Analyze coding & non-coding RNAs with InSyBio ncRNASeq

InSyBio
Intelligent Systems Biology

User Manual

July 2024

Insybio Suite v3.3

www.insybio.com

Introduction

ncRNASeq is an RNA analysis tool for the prediction and analysis of:

- Coding RNAs
- non-coding RNAs
- miRNA target genes
- Bulk RNA-sequencing data
- single-cell RNA-sequencing data

Non-coding RNA genes are RNA sequences transcribed from DNA, but not translated to proteins. Their identification as well as the identification of the genes they regulate is a promising research area.

InSyBio ncRNASeq enables users to analyze non-coding RNAs. Users can search and analyze the RNA sequence of their interest. They can also analyze a full sequences dataset derived from online available databases, experimental sequencing techniques or computational in silico techniques.

With InSyBio ncRNASeq you can predict and analyze RNA genes and miRNA target genes by combining a variety of sequential, structural and functional information, and using a high-performance machine-learning technique. The RNA analysis is conducted by the calculation of the 58 most informative features described in the literature, and the miRNA-miRNA targets analysis is conducted by the calculation of the 124 most informative ones. InSyBio ncRNASeq also provides results storage in its knowledge base, equipped with information retrieval tools, to allow users to produce and extract their datasets.

With InSyBio ncRNASeq you can:

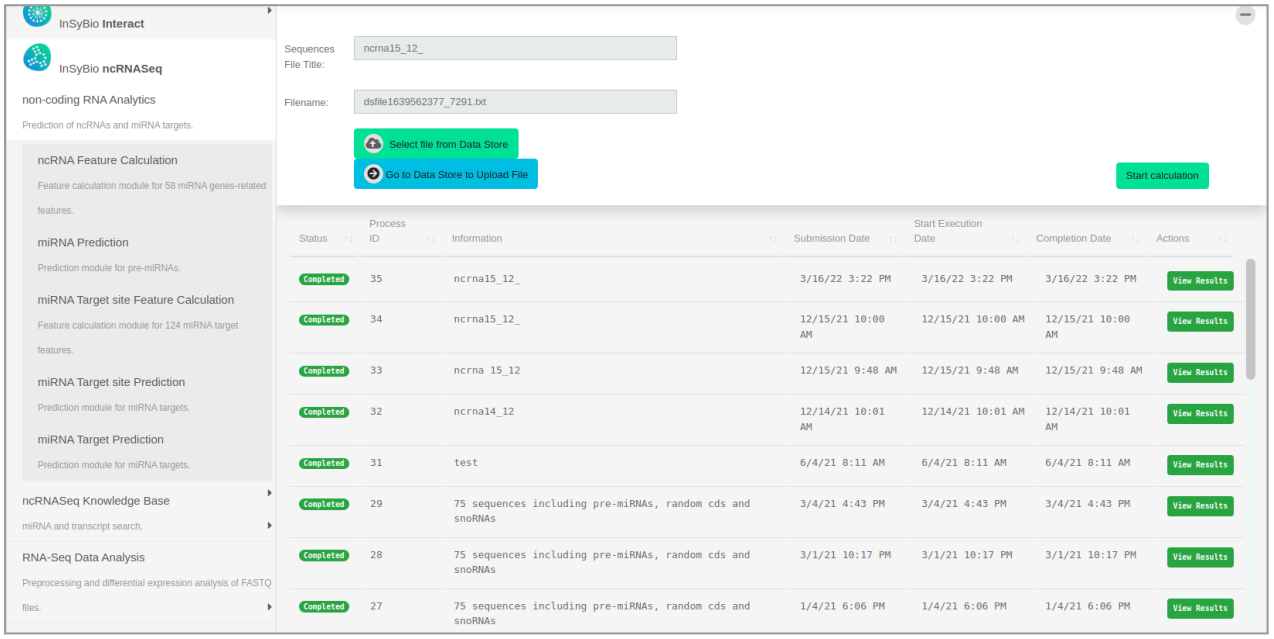
a) Calculate 58 RNA genes-related features
b) Predict miRNAs
c) Calculate 124 miRNA target site features
d) Predict miRNA target sites
e) Search stem-loop and mature miRNAs

f) Search transcripts and genes
g) Search transcripts and genes for potential miRNA targets
h) Predict miRNA targets
i) Apply our processing pipeline to your RNASeq data and perform Differential Expression Analysis
j) Identify different types of novel small non-coding RNAs (e.g. snoRNAs, miRNAs, tRNA fragments etc) from your raw RNA-sequencing data
k) Apply our processing pipeline to your single-cell RNASeq data and perform Differential Expression Analysis, cell clustering and additional analyses (eg. cell-cell communication, identification of cell differentiation patterns, deconvolution).

non -coding RNA Analytics

ncRNA Feature Calculation

You can calculate 58 informative features for non-coding RNAs by supplying their sequence in fasta format. These features include sequential, thermodynamical and structural properties of the RNA sequences.

