GENETICS

OFF TARGET SITES OF THE CRISPR CAS BABIES

14.12.2018

Unfortunately we don’t know the genome of the Chinese parents which would be necessary for any prediction of genome mutations in the twins . So far we only know one confirmed off target site from Dr. He’s Hongkong slides that reports 5 mismatches.

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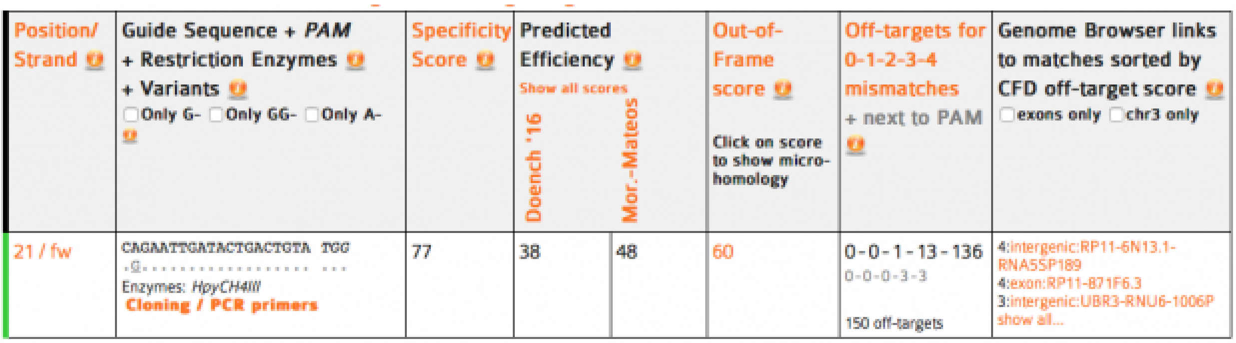
target       C A G A A T T G A T A C T G A C T G T A T G G
             | | | | | | | | | | | | | . . | | . . | | . | |
off -target  C A G A A T T T A T A C C C A C A C T A G G G
```

reported off target site

At least I would expect for statistical reasons, some 1 bp mismatch, some 2bp, some 3bp…, but not just one 5bp off target site.
So we could check several online services with the know sgRNA sequence that Dr. He used.

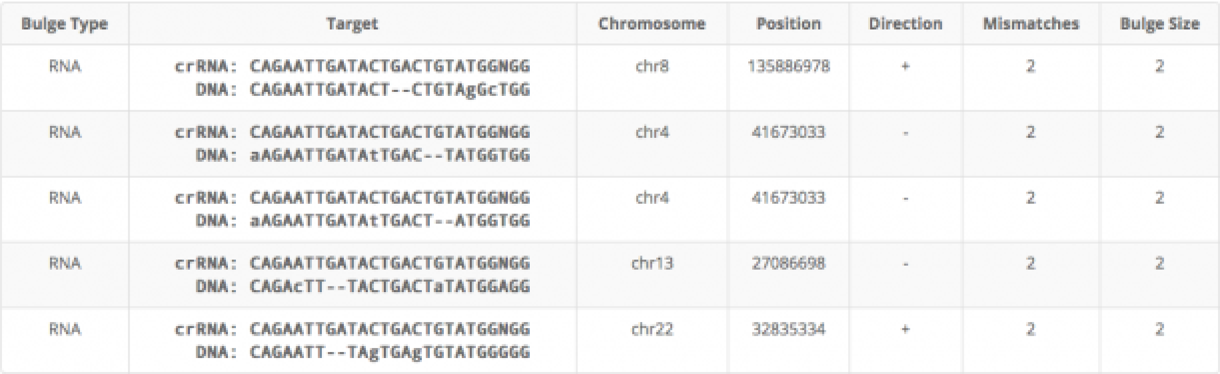
CRISPOR



Position/ Strand	Guide Sequence + *PAM* + Restriction Enzymes + Variants ☐Only G- ☐Only GG- ☐Only A-	Specificity Score	Predicted Efficiency Show all scores Doench '16	Mor.-Mateos	Out-of-Frame score Click on score to show micro-homology	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by CFD off-target score ☐exons only ☐chr3 only
21 / fw	CAGAATTGATACTGACTGTA *TGG* .G.................. ... Enzymes: *HpyCH4III* **Cloning / PCR primers**	77	38	48	60	0-0-1-13-136 0-0-0-3-3 150 off-targets	4:intergenic:RP11-6N13.1-RNA5SP189 4:exon:RP11-871F6.3 3:intergenic:UBR3-RNU6-1006P show all...

