

OffRisk User guide

OffRisk is a pipeline that comprise of two dockers – server docker and web UI docker.

The main function of the server is to analyze off-target and return the result of the analysis from different databases.

The web UI enables users to easily run analysis with offRisk server for two use cases:

1. Off-target analysis – input from the user for off-target location and returning the results of the analysis.
2. On-target analysis – user input gRNA sequences, and then using Cas-OFFinder and FlashFry the pipeline will search for the off-target locations and then analysis them and return the results.

Installation

Docker

To work with the docker files, docker and docker-compose must be installed.

<https://docs.docker.com/get-docker/>

If downloading for windows use the “WSL 2 backend” option

- Enable the WSL 2 feature on Windows. For detailed instructions, refer to the [Microsoft documentation \(https://learn.microsoft.com/en-us/windows/wsl/install\)](https://learn.microsoft.com/en-us/windows/wsl/install).
- Download and install the [Linux kernel update package \(https://learn.microsoft.com/en-us/windows/wsl/install-manual#step-4---download-the-linux-kernel-update-package\)](https://learn.microsoft.com/en-us/windows/wsl/install-manual#step-4---download-the-linux-kernel-update-package).
- [Set WSL 2 as your default version, as it is needed by the docker.](#)

[Restart computer and run “Docker desktop” from the start menu / desktop shortcut \(if you created one\)](#)

The dockers configurations are in docker-compose.yml file for easy work with both dockers. A modification to the volumes path needs to be done – please see explanation in chapter [Run on-target / off-target search on docker](#) .

To work with OffRisk the following file need to be downloaded:

<https://www.ise.bgu.ac.il/clusters/OffRisk-db.tar.gz>

This will require 8 GB. It contains the database folder, this user guide and docker-compose.yml file.

The docker are also available to download without the docker-compose.yml file from:

OffRisk dockers are on DockerHub: https://hub.docker.com/r/almaliahbgu/off_risk/tags

- Docker server will require 6.5 GB.
- Docker UI will require 1.61 GB.

The code is also available from GitHub in the following links:

- <https://github.com/gili311/OffRisk>

- <https://github.com/gili311/OffRisk-ui>

Usage

The web UI docker must communicate with the server docker.

The server docker can work as standalone and integrate with other tools by using API.

Validation on the input is done using python module pydantic and have the following structure:

General structure



Off-target

Has two types of objects:

```
OffTarget():
    chrom: str
    start: int
    end: int
    strand: str = None

OffTargetList:
    request_id: int
    organism: str = 'human'
    off_targets: List[OffTarget]
    on_target = OffTarget = None
    db_list: List[str] = ["all"]
```

OffTarget defines one off-target. "chrom" structure begins is a number of the chromosome or "X", "Y". "strand" can be "+" or "-".

OffTargetList is an object containing request id for API request, off_sites which is a list of OffTarget and db_list which contains specification on which databases to run the analysis.

