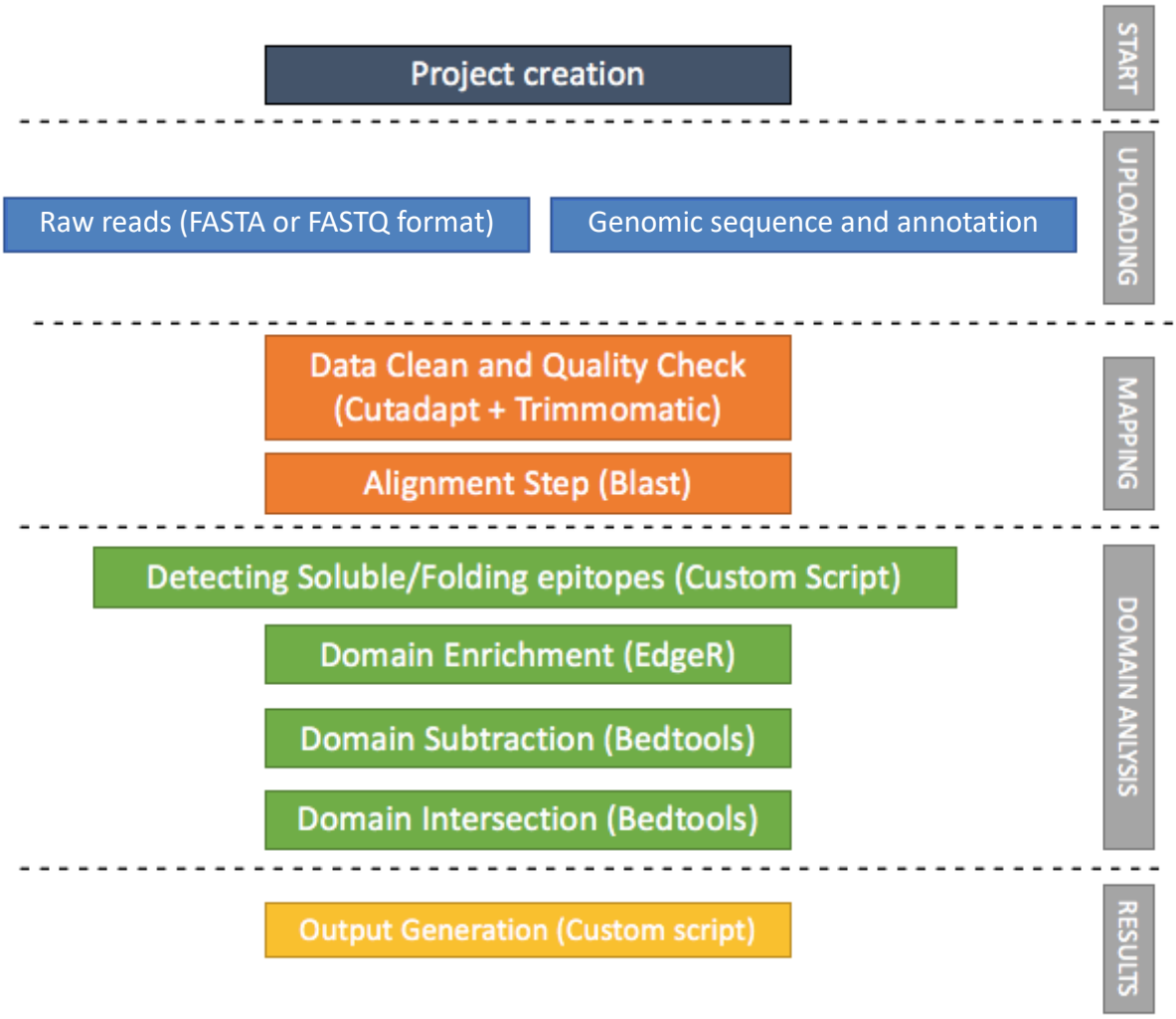
Prokaryote – User Guide

This introductory section provides an overview of **Prokaryote** pipeline drafting and design. The vertical gray rectangles correspond to the website sections.



Input Files

Mandatory inputs for **InteractomeSeq - Prokaryote** execution are:

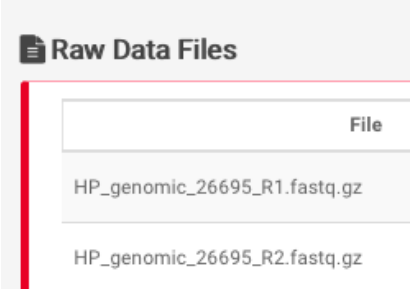
- genome reference file in FASTA format (either a custom annotation file or one selected from the drop-down menu) (Organism).
- a genome annotation (either a custom annotation file or one selected from the drop-down menu) (Organism).
- Raw Data files, FASTA or FASTQ format for query reads are allowed in the input, therefore the web interface additionally allows the submission of compressed files (gz format) to reduce the time of data upload (DataSets).

InteractomeSeq requires the user to upload at least two datasets. The input datasets must be generated with the same sequencing platform.

Raw Data Files

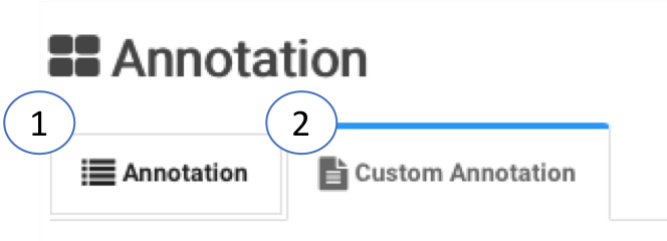
FASTA or FASTQ format are allowed as input, therefore the web interface additionally allows the submission of compressed files (gz format) to reduce the time of data upload (DataSets).

Input form is designed both for loading Single End or Paired-End sequencing. For Paired-End mode, as shown in the screen-shot below, the loading must be repeated both for the forward and reverse dataset.



Genome Sequence and Annotation

Genome annotation can be provided either as by selecting one of the annotations pre-loaded in the internal **InteractomeSeq** database (derived from bacterial genome annotations by the National Center for Biotechnology Information of the National Institute of Health) (NCBI Genome Annotation) (1) or a custom file (Custom Genome Annotation tab) (2).



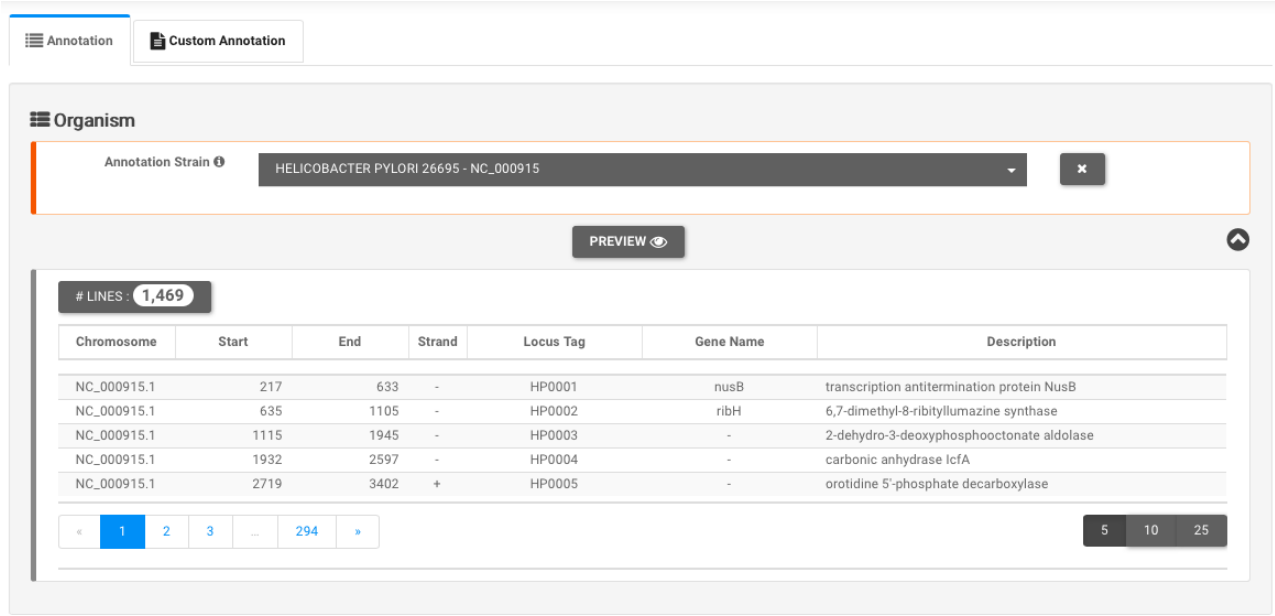
NCBI Genome Annotation

Users can select one of the pre-loaded NCBI bacteria genome annotations (NCBI ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/bacteria/, last updated on November 2019), containing complete information about 15593 bacterial strains (Complete Genome).
In order to select the proper strain from the drop-down menu, the user has to type just 3 or more characters of the strain name to activate the automatic search in the database.

Clicking on the "Preview" button 

allows quick check of the Genome Annotation selected.

Note: check that the “Gene Name” field contains the same values as the GED file, otherwise **InteractomeSeq** will not process the data.