CRISPOR finds one region with 2 mismatches (intergenic) and 13 with 3 mismatches (7 intergenic hits, 5x introns (ST8SIA6, CTD-2532D12.5, CNTN5, C2D3, RABGAP1) and 1x exon of ACACA). This doesn’t match the reported off target sequence.

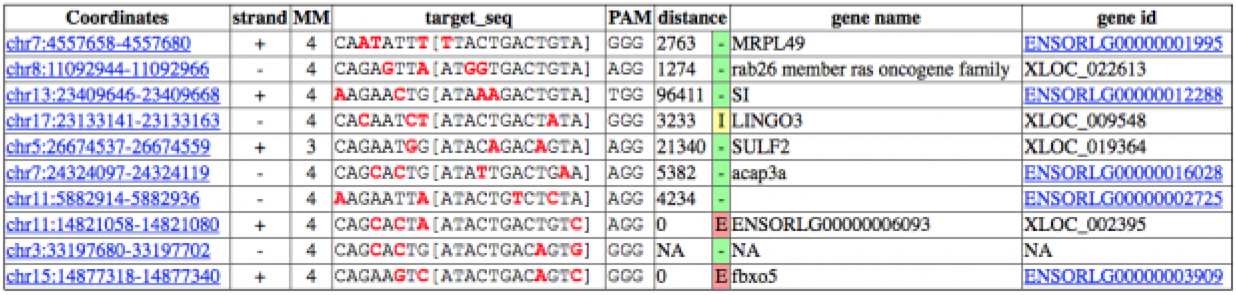
Cas-OFFinder



Bulge Type	Target	Chromosome	Position	Direction	Mismatches	Bulge Size
RNA	crRNA: CAGAATTGATACTGACTGTATGGNGG DNA: CAGAATTGATACT--CTGTAgGcTGG	chr8	135886978	+	2	2
RNA	crRNA: CAGAATTGATACTGACTGTATGGNGG DNA: aAGAATTGATAtTGAC--TATGGTGG	chr4	41673033	-	2	2
RNA	crRNA: CAGAATTGATACTGACTGTATGGNGG DNA: aAGAATTGATAtTGACT--ATGGTGG	chr4	41673033	-	2	2
RNA	crRNA: CAGAATTGATACTGACTGTATGGNGG DNA: CAGAcTT--TACTGACTaTATGGAGG	chr13	27086698	-	2	2
RNA	crRNA: CAGAATTGATACTGACTGTATGGNGG DNA: CAGAATT--TAgTGAgTGTATGGGGG	chr22	32835334	+	2	2

Cas-OFFinder identifies 5 regions with 2 mismatches (1x intergenic, 2x sites in LIMCH1, 1x intergenic, 1 in BPIFC).