On-target

Has two types of objects:

```
Site:
    sequence: str # include sequences and pam
    mismatch: int = 4

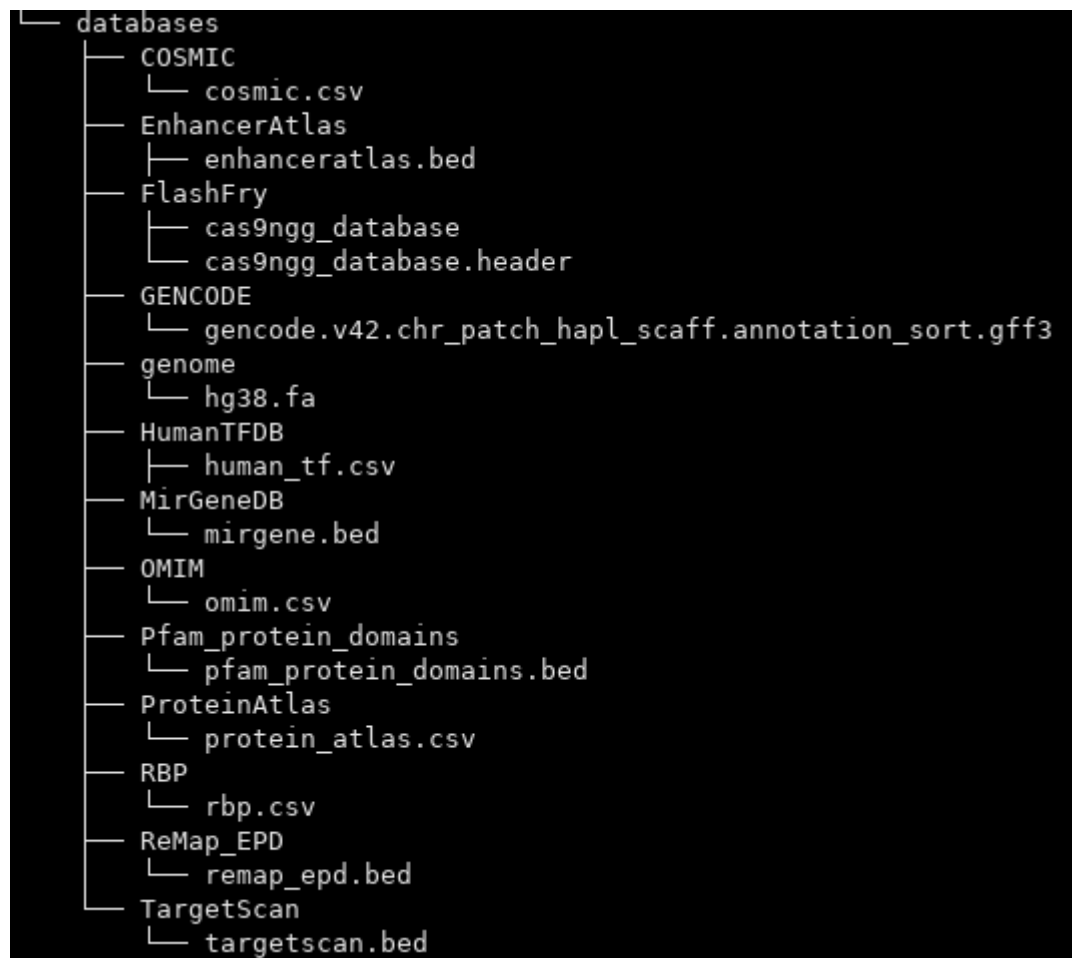
SitesList:
    request_id: int
    pattern: str = "NNNNNNNNNNNNNNNNNNNNNGG"
    pattern_dna_bulge: int = 0
    pattern_rna_bulge: int = 0
    sites: List[Site]
    db_list: List[str] = ["all"]
    search_tools: List[str] = ["flashfry"]
```

Site defines how a single gRNA we are searching for will look like. The sequence of the gRNA is mandatory and must include the PAM site, and mismatch is the number of allowed mismatches. By default, it will be 4.

SiteList is an object containing request id for API request, a list of sites to search for, db_list which contains specification on which databases to run the analysis. Search_tools it the tool to search for off-target and can be FlashFry or Cas-OFFinder. Finally, the different pattern options are as described in Cas-OFFinder documentation.

Database folder structure

Database folder structure:



“db_list” contains a list of supported databases to analyze.