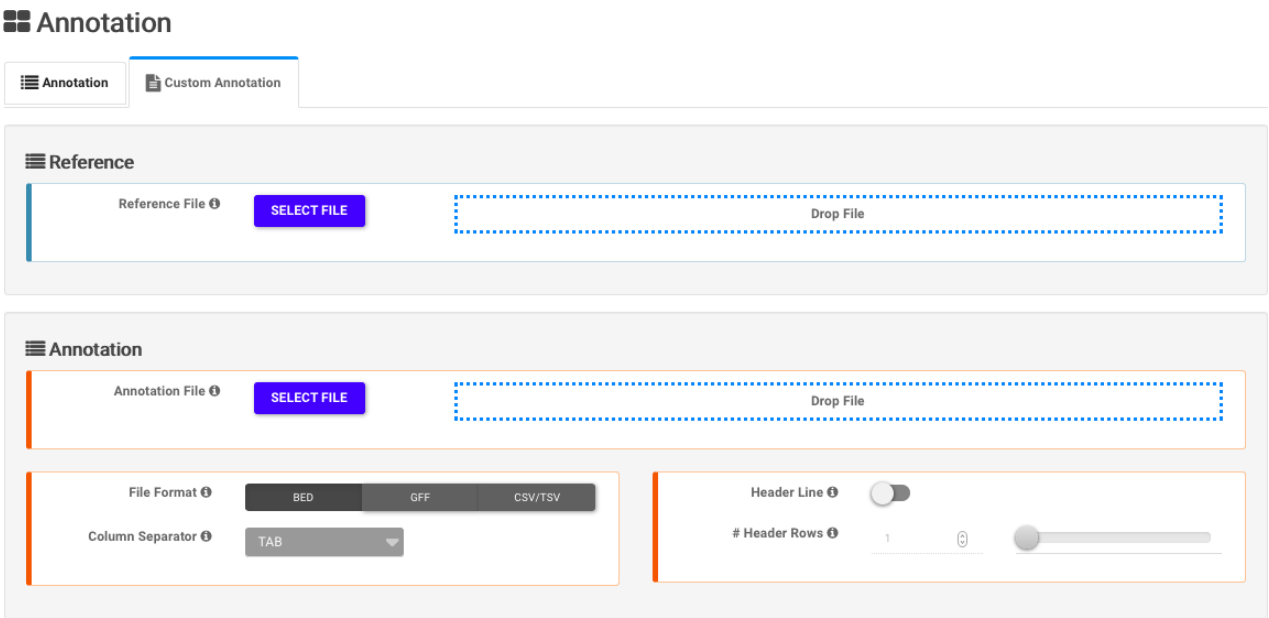


Custom Genome Sequence and Annotation

Alternatively, users can provide their own preferred genome sequence and annotation, provided the custom annotation file fulfills the specific requirements listed below.

Note: organisms with more than one chromosome or containing plasmids must be analyzed at the same time. Thus, when building custom genome annotations, users have to provide two files, one for nucleotide sequence (multi fasta) and one with gene annotation.

- Users can submit a custom nucleotide sequence file in one of the recognized file formats:
  - Fasta
  - Multi-Fasta
- Users can submit a custom gene annotation file in one of the recognized file formats:
  - BED
  - GFF
  - CSV/TSV
- Users can indicate (optionally):
  - Header Line and Rows (This file can optionally have a custom number of header lines at the top).
  - Column separator can be set selecting from TAB, SPACE, comma (“,”) or semicolon (“;”).



BED Format

Gene annotation in Browser Extensible Data format (BED) must be provided according to the UCSC standard, with at least 6 columns (also called "BED6" format) (https://genome.ucsc.edu/FAQ/FAQformat#format1).

BED file fields must contain the following information:

1. chrom – The name of the chromosome or scaffold.
2. chromStart – The starting position of the gene in the chromosome or scaffold. The first base in a chromosome is numbered 1.
3. chromEnd – The ending position of the gene in the chromosome or scaffold.
4. name – Defines the gene name.
5. score – not used. Please that the field cannot be empty, but must contain a value (set it to "0" or "." if the BED file has no score associated to the gene).
6. strand – Defines the transcription strand for each gene. Either "+" or "-".

An example of a valid Genome annotation BED6 file for use in **InteractomeSeq** is reported below.

locus	chr_start	chr_end	GeneName	score	strand
NC_007650	1	1188	BTH_II0001	0	+
NC_007650	1281	2324	BTH_II0002	0	-
NC_007650	2490	2870	BTH_II0003	0	-
NC_007650	2950	3558	BTH_II0004	0	-
NC_007650	3726	4925	BTH_II0005	0	+
NC_007650	4938	5969	BTH_II0006	0	+
NC_007650	6192	6740	BTH_II0007	0	-

GFF Format

Alternatively, annotations can be provided also in the General Feature Format (GFF) format, which has nine required fields that must be tab-separated. Please, refer to: https://genome.ucsc.edu/FAQ/FAQformat#format3 and http://gmod.org/wiki/GFF3 for the complete description of this format.

GFF file fields must contain the following information:

1. seqname – The name of the chromosome or scaffold.
2. source – not used. Please note that the field cannot be empty, but must contain a value (set it to "." if the GFF file has no value associated to this field).
3. feature – The feature type (e.g.: "gene", "CDS", "tRNA", etc.).
4. start – The starting position of the feature in the chromosome or scaffold. The first base is numbered 1.
5. end – The ending position of the feature in the chromosome or scaffold.
6. score – not used. Please note that the field cannot be empty, but must contain a value (set it to "0" or "." if the GFF file has no score associated to the feature).
7. strand – Valid entries are "+","-", or "." (for not available/not relevant).
8. frame – not used. If the feature is a coding exon, frame should be a number between 0-2 that represents the reading frame of the first base. If the feature is not a coding exon, the value should be ".". Please note that the field cannot be empty, but must contain a value.
9. attributes – A list of feature attributes in the format tag = value. Multiple tag = value pairs are separated by semicolons.

Note: Please check that records defining gene features have a pair: "locus_tag = LOCUS_NAME" in the "attributes" section (column 9), since InteractomeSeq infers the gene name from this tag-value pair.

Note: Valid GFF files are those downloaded from NCBI (https://www.ncbi.nlm.nih.gov/) and Patric (https://www.patricbrc.org/) and custom genome files formatted accordingly. Please, be sure to upload only plain text files to **InteractomeSeq** since it does not accept compressed formats (e.g.: zip, gzip or bzip archives).

Example of a GFF downloaded from NCBI

```
##gff-version 3
#!gff-spec-version 1.21
#!processor NCBI annotwriter
#!genome-build ASM852v1
#!genome-build-accession NCBI_Assembly:GCF_000008525.1
##sequence-region NC_000915.1 1 1667867
##species http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=85962
NC_000915.1	RefSeq	gene	217	633	0	-	0	ID=gene0;Dbxref=GeneID:898756;Name=nusB;gbkey=Gene;gene=nusB;gene_biotype=protein_coding;locus_tag=HP0001
NC_000915.1	RefSeq	CDS	217	633	0	-	0	ID=cds0;Parent=gene0;Dbxref=Genbank:NP_206803.1,GeneID:898756;Name=NP_206803.1;Note=Regulates rRNA biosynth
NC_000915.1	RefSeq	gene	635	1105	0	-	0	ID=gene1;Dbxref=GeneID:898768;Name=ribH;gbkey=Gene;gene=ribH;gene_biotype=protein_coding;locus_tag=HP0002
NC_000915.1	RefSeq	CDS	635	1105	0	-	0	ID=cds1;Parent=gene1;Dbxref=Genbank:NP_206804.1,GeneID:898768;Name=NP_206804.1;Note=RibE%3B 6%2C7-dimeth
NC_000915.1	RefSeq	gene	1115	1945	0	-	0	ID=gene2;Dbxref=GeneID:898773;Name=HP0003;gbkey=Gene;gene_biotype=protein_coding;locus_tag=HP0003
NC_000915.1	RefSeq	CDS	1115	1945	0	-	0	ID=cds2;Parent=gene2;Dbxref=Genbank:NP_206805.1,GeneID:898773;Name=NP_206805.1;Note=catalyzes the formation of
NC_000915.1	RefSeq	gene	1932	2597	0	-	0	ID=gene3;Dbxref=GeneID:898779;Name=HP0004;gbkey=Gene;gene_biotype=protein_coding;locus_tag=HP0004
NC_000915.1	RefSeq	CDS	1932	2597	0	-	0	ID=cds3;Parent=gene3;Dbxref=Genbank:NP_206806.1,GeneID:898779;Name=NP_206806.1;gbkey=CDS;product=carbonic a
NC_000915.1	RefSeq	gene	2719	3402	0	+	0	ID=gene4;Dbxref=GeneID:898802;Name=HP0005;gbkey=Gene;gene_biotype=protein_coding;locus_tag=HP0005
NC_000915.1	RefSeq	CDS	2719	3402	0	+	0	ID=cds4;Parent=gene4;Dbxref=Genbank:NP_206807.1,GeneID:898802;Name=NP_206807.1;Note=type 1 subfamily%3B invo
NC_000915.1	RefSeq	gene	3403	4233	0	+	0	ID=gene5;Dbxref=GeneID:898828;Name=panC;gbkey=Gene;gene=panC;gene_biotype=protein_coding;locus_tag=HP0006
NC_000915.1	RefSeq	CDS	3403	4233	0	+	0	ID=cds5;Parent=gene5;Dbxref=Genbank:NP_206808.1,GeneID:898828;Name=NP_206808.1;Note=catalyzes the formation of
NC_000915.1	RefSeq	gene	4250	4322	0	-	0	ID=gene6;Dbxref=GeneID:898829;Name=tRNA-Glu-1;gbkey=Gene;gene=tRNA-Glu-1;gene_biotype=tRNA;locus_tag=HPt01
NC_000915.1	RefSeq	tRNA	4250	4322	0	-	0	ID=rna0;Parent=gene6;Dbxref=GeneID:898829;gbkey=tRNA;gene=tRNA-Glu-1;product=tRNA-Glu
NC_000915.1	RefSeq	exon	4250	4322	0	-	0	ID=id1;Parent=rna0;Dbxref=GeneID:898829;gbkey=tRNA;gene=tRNA-Glu-1;product=tRNA-Glu
```

