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GGGenome & CRISPRdirect アップデート：塩基配列検索およびゲノム編集のためのウェブツール

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【要 旨】

高速塩基配列検索ソフトウェア GGGenome (https://GGGenome.dbcls.jp/) および CRISPR–Cas9 によるゲノム編集のためのガイド RNA 設計ソフトウェア CRISPRdirect (https://crispr.dbcls.jp/) を紹介する。GGGenome（ゲゲゲノム）は、ゲノムや転写産物などの塩基配列を高速に検索することができるツールである。十数塩基程度の短い配列の検索にも対応しており、ミスマッチや挿入・欠失を含む配列であっても検索漏れがないため、核酸医薬品や CRISPR–Cas9 のガイド RNA の特異性を確認するためにも役立つ。CRISPRdirect は、GGGenome による塩基配列検索を利用することにより、特異性の高いガイド RNA を簡便に設計できるツールである。

GGGenome および CRISPRdirect は、多数の実験動植物や作物などのゲノムに対応し、他のデータベースやソフトウェアとも容易に連携できるよう API を提供している。これらの全ての機能は商用・非商用を問わず無償で自由に利用できる。また、GGGenome や CRISPRdirect を企業等の保有するコンピュータ上で実行可能な Docker によるパッケージ版が、株式会社レトリバより発売されている。

GGGenome

塩基配列をさがす

https://GGGenome.dbcls.jp/

- **塩基配列を高速に検索**

  350種の生物種に対応

- **ミスマッチや挿入欠失を含む短い配列の検索に強い**

CRISPRdirect

CRISPRガイドRNA設計

https://crispr.dbcls.jp/

- **CRISPRガイドRNA設計**

  PAMに隣接する20塩基の選択

- **オフターゲット検索**

  powered by GGGenome

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GGGenome による塩基配列検索

https://GGGenome.dbcls.jp/

超絶高速ゲノム配列検索 Help | English

GGGenome

Human genome, GRCh37/hg19 (Feb, 2009)

許容するミスマッチ/ギャップの数：0 (検索する塩基配列の長さの25%まで)

● 双方向を検索 ○ +方向のみ検索 ○ −方向のみ検索

検索例：

- [TTCATTGACAACATT] …… 塩基配列を検索
- 詳細な使い方
- 遺伝子や転写産物のキーワード検索は GGRNA《ぐぐるな》へ
    - 例）ヒトの「nanog」を検索：http://GGRNA.dbcls.jp/hs/nanog

検索結果へのリンク：

- http[s]://GGGenome.dbcls.jp/db/k/[strand]/sequence[.format][.download]
    - db → hg19, mm10, dm3, ce10, TAIR10, pombe, refseq, etc. 省略時は hg19
      検索可能なデータベース一覧
    - k → 許容するミスマッチ/ギャップの数。あまり大きいとしぼうする。省略時は 0
    - strand → '+' ('plus') または '-' ('minus') で特定の方向のみ検索。省略時は両方向を検索
    - sequence → 塩基配列。大文字・小文字は区別しない
    - format → html, txt, csv, bed, gff, json。省略時は html
    - download → URLの最後に付加すると検索結果をファイルとしてダウンロードできる
- 例1：http://GGGenome.dbcls.jp/TTCATTGACAACATT
    - ヒトゲノム hg19 (省略可) で
    - ミスマッチ/ギャップを許容せず (省略可)
    - TTCATTGACAACATT を検索し
    - html 形式 (省略可) で結果を返す
- 例2：http://GGGenome.dbcls.jp/mm10/2/+/TTCATTGACAACATTGCGT.txt
    - マウスゲノム mm10 で
    - 2 ミスマッチ/ギャップまで許容して

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GGGenome による塩基配列検索

超絶高速ゲノム配列検索 Help | English

GGGenome

TTCACTGACAACATTGAGTA [検索] Human genome, GRCh37/hg19 (Feb, 2009)

許容するミスマッチ/ギャップの数：0 (検索する塩基配列の長さの25%まで)

● 双方向を検索 ○ +方向のみ検索 ○ −方向のみ検索

2017-06-28 06:20:32, GGGenome : Human genome, GRCh37/hg19 (Feb, 2009)

Summary:

- TTCACTGACAACATTGAGTA (1)
- TACTCAATGTTGTCAGTGAA (0)
- **TOTAL (1)**

Results:

検索語に色がつき スマッチ・挿入欠失)。

chr2:30898313-30898332 ▼30898313

```
GCCCCCAGCCACTTATATGTATTTTTTAAATTCACTGACAACATTGAGTAGAAAAGATAATTTTTTTTTTTTTGAGACAG
```

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1 件中

Data Export:

下記のフォーマットで最大100000件まで検索結果を取得できます。

- タブ区切りテキスト → 表示 | ダウンロード
- CSV形式 → 表示 | ダウンロード

エクセル等の表計算ソフトにて開くことができます

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GGGenome による塩基配列検索

超絶高速ゲノム配列検索 Help | English

GGGenome

TTCACTGACAACATT 検索 Human genome, GRCh37/hg19 (Feb, 2009)

許容するミスマッチ/ギャップの数：0 （検索する塩基配列の長さの25%まで）

● 双方向を検索 ○ ＋方向のみ検索 ○ －方向のみ検索

2017-06-28 06:20:47, GGGenome : Human genome, GRCh37/hg19 (Feb, 2009)

Summary:

- TTCACTGACAACATT (10)
- AATGTTGTCAGTGAA (7)
- **TOTAL (17)**

Results:

検索語に色がつきます（ミスマ 挿入欠失）。

chr1:173400829-173400843 ▼173400829
TTTTCACATGCACTACCTTGCCTGCTGTCCTTCACTGACAACATTTCAGGGTCTCGCAAGGCATGGAATACAATG

chr1:197463616-197463630 ▼197463616
AATGATCTTTACTGTTAACACTGCACTTTATTCACTGACAACATTACTTAAAGAAAGAGATTATGATTGGATAAA

chr2:30898313-30898327 ▼30898313
GCCCCCAGCCACTTATATGTATTTTTTAAATTCACTGACAACATTGAGTAGAAAAGATAATTTTTTTTTTTTTGA

chr2:163535086-163535100 ▼163535086
TGAGAAAGAGGACACCCTAAGAATTATGGCTTCACTGACAACATTAGAGAACATATCAAACTCAAACGAATATTC

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GGGenome による塩基配列検索

DBCLS
Database Center
for Life Science

超絶高速ゲノム配列検索 Help | English

GGGenome

TTCACTGACA [検索] Human genome, GRCh37/hg19 (Feb, 2009)

許容するミスマッチ/ギャップの数：0 (検索する塩基配列の長さの25%まで)

● 双方向を検索 ○ ＋方向のみ検索 ○ －方向のみ検索

2017-06-28 06:21:00, GGGenome : Human genome, GRCh37/hg19 (Feb, 2009)

Summary:

- TTCACTGACA (4957)
- TGTCAGTGAA (5068)
- **TOTAL (10025)**

Results:

検索語に色がつきます（ミスマ 挿入欠失）。

chr1:620618-620627 ▼620618
ATACTAGAATTTTAAAAGCAAATGTCTTTATTCACTGACAACATAATCATCTATAAAGAAAATCCTACAT

chr1:728363-728372 ▼728363
CCCTCAGCCACACAGCTGACGCTGTAGAGATTCACTGACATCCAGTCTGATGGACACTGTTTCTCCAGCC

chr1:1336263-1336272 ▼1336263
CCGCAGGGCTCTGCCACATCCCTGTCTTCCTTCACTGACATGAAACGCAGAAAAGGCAGCTTTGCCACAA

chr1:3274566-3274575 ▼3274566
AGAGGCCCACTCGTGAGCCTTGCTGTGCCCTTCACTGACATGCTCGCCCATGAGCTGAGTCTGCCTTGAC

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GGGenome による塩基配列検索

超絶高速ゲノム配列検索 Help | English

GGGenome

TTCACTGACAACATTGAGTA

Human genome, GRCh37/hg19 (Feb, 2009)

許容するミスマッチ/ギャップの数：2 (検索する塩基配列の長さの25%まで)

● 双方向を検索 ○ +方向のみ検索 ○ み検索

2017-06-28 06:21:13, GGGenome : H me, GRCh37/hg19 (Feb, 2009)

Summary:

- TTCACTGACAACATTGAGTA (36)
- TACTCAATGTTGTCAGTGAA (33)
- **TOTAL (69)**

Results:

検索語に色がつきます（ミスマッチ・挿入欠失）。

chr1:77930953-77930971 ▼77930953
AGCAGTACTCAAGATCACCTTTTTTTAAAGTTCACTG-CAACATTCAGTAGATGAAAAATGTGTAGCTGTAATTCATTGA

chr1:106947513-106947532 ▼106947513
TAGAAAACATACCACATAAAGAAGCAATCTTTCACTGCACAA-ATTGAGTATAAAATTTAACCCGTTTGAAAGGGTCATTA

chr2:30898313-30898332 ▼30898313
GCCCCCAGCCACTTATATGTATTTTTTAAATTCACTGACAACATTGAGTAGAAAAGATAATTTTTTTTTTTTTGAGACAG

chr2:141517099-141517116 ▼141517099
TTTATTTTCTTTAACACAGCCTTTGATAAATTCACTGAC-ACATTGAGT-TAGCCATAGTGTGTAATGGGAGACTGCCCA

TCAAACTCAAACGAATATTCTGG

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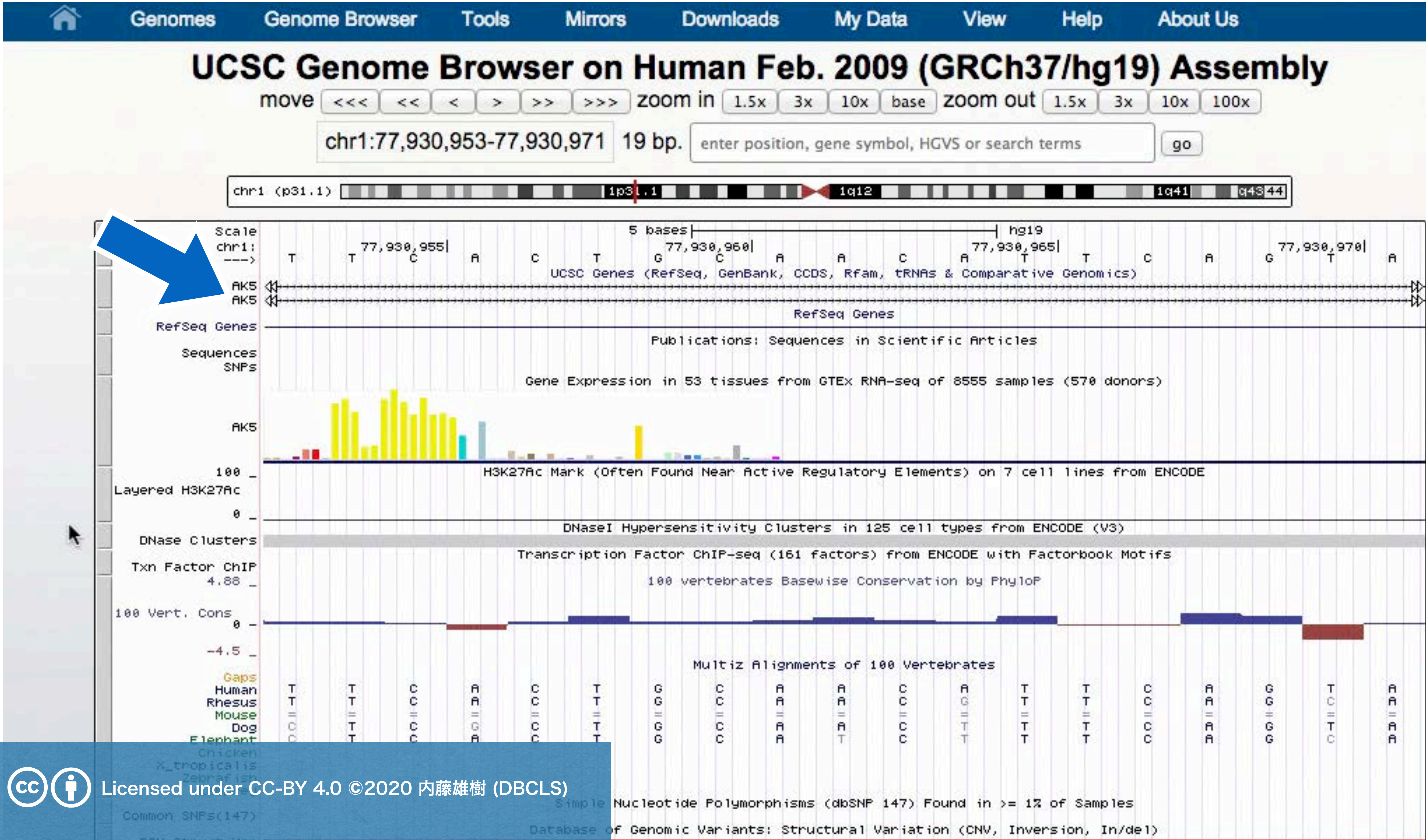
GGGenome による塩基配列検索



UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

chr1:77,930,953-77,930,971 19 bp.

UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)

RefSeq Genes

Publications: Sequences in Scientific Articles

Gene Expression in 53 tissues from GTEx RNA-seq of 8555 samples (570 donors)

H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE

DNaseI Hypersensitivity Clusters in 125 cell types from ENCODE (V3)

Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs

100 vertebrates Basewise Conservation by PhyloP

Multiz Alignments of 100 Vertebrates

Simple Nucleotide Polymorphisms (dbSNP 147) Found in >= 1% of Samples

Database of Genomic Variants: Structural Variation (CNV, Inversion, In/del)

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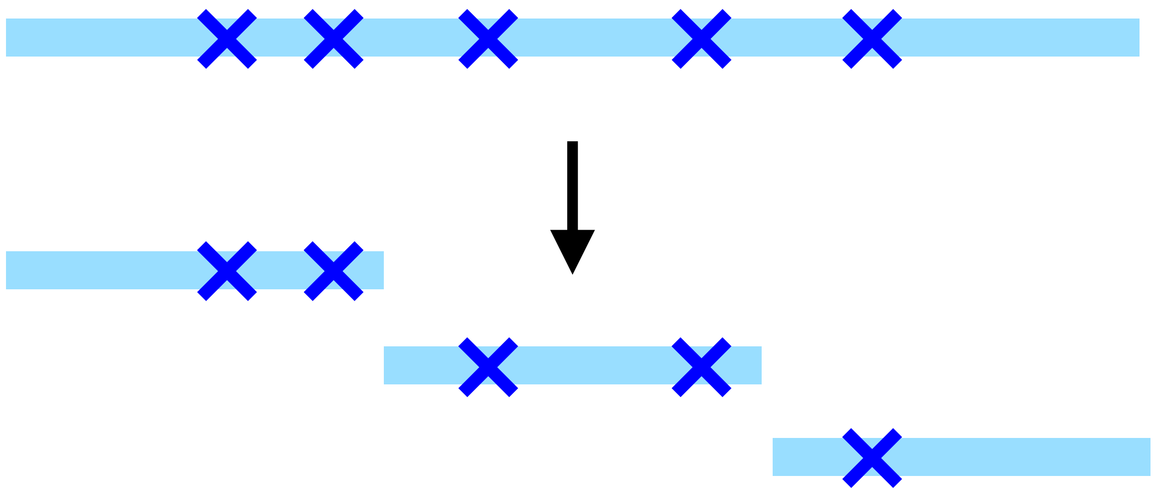
GGGenome のしくみ

- **分割統治法**



30 mer / **5ミスマッチ**以内

分割すると、どれかは必ず
10 mer / **1ミスマッチ**以内

- **接尾辞配列 (suffix array) ＋ FM-index**
- **インデックスをSSDに置くことにより高速化**

漏れのない検索を高速に実行

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GGGenome を他のツールから呼び出す

- アドレス ＝ 検索クエリ になっている
- 結果をさまざまな形式で出力できる

検索例：

http[**s**]://GGGenome.dbcls.jp/**hg38**/**2**/**ATAGGACCATA**[.**txt**]

暗号化通信に対応

①ヒトゲノム hg38に対し

②最大ミスマッチ・挿入欠失 ＝ 2 で

③この配列を検索

④結果はテキスト (txt) 形式で。

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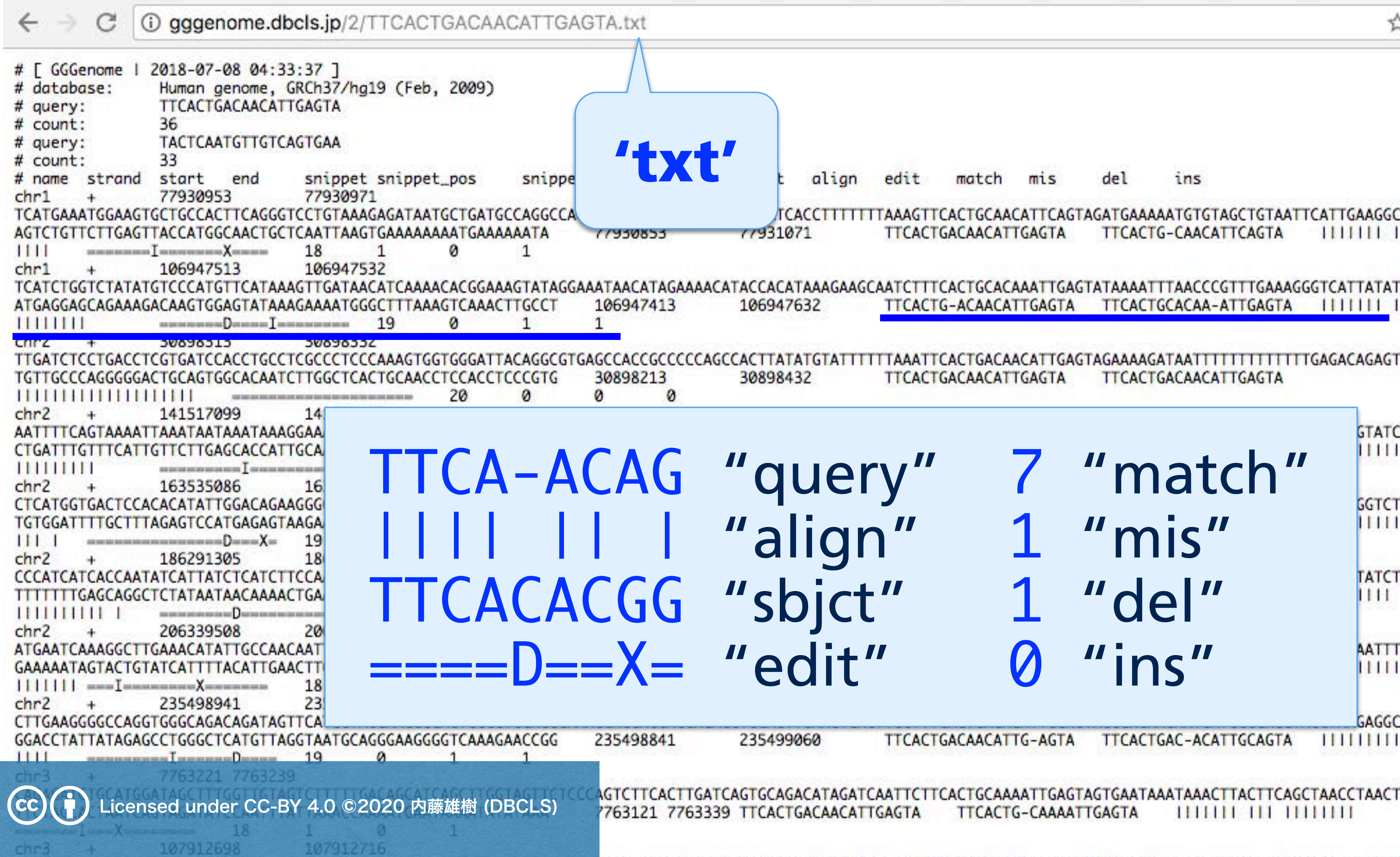
GGGenome を他のツールから呼び出す



```
# [ GGGenome | 2018-07-08 04:33:37 ]
# database:     Human genome, GRCh37/hg19 (Feb, 2009)
# query:        TTCACTGACAACATTGAGTA
# count:        36
# query:        TACTCAATGTTGTCAGTGAA
# count:        33
# name  strand  start   end     snippet snippet_pos     snippe                     t  align   edit    match   mis     del     ins
chr1    +       77930953        77930971
TCATGAAATGGAAGTGCTGCCACTTCAGGGTCCTGTAAAGAGATAATGCTGATGCCAGGCCA                  TCACCTTTTTTAAAGTTCACTGCAACATTCAGTAGATGAAAAATGTGTAGCTGTAATTCATTGAAGGC
AGTCTGTTCTTGAGTTACCATGGCAACTGCTCAATTAAGTGAAAAAAAATGAAAAAATA    77930853        77931071        TTCACTGACAACATTGAGTA    TTCACTG-CAACATTCAGTA    ||||||| |
||||    =======I========X====    18      1       0       1
chr1    +       106947513       106947532
TCATCTGGTCTATATGTCCCATGTTCATAAAGTTGATAACATCAAAACACGGAAAGTATAGGAAATAACATAGAAAACATACCACATAAAGAAGCAATCTTTCACTGCACAAATTGAGTATAAAATTTAACCCGTTTGAAAGGGTCATTATAT
ATGAGGAGCAGAAAGACAAGTGGAGTATAAAGAAAATGGGCTTTAAAGTCAAACTTGCCT    106947413       106947632       TTCACTG-ACAACATTGAGTA   TTCACTGCACAA-ATTGAGTA   ||||||| |
||||||||        =======D====I========   19      0       1       1
chr2    +       30898313        30898332
TTGATCTCCTGACCTCGTGATCCACCTGCCTCGCCCTCCCAAAGTGGTGGGATTACAGGCGTGAGCCACCGCCCCCAGCCACTTATATGTATTTTTAAATTCACTGACAACATTGAGTAGAAAAGATAATTTTTTTTTTTTGAGACAGAGT
TGTTGCCCAGGGGGACTGCAGTGGCACAATCTTGGCTCACTGCAACCTCCACCTCCCGTG    30898213        30898432        TTCACTGACAACATTGAGTA    TTCACTGACAACATTGAGTA
||||||||||||||||||||    ====================    20      0       0       0
chr2    +       141517099       14
AATTTTCAGTAAAATTAAATAATAAATAAAGGAA                                                                                                           GTATC
CTGATTTGTTTCATTGTTCTTGAGCACCATTGCA                                                                                                           |||||
|||||||||       ==========I======
chr2    +       163535086       16
CTCATGGTGACTCCACACATATTGGACAGAAGGG                                                                                                           GGTCT
TGTGGATTTTGCTTTAGAGTCCATGAGAGTAAGA                                                                                                           |||||
||| |   ================D===X=  19
chr2    +       186291305       18
CCCATCATCACCAATATCATTATCTCATCTTCCA                                                                                                           TATCT
TTTTTTTGAGCAGGCTCTATAATAACAAAACTGA                                                                                                           ||||
|||||||||| |    =========D=======
chr2    +       206339508       20
ATGAATCAAAGGCTTGAAACATATTGCCAACAAT                                                                                                           AATTT
GAAAAATAGTACTGTATCATTTTACATTGAACTT                                                                                                           |||||
||||||| ===I=========X========  18
chr2    +       235498941       23
CTTGAAGGGGCCAGGTGGGCAGACAGATAGTTCA                                                                                                           GAGGC
GGACCTATTATAGAGCCTGGGCTCATGTTAGGTAATGCAGGGAAGGGGTCAAAGAACCGG    235498841       235499060       TTCACTGACAACATTG-AGTA   TTCACTGAC-ACATTGCAGTA   |||||||||
||||    ===========I=======D====    19      0       1       1
chr3    +       7763221 7763239
                                                                               AGTCTTCACTTGATCAGTGCAGACATAGATCAATTCTTCACTGCAAAATTGAGTAGTGAATAAATAAACTTACTTCAGCTAACCTAACT
                                                                               7763121 7763339 TTCACTGACAACATTGAGTA    TTCACTG-CAAAATTGAGTA    ||||||| ||| ||||||||
========I===X=========  18      1       0       1
chr3    +       107912698       107912716
```

‘txt’

TTCA-ACAG	“query”	7	“match”
|||| || |	“align”	1	“mis”
TTCACACGG	“sbjct”	1	“del”
====D==X=	“edit”	0	“ins”

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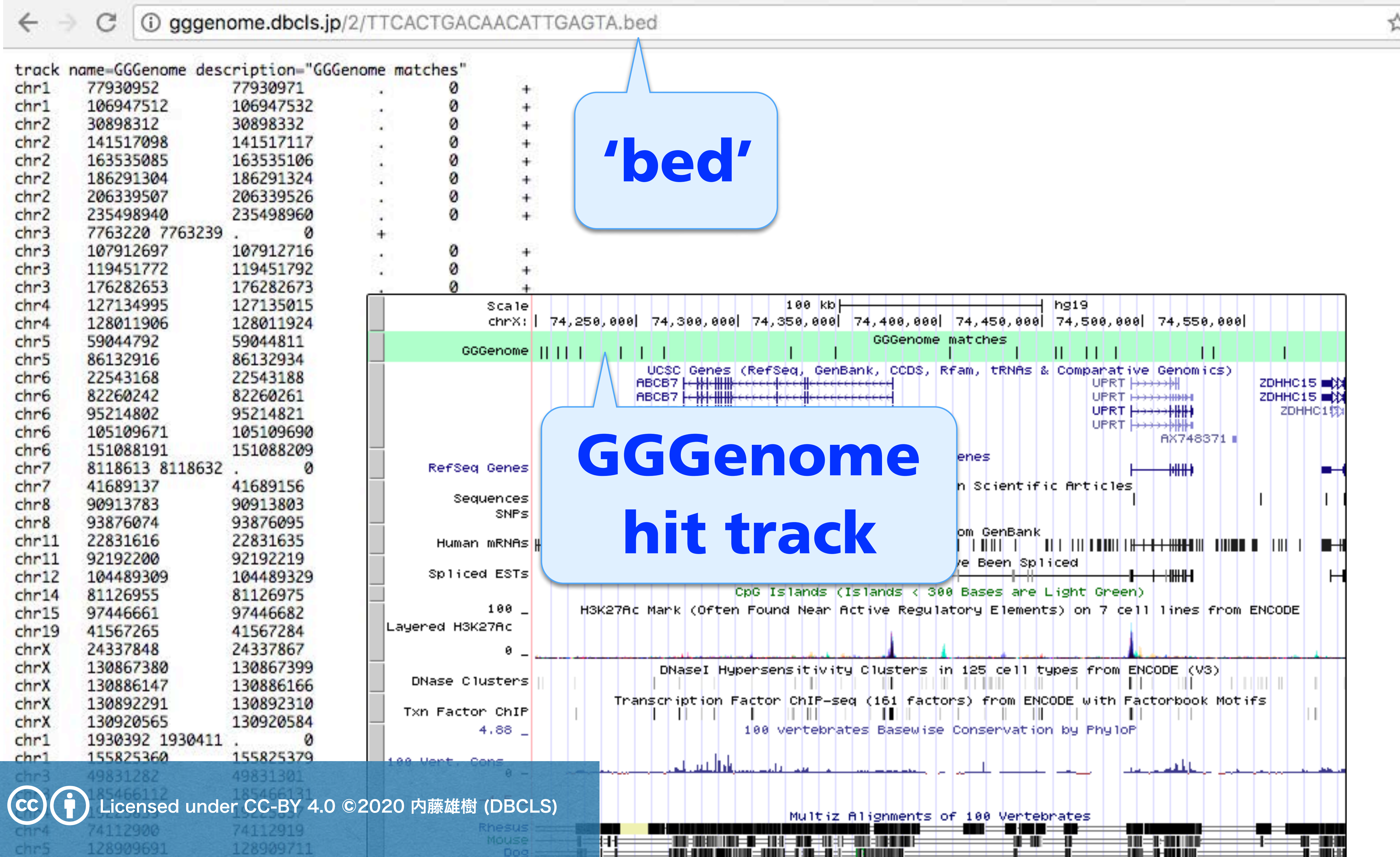
GGGenome を他のツールから呼び出す



```
track name=GGGenome description="GGGenome matches"
chr1    77930952        77930971        .       0       +
chr1    106947512       106947532       .       0       +
chr2    30898312        30898332        .       0       +
chr2    141517098       141517117       .       0       +
chr2    163535085       163535106       .       0       +
chr2    186291304       186291324       .       0       +
chr2    206339507       206339526       .       0       +
chr2    235498940       235498960       .       0       +
chr3    7763220 7763239 .       0       +
chr3    107912697       107912716       .       0       +
chr3    119451772       119451792       .       0       +
chr3    176282653       176282673       .       0       +
chr4    127134995       127135015
chr4    128011906       128011924
chr5    59044792        59044811
chr5    86132916        86132934
chr6    22543168        22543188
chr6    82260242        82260261
chr6    95214802        95214821
chr6    105109671       105109690
chr6    151088191       151088209
chr7    8118613 8118632 .       0
chr7    41689137        41689156
chr8    90913783        90913803
chr8    93876074        93876095
chr11   22831616        22831635
chr11   92192200        92192219
chr12   104489309       104489329
chr14   81126955        81126975
chr15   97446661        97446682
chr19   41567265        41567284
chrX    24337848        24337867
chrX    130867380       130867399
chrX    130886147       130886166
chrX    130892291       130892310
chrX    130920565       130920584
chr1    1930392 1930411 .       0
chr1    155825360       155825379
chr3    49831282        49831301
[illegible]     185466112       185466131
[illegible]
chr4    74112900        74112919
chr5    128909691       128909711
```