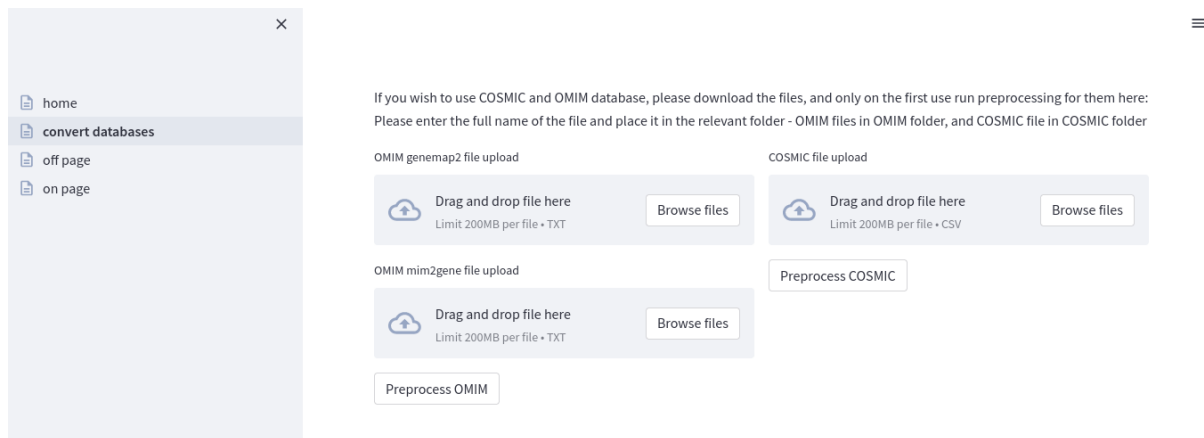
Currently supported databases:

```
"gencode", "mirgene", "remapepd", "enhanceratlas", "pfam", "targetscan",  
"omim", "humantf", "protein_atlas", "rbp", "cosmic"]
```

All relevant files for the databases are in the OffRisk-db.tar file except for OMIM and COSMIC. OMIM and COSMIC require licenses. therefore, users who want to use them need to download them from the website and convert them:

1. Download the file for COSMIC: from - <https://cancer.sanger.ac.uk/cosmic/download>, file **Cancer Gene Census**.
2. Download the files for OMIM. Both files are needed: from - <https://www.omim.org/contact>, files name: **mim2gene.txt** and **genemap2.txt**.
3. Navigate in OffRisk to the page **convert databases**, and upload the files there.
4. For COSMIC database conversion click on the button **Pre-process COSMIC**.
5. For OMIM database conversion click on the button **Pre-process OMIM**.
6. Download the result file (do not change the given name) and place it in the relevant folder on your database folders.

7. The file for COSMIC should be **cosmic.csv** in folder **COSMIC**.
8. The file for OMIM should be **omim.csv** in folder **OMIM**.



A script name preprocess.py is also available for converting new files and versions of the original database to the structure of OffRisk databases. The script should be run with name as

Run on-target / off-target search on docker

on the first use run the following:

1. Change the path of the volume for your database in docker-compose.yml under services->off-risk-server->volumes.
For example, if the path of the database is in /home/database, then the line should be: "/home/database:/databases"
2. Run: 'docker network create OffRisk-net'.
3. Run: 'docker-compuse up --no-build'

once both docker are up browse to the one of the URL presented like the following example (Network URL or External URL):

```
off-risk-ui_1 | Collecting usage statistics. To deactivate, set browser.gatherUsageStats to False.
off-risk-ui_1 |
off-risk-ui_1 | You can now view your Streamlit app in your browser.
off-risk-ui_1 |
off-risk-ui_1 | Network URL: http://172.19.0.2:8501
off-risk-ui_1 | External URL: http://194.90.225.101:8501
off-risk-ui_1 |
```

or to the following default URL: <http://localhost:8051/>.

In the UI side bar, there are 4 options under Navigation:

- Home – home page.
- Convert databases – for creating OMIM and COSMIC databases.
- Off-target – page to analyze input off-target sites from the user.
- On-target – page to search for off-target for a gRNA and then analyze the resulted locations.

Once choosing the relevant page follow the instruction:

Off-target page

Select the relevant server and database for this analysis. Options are CRISPR-IL, local or custom. For local use, the server should be **local**. The server will load on localhost, docker network OffRisk-net, port 8123.

This page receives off-target site location from the user in a tsv (tab delimiter file) format or written as text.

The tsv file should be in the following structure, each line is a site, and each value is separate with tab (chromosome, start position, end position, strand):

1	116905	116928	+
10	20041965	20041988	+

The text should be in the following structure, each line is a site, and each value is separate with a space:

```
1 116905 116928 +
10 20041965 20041988 +
```

Once you finish choosing the input click on **Run**, the results will be presented on the same page. For more information on the result please refer to the Result section.

On-target page

This page receives on-target site list from the user in json file format or filling the relevant fields.