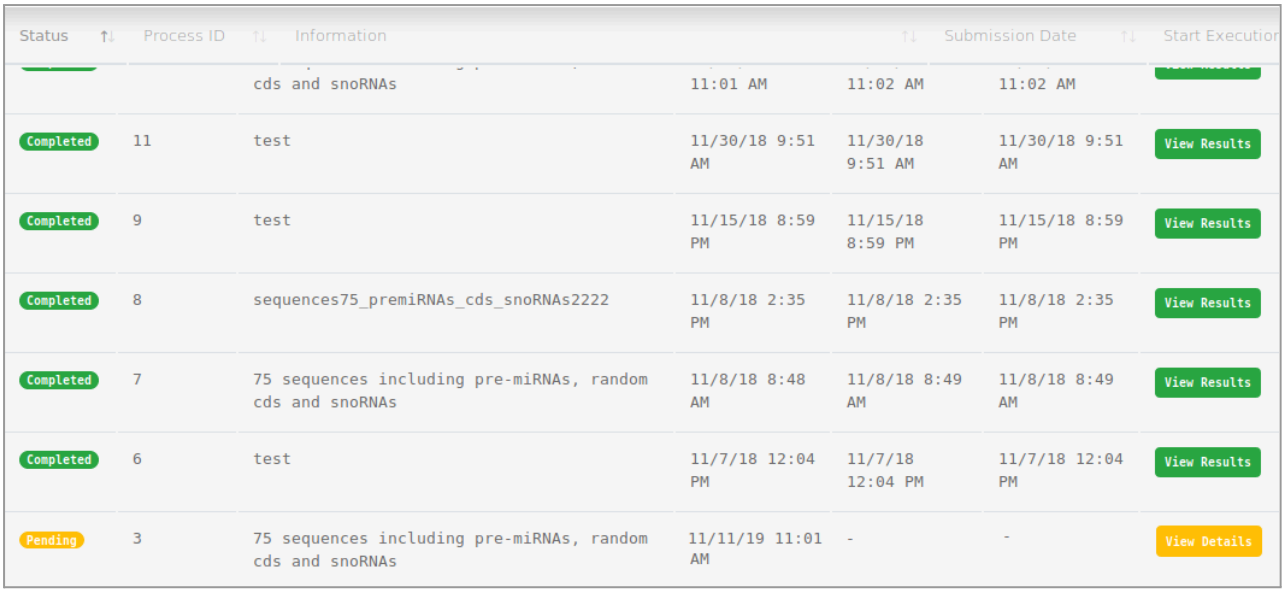


To start the calculation:

Select from the menu “Insybio ncRNASeq” → “non-coding RNA Analytics” → “ncRNA Feature Calculation”:

- Upload a new file of sequences in fasta format. You are redirected to the Data Store where step-by-step instructions guide you, or
- Select a file from the Data Store. There you can find your previously uploaded files or InSyBio pre-uploaded sample datasets.

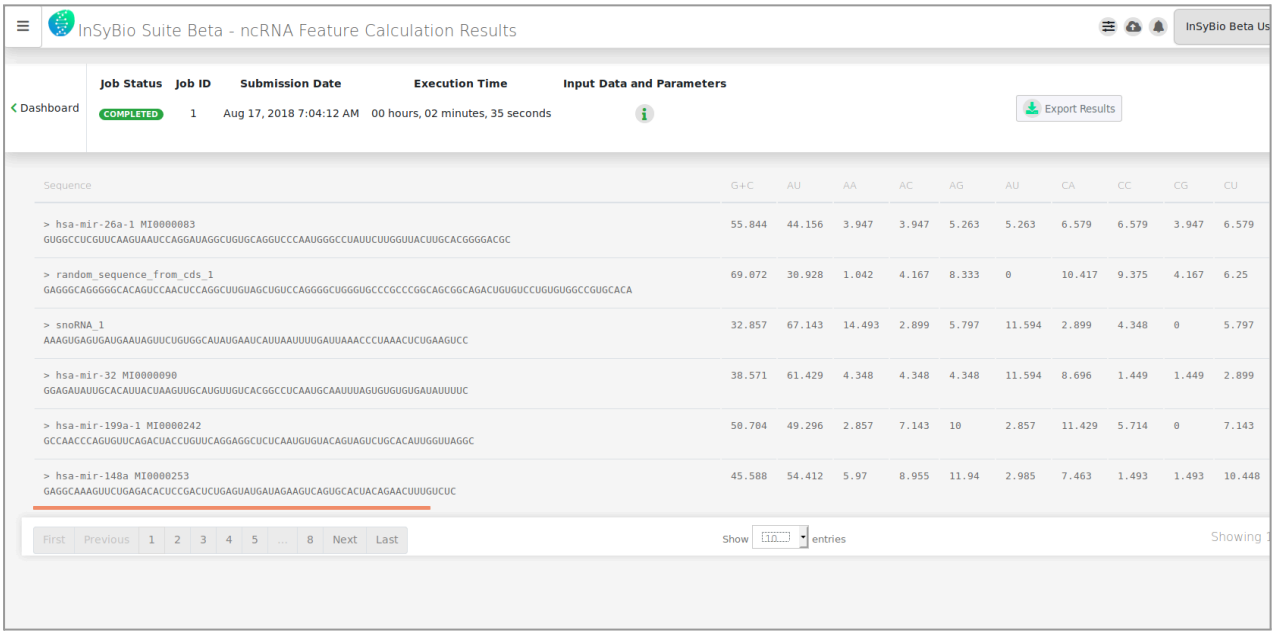
Batch calculations of many sequences are allowed. Just put the sequences in one file in fasta format.