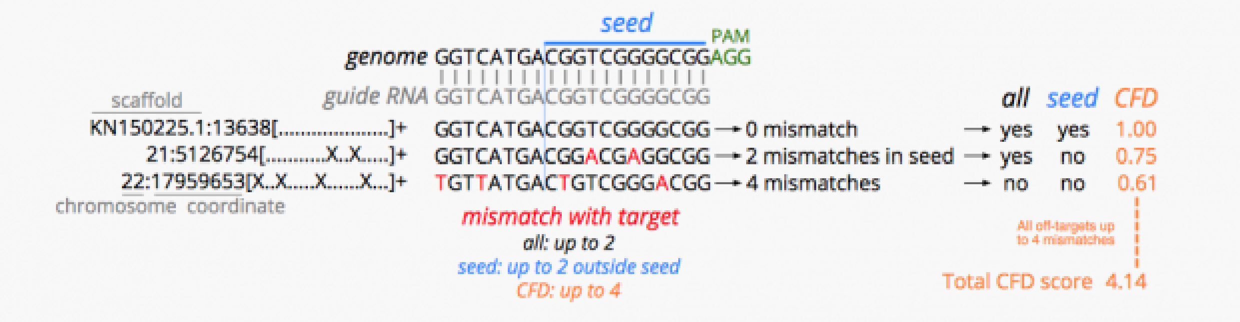
CCtop (yes, I was waiting for that name :-)



Coordinates	strand	MM	target_seq	PAM	distance		gene name	gene id
chr7:4557658-4557680	+	4	CAATATTT [TTACTGACTGTA]	GGG	2763	-	MRPL49	ENSORLG00000001995
chr8:11092944-11092966	-	4	CAGAGTTA [ATGGTGACTGTA]	AGG	1274	-	rab26 member ras oncogene family	XLOC_022613
chr13:23409646-23409668	+	4	AAGAACTG [ATAAAGACTGTA]	TGG	96411	-	SI	ENSORLG00000012288
chr17:23133141-23133163	-	4	CACAATCT [ATACTGACTATA]	GGG	3233	I	LINGO3	XLOC_009548
chr5:26674537-26674559	+	3	CAGAATGG [ATACAGACAGTA]	AGG	21340	-	SULF2	XLOC_019364
chr7:24324097-24324119	-	4	CAGCACTG [ATATTGACTGAA]	AGG	5382	-	acap3a	ENSORLG00000016028
chr11:5882914-5882936	-	4	AAGAATTA [ATACTGTCTCTA]	AGG	4234	-		ENSORLG00000002725
chr11:14821058-14821080	+	4	CAGCACTA [ATACTGACTGTC]	AGG	0	E	ENSORLG00000006093	XLOC_002395
chr3:33197680-33197702	-	4	CAGCACTG [ATACTGACAGTG]	GGG	NA	-	NA	NA
chr15:14877318-14877340	+	4	CAGAAGTC [ATACTGACAGTC]	GGG	0	E	fbxo5	ENSORLG00000003909

COS finds only one intergenic region with 3 mismatches.

CRISPRSCAN



has the best explanation of what it does but cannot search from sgRNA to off-target as many other online tools.

OFFspotter

Chrom	Strand	Start	End	Given query	Actual genomic hit	Number of mismatches	Pre-mRNA (Unspliced)	mRNA (5UTR)	mRNA (CDS)	mRNA (3UTR)	lincRNA (Unspliced)	lincRNA (Spliced)	GC content	External links
3	-	46414940	46414962	CAGAATTGATACTGACTGTA	CAGAATTGATACTGACTGTA-TGG	0	ENSG00000160791: (CCR5 - chemokine (C-C motif) receptor 5 (gene/pseudogene)) ENST00000343801 ENST00000292303 ENST00000445772	-	ENSG00000160791: (CCR5 - chemokine (C-C motif) receptor 5 (gene/pseudogene)) ENST00000292303 ENST00000343801 ENST00000445772	-	-	-	35.0%	UCSC Genome Browser
13	+	54263120	54263142	CAGAATTGATACTGACTGTA	tAGAATTGATACTGtCTGTA-TGG	2	-	-	-	-	-	-	30.0%	UCSC Genome Browser
8	-	139024559	139024581	CAGAATTGATACTGACTGTA	tAtAtTTGATACTGACTGTA-AGG	3	-	-	-	-	ENSG00000253288: (RP11-238K6.1) ENST00000518973	-	25.0%	UCSC Genome Browser
2	+	170946127	170946149	CAGAATTGATACTGACTGTA	aAGAATTGtTACaGACTGTA-GGG	3	-	-	-	-	-	-	30.0%	UCSC Genome Browser
13	+	88793606	88793628	CAGAATTGATACTGACTGTA	CAtAATTGATgCTcACTGTA-TGG	3	-	-	-	-	-	-	35.0%	UCSC Genome Browser

is spotting 1 intergenic region with 2 mismatches, 3 with 3 mismatches (2 intergenic, 1 in- RP11-238K6.1).

Taken these five different predictions together, I can’t make conclusion but think that we need to resequence the families with much higher coverage (150-200x fold coverage) and even compare the mutations with the phenotype of the children.