The different options are:

Pattern: Relevant for Cas-OFFinder-bulge run. Indicates the desired pattern including PAM site. Default is "NNNNNNNNNNNNNNNNNNNNNGG" (NGG in the end is the PAM site). For more information on this field please refer to Cas-OFFinder documentation.

pattern dna bulge: Relevant for Cas-OFFinder-bulge run. The desire DNA bulge size.

pattern rna bulge: Relevant for Cas-OFFinder-bulge run. The desire RNA bulge size.

Sites: A list of all desired sequences. Each site has the sequence and number of mismatches. The default number of mismatches is 4. For FlashFry the sequence needs to be with PAM.

db list: A list defining the databases to analyze. The default is "all". Options databases are: ["gencode", "mirgene", "remapepd", "enhanceratlas", "pfam", "targetscan", "omim", "humantf", "protein_atlas", "rbp", "cosmic"]

search tools: For on-target search, define which tool will search for off-target location – FlashFry or Cas-OFFinder-bulge. For more information on each please refer to the relevant guide. It is recommended to use FlashFry, since Cas-offinder can take longer and require more computational power

From UI:

Select the relevant server and database for this analysis.

On target page

Please choose server and databases for on-target analysis

Select server: Local

Select databases:

- ☒ database
- ☒ images
- ☒ messages
- ☒ newsletters
- ☒ users
- ☒ vlogs
- ☒ votes
- ☒ vusers
- ☒ votes_alot
- ☒ vlog

☒ Select all

Select search tool. The default is **FlashFry**.

off-target search tool

Please select off-target search tool

flashby x cas_offinder x 0

Fill in the desired Pattern, DNA and RNA bulge, sequence and number of mismatch.

Fill the following for on target analysis. Sequence is a mandatory field

Method	Mismatch	Coverage
Pattern	0	100%
Sequence	4	80%

From file

The Json format needs to be in SiteList object structure. An example:

```
{
  "pattern": "NNNNNNNNNNNNNNNNNNNNGG",
  "pattern_dna_bulge": 0,
  "pattern_rna_bulge": 0,
  "sites": [
    {
      "sequence": "CTTAAGAATACGCGTAGTCGAGG",
      "mismatch": 4
    },
    {
      "sequence": "ATGTCTGGTAAGACGCCCATCGG",
      "mismatch": 4
    }
  ],
  "db_list": ["all"],
  "search tools": [
```

Choose the file here. Must be a Json file.

Result

Circular view for off-target location in genome

This circular graph shows the result off-target locations on the different chromosomes

FlashFry score summary:

If FlashFry was chosen to run, the score result from it will be shown.

FlashFry Score Summary

Contig	Start	Stop	Target	Consent	OverTime_X	Orientation_X	Okcount_X	Dcount3054Okdtarget	DcountOkL_Hsant	DcountOkL_SpecOkLtycure	Dangerous_Gr	Dangerous_Polyt	Dangerous_It_Genome	Hu2013	BaseordFlacseqd03
sequence_9	0	23	GAGATCAAAATCGTGAATAGG	NONE	OK	FRD	143		0.4875000002	0.054761026	NONE	NONE	BL_GENOME+1	84.202202055	3