Status	Process ID	Information		Submission Date	Start Execution
		cds and snoRNAs	11:01 AM	11:02 AM	11:02 AM
Completed	11	test	11/30/18 9:51 AM	11/30/18 9:51 AM	11/30/18 9:51 AM View Results
Completed	9	test	11/15/18 8:59 PM	11/15/18 8:59 PM	11/15/18 8:59 PM View Results
Completed	8	sequences75_premiRNAs_cds_snoRNAs2222	11/8/18 2:35 PM	11/8/18 2:35 PM	11/8/18 2:35 PM View Results
Completed	7	75 sequences including pre-miRNAs, random cds and snoRNAs	11/8/18 8:48 AM	11/8/18 8:49 AM	11/8/18 8:49 AM View Results
Completed	6	test	11/7/18 12:04 PM	11/7/18 12:04 PM	11/7/18 12:04 PM View Results
Pending	3	75 sequences including pre-miRNAs, random cds and snoRNAs	11/11/19 11:01 AM	-	- View Details

To view the results:

By starting a calculation the ncRNA Feature Calculation dashboard is updated with the submitted job, there you can view the status of your current and previous ncRNA feature calculations. You can select the View Details at the Actions column and view the calculated features at completion of the calculation.



The results are presented on your screen in a browse-able table or you can download them as a TAB delimited txt file.

For each non-coding RNA, its sequence and its 58 features are presented.

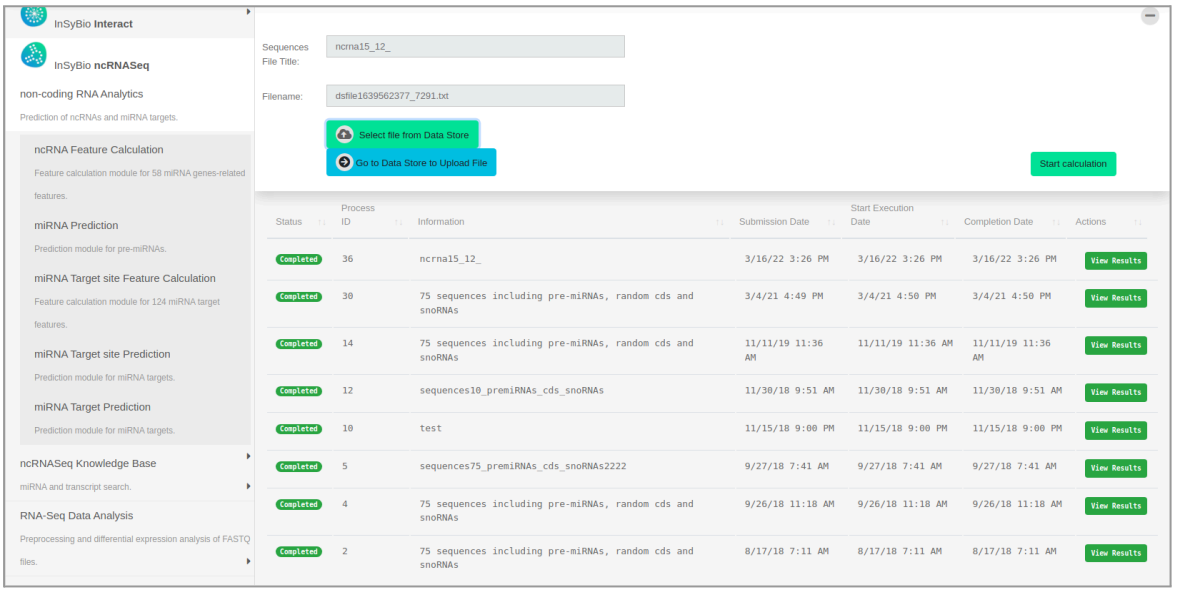
The description of the supported features for the characterization of the non-coding RNAs is the following:

Feature	ABBR
2 Aggregate Dinucleotide Frequencies (%G+C ratio, %A+U ratio)	G + C, A + U
16 dinucleotide frequencies (%XY) such that X,Y e Σ[A,C,G,U]	AA, AC, AG, AU, CA, CC, CG, CU, GA, GC, GG, GU, UA, UC, UG, UU
MFE Index 1 = dG/%(C+G)	MFE1
MFE Index 2 = dG/number_of_stems, where each stem is at least 3 continuous base pairs in the structure	MFE2
MFE Index 3 = dG/number_of_loops , where number_of_loops is the number of the loops in the secondary structure	MFE3
MFE Index 4 = dG/total_bases	MFE4
MFE Index 5 = dG/%(A+U) ratio	MFE5
Adjusted Minimum Free Energy of folding dG = MFE/L, where MFE is the minimum free energy of the structure as calculated by the Vienna fold routine	dG
Adjusted base pairing propensity dP = total_bases/L, where L is the length of the structure and total_bases the number of base pairs in the structure	dP
Adjusted base pair distance dD	dD
Adjusted shannon entropy dQ	dQ
Positional Entropy dPs: a new introduced attribute which estimates the structural volatility of the secondary structure	PosEntropy
Normalized Ensemble Free Energy	EAFE
Structural Diversity	Div/ty
Frequency of MFE structure	Freq

