGTAGAAAAAT
CAGTAACATCTACAAACGGAGAGCAAGAAAAAGTAGAAGGTTACGTTTCTAAATCAGTAGACACTGAA
GAACAAGCTGAAACTGGTGAAGCAAAATCAGAAGAAGCTGAAGAAGTACAAGAAGATAACACATTTA
AAGGATTAAGTCAAGAAGAACGAACTAAGTTCATGGATTCTTACAAAGCACAAGCTAAAGACCCTAGA
GCTTCTAAACATGACTTACAATCAGCTTACCAATCTTACTTGAACATTAACACTGACCCTACTAATGCAT
CAGAGAAAGATATTTAAACTGTAAAAGACTTTGCACAAATTTAATTAATGCACAAAAGTTGTGTTATATT
ATACGGTGTAACTAAAGAATATAAATAGGGTACATTTTACTGTACCCTACATAAAAAAAGAACACA
AATGAAAGGTGATAAATTTATATGACTATCGAAAAGAACCTGTCAGACGTTCAACAAAAGTACGCTGA
CCAATTCCAAGAA
```

4. Otevřete skript MainScript.py ve vhodném editoru, můžete použít například Notepad. Upravte sekvenci tag, pokud využíváte jiný Cas editační systém a Váš tag se liší.

```
MainScript_MCP - Poznámkový blok
Soubor Úpravy Formát Zobrazení Nápověda

import GNfunctions

tag = ['A', 'C', 'G', 'A', 'G', 'A', 'A', 'C'] # Corresponding (Reverse Complemented)
mask = [nuc for nuc in reversed(GNfunctions.comp(tag))]
print(mask)

gene =
"ATGACTATCGAAAAGAACCTGTGACAGCTTCAACAAAAGTACGCTGACCAATTC AAGAAGACGTAGTAAAGTCATTCCAACCTGGTTATGGAATCACTCTGATACACAAATGACG
GTGATATCTCACGCCGCTCTGCTCAATCTACAGTAGTAAAATACGACCAATATTTACGTCATGGTAACGTAGGTCACCTCGTTTCGTTAAAGAAATCGGAGTAGCACCAGTATCTGAC
AATAACATTGCTGACCCATCACAAATCCTTACAGAAGATGCTATCGCAGTTGTTGCAAAAACAATGAGTGGGCTTCATTCTACGGTGACGCTTCATTAACCTCTGAAGTTGAAGGTGA
GAAACACTTAAATGAGCGCCGGTACGTATCGGTAAGGTTTCGGTACAGCTACAGATGCTTACATGCCTATCGGTGTACACGCAGACTTCGTTAACTCAATCTTAGGTCGTC AATGTC
AATTACATGGTTCTACAGTAATGGAAAATGAACTAATCTTAGATGAATCATTACAACCATACCAAATGCTCCACAACCTGC TAAAGTTACAGCTACTGTTGAACTAAGCAAAAAGGT
TCTGAAGAAGTAAACAGCTACAGTATCTAACGTAGACGATGGTGTAAACTTTCAATTAATGTTAACGCATGTACCAACAACAACCAATTCGTTTCTATCTACCGTCAAGGTAAGA
GAACGAAACATTGCCTGAAACAGCAGACGTATTTGTTGGTAAAATGTCACCACAAGTAGTTCACCTATTTCGAATTACTTCCAATGATGAAATTACCATTAGCTCAAATTAATGCTTCTA
ATATCGCAGTTTAA"
poscount, Data = GNfunctions.AnalyzeSpacer(mask, gene)

#poscount represents the number of possible targets in the gene

# WRITE FILE

file = open("Results_MCP.txt", "w")
```

5. Dále vložte sekvenci MCP a okolního lokusu jako plain text do proměnné gene. Nezapomeňte ponechat uvozovky.

```
MainScript_MCP - Poznámkový blok
Soubor Úpravy Formát Zobrazení Nápověda

import GNfunctions

tag = ['A', 'C', 'G', 'A', 'G', 'A', 'A', 'C'] # Corresponding (Reverse Complemented)
mask = [nuc for nuc in reversed(GNfunctions.comp(tag))]
print(mask)

gene =
"ATGACTATCGAAAAGAACCTGTGACAGCTTCAACAAAAGTACGCTGACCAATTC AAGAAGACGTAGTAAAGTCATTCCAACCTGGTTATGGAATCACTCTGATACACAAATGACG
GTGATATCTCACGCCGCTCTGCTCAATCTACAGTAGTAAAATACGACCAATATTTACGTCATGGTAACGTAGGTCACCTCGTTTCGTTAAAGAAATCGGAGTAGCACCAGTATCTGAC
AATAACATTGCTGACCCATCACAAATCCTTACAGAAGATGCTATCGCAGTTGTTGCAAAAACAATGAGTGGGCTTCATTCTACGGTGACGCTTCATTAACCTCTGAAGTTGAAGGTGA
GAAACACTTAAATGAGCGCCGGTACGTATCGGTAAGGTTTCGGTACAGCTACAGATGCTTACATGCCTATCGGTGTACACGCAGACTTCGTTAACTCAATCTTAGGTCGTC AATGTC
AATTACATGGTTCTACAGTAATGGAAAATGAACTAATCTTAGATGAATCATTACAACCATACCAAATGCTCCACAACCTGC TAAAGTTACAGCTACTGTTGAACTAAGCAAAAAGGT
TCTGAAGAAGTAAACAGCTACAGTATCTAACGTAGACGATGGTGTAAACTTTCAATTAATGTTAACGCATGTACCAACAACAACCAATTCGTTTCTATCTACCGTCAAGGTAAGA
GAACGAAACATTGCCTGAAACAGCAGACGTATTTGTTGGTAAAATGTCACCACAAGTAGTTCACCTATTTCGAATTACTTCCAATGATGAAATTACCATTAGCTCAAATTAATGCTTCTA
ATATCGCAGTTTAA"
poscount, Data = GNfunctions.AnalyzeSpacer(mask, gene)