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Off Target Summary

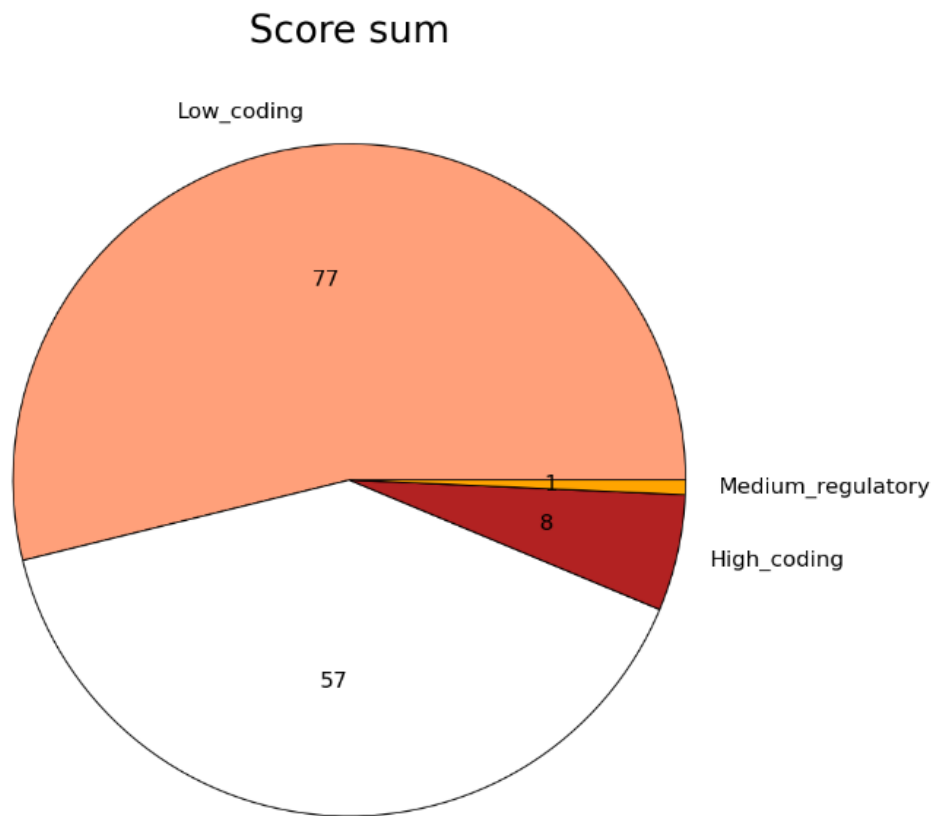
Off target database intersection result. Each off-target is colored according to the risk score that was calculated. If on-target mode was used with FlashFry, the information on the number of mismatch and occurrence will also be available.

Off-targets Summary

Chromosome	Start	End	OPF_Target_M	Gene	Strand	Attributes	M	Sequence	Gene_Zoomout_M	Gene_Symbol	Segment	Reorg_Spt_Gene_Zoomout_M	Enhancer_Active_Gene_Zoomout_M	Disease_Associated
5	58300019	58300021	0	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	-	-	-	-	-	-
10	8960618	8960641	1	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	ENSG00000007845	GATA3	transcript	-	-	Hypogardylphallos, ...
14	87923817	87923840	2	0	+	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	ENSG00000008807	transcript	transcript	-	-	-
9	77334676	77335001	3	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	ENSG00000007969	VP33A	transcript	-	-	-
8	52000019	52000020	4	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	ENSG00000007969	VP33A	transcript	ENSG00000007969	ENSG000000012908	Chromosome position, 200 ...
3	94600004	94600012	5	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	ENSG00000001036	PTC1	gene	-	-	Receptor signaling M, 11 ...
20	90700004	90700014	6	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	-	-	-	-	-	-
21	96620061	96620164	7	0	+	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	-	-	-	-	-	-
9	64600013	64600036	8	0	+	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	-	-	-	-	-	-
14, CL000003461_random	47321	47340	9	0	+	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	-	-	-	-	-	-
8	66000000	66000012	10	0	-	contig=sequence.3:orange-GAGATCAAAATCGCTGAATAGG	-	-	-	-	-	-	-	-

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Score sum



GENCODE summary

This section contains the complete result from GENCODE intersection. First will be the complete result table, and then pie graph with the different distribution for gene type, segment that are coding and segment that are not.