'bed'

GGGenome hit track

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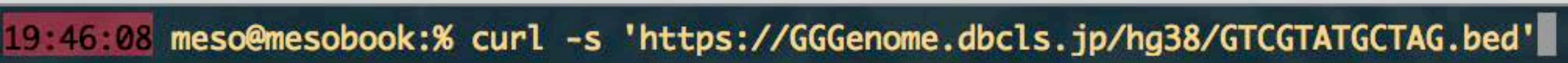
GGGenome を他のツールから呼び出す

gggenome.dbcls.jp/2/TTCACTGACAACATTGAGTA.json

‘json’

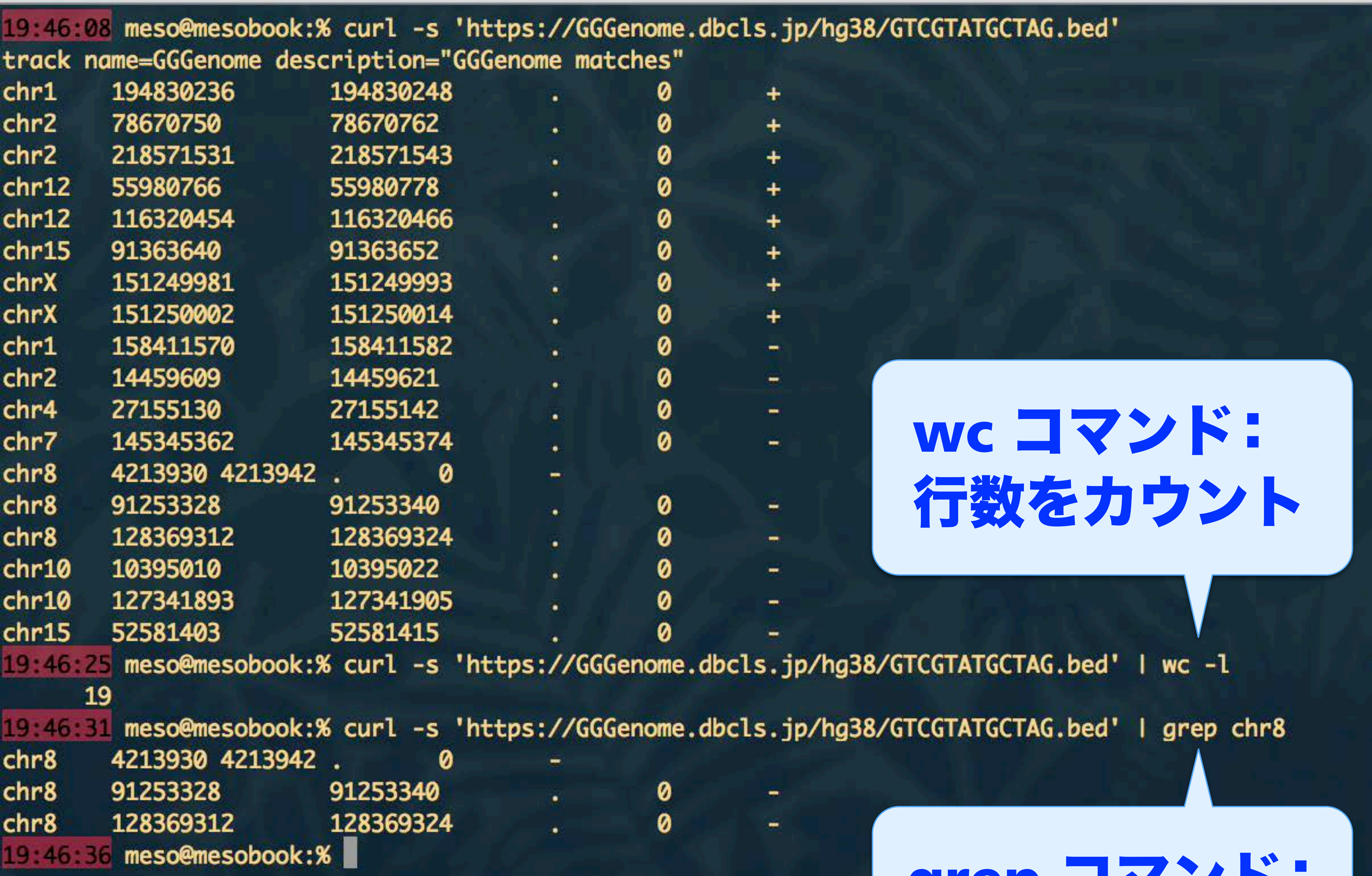
```
-
{ ···

  • database: "Human genome, GRCh37/hg19 (Feb, 2009)",
  • error: "none",
  • results:
     -
    [ ···

      ○  -
        { ···

          ▪ align: "||||||| ||||||| ||||",
          ▪ del: 0,
          ▪ edit: "=======I=======X====",
          ▪ ins: 1,
          ▪ match: 18,
          ▪ mis: 1,
          ▪ name: "chr1",
          ▪ position: 77930953,
          ▪ position_end: 77930971,
          ▪ query: "TTCACTGACAACATTGAGTA",
          ▪ sbjct: "TTCACTG-CAACATTCAGTA",
          ▪ snippet: "TCATGAAATGGAAGTGCTGCCACTTCAGGGTCCTGTAAAGAGATAATGCTGATGCCAGGCCAGGGAAAAAAGCAGTACTCAAGATCACCTTTTTTAAAGTTCACTGCAAC
          ▪ snippet_end: 77931071,
          ▪ snippet_pos: 77930853,
          ▪ strand: "+"
        },
      ○  -
        { ···

          ▪ align: "||||||| |||| ||||||||",
          ▪ del: 1,
          ▪ edit: "=======D====I========",
          ▪ ins: 1,
          ▪ match: 19,
          ▪ mis: 0,
          ▪ name: "chr1",
          ▪ position: 106947513,
          ▪ position_end: 106947532,
          ▪ query: "TTCACTG-ACAACATTGAGTA",
          ▪ sbjct: "TTCACTGCACAA-ATTGAGTA",
```



```
19:46:08 meso@mesobook:% curl -s 'https://GGGenome.dbcls.jp/hg38/GTCGTATGCTAG.bed'
```

curl コマンド：
webから情報を取得

ターミナルから塩基配列検索



```
19:46:08 meso@mesobook:% curl -s 'https://GGGenome.dbcls.jp/hg38/GTCGTATGCTAG.bed'
track name=GGGenome description="GGGenome matches"
chr1    194830236       194830248       .       0       +
chr2    78670750        78670762        .       0       +
chr2    218571531       218571543       .       0       +
chr12   55980766        55980778        .       0       +
chr12   116320454       116320466       .       0       +
chr15   91363640        91363652        .       0       +
chrX    151249981       151249993       .       0       +
chrX    151250002       151250014       .       0       +
chr1    158411570       158411582       .       0       -
chr2    14459609        14459621        .       0       -
chr4    27155130        27155142        .       0       -
chr7    145345362       145345374       .       0       -
chr8    4213930 4213942 .       0       -
chr8    91253328        91253340        .       0       -
chr8    128369312       128369324       .       0       -
chr10   10395010        10395022        .       0       -
chr10   127341893       127341905       .       0       -
chr15   52581403        52581415        .       0       -
19:46:25 meso@mesobook:% curl -s 'https://GGGenome.dbcls.jp/hg38/GTCGTATGCTAG.bed' | wc -l
      19
19:46:31 meso@mesobook:% curl -s 'https://GGGenome.dbcls.jp/hg38/GTCGTATGCTAG.bed' | grep chr8
chr8    4213930 4213942 .       0       -
chr8    91253328        91253340        .       0       -
chr8    128369312       128369324       .       0       -
19:46:36 meso@mesobook:%
```

wc コマンド：
行数をカウント

grep コマンド：
キーワード抽出

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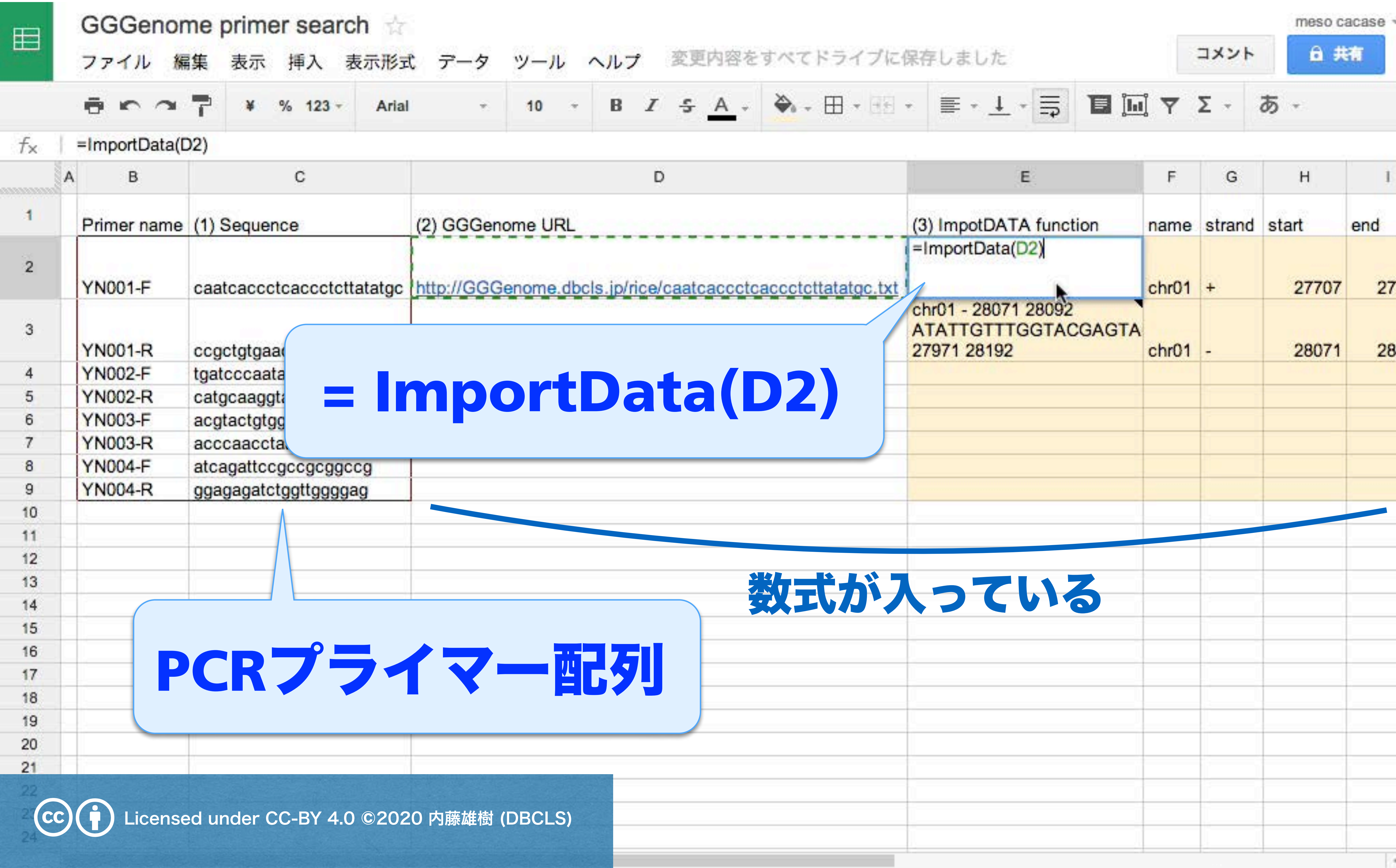
表計算ソフトから GGGenome 検索

DBCLS
Database Center for Life Science



=ImportData(D2)