Example of a GFF downloaded from Patric

```
##gff-version 3
#Genome: 400667.7|Acinetobacter baumannii ATCC 17978
#Date:02/24/2015

##sequence-region	accn|NC_009083	1	13408
accn|NC_009083	RefSeq	CDS	1	957	0	+	0	ID=A1S_3471;locus_tag=A1S_3471;product=hypothetical protein
accn|NC_009083	RefSeq	CDS	950	1504	0	+	0	ID=A1S_3461;locus_tag=A1S_3461;product=DNA replication protein
accn|NC_009083	RefSeq	CDS	2523	3437	0	-	0	ID=A1S_3462;locus_tag=A1S_3462;product=hypothetical protein
accn|NC_009083	RefSeq	CDS	3538	4788	0	+	0	ID=A1S_3463;locus_tag=A1S_3463;product=diaminopimelate decarboxylase;ec_number=4.1.1.20
accn|NC_009083	RefSeq	CDS	5039	5629	0	+	0	ID=A1S_3464;locus_tag=A1S_3464;product=Cro-like protein
accn|NC_009083	RefSeq	CDS	6340	6906	0	-	0	ID=A1S_3465;locus_tag=A1S_3465;product=hypothetical protein
accn|NC_009083	RefSeq	CDS	7074	7685	0	-	0	ID=A1S_3466;locus_tag=A1S_3466;product=resolvase
accn|NC_009083	RefSeq	CDS	8602	9732	0	-	0	ID=A1S_3467;locus_tag=A1S_3467;product=hypothetical protein
accn|NC_009083	RefSeq	CDS	10072	10374	0	-	0	ID=A1S_3468;locus_tag=A1S_3468;product=putative lipoprotein
accn|NC_009083	RefSeq	CDS	10367	10723	0	-	0	ID=A1S_3469;locus_tag=A1S_3469;product=diaminopimelate decarboxylase
accn|NC_009083	RefSeq	CDS	12076	12444	0	-	0	ID=A1S_3470;locus_tag=A1S_3470;product=regulatory protein LysR
```

CSV/TSV Format

Finally, annotations can be provided also as a separated-columns text. Column separator can be chosen among TAB, SPACE, comma (“,”) or semicolon (“;”). This file must have 5 columns.

CSV file fields must contain the following information:

1. chrom – The name of the chromosome or scaffold.
2. chromStart – The starting position of the gene in the chromosome or scaffold. The first base in a chromosome is numbered 1.
3. chromEnd – The ending position of the gene in the chromosome or scaffold.
4. strand – Defines the transcription strand for each gene. Either "+" or "-".
5. name – Defines the gene name.

An example of a valid Genome annotation CSV file for use in **InteractomeSeq** is reported below.

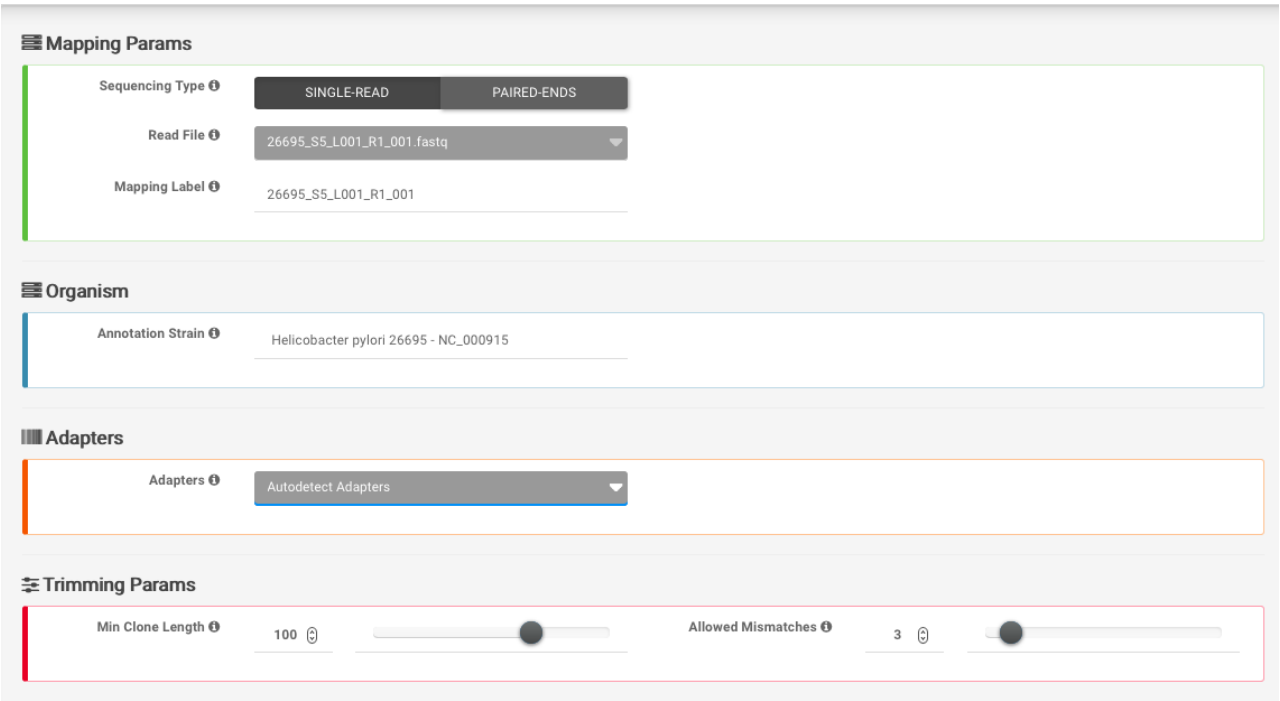
```
NC_011334.1,485,2020,+,HPG27_RS07980
NC_011333.1,899,1729,-,HPG27_RS00015
NC_011333.1,1716,2381,-,HPG27_RS00020
NC_011334.1,2065,2799,+,HPG27_RS07985
NC_011333.1,2503,3186,+,HPG27_RS00025
NC_011334.1,2849,3553,+,HPG27_RS07990
NC_011333.1,3187,4017,+,HPG27_RS00030
NC_011334.1,3672,4835,-,HPG27_RS07995
NC_011333.1,4031,4106,-,HPG27_RS00035
NC_011333.1,4179,4255,-,HPG27_RS00040
NC_011333.1,4306,4381,-,HPG27_RS00045
NC_011333.1,4423,4497,-,HPG27_RS00050
```

Mapping

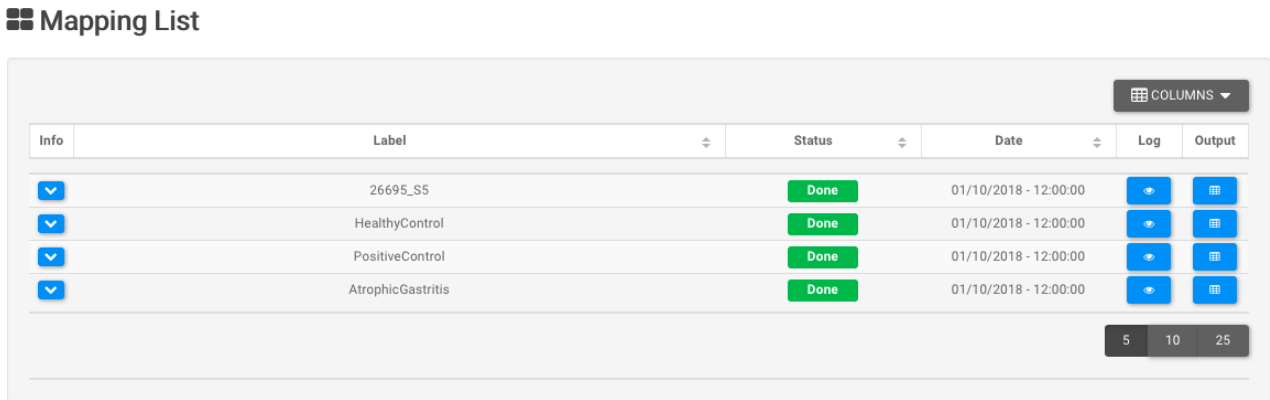
By clicking on the botton Mapping 

4 sub-sections will appear on the screen.

1. **Mapping Params.** Selection of sequencing type among paired-end reads or single-end.
2. **Organism.** Selected FASTA file that will be used as reference to align the sequences.
3. **Adapters.** Selection of adapters to remove from input sequences. User can select between three options: i) **Autodetec Adapters**; ii) **Custom Adapters**; iii) **Illumina Adapters**
4. **Trimming Params.** Selection of minimum length of sequence and number of mismatch allows, reads below this threshold will be discarded.



Note: Mapping step should be repeated for each input dataset. For each mapping file generated, user can check the log file associated and the Status message.