Detailed biological information

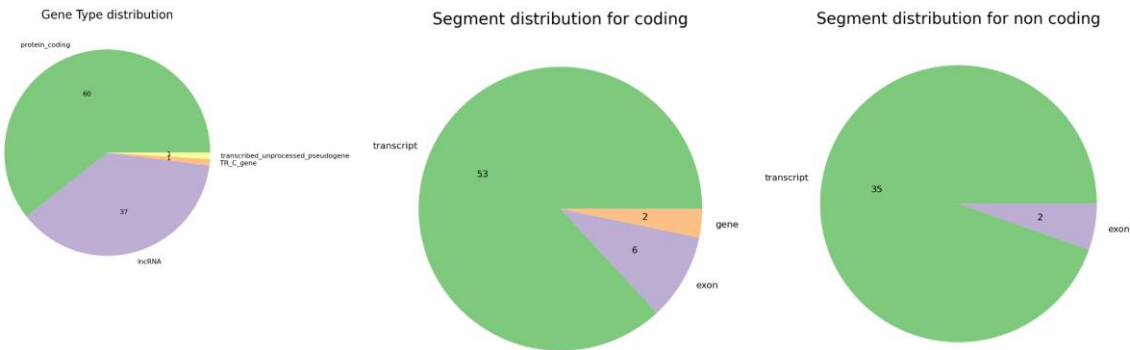
GENCODE database

Off Target (OT) ID	OT Chromosome	OT Start	OT End	Gene Ensembl ID	Gene Symbol	Feature (F)	F Start	F End	F Strand	Gene Type	Transcript Ensembl ID	Transcript Type	Transcript Symbol
2	10	116159111	116159134	ENSG00000151893	GFRAL	transcript	116056025	116273206	-	protein_coding	ENST00000355422.11	protein_coding	GFRAL-201
3	10	21110550	21110573	ENSG00000078114	NEBL	transcript	20780050	21253011	-	protein_coding	ENST00000675702.1	protein_coding, CDS_not_defined	NEBL-221
4	10	79560000	79560023	ENSG00000185303	SFTPA2	exon	79559969	79560163	-	protein_coding	ENST00000417041.1	protein_coding	SFTPA2-203
5	12	40294380	40294403	ENSG00000188906	LSRK2	transcript	40224597	40365285	+	protein_coding	ENST00000298910.12	protein_coding	LSRK2-201
7	14	22547572	22547595	ENSG00000277734	TRAC	exon	22547506	22547778	+	TR_C_gene	ENST00000611116.2	TR_C_gene	TRAC-201
8	14	52741736	52741759	ENSG00000158252	STYX	transcript	52730166	52774989	+	protein_coding	ENST00000354586.5	protein_coding	STYX-201
9	16	21747344	21747367	ENSG00000155719	OTOA	transcript	21663968	21760729	+	protein_coding	ENST00000646100.2	protein_coding	OTOA-208
10	16	22563467	22563490	ENSG00000290866	OTOP1	transcript	22546598	22576865	+	lncRNA	ENST00000505753.5	lncRNA	OTOP1-202
10	16	22563467	22563490	ENSG00000257838	OTOP1	transcript	22546564	22576682	+	transcribed_unprocessed_pseudogene	ENST00000548889.1	transcribed_unprocessed_pseudogene	OTOP1-201
11	15	74016587	74016610	ENSG00000140464	PML	transcript	73994673	74043337	+	protein_coding	ENST00000395135.7	protein_coding	PML-206
12	14	80418379	80418402	ENSG00000258766	DIO2-AS1	transcript	80211419	80455469	+	lncRNA	ENST0000053979.1	lncRNA	DIO2-AS1-201

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MirGene DB summary

This section contains the complete result from MirGene intersection.

MirGene database

Off Target (OT) ID	OT Chromosome	OT Start	OT End	MIRNA symbol	MIRNA start	MIRNA end	MIRNA strand
6	1	98046084	98046107	Hsa-Mi-137-P1-v1_pri	98046060	98046179	-
7	1	1169010	1169033	Hsa-Mi-8-P3a_pri	1168989	1169106	+

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ReMap & EPD summary

This section contains the complete result table from ReMap & EPD intersection.

MirGene database

There are no Result for this database

ReMap and Eukaryotic Promoter Database database

Off Target (OT) ID	OT Chromosome	OT Start	OT End	Gene with Promoter (ENSG)	Epd Gene Symbol	Binding Site (BS) Start	BS End	BS Strand	Binding Factor (Biotype)	EPD Coding/Non-Coding (L/R)
4	10	79560000	79560023	ENSG00000185303	SFTPA2_1	79559875	79560534	-	FOXCPAC-1	1
4	10	79560000	79560023	ENSG00000185303	SFTPA2_1	79559881	79560709	-	JARIDCPAC-1	1
4	10	79560000	79560023	ENSG00000185303	SFTPA2_1	79559996	79560583	-	MED1VCaP	1