Feature	ABBR
Diff = |MFE-EFE|/L where, EFE is the ensemble free energy	Diff
Structure Enthalpy dH	dH
Normalized Structure Enthalpy dH/L	dH/L
Structure Entropy dS	dS
Normalized Structure Entropy dS/L	dS/L
Melting Temperature Tm	Tm
Normalized Structure Enthalpy TH/L	Tm/L
|X-Y| is the number of (X-Y) base pairs in the secondary structure	|A-U|/L, |G-C|/L, |G-U|/L
Average base pair per stem	Avg_BP_stems
%(A-U)/n_stems, %(G-C)/n_stems, %(G-U)/n_stems.	(A-U)/n_stems, (G-C)/n_stems, (G-U)/n_stems
Ratio G/C ,where G,C is the number of G,C bases	G/C
BP is the total number of base pairs and GC,GU,AU the number of respective base pairs	BP/GC, BP/GU, BP/AU
Length of the sequence	Len
Centroid Energy: RNA folding related attribute calculated by the Vienna RNA package	DE/L
Centroid Distance: RNA folding related attribute calculated by the Vienna RNA package	CE_dist
5 statistical features	zG, zP, zD, zQ, zSP
Topological descriptor dF	dF

miRNA Prediction

You can predict pre-miRNAs and discriminate them between pseudo-hairpins and other molecules providing RNA sequences in fasta format. The prediction of pre-miRNAs and pseudo-hairpins is accomplished through the application of a novel methodology which combines Genetic Algorithms with epsilon-SVR techniques. Genetic Algorithms were used to optimize the feature subset which should be used as inputs and the parameters C, sigma and epsilon of epsilon SVR models. The accuracy of this technique in predicting pre-miRNAs is 95%. A sequence is predicted as other if the minimum free energy is more than -15 kcal/mol or the number of base pairs is less than 18.

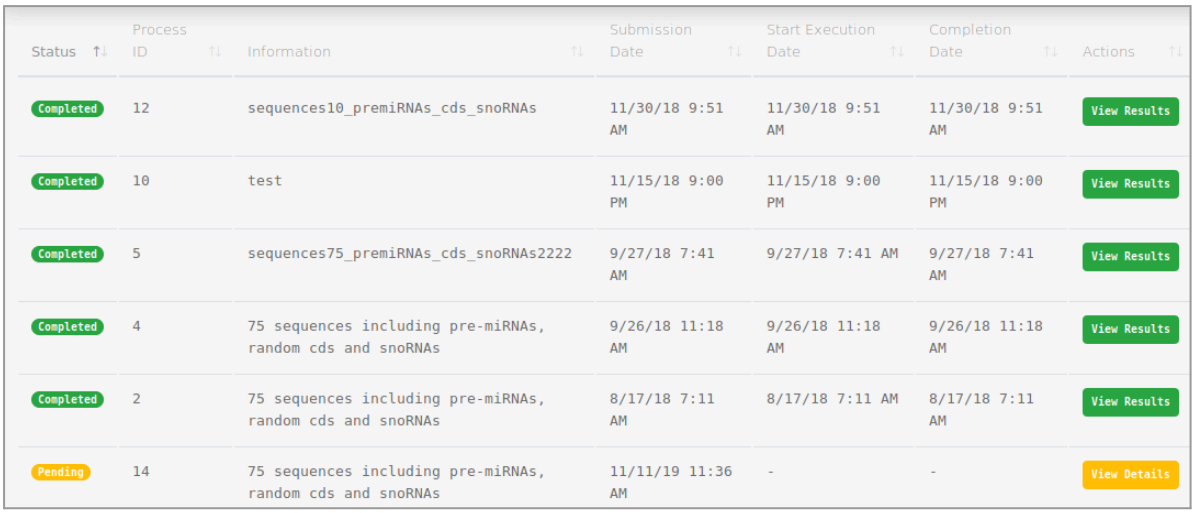


To start the calculation:

Select from the menu “Insybio ncRNASeq” → “non-coding RNA Analytics” → “miRNA Prediction”:

- Upload a new file of sequences in fasta format. You are redirected to the Data Store where step-by-step instructions guide you.
- Select a file from the Data Store. There you can find your previously uploaded files or InSyBio pre-uploaded sample datasets.

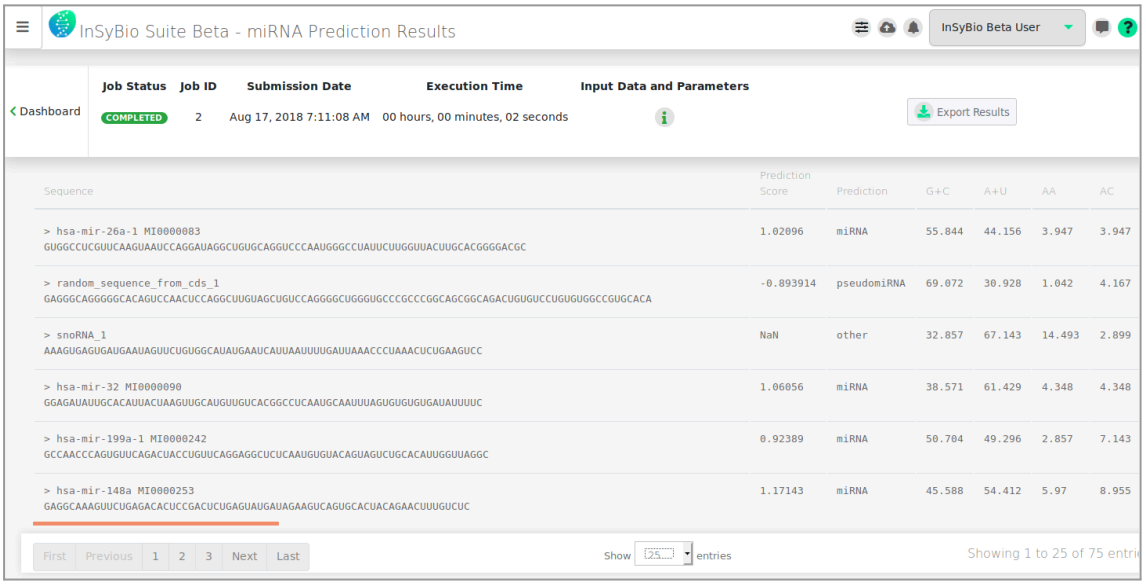
Batch calculations of many sequences are allowed. Just put the sequences in one file in fasta format.



Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	12	sequences10_premiRNAs_cds_snoRNAs	11/30/18 9:51 AM	11/30/18 9:51 AM	11/30/18 9:51 AM	View Results
Completed	10	test	11/15/18 9:00 PM	11/15/18 9:00 PM	11/15/18 9:00 PM	View Results
Completed	5	sequences75_premiRNAs_cds_snoRNAs2222	9/27/18 7:41 AM	9/27/18 7:41 AM	9/27/18 7:41 AM	View Results
Completed	4	75 sequences including pre-miRNAs, random cds and snoRNAs	9/26/18 11:18 AM	9/26/18 11:18 AM	9/26/18 11:18 AM	View Results
Completed	2	75 sequences including pre-miRNAs, random cds and snoRNAs	8/17/18 7:11 AM	8/17/18 7:11 AM	8/17/18 7:11 AM	View Results
Pending	14	75 sequences including pre-miRNAs, random cds and snoRNAs	11/11/19 11:36 AM	-	-	View Details

To view the results:

By starting a calculation the “miRNA Prediction” dashboard is updated with the status of the new job, there you can view the status of your current and previous miRNA prediction. At completion of the prediction, you can select the View Results in the Actions column and view the calculated features.

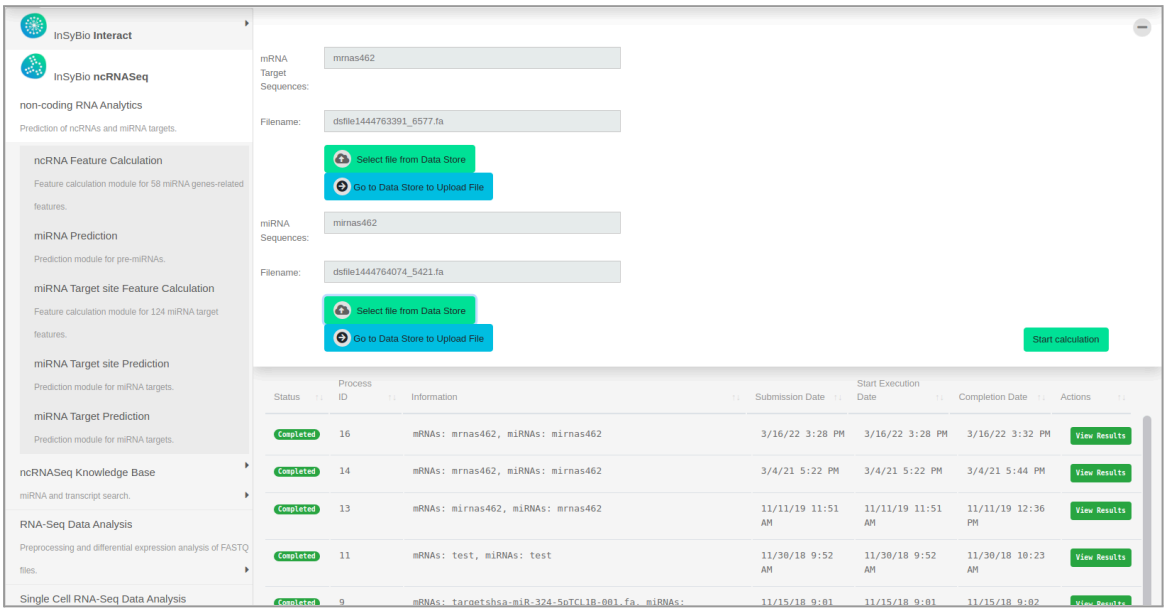


The results are presented on your screen in a browseable table or you can download them as a TAB delimited txt file.

For each non-coding RNA, its sequence, its calculated confidence score, the prediction of whether it is a miRNA, a pseudo-hairpin or other and its 58 features are presented.

miRNA Target site Feature Calculation

You can calculate 124 features for every pair of a miRNA and its potential target site within an mRNA. These features include sequential, thermodynamical and structural properties of the miRNA:mRNA pair.