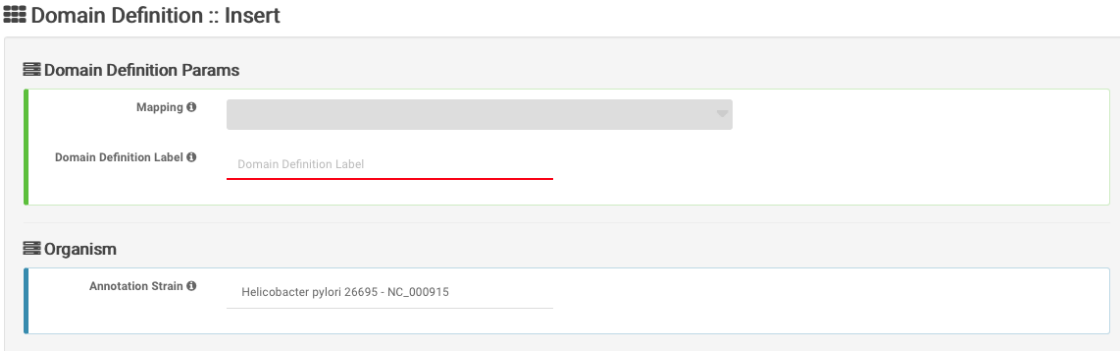


Domain Analysis

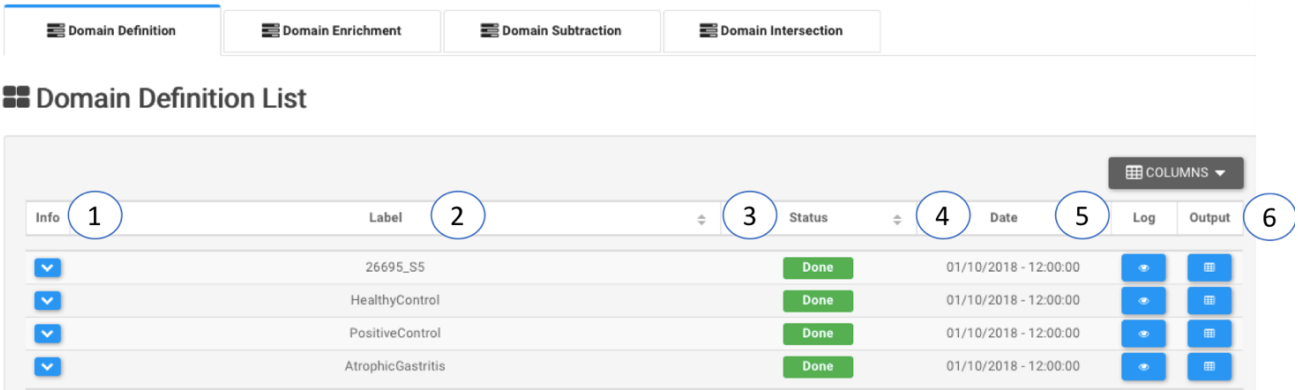
Domain Anlysis is composed by four sheets:

1. **Domain Definition**
2. **Domain Enrichment**
3. **Domain Subtraction**
4. **Domain Intersection**

1. **Domain Definition** takes as input the mapping file previously generated.



Domain Definition back-end script launches "bedtool genomomecov" to computes coverage depth at each genome position of the coding regions (CDS). Next with a custom script it calculates the average depth coverage and only the genome positions that have a depth coverage greater than the average depth coverage are taken into account. Afterwards the epitopes are defining by combining consecutive bases that have a valid depth coverage. An epitope will be defined by at least 10 consecutive bases. When the computational steps are complete, user can check the status of his analysis.



1. **Info** – Drop-down menu with information of Mapping input file**.**
2. **Label -** Sample label.
3. **Status** – When the execution ends successfully, the button turns green, otherwise, it turns red.

4. **Date** - Day and time of analysis execution
5. **Log** – Button that hide/open a box with execution log file.

Domain Definition :: Log :: 26695_S5

STATUS | Domain Definition Done | Completed Processing

```
Prokaryote Domain Definition - Start * Wednesday June 20, 2018 - 15:43:01
Conversion Tabular ==>FastA complete.
Representative clones picking complete.
Blastn analysis complete.
Output Bed parsed complete.
Checking clones gene intersection complete.
Filtering Clones outside CDS.
Clone cluster count.Complete
Filtering cluster complete.index file 26695_S5.Reference.fasta.fai not found, generating...
Filtering cluster complete.
Pybedtools getfasta complete.
Add gene description complete.
Conversion FastA ==>Tabular complete.
Add nucleotide sequence complete.
Prokaryote Domain Definition - End * Wednesday June 20, 2018 - 15:44:56
```

6. **Output** – Hide/open panel with output preview

Domain Definition :: Output :: 26695_S5

Domain Definition Output File | DOWNLOAD

TOTAL : 2,986 | COLUMNS

Info	Chromosome	Clone Start	Clone End	Clone Length	Start	End	Gene	Strand
	NC_000915.1	346	526	180	217	633	HP0001	-
	NC_000915.1	724	1073	349	635	1105	HP0002	-
	NC_000915.1	1178	1721	543	1115	1945	HP0003	-
	NC_000915.1	1775	1955	170	1115	1945	HP0003	-
	NC_000915.1	1983	2463	480	1932	2597	HP0004	-
	NC_000915.1	2751	3018	267	2719	3402	HP0005	+
	NC_000915.1	3060	3168	108	2719	3402	HP0005	+
	NC_000915.1	3789	3897	108	3403	4233	HP0006	+
	NC_000915.1	3900	4035	135	3403	4233	HP0006	+
	NC_000915.1	5743	5985	242	5241	7145	HP0009	-

2. **Domain Enrichment** takes as input the Genomic and Target output of Domain Definition step.

Domain Enrichment List

DOMAIN ENRICHMENT | COLUMNS

Info	Label	Status	Date	Log	Output	Edit	Delete

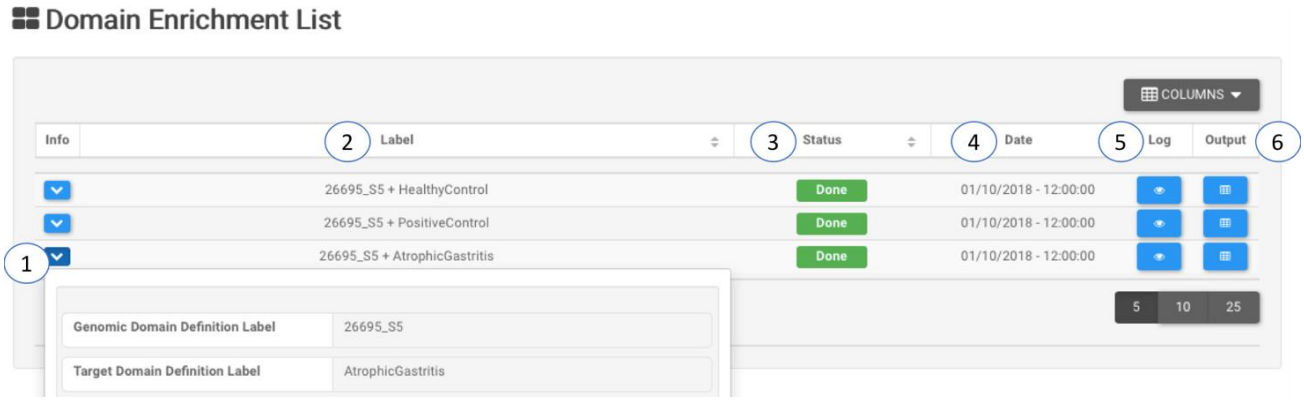
Domain Enrichment :: Insert

Genomic Domain Definition

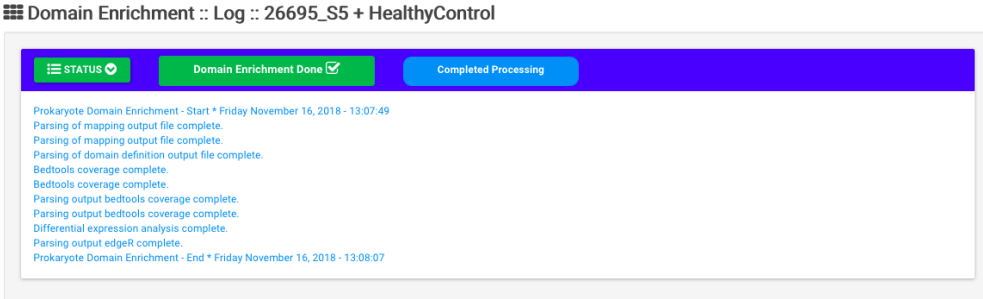
Target Domain Definition

Domain Enrichment Label — Domain Enrichment Label

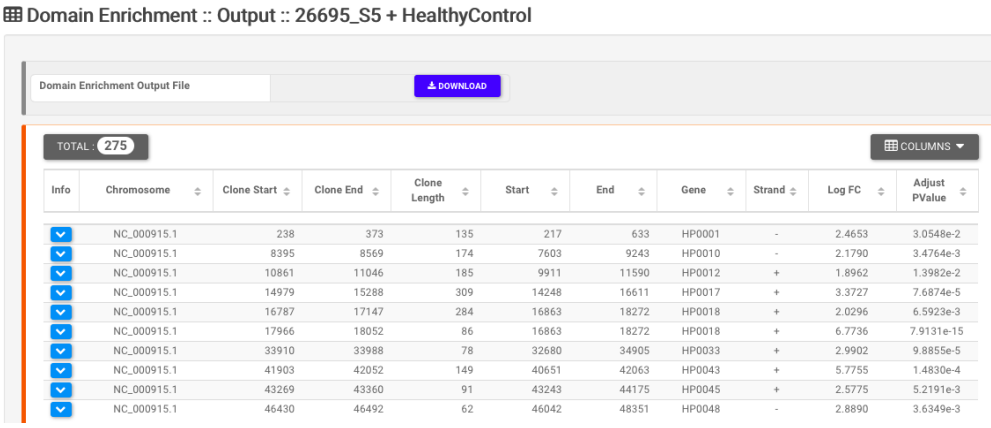
Domain Enrichment back-end script launches “bedtools genomecov” to compute the number of feature (reads) that map inside the epitope regions. After counting, the epitopes counts are normalized in TPM (transcription per milion) and with R-package EdgeR establish the differentially epitopes of target sample (Target Domain Definition) compare to the background (Genomic Domain Definition). When the computational steps are complete, user can check the status of his analysis.



1. **Info** – Drop-down menu with information of Domain Definition input file**.**
2. **Label -** Sample label.
3. **Status** – When the execution ends successfully, the button turns green, otherwise, it turns red.
4. **Date** - Day and time of analysis execution
5. **Log** – Button that hide/open a box with execution log file.