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Enhancer Atlas summary

This section contains the complete result table from Enhancer Atlas intersection

EnhancerAtlas database

OFF Target (OT) Id	OT Chromosome	OT Start	OT End	Gene Ensembl Id	Gene Symbol	Gene Start	Enh Start	Enh Stop	Enh-Gene Score	Tissue
3	10	21110550	21110573	ENSG00000211920	RP11-969H13.2	21462943	21109631	21111221	1.490428	AS49
3	10	21110550	21110573	ENSG00000218860	RP11-969H13.3	21506820	21109631	21111221	0.517181	AS49
3	10	21110550	21110573	ENSG00000204683	C10orf113	21439488	21109631	21111221	1.352665	AS49
3	10	21110550	21110573	ENSG00000078114	NEBL	21463116	21109631	21111221	1.688397	AS49
14	15	39586806	39586829	ENSG00000137801	THBS1	39873280	39585559	39587339	3.361306	HFF
14	15	39586806	39586829	ENSG00000137801	THBS1	39873280	39585559	39587339	3.177532	MCF10A
14	15	39586806	39586829	ENSG00000137801	THBS1	39873280	39585559	39587339	3.158810	AS49
14	15	39586806	39586829	ENSG00000246863	RP11-329N19.3	40213243	39585559	39587339	1.059056	NHEK
14	15	39586806	39586829	ENSG00000137801	THBS1	39873280	39585559	39587339	2.770241	VCAF
14	15	39586806	39586829	ENSG00000128829	EPJAW4	40226347	39585559	39587339	2.866988	HFF
14	15	39586806	39586829	ENSG00000137801	THBS1	39873280	39585559	39587339	3.412227	T98G

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Pfam summary

This section contains the complete result table from Pfam intersection.

Pfam Protein Domains database

OFF Target (OT) Id	OT Chromosome	OT Start	OT End	Gene Ensembl Id	Gene Symbol	Domain start	Domain End	Pfam Domain Name
0	1	69168	69969	ENSG00000186092	OR4F5	69168	69969	7tm_4
0	1	69168	69969	ENSG00000186092	OR4F5	69183	69816	7TM_GPCR_Srv
0	1	69168	69969	ENSG00000186092	OR4F5	69189	69930	7tm_1

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TargetScan summary

This section contains the complete result from table TargetScan intersection.

TargetScan database

OFF Target (OT) Id	OT Chromosome	OT Start	OT End	Gene Ensembl Id	Gene Symbol	miRNA Symbol
5	1	965219	965226	ENSG00000187961	KU4L17	miR-299-3p
5	1	965219	965226	ENSG00000187961	KU4L17	miR-124-3p.1
5	1	965219	965226	ENSG00000187961	KU4L17	miR-124-3p.2/508-3p

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OMIM summary

This section contains the complete result table from OMIM intersection.

Online Mendelian Inheritance in Man database

OFF Target (OT) Id	Gene Ensembl Id	Gene Symbol	OMIM ID	Phenotype	Inheritance Model
104	ENSG00000135821	GLUL	138290	Glutamine deficiency, congenital, 610015 (3)	Autosomal recessive
108	ENSG00000169764	UGP2	191760	Developmental and epileptic encephalopathy 83, 618744 (3)	Autosomal recessive
11	ENSG00000140464	PML	102578	Leukemia, acute promyelocytic, PML/RARA type (3)	
110	ENSG00000164619	BMPER	608699	Diaphanospondylosis, 608022 (3)	Autosomal recessive
114	ENSG00000147408	CSGALNACT1	616615	Skeletal dysplasia, mild, with joint laxity and advanced bone age, 618870 (3)	Autosomal recessive
118	ENSG00000181230	CTNNA3	607667	Arrhythmogenic right ventricular dysplasia, familial, 13, 615616 (3)	Autosomal dominant
23	ENSG00000101333	PLCB4	600810	Auriculocondylar syndrome 2, 614669 (3)	Autosomal dominant
31	ENSG00000100412	AC02	100850	Infantile cerebellar-retinal degeneration, 614559 (3), 17C optic atrophy 9, 614289 (3)	Autosomal recessive
4	ENSG00000185303	SFTPA2	178642	Pulmonary fibrosis, idiopathic, 178500 (3)	Autosomal dominant
45	ENSG00000079482	OPHN1	300127	Mental retardation, X-linked, with cerebellar hypoplasia and distinctive facial appearance, 300486 (3)	X-linked recessive
49	ENSG00000107485	GATA3	131320	Hypoparathyroidism, sensorimotor deafness, and renal dysplasia, 146255 (3)	Autosomal dominant

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Human TF summary

This section contains the complete result table from Human TF intersection.