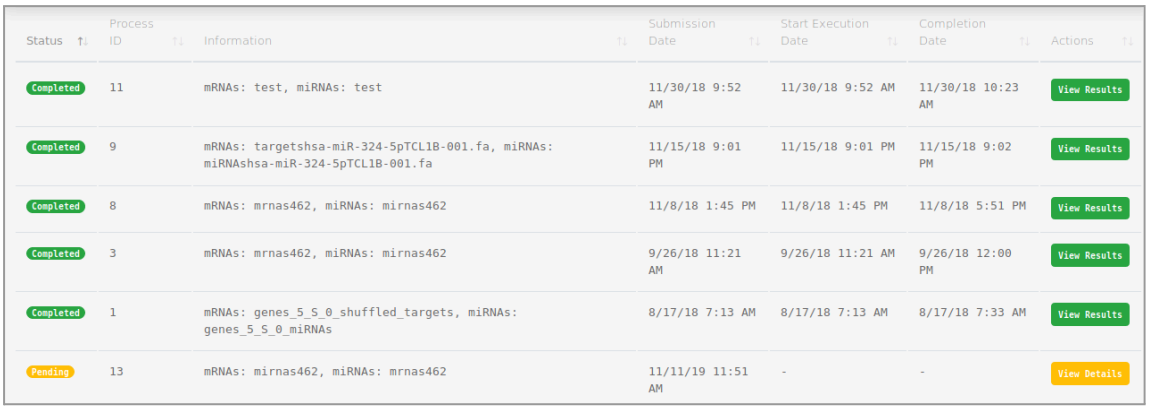


To start the calculation:

Select from the menu “InSyBio ncRNASeq” → “non-coding RNA Analytics” → “miRNA Target Features Calculation” and then:

- Upload a new file of mRNA binding sites sequences and a new file of miRNA sequences, both in fasta format. The mRNA target site of the first file and every miRNA of the second file are considered as a miRNA:mRNA pair. You are redirected to the Data Store where step-by-step instructions guide you for both files uploading.
- Or Select a file of mRNA binding sites sequences and a file of miRNA sequences, both in fasta format from the Data Store. There you can find your previously uploaded files or InSyBio pre-uploaded sample datasets.

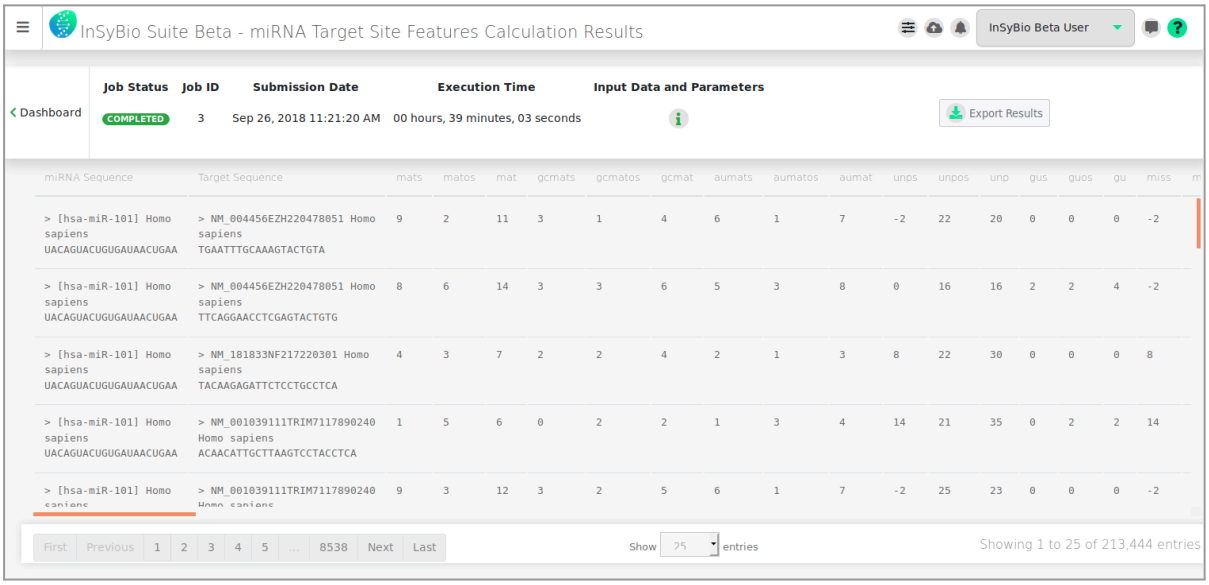
Batch feature calculation of many miRNA:mRNA pairs with a single run is allowed. Just put the mRNA binding sites sequences in the first file and miRNA sequences in the second file in fasta format.



Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	11	mRNAs: test, miRNAs: test	11/30/18 9:52 AM	11/30/18 9:52 AM	11/30/18 10:23 AM	View Results
Completed	9	mRNAs: targetshsa-miR-324-5pTCL1B-001.fa, miRNAs: miRNAshsa-miR-324-5pTCL1B-001.fa	11/15/18 9:01 PM	11/15/18 9:01 PM	11/15/18 9:02 PM	View Results
Completed	8	mRNAs: mrnas462, miRNAs: mirnas462	11/8/18 1:45 PM	11/8/18 1:45 PM	11/8/18 5:51 PM	View Results
Completed	3	mRNAs: mrnas462, miRNAs: mirnas462	9/26/18 11:21 AM	9/26/18 11:21 AM	9/26/18 12:00 PM	View Results
Completed	1	mRNAs: genes_5_S_0_shuffled_targets, miRNAs: genes_5_S_0_miRNAs	8/17/18 7:13 AM	8/17/18 7:13 AM	8/17/18 7:33 AM	View Results
Pending	13	mRNAs: mirnas462, miRNAs: mrnas462	11/11/19 11:51 AM	-	-	View Details

To view the results:

By starting a new calculation the “miRNA Target Site Feature Calculation” dashboard is updated with the new job, there you can view the status of your current and previous miRNA Target Features Calculations. At completion of the calculation, you can select the View Results in the Actions column and view the calculated features.