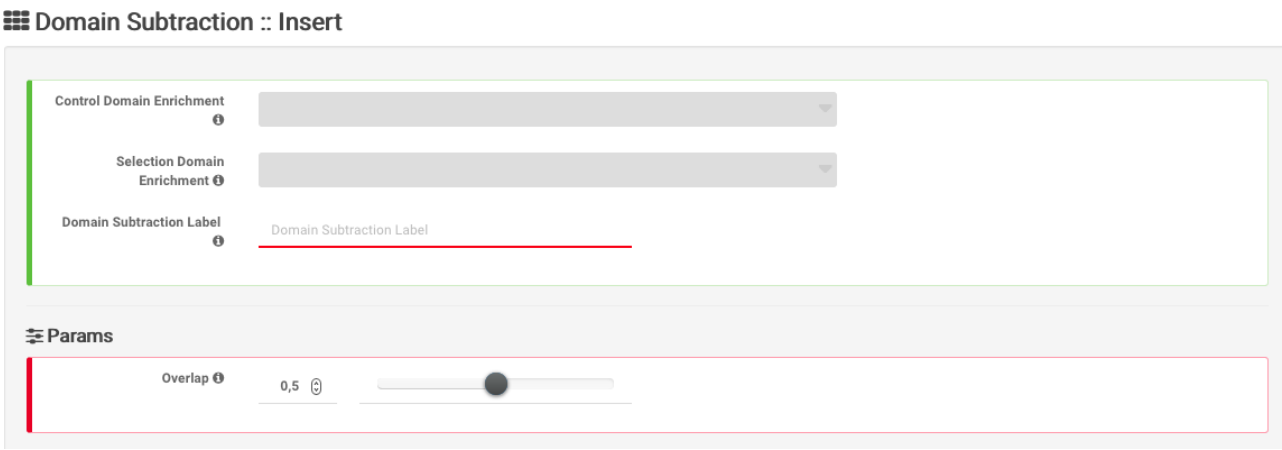
6. **Output** – Hide/open panel with output preview



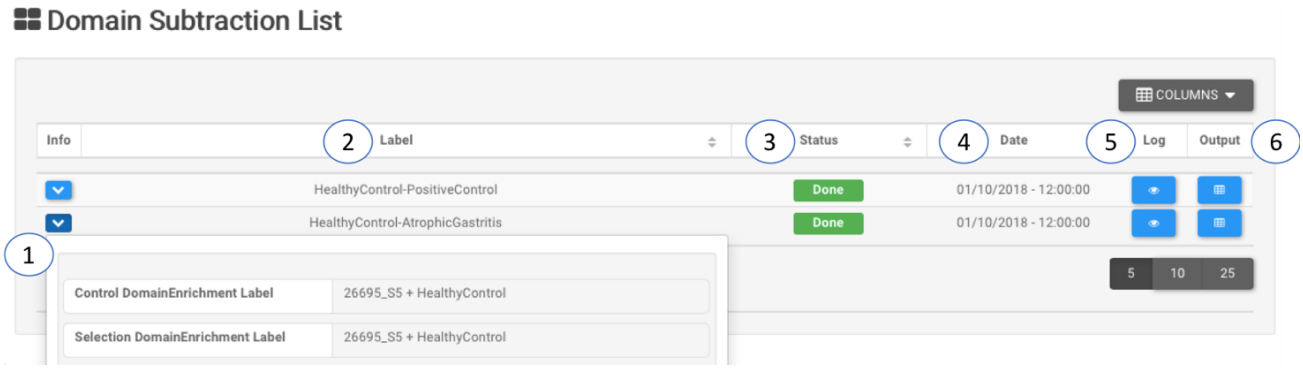
Info	Chromosome	Clone Start	Clone End	Clone Length	Start	End	Gene	Strand	Log FC	Adjust PValue
	NC_000915.1	238	373	135	217	633	HP0001	-	2.4653	3.0548e-2
	NC_000915.1	8395	8569	174	7603	9243	HP0010	-	2.1790	3.4764e-3
	NC_000915.1	10861	11046	185	9911	11590	HP0012	+	1.8962	1.3982e-2
	NC_000915.1	14979	15288	309	14248	16611	HP0017	+	3.3727	7.6874e-5
	NC_000915.1	16787	17147	284	16863	18272	HP0018	+	2.0296	6.5923e-3
	NC_000915.1	17966	18052	86	16863	18272	HP0018	+	6.7736	7.9131e-15
	NC_000915.1	33910	33988	78	32680	34905	HP0033	+	2.9902	9.8855e-5
	NC_000915.1	41903	42052	149	40651	42063	HP0043	+	5.7755	1.4830e-4
	NC_000915.1	43269	43360	91	43243	44175	HP0045	+	2.5775	5.2191e-3
	NC_000915.1	46430	46492	62	46042	48351	HP0048	-	2.8890	3.6349e-3

Note: The Domain Enrichment step requires that the design of the experiment includes InteractomeSequencing of both the bacterial genome and the selections with patient sera.

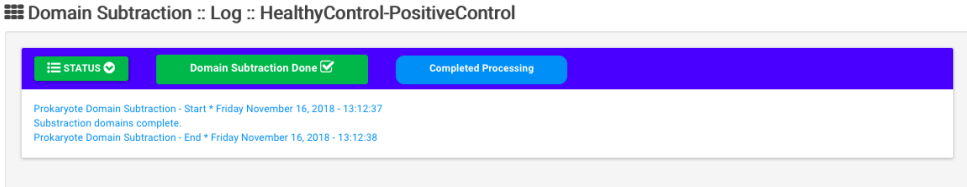
3. **Domain Subtraction** takes as input two differentially enriched epitopes/domains lists, one defined as Control Domain Enrichment and one defined as Selection Domain Enrichment.



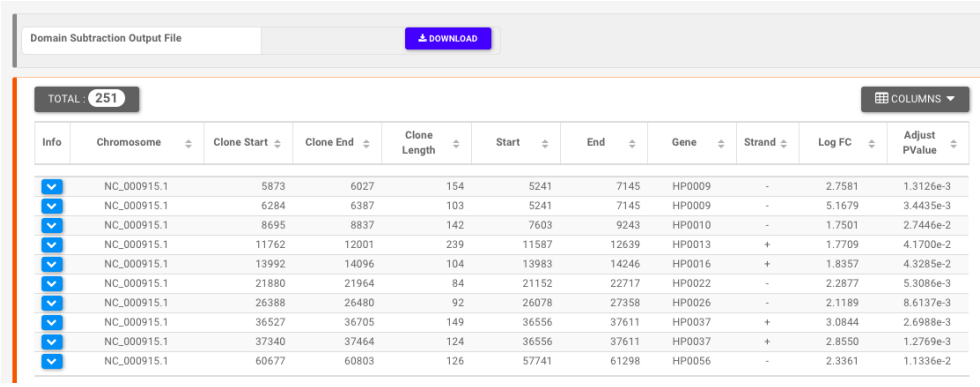
Domain Subtraction back-end script launches “bedtools subtract” that searches for domains in Control Enrichment file that overlap with those of Selection Enrichment file. If an overlapping feature is found in Control Enrichment file, the overlapping portion is removed from Selection Enrichment file and the remaining portion of Selection Enrichment domains are reported. When the computational steps are complete, user can check the status of his analysis.



1. **Info** – Drop-down menu with information of Domain Enrichment input file**.**
2. **Label -** Sample label.
3. **Status** – When the execution ends successfully, the button turns green, otherwise, it turns red.
4. **Date** - Day and time of analysis execution
5. **Log** – Button that hide/open a box with execution log file.