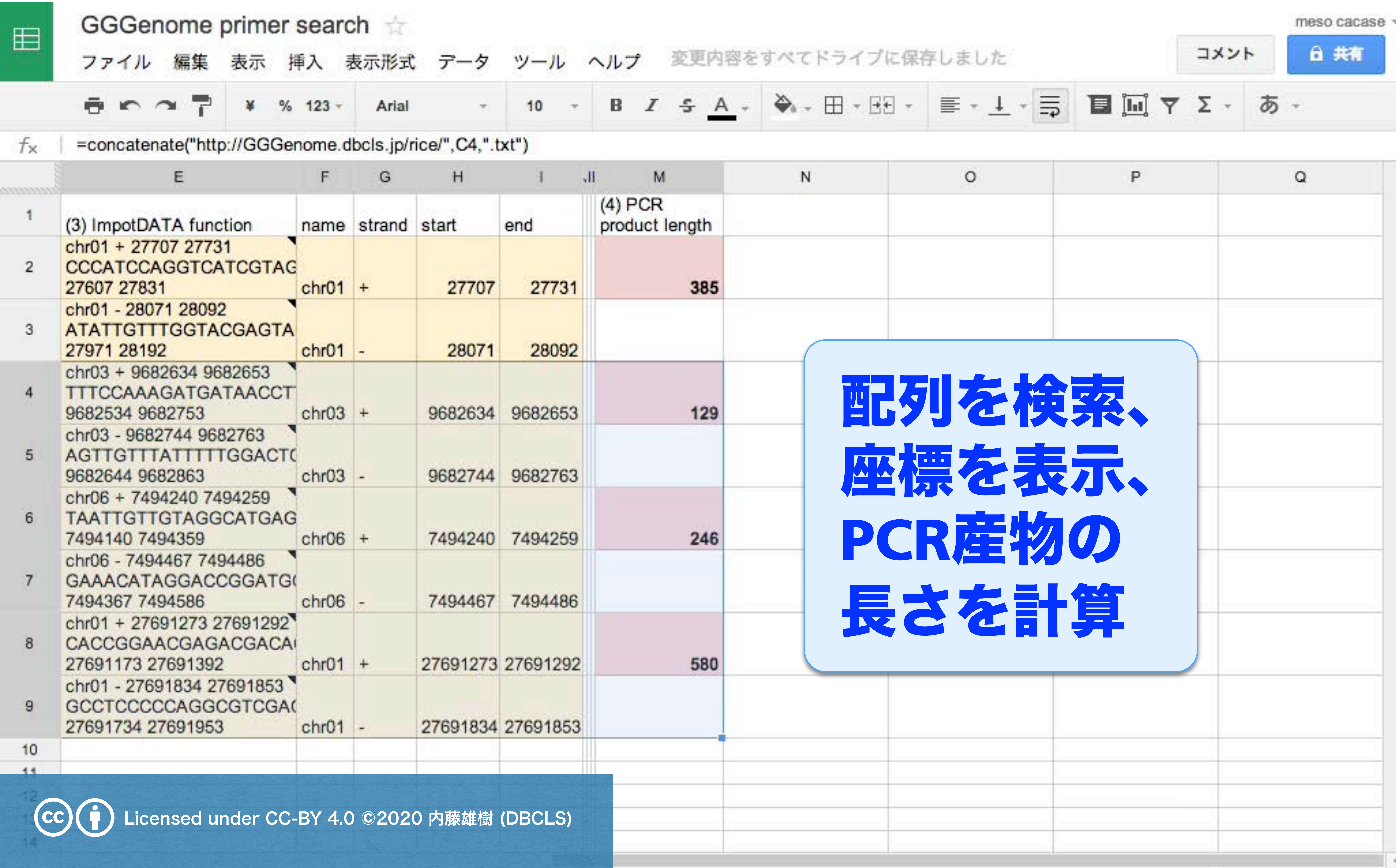
	A	B	C	D	E	F	G	H	I
1		Primer name	(1) Sequence	(2) GGGenome URL	(3) ImpotDATA function	name	strand	start	end
2		YN001-F	caatcaccctcaccctcttatatgc	http://GGGenome.dbcls.jp/rice/caatcaccctcaccctcttatatgc.txt	=ImportData(D2)	chr01	+	27707	27
3		YN001-R	ccgctgtgaa		chr01 - 28071 28092 ATATTGTTTGGTACGAGTA 27971 28192	chr01	-	28071	28
4		YN002-F	tgatcccaata						
5		YN002-R	catgcaaggt						
6		YN003-F	acgtactgtgg						
7		YN003-R	acccaaccta						
8		YN004-F	atcagattccgccgcggccg						
9		YN004-R	ggagagatctggttggggag						

= ImportData(D2)

数式が入っている

PCRプライマー配列

表計算ソフトから GGGenome（ゲゲゲ）検索



=concatenate("http://GGGenome.dbcls.jp/rice/",C4,".txt")

	E	F	G	H	I	M
1	(3) ImpotDATA function	name	strand	start	end	(4) PCR product length
2	chr01 + 27707 27731 CCCATCCAGGTCATCGTAG 27607 27831	chr01	+	27707	27731	385
3	chr01 - 28071 28092 ATATTGTTTGGTACGAGTA 27971 28192	chr01	-	28071	28092	
4	chr03 + 9682634 9682653 TTTCCAAAGATGATAACCT 9682534 9682753	chr03	+	9682634	9682653	129
5	chr03 - 9682744 9682763 AGTTGTTTATTTTTGGACTC 9682644 9682863	chr03	-	9682744	9682763	
6	chr06 + 7494240 7494259 TAATTGTTGTAGGCATGAG 7494140 7494359	chr06	+	7494240	7494259	246
7	chr06 - 7494467 7494486 GAAACATAGGACCGGATG 7494367 7494586	chr06	-	7494467	7494486	
8	chr01 + 27691273 27691292 CACCGGAACGAGACGACA 27691173 27691392	chr01	+	27691273	27691292	580
9	chr01 - 27691834 27691853 GCCTCCCCCAGGCGTCGA 27691734 27691953	chr01	-	27691834	27691853	

配列を検索、座標を表示、PCR産物の長さを計算

ゲ ゲ ゲ

GGGenome まとめ

DBCLS
Database Center
for Life Science

https://GGGenome.dbcls.jp/

- **ゲノムを高速に検索可能**

  ゲノム、転写産物 (RefSeq, GENCODE)、pre-mRNA (理研D3G)

- **短い塩基配列の検索に強い**

  ミスマッチや挿入欠失が多くても見落としのない検索

- **他のツールから呼び出すことができる**（REST API）

  GoogleスプレッドシートのImportData() 関数

  Excel 2013以降の WEBSERVICE() 関数

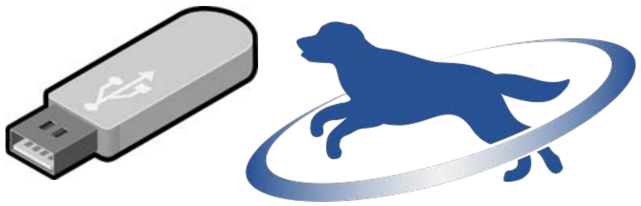
  R、Ruby、Galaxy、ゲノムブラウザ・・・

- **ウェブ版は無償で自由に利用可能**

  商用利用であっても無償。

  利用記録は当センターにおいて機密扱い。

- **Docker版をレトリバ社から販売開始** →



GGGenome

塩基配列をさがす

https://GGGenome.dbcls.jp/

- **塩基配列を高速に検索**

  350種の生物種に対応
- **ミスマッチや挿入欠失を含む短い配列の検索に強い**

CRISPRdirect

CRISPRガイドRNA設計

https://crispr.dbcls.jp/

- **CRISPRガイドRNA設計**

  PAMに隣接する20塩基の選択
- **オフターゲット検索**

  powered by GGGenome

CRISPR-Cas9 のガイド RNA 設計

PAMに隣接する配列の選択

ガイドRNA
設計支援ツール

CRISPRdirect

オフターゲット配列検索

GGGenome

高速で正確な塩基配列検索ツール

```
GAAGGCAGTCCAGTGAAATG
|||| |||| |||| |||||
GAAGCCAGTACAGAGAAATG
     ←→   ←→
```

wordが完全一致せず
BLASTで検索できない

CRISPRdirect によるガイド RNA 設計

CRISPRdirect — Rational design of CRISPR/Cas target. Help

Enter an accession number (e.g. NM_006299) or genome location (e.g. hg19:chr7:

retrieve sequence

or Paste a nucleotide sequence: ?

```
agtgcacctcccgttcgcctcgctcgcgagctcgcgttactttacaccgccgccgccgagctctccagactccggaggaggaagcg
atcgttacacgtacgcctcgtcaagcagaagcagaacatctgaagaatggctgacgaggatattcaacctattgtgtgcgacaatg
gcactggaatggtcaaggcaggttttgctggtgatgatgcaccaagggccgtcttccctagcattgtagggagaccacgtcacacc
ggtgtcatggttgggatgggccaaaaggatgcctatgtgggtgatgaagctcaggcaaaaaggggtatcctgactctaaagtaccc
aattgaacatggaattgtcaataactgggatgacatggagaaaatatggcaccacaccttctacaatgagcttcgtgttgcacctg
aagatcaccctgtattactaactgaagcccctctcaatcccaaagccaacagagagaagatgacacagataatgtttgagaccttc
aattgcccagcaatgtatgtcgcaatccaggctgttctatccttgt
```

or upload sequence file: ? ファイルを選択 選択されていません

PAM sequence requirement: NGG (e.g. NGG, NRG) ?

Specificity check: Human (Homo sapiens) genome, GRCh37/hg19 (Feb, 2009) ?

design

- 2017-06-19 Added 8 species - List

https://crispr.dbcls.jp/

Accession番号

または

塩基配列 を入力

CRISPRdirect によるガイド RNA 設計

or Paste a nucleotide sequence: ?

```
agtgcacctcccgttcgcctcgctcgcgagctcgcgttactttacaccgccgccgccgagctctccagactccggaggaggaagcg
atcgttacacgtacgcctcgtcaagcagaagcagaacatctgaagaatggctgacgaggatattcaacctattgtgtgcgacaatg
gcactggaatggtcaaggcaggttttgctggtgatgatgcaccaagggccgtcttccctagcattgtagggagaccacgtcacacc
ggtgtcatggttgggatgggccaaaaggatgcctatgtgggtgatgaagctcaggcaaaaaggggtatcctgactctaaagtaccc
aattgaacatggaattgtcaataactgggatgacatggagaaaatatggcaccacaccttctacaatgagcttcgtgttgcacctg
aagatcaccctgtattactaactgaagcccctctcaatcccaaagccaacagagagaagatgacacagatcatgtttgagaccttc
aattgcccagcaatgtatgtcgcaatccaggctgttctatccttgt
```

or uplo

PAM sequence requirem t: NGG (e.g. NGG, NRG) ?

Specificity check: rice ?

数文字ほど入力すると

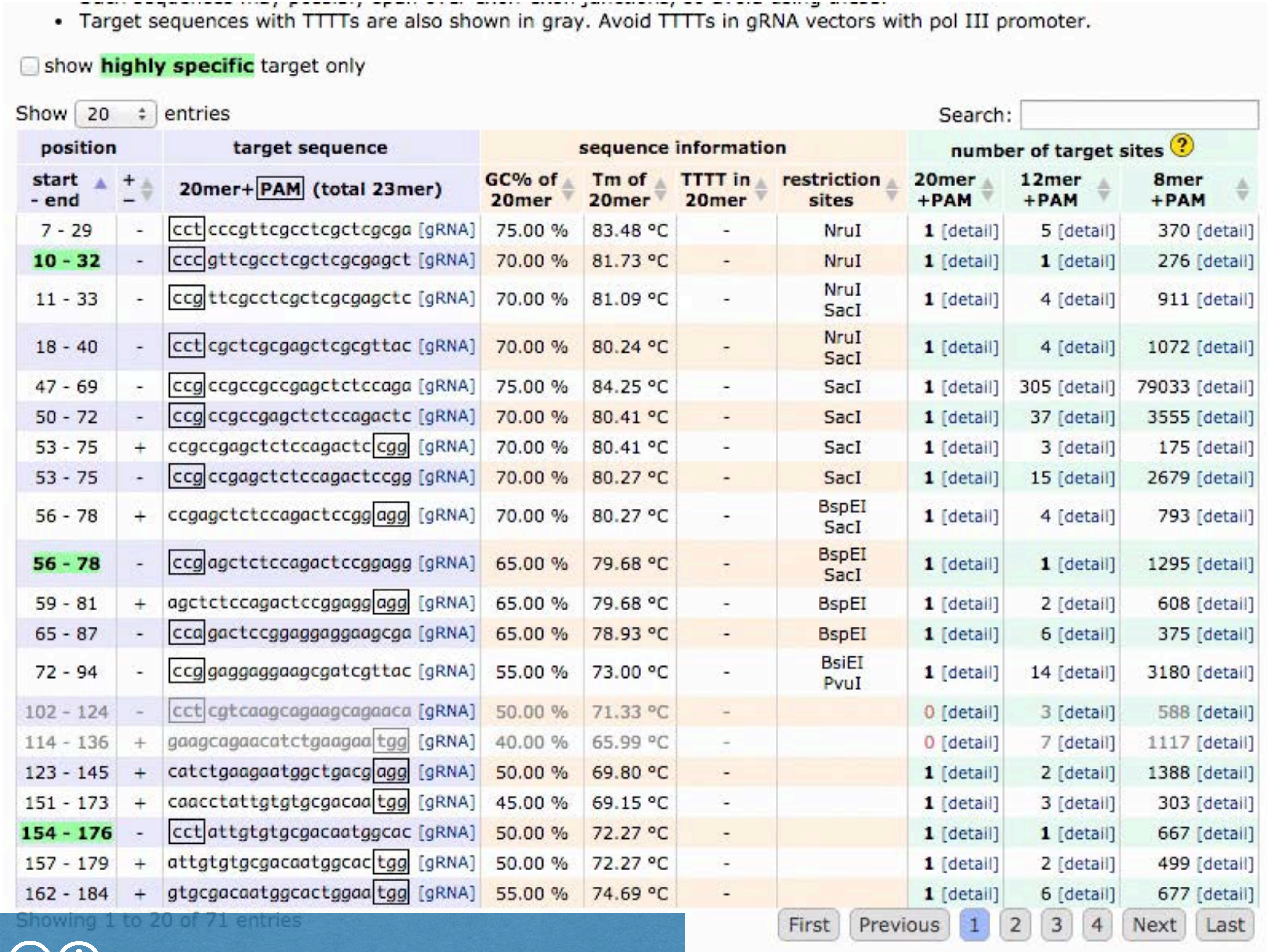
生物種を検索 できる

Rice (Oryza sativa ssp. japonica) genome, Os-Nipponbare-Reference-IRGSP-1.0 (Oct, 2011)
Chinese hamster (Cricetulus griseus) genome, C_griseus_v1.0/criGri1 (Jul, 2013)
Golden hamster (Mesocricetus auratus) genome, MesAur1.0 (Mar, 2013)
African wild rice (Oryza barthii) genome, Obart_v1.0 (Apr, 2014)
African wild rice (Oryza brachyantha) genome, Oryza_brachyantha.v1.4b (May, 2011)
African wild rice (Oryza glaberrima) genome, AGI1.1 (May, 2011)
Brazilian wild rice (Oryza glumaepatula) genome, ALNU02000000 (Aug, 2013)
Longstamen rice (Oryza longistaminata) genome, v0117-2013Aug (Aug, 2013)
Australian wild rice (Oryza meridionalis) genome, Oryza_meridionalis_v1.3 (Oct, 2014)
Indian wild rice (Oryza nivara) genome, AWHD00000000 (Aug, 2013)
Red rice (Oryza punctata) genome, AVCL00000000 (Aug, 2013)
7 (Aug, 2013)
Rice (Oryza sativa ssp. indica) genome, ASM465v1 (Jan, 2005)

Whats' new:

- 2017-06-1
- 2017-06-0
- 2017-02-17
- 2017-01-2
- 2016-12-1
- 2016-09-0
- 2016-08-3
- 2016-06-1
- 2015-10-0
- 2015-01-1
- 2014-11-2

CRISPRdirect によるガイド RNA 設計



- Target sequences with TTTTs are also shown in gray. Avoid TTTTs in gRNA vectors with pol III promoter.