The results are presented on your screen in a browse-able table or you can download them as a TAB delimited txt file.

For each miRNA:mRNA pair, the miRNA sequence, the mRNA binding site sequence and the 124 miRNA::mRNA pair features are presented.

The description of the supported features for the characterization of the miRNA::mRNA pair is the following:

Feature	ABBR	Category
number of matches in seed part	mats	structural
number of matches in out-seed part	matos	structural
total number of matches	mat	structural
number of GC matches in seed part	gcmats	structural
number of GC matches in out-seed part	gcmatos	structural
total number of GC matches	gcmat	structural
number of AU matches in seed part	aumats	structural
number of AU matches in out-seed part	aumatos	structural
total number of AU matches	aumat	structural
number of mismatches in seed part	unps	structural
number of mismatches in out-seed part	unpos	structural
total number of mismatches	unp	structural
number of GU wobble pairs in seed part	gus	structural
number of GU wobble pairs in out-seed part	guos	structural
total number of GU wobble pairs	gu	structural
number of other mismatches in seed part	miss	structural
number of other mismatches in out-seed part	misos	structural
total number of other mismatches	mis	structural
number of bulges in seed part	buls	structural

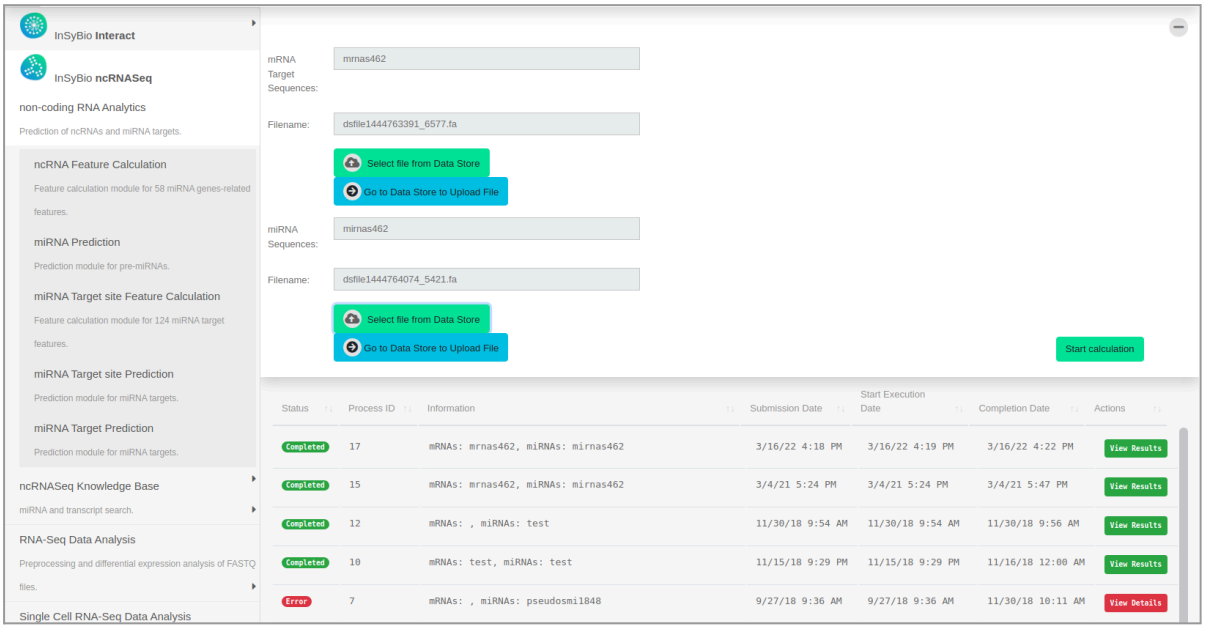
Feature	ABBR	Category
number of bulges in out-seed part	bulos	structural
total number of bulges	bul	structural
number of loops in seed part	symls	structural
number of loops in out-seed part	symlos	structural
total number of loops	syml	structural
number of asymmetric loops in seed part	asymls	structural
number of asymmetric loops in out-seed part	asymlos	structural
total number of asymmetric loops	asyml	structural
length of largest bulge	maxbul	structural
number of bulges of length 1-7 and greater than 7 in seed part (8 features)	cbul1s, cbul2s, cbul3s, cbul4s, cbul5s, cbul6s, cbul7s, cbul8s	structural
number of bulges of length 1-7 and greater than 7 in out-seed part (8 features)	cbul1os, cbul2os, cbul3os, cbul4os, cbul5os, cbul6os, cbul7os, cbul8os	structural
number of symmetric loops of length 1-7 and greater than 7 in seed part (8 features)	csl1s, csl2s, csl3s, csl4s, csl5s, csl6s, csl7s, csl8s	structural
number of symmetric loops of length 1-7 and greater than 7 in out-seed part (8 features)	csl1os, csl2os, csl3os, csl4os, csl5os, csl6os, csl7os, csl8os	structural
number of asymmetric loops of length 1-7 and greater than 7 in seed part (8 features)	casl1s, casl2s, casl3s, casl4s, casl5s, casl6s, casl7s, casl8s	structural
number of asymmetric loops of length 1-7 and greater than 7 in out-seed part (8 features)	casl1os, casl2os, casl3os, casl4os, casl5os, casl6os, casl7os, casl8os	structural
proportion of A, C, G, U in the target sequence (4	aper, cper, gper,	structural