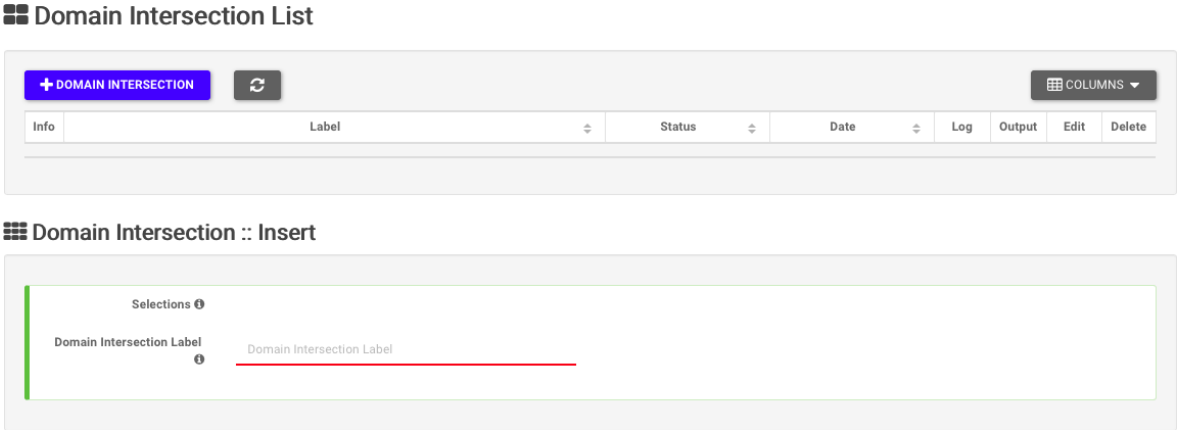


6. **Output** – Hide/open panel with output preview

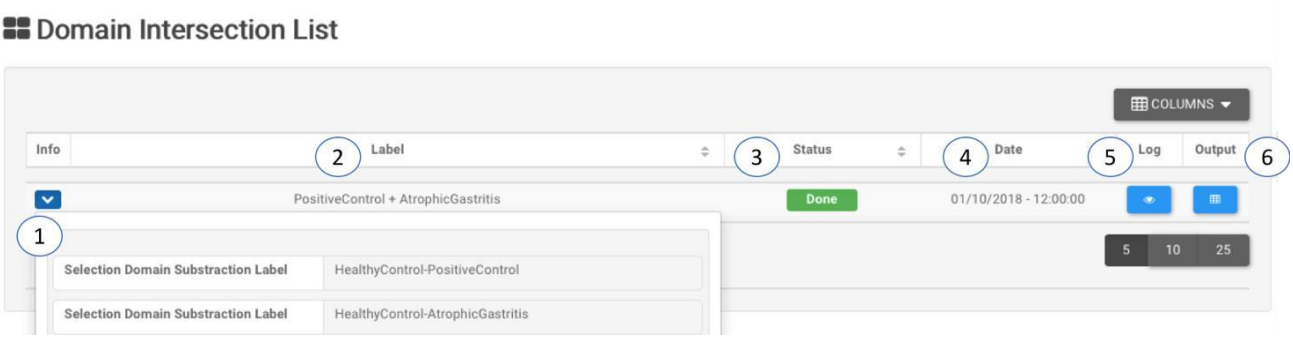


Info	Chromosome	Clone Start	Clone End	Clone Length	Start	End	Gene	Strand	Log FC	Adjust PValue
	NC_000915.1	5873	6027	154	5241	7145	HP0009	-	2.7581	1.3126e-3
	NC_000915.1	6284	6387	103	5241	7145	HP0009	-	5.1679	3.4435e-3
	NC_000915.1	8695	8837	142	7603	9243	HP0010	-	1.7501	2.7446e-2
	NC_000915.1	11762	12001	239	11587	12639	HP0013	+	1.7709	4.1700e-2
	NC_000915.1	13992	14096	104	13983	14246	HP0016	+	1.8357	4.3285e-2
	NC_000915.1	21880	21964	84	21152	22717	HP0022	-	2.2877	5.3086e-3
	NC_000915.1	26388	26480	92	26078	27358	HP0026	-	2.1189	8.6137e-3
	NC_000915.1	36527	36705	149	36556	37611	HP0037	+	3.0844	2.6988e-3
	NC_000915.1	37340	37464	124	36556	37611	HP0037	+	2.8550	1.2769e-3
	NC_000915.1	60677	60803	126	57741	61298	HP0056	-	2.3361	1.1336e-2

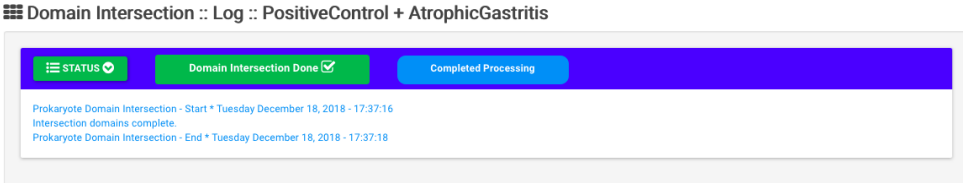
4. **Domain Intersection** takes as input two differentially enriched epitopes/domains lists output of Domain Definition step.



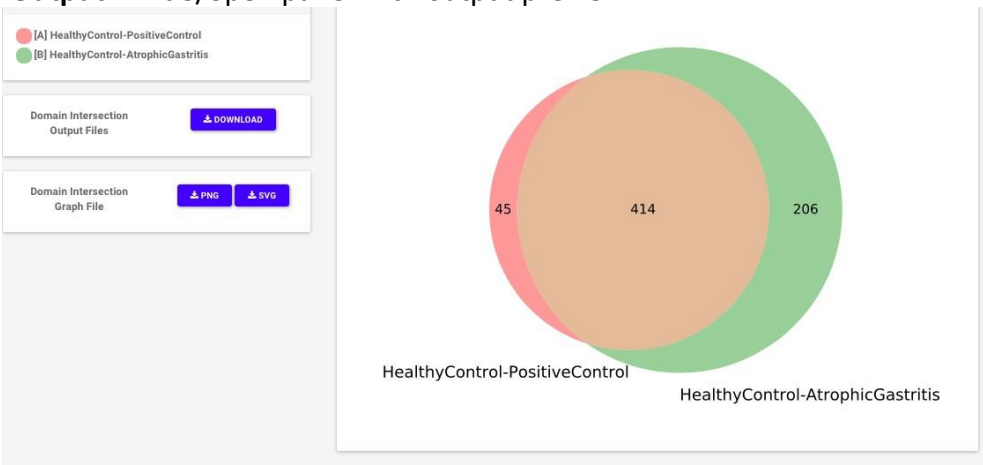
Domain Intersection allows one to screen for overlaps between two sets of epitopes/domains lists.



1. **Info** – Drop-down menu with information of Domain Enrichment input file**.**
2. **Label -** Sample label.
3. **Status** – When the execution ends successfully, the button turns green, otherwise, it turns red.
4. **Date** - Day and time of analysis execution
5. **Log** – Button that hide/open a box with execution log file.

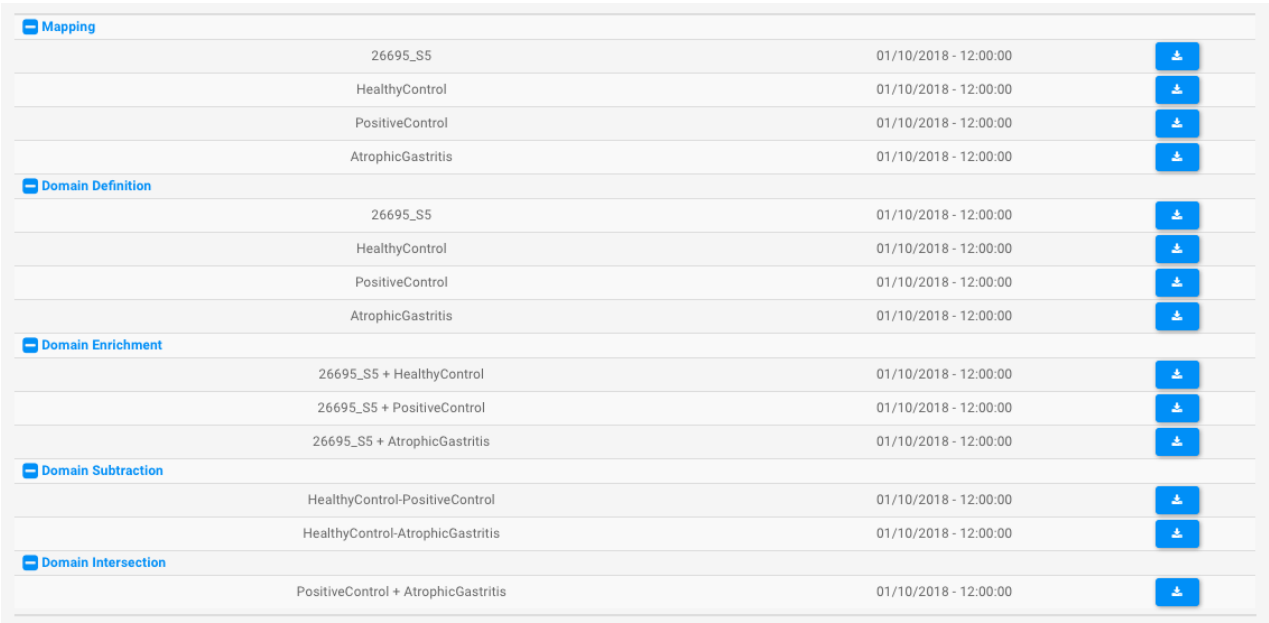


6. **Output** – Hide/open panel with output preview



Results

In this section user will find a summary of the output files generated by the execution. Download of the data can be activated clicking on the corresponding button. Data are in zip-compressed archives and can be opened and edited as tab-separated files.



Mapping		
26695_S5	01/10/2018 - 12:00:00	
HealthyControl	01/10/2018 - 12:00:00	
PositiveControl	01/10/2018 - 12:00:00	
AtrophicGastritis	01/10/2018 - 12:00:00	
Domain Definition		
26695_S5	01/10/2018 - 12:00:00	
HealthyControl	01/10/2018 - 12:00:00	
PositiveControl	01/10/2018 - 12:00:00	
AtrophicGastritis	01/10/2018 - 12:00:00	
Domain Enrichment		
26695_S5 + HealthyControl	01/10/2018 - 12:00:00	
26695_S5 + PositiveControl	01/10/2018 - 12:00:00	
26695_S5 + AtrophicGastritis	01/10/2018 - 12:00:00	
Domain Subtraction		
HealthyControl-PositiveControl	01/10/2018 - 12:00:00	
HealthyControl-AtrophicGastritis	01/10/2018 - 12:00:00	
Domain Intersection		
PositiveControl + AtrophicGastritis	01/10/2018 - 12:00:00	

Outputs

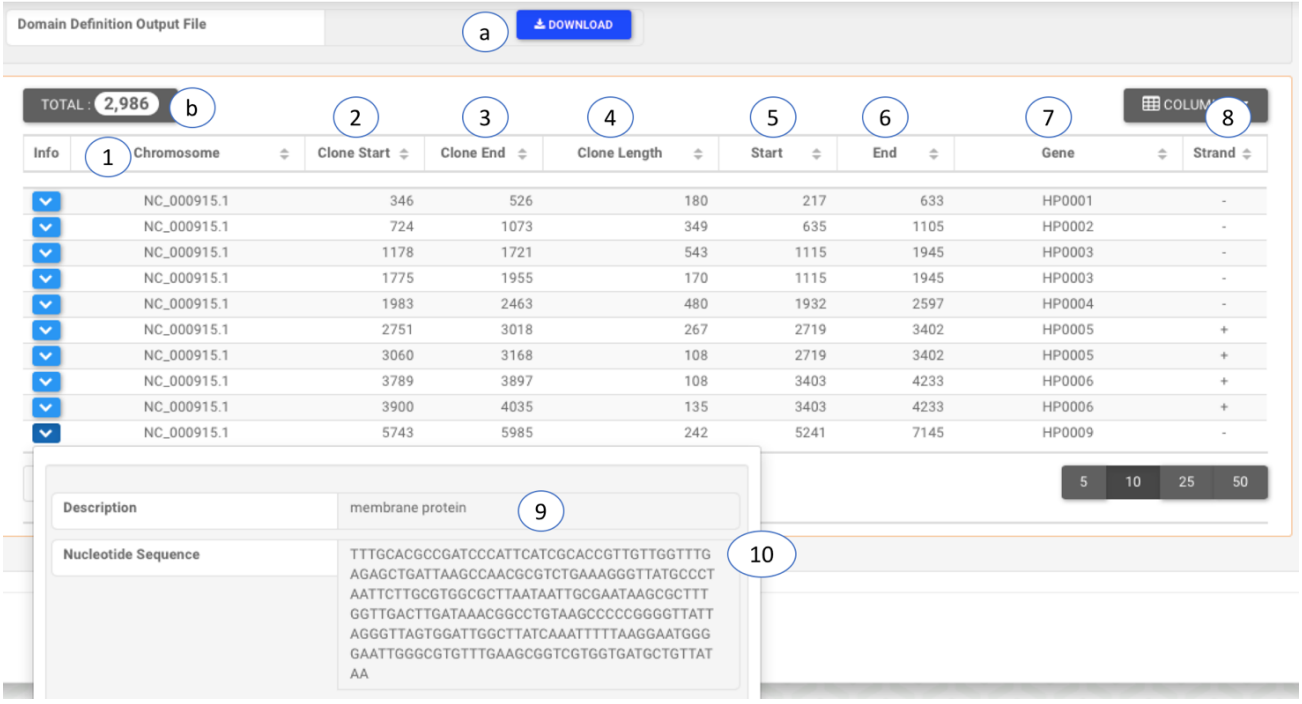
- **Mapping**. Tabular output file is composed by 13 columns and in each row is stored the information about uniquely mapping reads.