☐ show **highly specific** target only

Show 20 entries

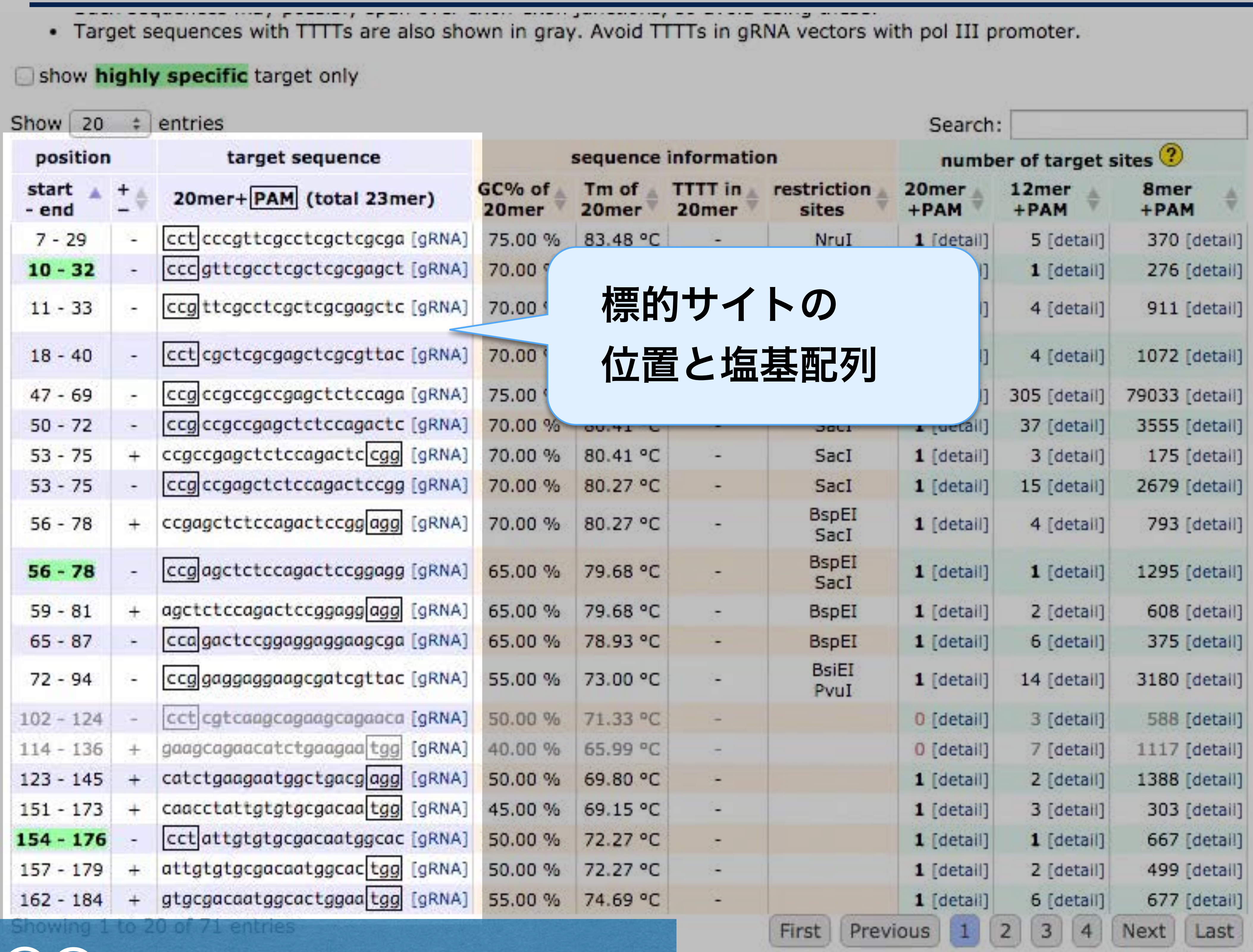
Search:

position		target sequence	sequence information				number of target sites		
start - end	+ −	20mer+PAM (total 23mer)	GC% of 20mer	Tm of 20mer	TTTT in 20mer	restriction sites	20mer +PAM	12mer +PAM	8mer +PAM
7 - 29	-	cct cccgttcgcctcgctcgcga [gRNA]	75.00 %	83.48 °C	-	NruI	1 [detail]	5 [detail]	370 [detail]
10 - 32	-	ccc gttcgcctcgctcgcgagct [gRNA]	70.00 %	81.73 °C	-	NruI	1 [detail]	1 [detail]	276 [detail]
11 - 33	-	ccg ttcgcctcgctcgcgagctc [gRNA]	70.00 %	81.09 °C	-	NruI SacI	1 [detail]	4 [detail]	911 [detail]
18 - 40	-	cct cgctcgcgagctcgcgttac [gRNA]	70.00 %	80.24 °C	-	NruI SacI	1 [detail]	4 [detail]	1072 [detail]
47 - 69	-	ccg ccgccgccgagctctccaga [gRNA]	75.00 %	84.25 °C	-	SacI	1 [detail]	305 [detail]	79033 [detail]
50 - 72	-	ccg ccgccgagctctccagactc [gRNA]	70.00 %	80.41 °C	-	SacI	1 [detail]	37 [detail]	3555 [detail]
53 - 75	+	ccgccgagctctccagactc cgg [gRNA]	70.00 %	80.41 °C	-	SacI	1 [detail]	3 [detail]	175 [detail]
53 - 75	-	ccg ccgagctctccagactccgg [gRNA]	70.00 %	80.27 °C	-	SacI	1 [detail]	15 [detail]	2679 [detail]
56 - 78	+	ccgagctctccagactccgg agg [gRNA]	70.00 %	80.27 °C	-	BspEI SacI	1 [detail]	4 [detail]	793 [detail]
56 - 78	-	ccg agctctccagactccggagg [gRNA]	65.00 %	79.68 °C	-	BspEI SacI	1 [detail]	1 [detail]	1295 [detail]
59 - 81	+	agctctccagactccggagg agg [gRNA]	65.00 %	79.68 °C	-	BspEI	1 [detail]	2 [detail]	608 [detail]
65 - 87	-	cca gactccggaggaggaagcga [gRNA]	65.00 %	78.93 °C	-	BspEI	1 [detail]	6 [detail]	375 [detail]
72 - 94	-	ccg gaggaggaagcgatcgttac [gRNA]	55.00 %	73.00 °C	-	BsiEI PvuI	1 [detail]	14 [detail]	3180 [detail]
102 - 124	-	cct cgtcaagcagaagcagaaca [gRNA]	50.00 %	71.33 °C	-		0 [detail]	3 [detail]	588 [detail]
114 - 136	+	gaagcagaacatctgaagaa tgg [gRNA]	40.00 %	65.99 °C	-		0 [detail]	7 [detail]	1117 [detail]
123 - 145	+	catctgaagaatggctgacg agg [gRNA]	50.00 %	69.80 °C	-		1 [detail]	2 [detail]	1388 [detail]
151 - 173	+	caacctattgtgtgcgacaa tgg [gRNA]	45.00 %	69.15 °C	-		1 [detail]	3 [detail]	303 [detail]
154 - 176	-	cct attgtgtgcgacaatggcac [gRNA]	50.00 %	72.27 °C	-		1 [detail]	1 [detail]	667 [detail]
157 - 179	+	attgtgtgcgacaatggcac tgg [gRNA]	50.00 %	72.27 °C	-		1 [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaa tgg [gRNA]	55.00 %	74.69 °C	-		1 [detail]	6 [detail]	677 [detail]

Showing 1 to 20 of 71 entries

Graphical View:

CRISPRdirect によるガイド RNA 設計



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7 - 29	-	cct cccgttcgcctcgctcgcga [gRNA]	75.00 %	83.48 °C	-	NruI	**1** [detail]	5 [detail]	370 [detail]
10 - 32	-	ccc gttcgcctcgctcgcgagct [gRNA]	70.00					**1** [detail]	276 [detail]
11 - 33	-	ccg ttcgcctcgctcgcgagctc [gRNA]	70.00					4 [detail]	911 [detail]
18 - 40	-	cct cgctcgcgagctcgcgttac [gRNA]	70.00					4 [detail]	1072 [detail]
47 - 69	-	ccg ccgccgccgagctctccaga [gRNA]	75.00					305 [detail]	79033 [detail]
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65 - 87	-	cca gactccggaggaggaagcga [gRNA]	65.00 %	78.93 °C	-	BspEI	**1** [detail]	6 [detail]	375 [detail]
72 - 94	-	ccg gaggaggaagcgatcgttac [gRNA]	55.00 %	73.00 °C	-	BsiEI PvuI	**1** [detail]	14 [detail]	3180 [detail]
102 - 124	-	cct cgtcaagcagaagcagaaca [gRNA]	50.00 %	71.33 °C	-		0 [detail]	3 [detail]	588 [detail]
114 - 136	+	gaagcagaacatctgaagaa tgg [gRNA]	40.00 %	65.99 °C	-		0 [detail]	7 [detail]	1117 [detail]
123 - 145	+	catctgaagaatggctgacg agg [gRNA]	50.00 %	69.80 °C	-		**1** [detail]	2 [detail]	1388 [detail]
151 - 173	+	caacctattgtgtgcgacaa tgg [gRNA]	45.00 %	69.15 °C	-		**1** [detail]	3 [detail]	303 [detail]
154 - 176	-	cct attgtgtgcgacaatggcac [gRNA]	50.00 %	72.27 °C	-		**1** [detail]	**1** [detail]	667 [detail]
157 - 179	+	attgtgtgcgacaatggcac tgg [gRNA]	50.00 %	72.27 °C	-		**1** [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaa tgg [gRNA]	55.00 %	74.69 °C	-		**1** [detail]	6 [detail]	677 [detail]

Showing 1 to 20 of 71 entries

標的サイトの
位置と塩基配列

CRISPRdirect によるガイド RNA 設計

- Target sequences with TTTTs are also shown in gray. Avoid TTTTs in gRNA vectors with pol III promoter.

☐ show **highly specific** target only

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10 - 32	-	ccc gttcgcctcgctcgcgagct [gRNA]	70.00 %	81.73 °C	-	NruI	1 [detail]	1 [detail]	276 [detail]
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123 - 145	+	catctgaagaatggctgacg agg [gRNA]	50.00 %	69.80 °C	-		1 [detail]	2 [detail]	1388 [detail]
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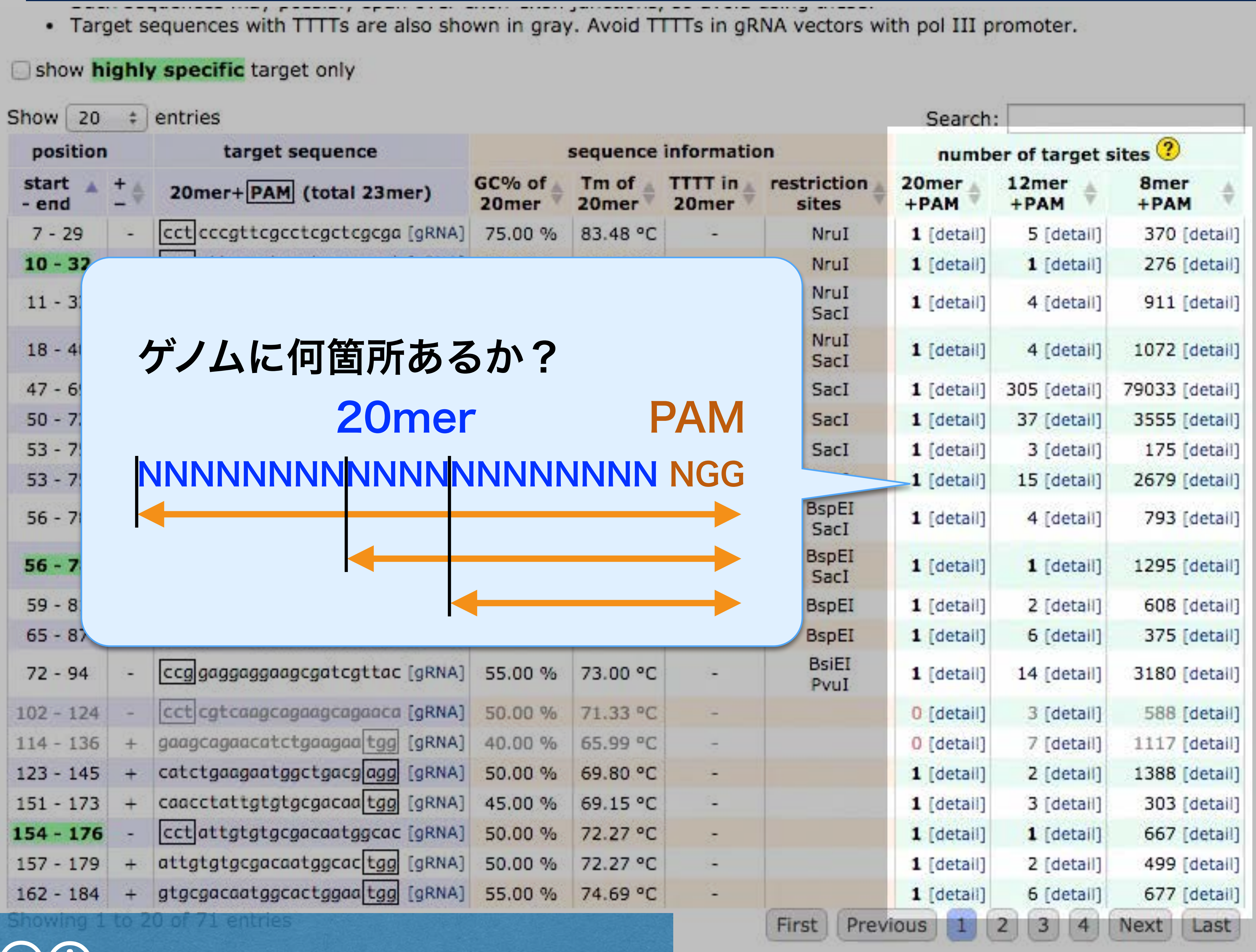
157 - 179	+	attgtgtgcgacaatggcac tgg [gRNA]	50.00 %	72.27 °C	-		1 [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaa tgg [gRNA]	55.00 %	74.69 °C	-		1 [detail]	6 [detail]	677 [detail]