Human transcription factors database

Off Target (OT) ID	Gene Ensembl ID	Gene Symbol	Family	HumanTF source
11	ENSG00000140464	PMEL	Others	TF cofactors
18	ENSG00000141376	BCAS3	Others	TF cofactors
25	ENSG00000179263	SP140	SAND	TF
49	ENSG00000107485	GATA3	zif-GATA	TF
6	ENSG00000188956	LRN2	Other_CTF	TF cofactors
64	ENSG00000159216	RUNX1	Runt	TF

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Protein Atlas summary

This section contains the complete result from Protein Atlas intersection. First will be presented the table, and then a heatmap colored according to the level of expression.

The Human Protein Atlas database

Gene Ensembl ID	Gene Symbol	adipose tissue adipocytes	adrenal gland cells in zona fasciculata	adrenal gland cells in zona glomerulosa	adrenal gland cells in zona reticularis	adrenal gland glandular cells	adrenal gland medullary cells	appendix endocrine cells	appendix enterocytes
ENSG00000135821	GLUL	High				Not detected			
ENSG00000107242	RHOB1	Medium				High			
ENSG00000109764	UGF2	Low				Low		Not detected	Low
ENSG00000140464	PMEL	High				Medium			
ENSG00000108619	ENC1B	Low				Medium			
ENSG00000149503	INCENP	Not detected				Low		High	Medium
ENSG00000131230	CTNNA3	Not detected				Not detected			
ENSG00000136236	TUBGCP3	Low				High			
ENSG00000175048	ZDNAC14	Not detected				Not detected			
ENSG00000138853	NDST4								
ENSG00000188822	CNR2	Low				Medium			

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RBP Summary

This section contains the complete result table from RBP intersection. First will be the table, and then a heatmap showing the gene function.

RNA binding proteins database

Off Target (OT) Id	Gene Ensembl Id	Gene Symbol	Essential Genes	Splicing regulation	Spliceosome	RNA modification	3' end processing	rRNA processing	Ribosome & basic translation	RNA stability & decay	microRNA processing	RNA localization
29	ENSG00000148642	RBMS3	0	0	0	0	0	0	0	1	0	

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COSMIC summary

This section contains the complete result table from COSMIC intersection.

COSMIC database database

Off Target (OT) Id	Gene Ensembl Id	Gene Symbol	Gene Name	Somatic	Germline	Tumour Types(Somatic)	Tumour Types(Germline)	Molecular Genetics	Role in Cancer
11	ENSG00000140464	PHL	promyelocytic leukemia	yes		APL, ALL		Dom	TSG, fusion
49	ENSG00000107485	GATA3	GATA binding protein 3	yes		breast		Rec	oncogene, TSG
64	ENSG00000159216	RLN1	runx-related transcription factor 1 (AML1)	yes		AML, pre B-ALL, T-ALL		Dom	oncogene, TSG, fusion
72	ENSG00000125354	SEPT6	septin 6	yes		AML		Dom	fusion

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No result summary

All the off-targets that got no hit in the different database will be presented in the final table.

The following off-target ID did not match in any DB:

chromosome	start	end	off_target_id
1	4888228	4888251	0
1	19967919	19967942	1
12	87147601	87147624	5
16	75830134	75830157	16
2	7150543	7150566	19
20	21476782	21476805	24
3	24075070	24075093	28
4	149997362	149997385	32
4	165639738	165639761	33
6	113854393	113854416	35
7	21313185	21313208	36
9	41276235	41276258	40
9	62840601	62840624	41

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