seq1:1:15	NC_000915.1	100.0	172	0	0	1	172	273366	273537	3.34e-88	318.0	GAAATACAGAGGCGAGTTTGAAGAGCGCTTGAAAAAG
seq1:1:16	NC_000915.1	100.0	100	0	0	1	100	922010	921911	2.11e-48	185.0	GAATTTAAACGCTGGAAGGCATGCCGATCAAACAGCG
seq1:1:17	NC_000915.1	99.59	244	1	0	1	244	1349358	1349601	2.03e-126	446.0	TAAAGATTCCCCTTTGATCCAAAAAACGCTCAATGTC
seq1:1:19	NC_000915.1	99.465	187	1	0	1	187	941000	941186	7.68e-95	340.0	TCCTTTCGCTCAACCTAGCGCCACTCCTAATTTAGTC
seq1:1:23	NC_000915.1	98.889	90	1	0	1	90	943437	943348	3.23e-41	161.0	CAAAGTTTGAGCTTGGGGATTAACCCGGTGCTGTTGC
seq1:1:24	NC_000915.1	100.0	196	0	0	1	196	1292807	1292612	1.72e-101	363.0	CGCGCGCTATTTTAGGAATTACACCCAATATGTCAAA
1	2	3	4	5	6	7	8	9	10	11	12	13

Tabular data report the following fields:

1. Sequence Id
2. Reference mapped strand ID used for mapping
3. Percentage of sequence aligned
4. Sequence length
5. Number of mismatch
6. Number of gap openings
7. Chromosome number
8. Alignment length
9. Genomic start of alignment
10. Genomic end of alignment
11. Expected value
12. Bit score
13. Aligned part of query sequence

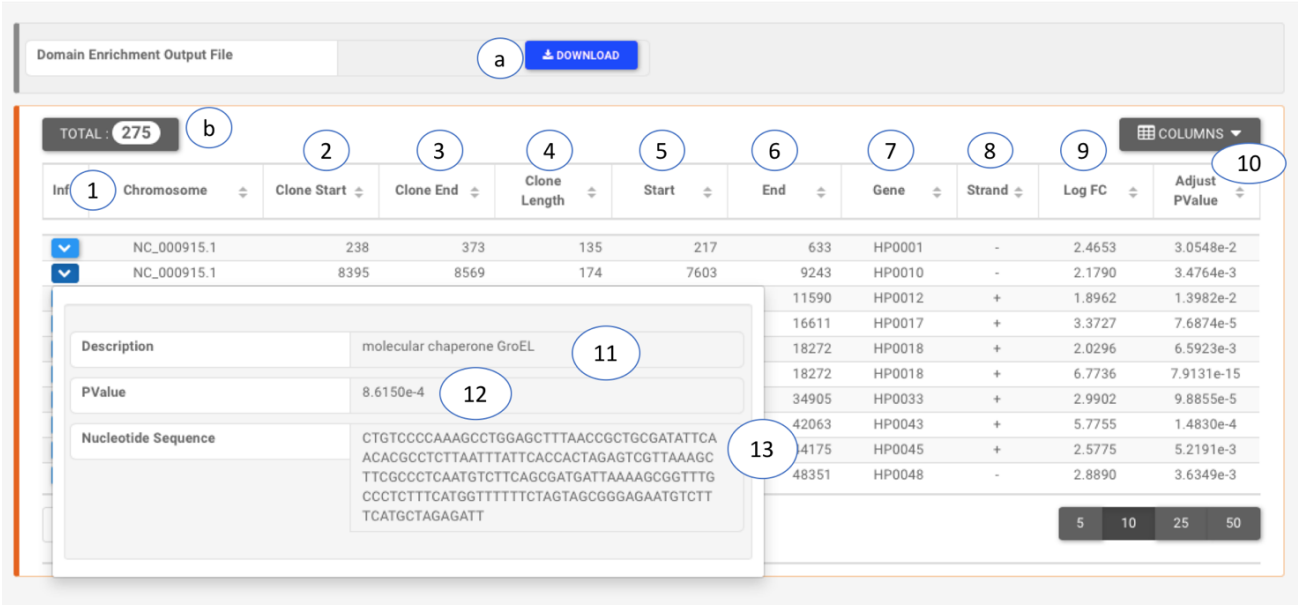
- **Domain Definition**. The domain definition step provides output in tabular format. In this file all soluble domains/epitopes detected are listed and the protein coding associated information are provided. As shown in the figure below, download of the data can be activated by clicking on the corresponding button (a) and in box (b) user can quickly check how many domains are detected.



Tabular data report the following fields:

1. Chromosome - Reference mapped strand ID used for mapping
2. Clone Start - Genomic start of soluble folding domain/epitope
3. Clone End - Genomic end of soluble folding domain/epitope
4. Clone Length - Domain/epitope length
5. Start – Starting coordinate of the gene associated with the domain/epitope on the chromosome.
6. End - Ending coordinate of the gene associated with the domain/epitope on the chromosome.
7. Gene – Gene ID
8. Strand – Strand associated with gene annotation
9. Description – Gene associated with the domain/epitope annotation
10. Nucleotide Sequence - Domain/epitope nucleotide sequence.

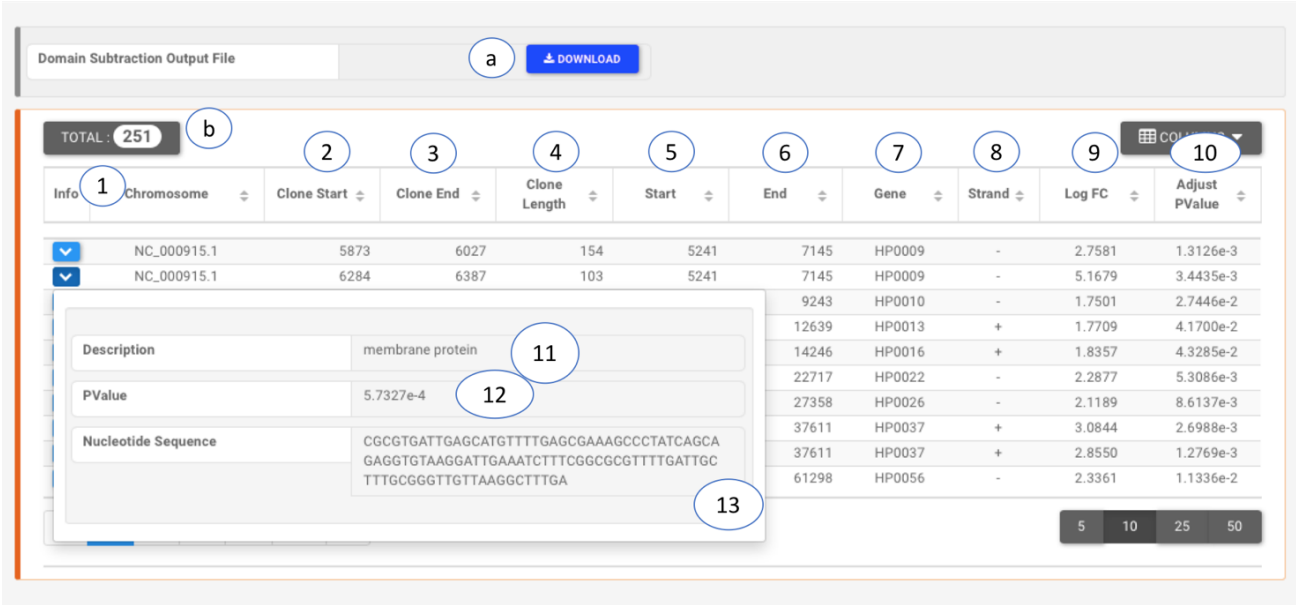
- **Domain Enrichment**. The domain enrichment step provides output in tabular format. In this file the expression of all soluble domains/epitopes detected from the Selection sample are statistically tested (edgeR) against Genomic domains/epitopes. As shown in the figure below, download of the data can be activated by clicking on the corresponding button (a) and in box (b) user can quickly check how many domains are detected.