Showing 1 to 20 of 71 entries

配列に関する情報

制限酵素サイト も

CRISPRdirect によるガイド RNA 設計



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10 - 32						NruI	1 [detail]	**1** [detail]	276 [detail]
11 - 3						NruI SacI	1 [detail]	4 [detail]	911 [detail]
18 - 4						NruI SacI	1 [detail]	4 [detail]	1072 [detail]
47 - 6						SacI	1 [detail]	305 [detail]	79033 [detail]
50 - 7						SacI	1 [detail]	37 [detail]	3555 [detail]
53 - 7						SacI	1 [detail]	3 [detail]	175 [detail]
53 - 7							1 [detail]	15 [detail]	2679 [detail]
56 - 7						BspEI SacI	1 [detail]	4 [detail]	793 [detail]
56 - 7						BspEI SacI	1 [detail]	**1** [detail]	1295 [detail]
59 - 8						BspEI	1 [detail]	2 [detail]	608 [detail]
65 - 87						BspEI	1 [detail]	6 [detail]	375 [detail]
72 - 94	-	ccg gaggaggaagcgatcgttac [gRNA]	55.00 %	73.00 °C	-	BsiEI PvuI	1 [detail]	14 [detail]	3180 [detail]
102 - 124	-	cct cgtcaagcagaagcagaaca [gRNA]	50.00 %	71.33 °C	-		0 [detail]	3 [detail]	588 [detail]
114 - 136	+	gaagcagaacatctgaagaa tgg [gRNA]	40.00 %	65.99 °C	-		0 [detail]	7 [detail]	1117 [detail]
123 - 145	+	catctgaagaatggctgacg agg [gRNA]	50.00 %	69.80 °C	-		1 [detail]	2 [detail]	1388 [detail]
151 - 173	+	caacctattgtgtgcgacaa tgg [gRNA]	45.00 %	69.15 °C	-		1 [detail]	3 [detail]	303 [detail]
154 - 176	-	cct attgtgtgcgacaatggcac [gRNA]	50.00 %	72.27 °C	-		1 [detail]	**1** [detail]	667 [detail]
157 - 179	+	attgtgtgcgacaatggcac tgg [gRNA]	50.00 %	72.27 °C	-		1 [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaa tgg [gRNA]	55.00 %	74.69 °C	-		1 [detail]	6 [detail]	677 [detail]

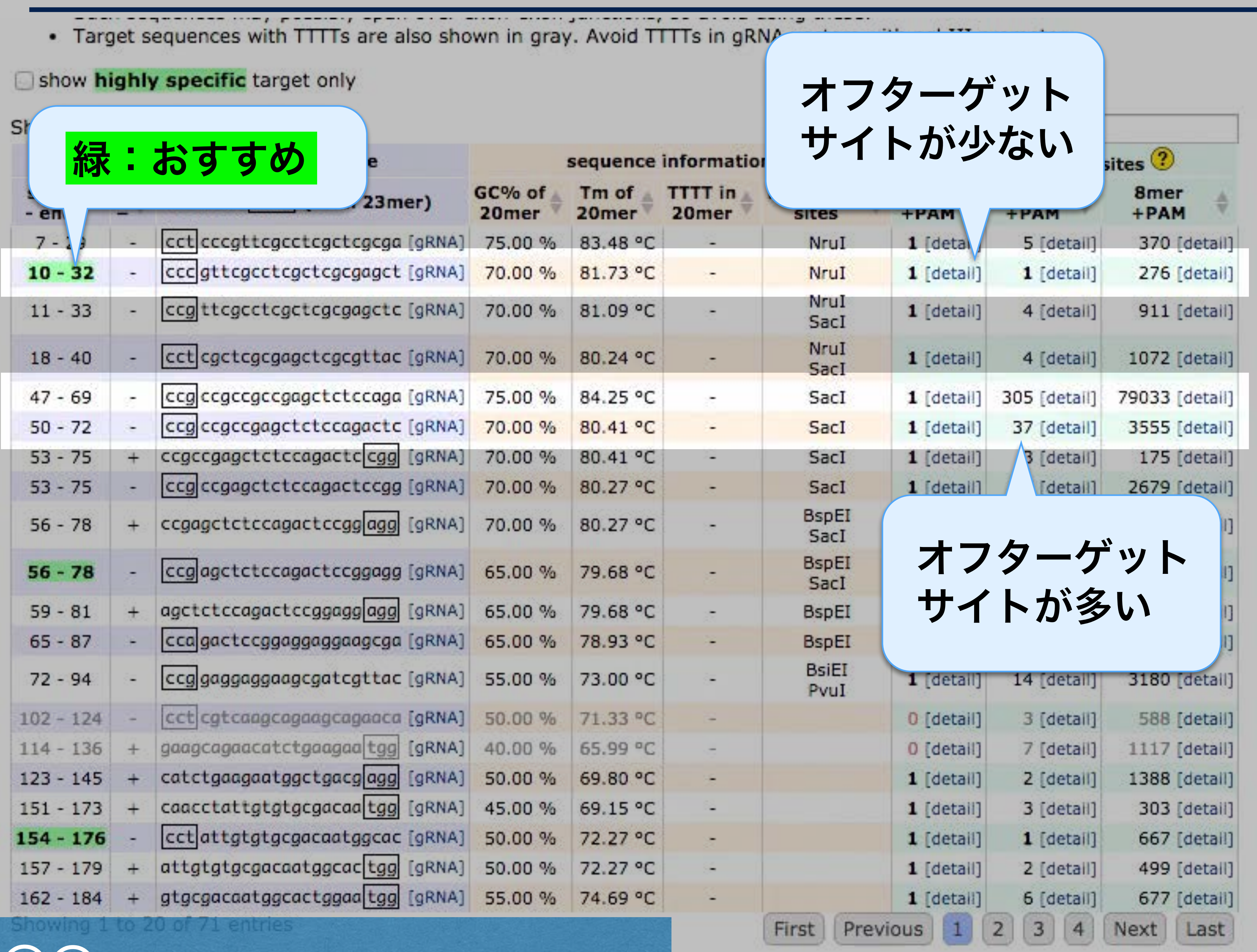
Showing 1 to 20 of 71 entries

ゲノムに何箇所あるか？

20mer PAM

NNNNNNNNNNNNNNNNNNNN NGG

CRISPRdirect によるガイド RNA 設計



- Target sequences with TTTTs are also shown in gray. Avoid TTTTs in gRNA

☐ show **highly specific** target only

- en		23mer)	sequence information GC% of 20mer	Tm of 20mer	TTTT in 20mer	sites	sites ? +PAM	+PAM	8mer +PAM
7 - 29	-	cct cccgttcgcctcgctcgcga [gRNA]	75.00 %	83.48 °C	-	NruI	1 [detail]	5 [detail]	370 [detail]
10 - 32	-	ccc gttcgcctcgctcgcgagct [gRNA]	70.00 %	81.73 °C	-	NruI	1 [detail]	1 [detail]	276 [detail]
11 - 33	-	ccg ttcgcctcgctcgcgagctc [gRNA]	70.00 %	81.09 °C	-	NruI SacI	1 [detail]	4 [detail]	911 [detail]
18 - 40	-	cct cgctcgcgagctcgcgttac [gRNA]	70.00 %	80.24 °C	-	NruI SacI	1 [detail]	4 [detail]	1072 [detail]
47 - 69	-	ccg ccgccgccgagctctccaga [gRNA]	75.00 %	84.25 °C	-	SacI	1 [detail]	305 [detail]	79033 [detail]
50 - 72	-	ccg ccgccgagctctccagactc [gRNA]	70.00 %	80.41 °C	-	SacI	1 [detail]	37 [detail]	3555 [detail]
53 - 75	+	ccgccgagctctccagactc cgg [gRNA]	70.00 %	80.41 °C	-	SacI	1 [detail]	3 [detail]	175 [detail]
53 - 75	-	ccg ccgagctctccagactccgg [gRNA]	70.00 %	80.27 °C	-	SacI	1 [detail]	[illegible] [detail]	2679 [detail]
56 - 78	+	ccgagctctccagactccgg agg [gRNA]	70.00 %	80.27 °C	-	BspEI SacI	[illegible]	[illegible]	[illegible]
56 - 78	-	ccg agctctccagactccggagg [gRNA]	65.00 %	79.68 °C	-	BspEI SacI	[illegible]	[illegible]	[illegible]
59 - 81	+	agctctccagactccggagg agg [gRNA]	65.00 %	79.68 °C	-	BspEI	[illegible]	[illegible]	[illegible]
65 - 87	-	cca gactccggaggaggaagcga [gRNA]	65.00 %	78.93 °C	-	BspEI	[illegible]	[illegible]	[illegible]
72 - 94	-	ccg gaggaggaagcgatcgttac [gRNA]	55.00 %	73.00 °C	-	BsiEI PvuI	1 [detail]	14 [detail]	3180 [detail]
102 - 124	-	cct cgtcaagcagaagcagaaca [gRNA]	50.00 %	71.33 °C	-		0 [detail]	3 [detail]	588 [detail]
114 - 136	+	gaagcagaacatctgaagaa tgg [gRNA]	40.00 %	65.99 °C	-		0 [detail]	7 [detail]	1117 [detail]
123 - 145	+	catctgaagaatggctgacg agg [gRNA]	50.00 %	69.80 °C	-		1 [detail]	2 [detail]	1388 [detail]
151 - 173	+	caacctattgtgtgcgacaa tgg [gRNA]	45.00 %	69.15 °C	-		1 [detail]	3 [detail]	303 [detail]
154 - 176	-	cct attgtgtgcgacaatggcac [gRNA]	50.00 %	72.27 °C	-		1 [detail]	1 [detail]	667 [detail]
157 - 179	+	attgtgtgcgacaatggcac tgg [gRNA]	50.00 %	72.27 °C	-		1 [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaa tgg [gRNA]	55.00 %	74.69 °C	-		1 [detail]	6 [detail]	677 [detail]

Showing 1 to 20 of 71 entries

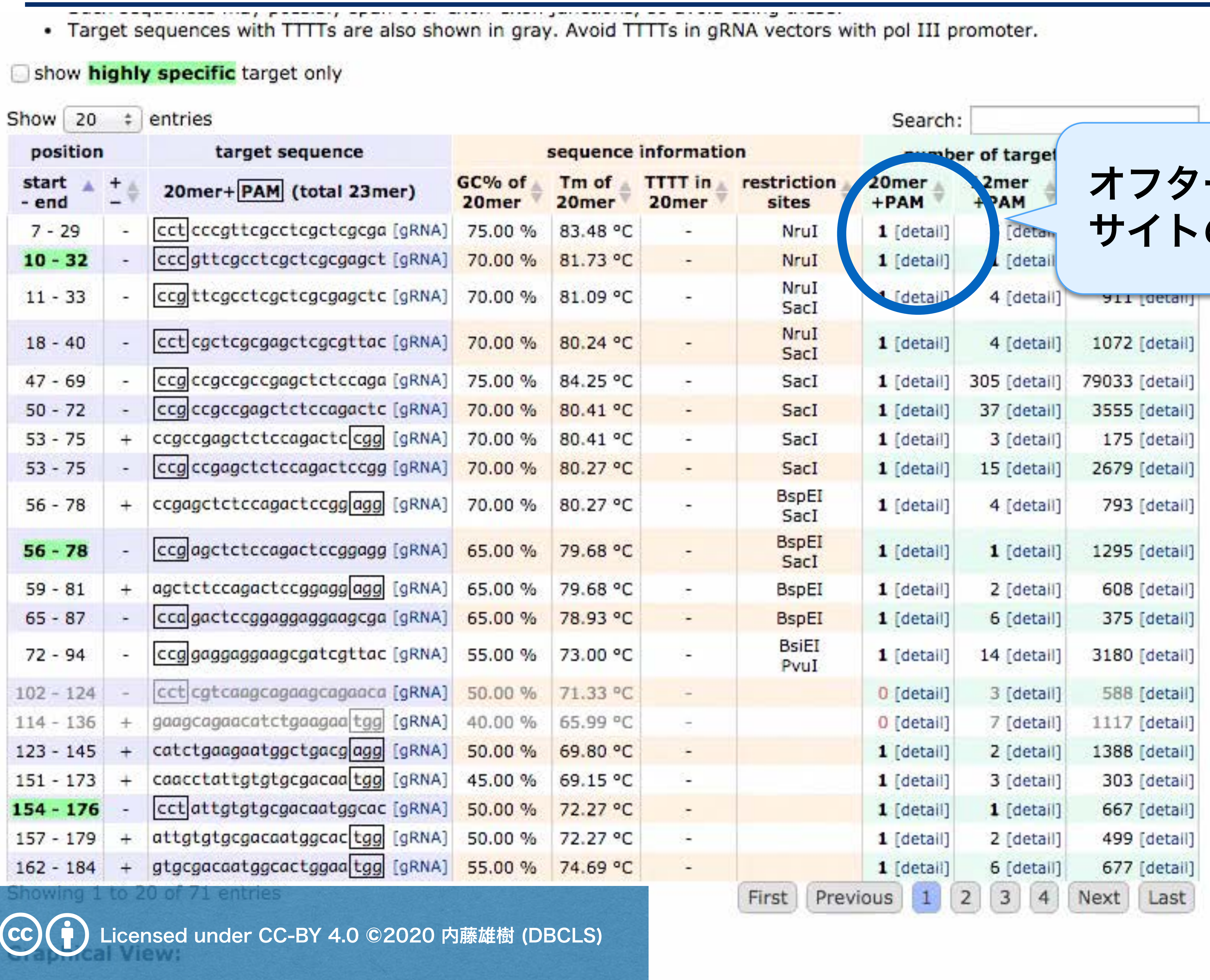
Graphical View:

緑：おすすめ

オフターゲット
サイトが少ない

オフターゲット
サイトが多い

CRISPRdirect によるガイド RNA 設計



- Target sequences with TTTTs are also shown in gray. Avoid TTTTs in gRNA vectors with pol III promoter.