#poscount represents the number of possible targets in the gene

# WRITE FILE

file = open("Results_MCP.txt", "w")
```

6. Uložte změny a spusťte skript.
7. Ve stejné složce jako jsou uloženy skripty by se vám měl zobrazit soubor Results.txt, ve kterém naleznete sekvence možných protospacerů.

Length of Sequence: 324

Possible Protospacers: 57

Tag Input: ACGAGAAC

Sequence Input:

ATGGCAGAAGAAGAAAAATTATTAAGAAGAACCAACGAATGAAGAAACAGAACAACCAGAAAAATTGAAAGTGCAGAAGATGTTGTAAC TGAA  
 AGAAGAAAAATCAGAAGCTTTGTACAATTAGAACAACGTATATCTTCTTTAGAACAAGATTAATAACTTAGAATCACAACCACAACCAACGCA/  
 ATTTTGAAGATAAACAGTACCAACTGAAGTTGATGACAATCAAGAAACAGACGGTATTGAATCAAGTGAAGAAATTAACAAATGTTAAATTTAT/

1	ATGGCAGAAGAAGAAAAATTATTAAGAAGAACC	AACGAATG	GGTCTCTCTTTAATAATTTTTCTCTTCTGCCAT
7	GAAGAAGAAAAATTATTAAGAAGAACCAACGAA	TGAAGAAA	TTCGTTGGTTCTTCTTAATAATTTTTCTTCTTC
9	AGAAGAAAAATTATTAAGAAGAACCAACGAATG	AAGAAACA	CATTCTGTTGGTTCTTCTTAATAATTTTTCTTCT
13	GAAAAATTATTAAGAAGAACCAACGAATGAAGA	AACAGAAC	TCTTCATTCTGTTGGTTCTTCTTAATAATTTTTCT
14	AAAAATTATTAAGAAGAACCAACGAATGAAGAA	ACAGAACA	TTCTTCATTCTGTTGGTTCTTCTTAATAATTTTTCT
16	AAAATTATTAAGAAGAACCAACGAATGAAGAAAC	AGAACAAC	GTTTCTTCATTCTGTTGGTTCTTCTTAATAATTTTT
22	ATTAAGAAGAACCAACGAATGAAGAAACAGAACA	ACCAGAAA	TGTTCTGTTTCTTCATTCTGTTGGTTCTTCTTAAT
23	TAAAGAAGAACCAACGAATGAAGAAACAGAACA	CCAGAAAA	TTGTTCTGTTTCTTCATTCTGTTGGTTCTTCTTA
24	TAAAGAAGAACCAACGAATGAAGAAACAGAACA	CAGAAAAA	GTTGTTCTGTTTCTTCATTCTGTTGGTTCTTCTTA
25	AAAGAAGAACCAACGAATGAAGAAACAGAACAAC	AGAAAAAA	GGTTGTTCTGTTTCTTCATTCTGTTGGTTCTTCTTT
28	GAAGAACCAACGAATGAAGAAACAGAACAACCA	AAAAATTG	TCTGGTTGTTCTGTTTCTTCATTCTGTTGGTTCTTC
34	CCAACGAATGAAGAAACAGAACAACCAGAAAAAT	TGAAAGTG	ATTTTTCTGGTTGTTCTGTTTCTTCATTCTGTTGG
40	AATGAAGAAACAGAACAACCAGAAAAATTGAAAG	TGCAGAAG	CTTTCAATTTTTCTGGTTGTTCTGTTTCTTCATT
43	GAAGAAACAGAACAACCAGAAAAATTGAAAGTGC	AGAAGATG	GCACTTCAATTTTTCTGGTTGTTCTGTTTCTTC
46	GAAACAGAACAACCAGAAAAATTGAAAGTGCAGA	AGATGTTG	TCTGCACTTCAATTTTTCTGGTTGTTCTGTTTCT
55	CAACCAGAAAAATTGAAAGTGCAGAAGATGTTGT	AACTGAAC	ACAACATCTTCTGCACTTTCATTTTTCTGTTG
61	GAAAAATTGAAAGTGCAGAAGATGTTGTAAC TGA	ACCTGAAA	TCAGTTACAACATCTTCTGCACTTTCATTTTTCT