Tabular data report the following fields:

1. Chromosome - Reference mapped strand ID used for mapping
2. Clone Start - Genomic start of soluble folding domain/epitope
3. Clone End - Genomic end of soluble folding domain/epitope
4. Clone Length - Domain/epitope length
5. Start – Starting coordinate of the gene associated with the domain/epitope on the chromosome.
6. End - Ending coordinate of the gene associated with the domain/epitope on the chromosome.
7. Gene – Gene ID
8. Strand – Strand associated with gene annotation
9. LogFC – log2 fold change estimation
10. Adjust Pvalue – pvalue adjusted for FDR
11. Description – Gene associated with the domain/epitope annotation
12. Pvalue
13. Nucleotide Sequence - Domain/epitope nucleotide sequence.

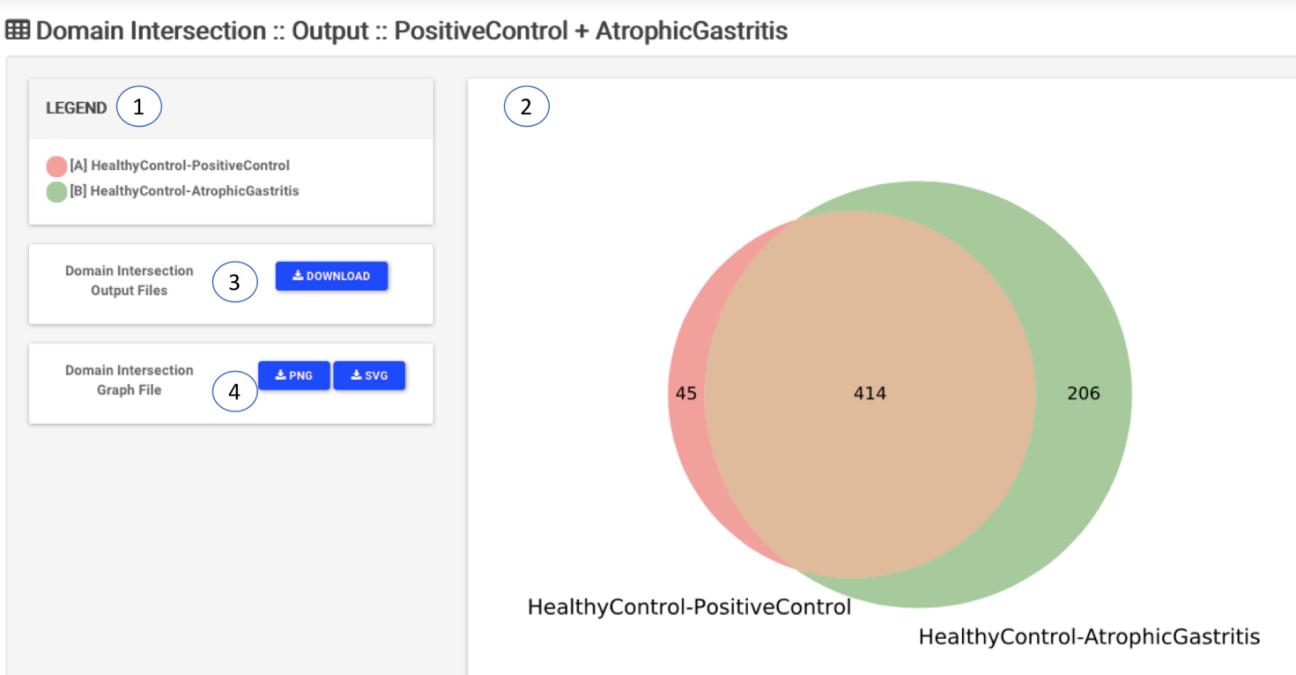
- **Domain Subtraction**. The domain subtraction step provides output in tabular format. In this file are listed the soluble domains/epitopes that result from the subtraction between two differentially enriched Selections. As shown in the figure below, download of the data can be activated by clicking on the corresponding button (a) and in box (b) user can quickly check how many domains remain after the subtraction.



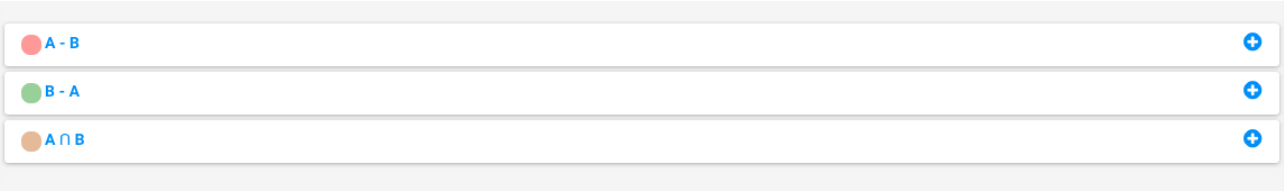
Tabular data report the following fields:

1. Chromosome - Reference mapped strand ID used for mapping
2. Clone Start - Genomic start of soluble folding domain/epitope
3. Clone End - Genomic end of soluble folding domain/epitope
4. Clone Length - Domain/epitope length
5. Start – Starting coordinate of the gene associated with the domain/epitope on the chromosome.
6. End - Ending coordinate of the gene associated with the domain/epitope on the chromosome.
7. Gene – Gene ID
8. Strand – Strand associated with gene annotation
9. LogFC – log2 fold change estimation
10. Adjust Pvalue – pvalue adjusted for FDR
11. Description – Gene associated with the domain/epitope annotation
12. Pvalue
13. Nucleotide Sequence - Domain/epitope nucleotide sequence.

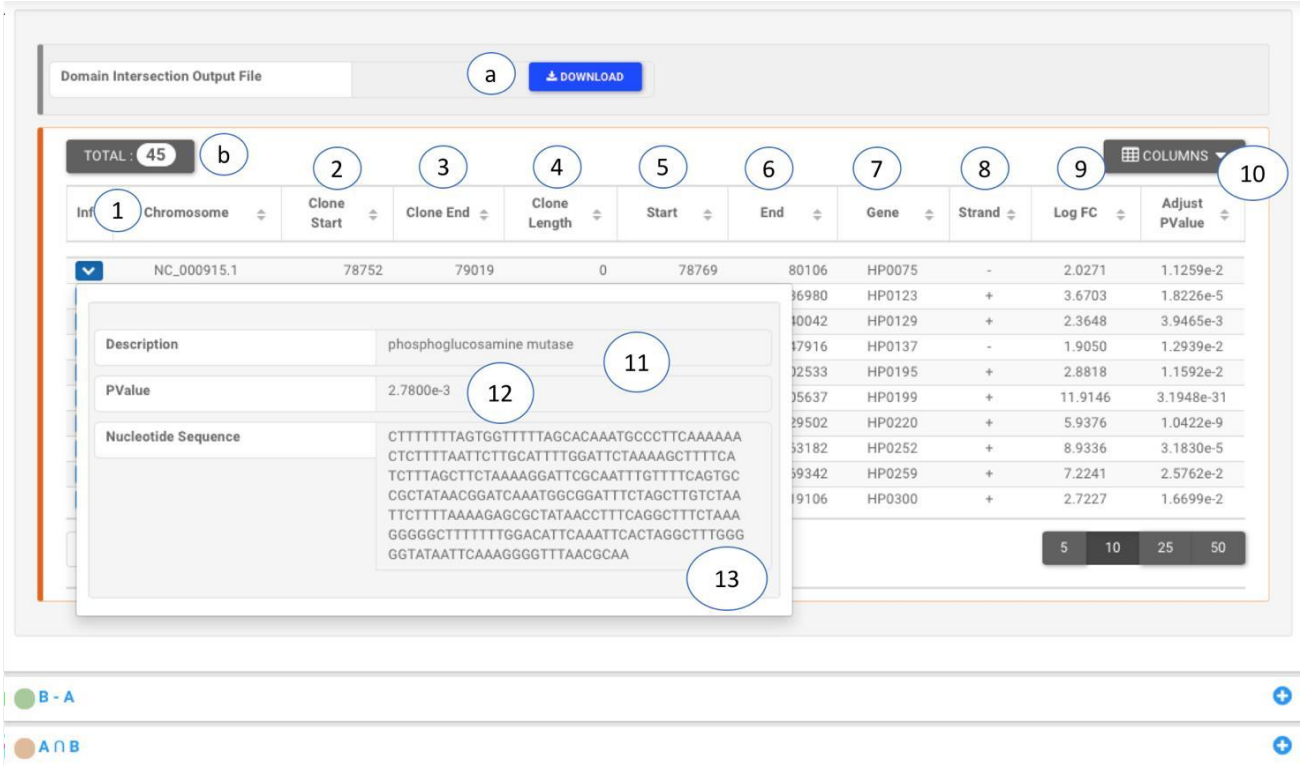
- **Domain Intersection**. The domain subtraction step provides two outputs, on in tabular format and the second is a Venn plot. This outputs represents the unique e common soluble domains/epitopes that result from the intersection of two or three differentially enriched Selections. As shown in the figure below, the unique and common domains lists have different name (1) and the circle of the plot is proportionally to the dimensions of the lists (2). The download of the data can be activated by clicking on the corresponding button (3) that will activate the download of all lists generated. The Venn plot can be saved in png or svg format by clicking the desired format button (4).



The preview of unique and common domains/epitopes, can be activated by clicking on the button **+**



As shown in the image below, download of the data can be activated by clicking on the corresponding button (a) and in box (b) user can quickly check the number of domains that are common or unique between Selections.



Tabular data report the following fields:

1. Chromosome - Reference mapped strand ID used for mapping
2. Clone Start - Genomic start of soluble folding domain/epitope
3. Clone End - Genomic end of soluble folding domain/epitope
4. Clone Length - Domain/epitope length
5. Start – Starting coordinate of the gene associated with the domain/epitope on the chromosome.
6. End - Ending coordinate of the gene associated with the domain/epitope on the chromosome.
7. Gene – Gene ID
8. Strand – Strand associated with gene annotation
9. LogFC – log2 fold change estimation
10. Adjust Pvalue – pvalue adjusted for FDR
11. Description – Gene associated with the domain/epitope annotation
12. Pvalue
13. Nucleotide Sequence - Domain/epitope nucleotide sequence.