☐ show **highly specific** target only

Show 20 entries

Search:

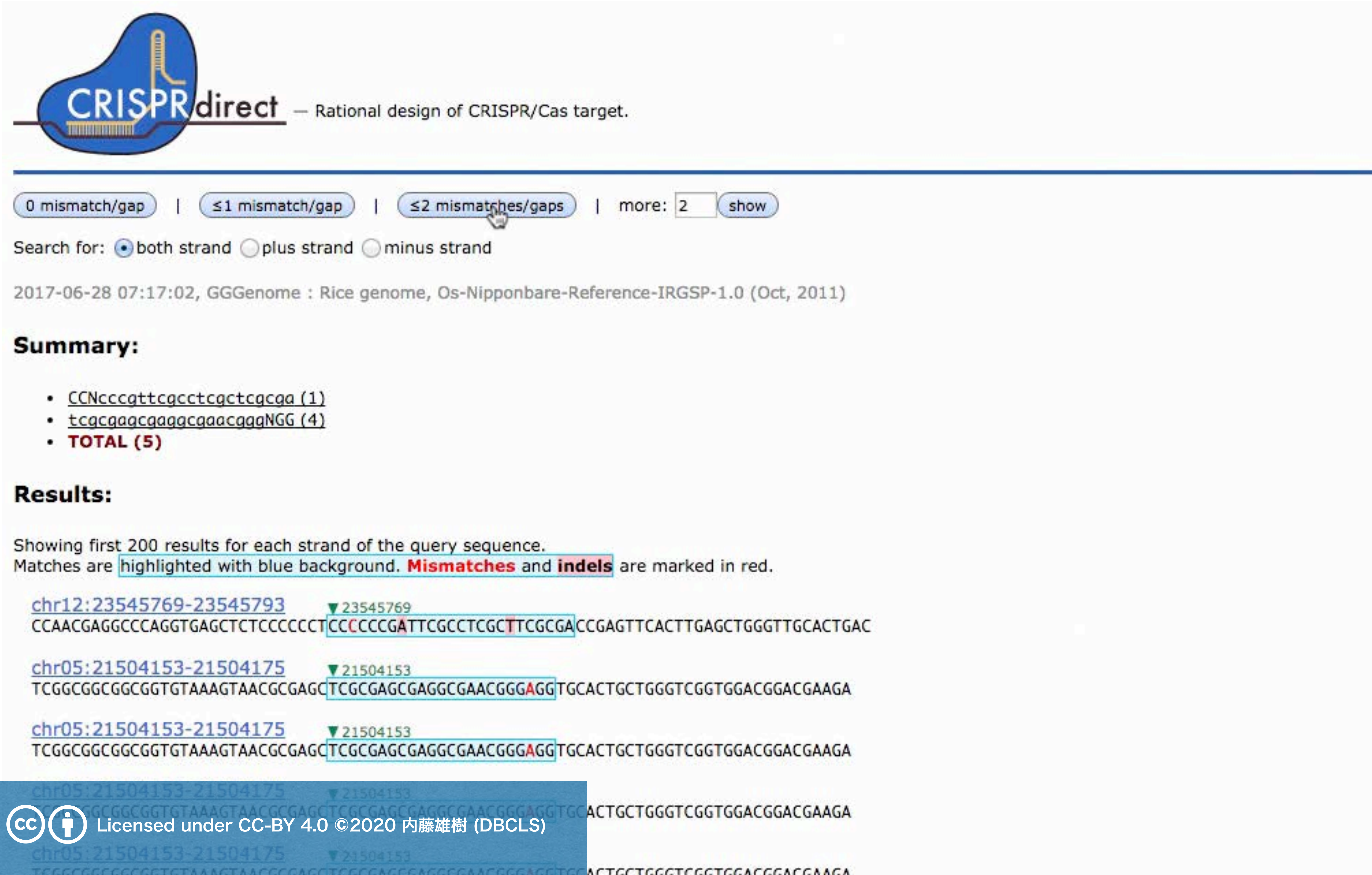
position		target sequence	sequence information				number of target		
start - end	+ −	20mer+PAM (total 23mer)	GC% of 20mer	Tm of 20mer	TTTT in 20mer	restriction sites	20mer +PAM	12mer +PAM	
7 - 29	-	cct cccgttcgcctcgctcgcga [gRNA]	75.00 %	83.48 °C	-	NruI	1 [detail]	[illegible] [detail]	[illegible]
10 - 32	-	ccc gttcgcctcgctcgcgagct [gRNA]	70.00 %	81.73 °C	-	NruI	1 [detail]	1 [detail]	[illegible]
11 - 33	-	ccg ttcgcctcgctcgcgagctc [gRNA]	70.00 %	81.09 °C	-	NruI SacI	1 [detail]	4 [detail]	911 [detail]
18 - 40	-	cct cgctcgcgagctcgcgttac [gRNA]	70.00 %	80.24 °C	-	NruI SacI	1 [detail]	4 [detail]	1072 [detail]
47 - 69	-	ccg ccgccgccgagctctccaga [gRNA]	75.00 %	84.25 °C	-	SacI	1 [detail]	305 [detail]	79033 [detail]
50 - 72	-	ccg ccgccgagctctccagactc [gRNA]	70.00 %	80.41 °C	-	SacI	1 [detail]	37 [detail]	3555 [detail]
53 - 75	+	ccgccgagctctccagactc cgg [gRNA]	70.00 %	80.41 °C	-	SacI	1 [detail]	3 [detail]	175 [detail]
53 - 75	-	ccg ccgagctctccagactccgg [gRNA]	70.00 %	80.27 °C	-	SacI	1 [detail]	15 [detail]	2679 [detail]
56 - 78	+	ccgagctctccagactccgg agg [gRNA]	70.00 %	80.27 °C	-	BspEI SacI	1 [detail]	4 [detail]	793 [detail]
56 - 78	-	ccg agctctccagactccggagg [gRNA]	65.00 %	79.68 °C	-	BspEI SacI	1 [detail]	1 [detail]	1295 [detail]
59 - 81	+	agctctccagactccggagg agg [gRNA]	65.00 %	79.68 °C	-	BspEI	1 [detail]	2 [detail]	608 [detail]
65 - 87	-	cca gactccggaggaggaagcga [gRNA]	65.00 %	78.93 °C	-	BspEI	1 [detail]	6 [detail]	375 [detail]
72 - 94	-	ccg gaggaggaagcgatcgttac [gRNA]	55.00 %	73.00 °C	-	BsiEI PvuI	1 [detail]	14 [detail]	3180 [detail]
102 - 124	-	cct cgtcaagcagaagcagaaca [gRNA]	50.00 %	71.33 °C	-		0 [detail]	3 [detail]	588 [detail]
114 - 136	+	gaagcagaacatctgaagaa tgg [gRNA]	40.00 %	65.99 °C	-		0 [detail]	7 [detail]	1117 [detail]
123 - 145	+	catctgaagaatggctgacg agg [gRNA]	50.00 %	69.80 °C	-		1 [detail]	2 [detail]	1388 [detail]
151 - 173	+	caacctattgtgtgcgacaa tgg [gRNA]	45.00 %	69.15 °C	-		1 [detail]	3 [detail]	303 [detail]
154 - 176	-	cct attgtgtgcgacaatggcac [gRNA]	50.00 %	72.27 °C	-		1 [detail]	1 [detail]	667 [detail]
157 - 179	+	attgtgtgcgacaatggcac tgg [gRNA]	50.00 %	72.27 °C	-		1 [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaa tgg [gRNA]	55.00 %	74.69 °C	-		1 [detail]	6 [detail]	677 [detail]

Showing 1 to 20 of 71 entries

Graphical View:

オフターゲット
サイトのリスト

CRISPRdirect によるガイド RNA 設計



CRISPRdirect — Rational design of CRISPR/Cas target.

Search for: ● both strand ○ plus strand ○ minus strand

2017-06-28 07:17:02, GGGenome : Rice genome, Os-Nipponbare-Reference-IRGSP-1.0 (Oct, 2011)

Summary:

- CCNcccgttcgcctcgctcgcga (1)
- tcgcgagcgaggcgaacgggNGG (4)
- **TOTAL (5)**

Results:

Showing first 200 results for each strand of the query sequence.
Matches are highlighted with blue background. **Mismatches** and **indels** are marked in red.

chr12:23545769-23545793 ▼23545769
CCAACGAGGCCCAGGTGAGCTCTCCCCCCTCCCCCCGATTCGCCTCGCTTCGCGACCGAGTTCACTTGAGCTGGGTTGCACTGAC

chr05:21504153-21504175 ▼21504153
TCGGCGGCGGCGGTGTAAAGTAACGCGAGCTCGCGAGCGAGGCGAACGGGAGGTGCACTGCTGGGTCGGTGGACGGACGAAGA

chr05:21504153-21504175 ▼21504153
TCGGCGGCGGCGGTGTAAAGTAACGCGAGCTCGCGAGCGAGGCGAACGGGAGGTGCACTGCTGGGTCGGTGGACGGACGAAGA

chr05:21504153-21504175 ▼21504153
TCGGCGGCGGCGGTGTAAAGTAACGCGAGCTCGCGAGCGAGGCGAACGGGAGGTGCACTGCTGGGTCGGTGGACGGACGAAGA

chr05:21504153-21504175 ▼21504153

CRISPRdirect によるガイド RNA 設計

151 - 173	+	caacctattgtgtgcgacaatgg	[gRNA]	45.00 %	69.15 °C	-		1 [detail]	3 [detail]	303 [detail]
154 - 176	-	cctattgtgtgcgacaatggcac	[gRNA]	50.00 %	72.27 °C	-		1 [detail]	1 [detail]	667 [detail]
157 - 179	+	attgtgtgcgacaatggcactgg	[gRNA]	50.00 %	72.27 °C	-		1 [detail]	2 [detail]	499 [detail]
162 - 184	+	gtgcgacaatggcactggaatgg	[gRNA]	55.00 %	74.69 °C	-		1 [detail]	6 [detail]	677 [detail]

Showing 1 to 20 of 71 entries

Graphical View:

```
        10        20        30        40        50        60        70        80        90       100
agtgcacctcccgttcgcctcgctcgcgagctcgcgttactttacaccgccgccgagctctccagactccggaggaggaagcgatcgttacacgtac
                                                    >  >  >
                            <  <<      <                            <  <  <  <        <      <
       110       120       130       140       150       160       170       180       190       200
gcctcgtcaagcagaagcagaacatctgaagaatggctgacgaggatattcaacctattgtgtgcgacaatggcactggaatggtcaaggcaggtttgc
             >        >                           >     >    >     >   >        >               >>
                       <                                                   <
       210       220       230       240       250       260       270       280       290       300
tggtgatgatgcaccaagggccgtcttccctagcattgtagggagaccacgtcacaccggtgtcatggttgggatgggccaaaggatgcctatgtgggt
                   >>                >       >   >>   >>       >           >>             >       >>
                                   <      <      <<                 <         <
       310       320       330       340       350       360       370       380       390       400
gatgaagctcaggcaaaaaggggtatcctgactctaaagtacccaattgaacatggaattgtcaataactgggatgacatggagaaaatatggcaccaca
>                                >               >>        >          >
<          <                                    <              <<
       410       420       430       440       450       460       470       480       490       500
ccttctacaatgagcttcgtgttgcacctgaagatcaccctgtattactaactgaagcccctctcaatcccaaagccaacagagagaagatgacacagat

                 <    <                         <          <<                  <<<        <<     <
       510       520       530       540       550       560
catgtttgagaccttcaattgcccagcaatgtatgtcgcaatccaggctgttctatccttgt
                        >
                                 <         <<
```

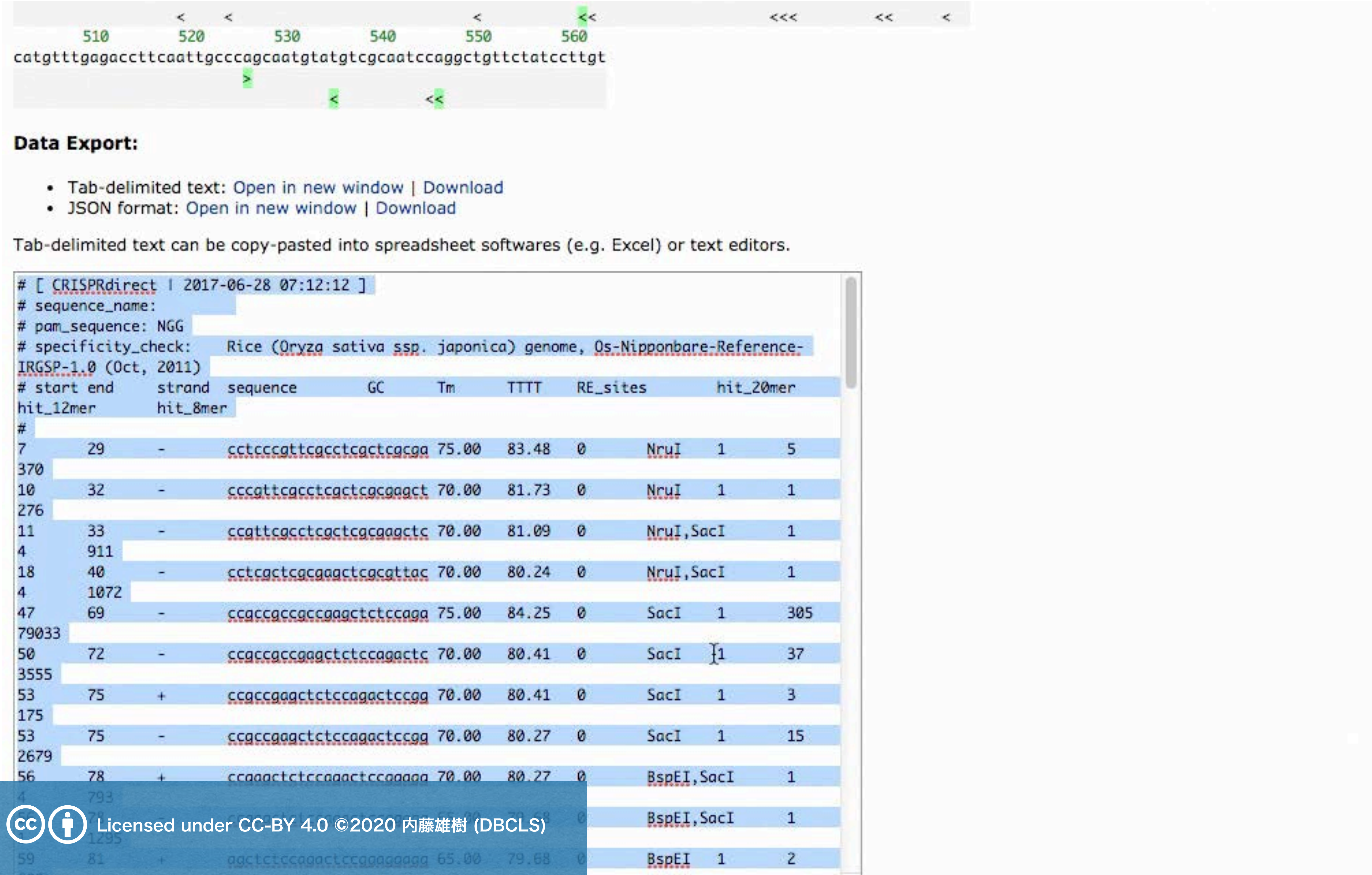
Data Export:

- Tab-delimited text: Open in new window | Download
- JSON format: Open in new window | Download

Tab-delimited text can be copy-pasted into spreadsheet softwares (e.g. Excel) or text editors.

```
# [ CRISPRdirect | 2017-06-28 07:12:12 ]
```

CRISPRdirect によるガイド RNA 設計



```
                <     <                             <         <<                  <<<         <<     <
         510        520        530        540        550        560
catgtttgagaccttcaattgcccagcaatgtatgtcgcaatccaggctgttctatccttgt
                        >
                                 <          <<
```

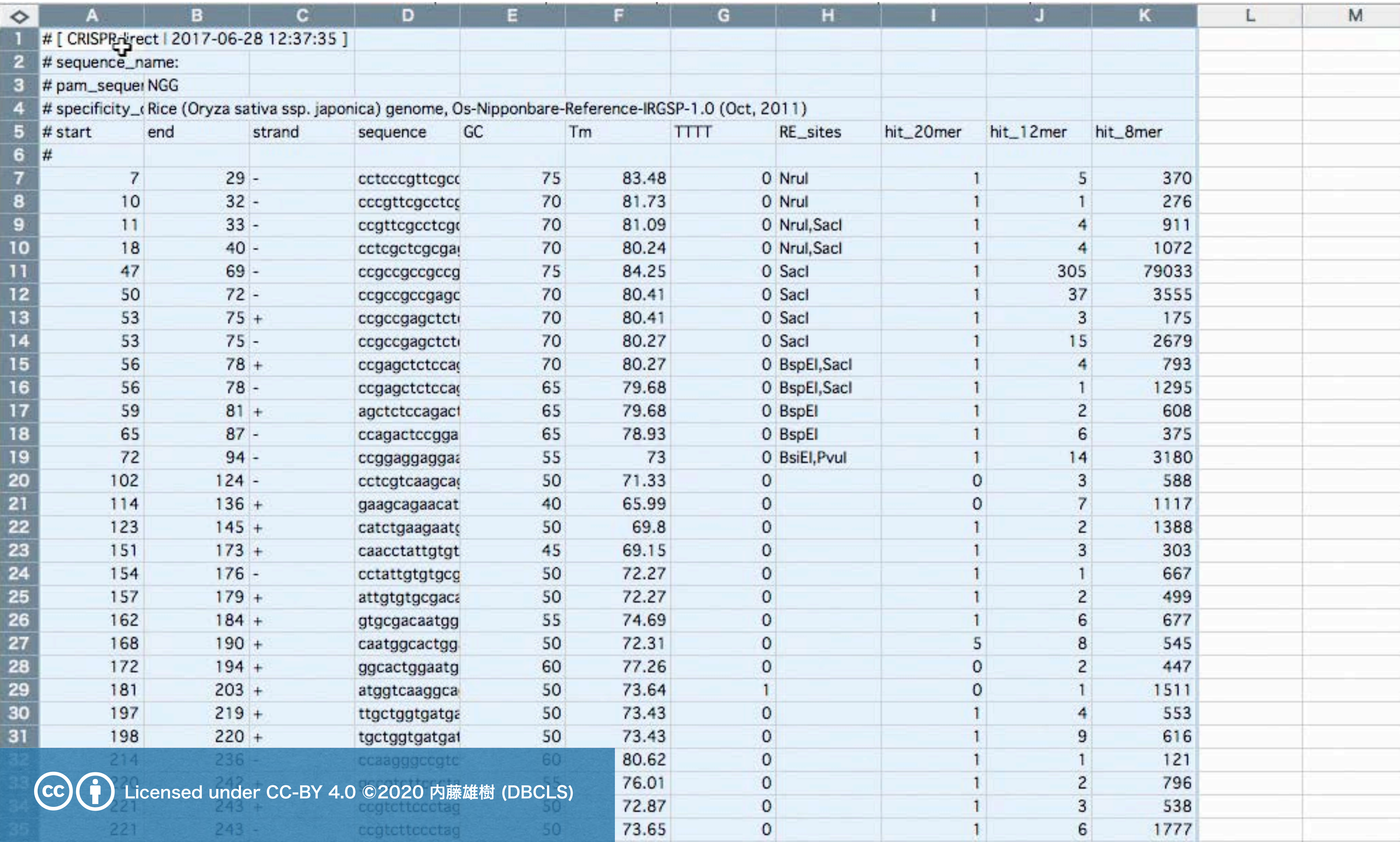
Data Export:

- Tab-delimited text: Open in new window | Download
- JSON format: Open in new window | Download

Tab-delimited text can be copy-pasted into spreadsheet softwares (e.g. Excel) or text editors.

```
# [ CRISPRdirect | 2017-06-28 07:12:12 ]
# sequence_name:
# pam_sequence: NGG
# specificity_check:	Rice (Oryza sativa ssp. japonica) genome, Os-Nipponbare-Reference-IRGSP-1.0 (Oct, 2011)
# start	end	strand	sequence	GC	Tm	TTTT	RE_sites	hit_20mer	hit_12mer	hit_8mer
#
7	29	-	cctcccgttcgcctcgctcgcga	75.00	83.48	0	NruI	1	5	370
10	32	-	cccgttcgcctcgctcgcgagct	70.00	81.73	0	NruI	1	1	276
11	33	-	ccgttcgcctcgctcgcgagctc	70.00	81.09	0	NruI,SacI	1	4	911
18	40	-	cctcgctcgcgagctcgcgttac	70.00	80.24	0	NruI,SacI	1	4	1072
47	69	-	ccgccgccgccgagctctccaga	75.00	84.25	0	SacI	1	305	79033
50	72	-	ccgccgccgagctctccagactc	70.00	80.41	0	SacI	1	37	3555
53	75	+	ccgccgagctctccagactccgg	70.00	80.41	0	SacI	1	3	175
53	75	-	ccgccgagctctccagactccgg	70.00	80.27	0	SacI	1	15	2679
56	78	+	ccgagctctccagactccggagg	70.00	80.27	0	BspEI,SacI	1	4	793
56	78	-	[illegible]	65.00	79.68	0	BspEI,SacI	1	1	1295
59	81	+	agctctccagactccggaggagg	65.00	79.68	0	BspEI	1	2
```

CRISPRdirect によるガイド RNA 設計



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	# [CRISPRdirect | 2017-06-28 12:37:35]												
2	# sequence_name:												
3	# pam_seque	NGG											
4	# specificity_	Rice (Oryza sativa ssp. japonica) genome, Os-Nipponbare-Reference-IRGSP-1.0 (Oct, 2011)											
5	# start	end	strand	sequence	GC	Tm	TTTT	RE_sites	hit_20mer	hit_12mer	hit_8mer		
6	#												
7	7	29	-	cctcccgttcgcc	75	83.48	0	NruI	1	5	370		
8	10	32	-	cccgttcgcctc	70	81.73	0	NruI	1	1	276		
9	11	33	-	ccgttcgcctcg	70	81.09	0	NruI,SacI	1	4	911		
10	18	40	-	cctcgctcgcga	70	80.24	0	NruI,SacI	1	4	1072		
11	47	69	-	ccgccgccgccg	75	84.25	0	SacI	1	305	79033		
12	50	72	-	ccgccgccgagc	70	80.41	0	SacI	1	37	3555		
13	53	75	+	ccgccgagctct	70	80.41	0	SacI	1	3	175		
14	53	75	-	ccgccgagctct	70	80.27	0	SacI	1	15	2679		
15	56	78	+	ccgagctctcca	70	80.27	0	BspEI,SacI	1	4	793		
16	56	78	-	ccgagctctcca	65	79.68	0	BspEI,SacI	1	1	1295		
17	59	81	+	agctctccagact	65	79.68	0	BspEI	1	2	608		
18	65	87	-	ccagactccgga	65	78.93	0	BspEI	1	6	375		
19	72	94	-	ccggaggaggaa	55	73	0	BsiEI,PvuI	1	14	3180		
20	102	124	-	cctcgtcaagca	50	71.33	0		0	3	588		
21	114	136	+	gaagcagaacat	40	65.99	0		0	7	1117		
22	123	145	+	catctgaagaatg	50	69.8	0		1	2	1388		
23	151	173	+	caacctattgtgt	45	69.15	0		1	3	303		
24	154	176	-	cctattgtgtgcg	50	72.27	0		1	1	667		
25	157	179	+	attgtgtgcgaca	50	72.27	0		1	2	499		
26	162	184	+	gtgcgacaatgg	55	74.69	0		1	6	677		
27	168	190	+	caatggcactgg	50	72.31	0		5	8	545		
28	172	194	+	ggcactggaatg	60	77.26	0		0	2	447		
29	181	203	+	atggtcaaggca	50	73.64	1		0	1	1511		
30	197	219	+	ttgctggtgatga	50	73.43	0		1	4	553		
31	198	220	+	tgctggtgatgat	50	73.43	0		1	9	616		
32	214	236	-	ccaagggccgtc	60	80.62	0		1	1	121		
33	220	242	+	gccgtcttccctg	55	76.01	0		1	2	796		
34	221	243	+	ccgtcttccctag	50	72.87	0		1	3	538		
35	221	243	-	ccgtcttccctag	50	73.65	0		1	6	1777		

CRISPRdirect まとめ

https://crispr.dbcls.jp/

- **PAMに隣接し、特異性の高い20塩基を選択**

  ゲノムのほかの領域とは塩基配列が一致せず、標的とする部位とだけ完全一致する配列を選択。特にPAM近傍のseed (12塩基または8塩基) の特異性を重視。

- **大量設計にも対応**

  ヘルプページにてスクリプトを公開

- **多くの生物種に対応**

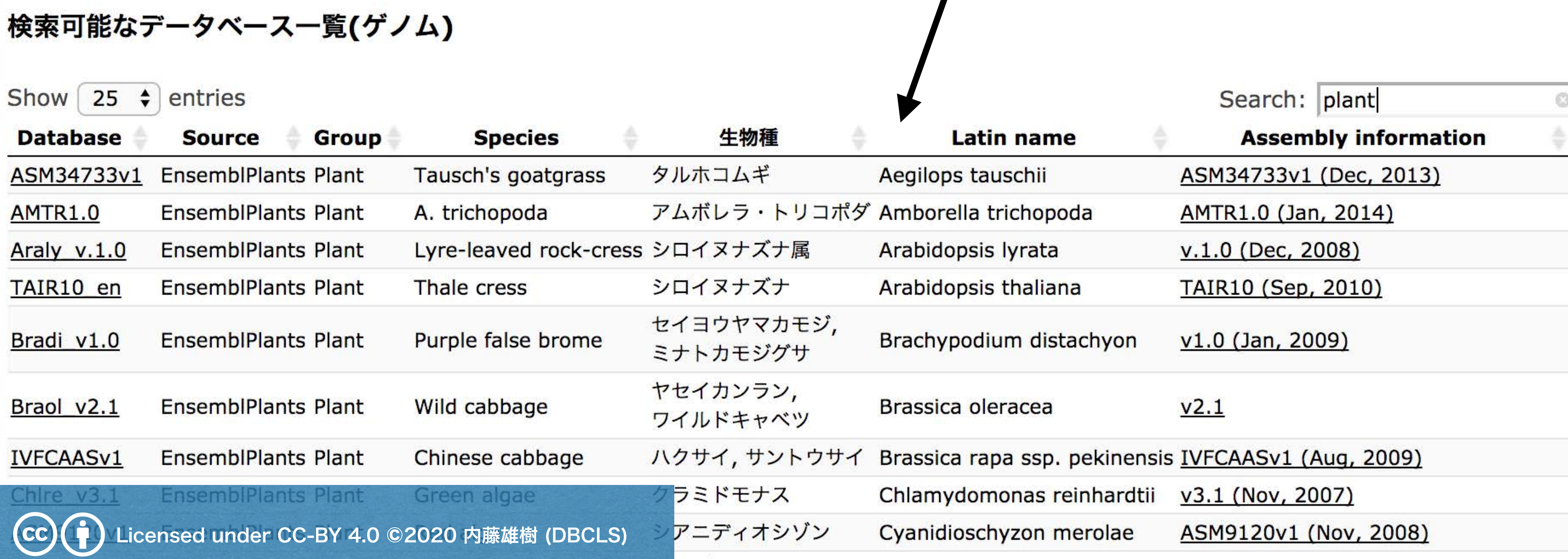
  350種の生物種に対応 (GGGenomeと同じ)

- **無償で自由に利用可能**

  商用利用であっても無償。
  利用記録は当センターにおいて機密扱い。

生物種の追加について

ゲノムが公開されていれば対応します。
ゲノム配列の所在、生物種に関する情報
をご連絡ください（項目はヘルプページ
の一覧を参照）



検索可能なデータベース一覧(ゲノム)

Show 25 entries

Search: plant

Database	Source	Group	Species	生物種	Latin name	Assembly information
ASM34733v1	EnsemblPlants	Plant	Tausch's goatgrass	タルホコムギ	Aegilops tauschii	ASM34733v1 (Dec, 2013)
AMTR1.0	EnsemblPlants	Plant	A. trichopoda	アムボレラ・トリコポダ	Amborella trichopoda	AMTR1.0 (Jan, 2014)
Araly_v.1.0	EnsemblPlants	Plant	Lyre-leaved rock-cress	シロイヌナズナ属	Arabidopsis lyrata	v.1.0 (Dec, 2008)
TAIR10_en	EnsemblPlants	Plant	Thale cress	シロイヌナズナ	Arabidopsis thaliana	TAIR10 (Sep, 2010)
Bradi_v1.0	EnsemblPlants	Plant	Purple false brome	セイヨウヤマカモジ, ミナトカモジグサ	Brachypodium distachyon	v1.0 (Jan, 2009)
Braol_v2.1	EnsemblPlants	Plant	Wild cabbage	ヤセイカンラン, ワイルドキャベツ	Brassica oleracea	v2.1
IVFCAASv1	EnsemblPlants	Plant	Chinese cabbage	ハクサイ, サントウサイ	Brassica rapa ssp. pekinensis	IVFCAASv1 (Aug, 2009)
Chlre_v3.1	EnsemblPlants	Plant	Green algae	クラミドモナス	Chlamydomonas reinhardtii	v3.1 (Nov, 2007)
[illegible]	[illegible]	[illegible]	[illegible]	シアニディオシゾン	Cyanidioschyzon merolae	ASM9120v1 (Nov, 2008)