features)	upper	
distance from the start of the seed part to the last match of the out-seed part	dist	structural
seed score obtained by the sum of pair scores in the seed region. GC and AU with 5, GU with 2 and the others with -3	scores	structural
out-seed score obtained by the sum of pair scores in the out-seed region. GC and AU with 5, GU with 2 and the others with -3	scoreos	structural
free energy of the seed part	mfes	thermodynamic
free energy of the out-seed part	mfeos	thermodynamic
free energy of the total miRNA-mRNA alignment structure	mfe	thermodynamic
free energy of the target sequence	mfet	thermodynamic
normalized free energy of the target sequence=(-1 * free energy of the target sequence)/log(length of target * length of miRNA)	nmfe	thermodynamic
difference in the free energies of the total miRNA-perfect target alignment structure and the total miRNA-mRNA alignment structure	dmfe	thermodynamic
positions from 1 to 20 with a GC match, an AU match, a GU match or a mismatch (20 features)	pos1, pos2, pos3, pos4, pos5, pos6, pos7, pos8, pos9, pos10, pos11, pos12, pos13, pos14, pos15, pos16, pos17, pos18, pos19, pos20	positional
terminal (position 8) base match	match8	positional
positional pair score obtained by the sum of the product of the weight and the corresponding pair score throughout the total miRNA-mRNA alignment structure. G:C and A:U are awarded with 5, G:U with 1, all other mismatches with -3 and the mismatches containing gaps with -1. Positional weight is 1 for all non-seed positions and 2 for all seed positions.	s106	positional

Feature	ABBR	Category
matrix score obtained by the sum of the diagonal elements in the matrix formed by the miRNA and its target. WC pairs: 5, Wobble pairs: 2, Inserts: -1, Deletes: -1, Symmetric mismatches: -3, Mismatches: -2	score	positional
deviation of the positional pair score with the score obtained with a perfect target	ds108	positional
deviation of the matrix score with the score obtained with a perfect target	ds109	positional
existence of the 10 most frequent nucleotide sequence 'words' with lengths 4, 5, 6, 7, 8 from the seed sequence of the miRNAs of our dataset	ugag, cagu, agug, agguag, aggua, aggu, gguag, ggua, guag, ugcu	'motif'

miRNA Target site Prediction

You can computationally validate miRNA targets. The computational intelligent technique, which was applied for the prediction of miRNAs (hybrid combination of Genetic Algorithms and epsilon-SVRs), and 124 informative features are used.

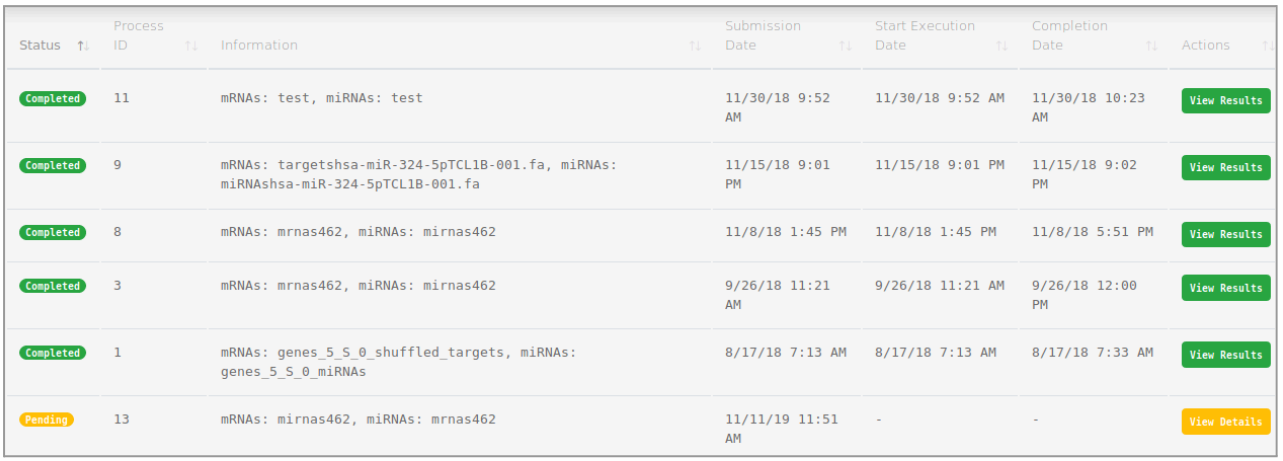


To start the prediction:

Select from the menu “InSyBio ncRNASeq” → “non-coding RNA Analytics” → “miRNA Target Site Prediction” and then:

- Upload a new file of candidate mRNA target binding sites sequences and a new file of miRNA sequences, both in fasta format. The mRNA target site of the first file and every miRNA of the second file are considered as a miRNA:mRNA pair. You are redirected to the Data Store where step-by-step instructions guide you for both files uploading.
- Or Select a file of candidate mRNA target binding sites sequences and a file of miRNA sequences, both in fasta format from the Data Store. There you can find your previously uploaded files or InSyBio pre-uploaded sample datasets.

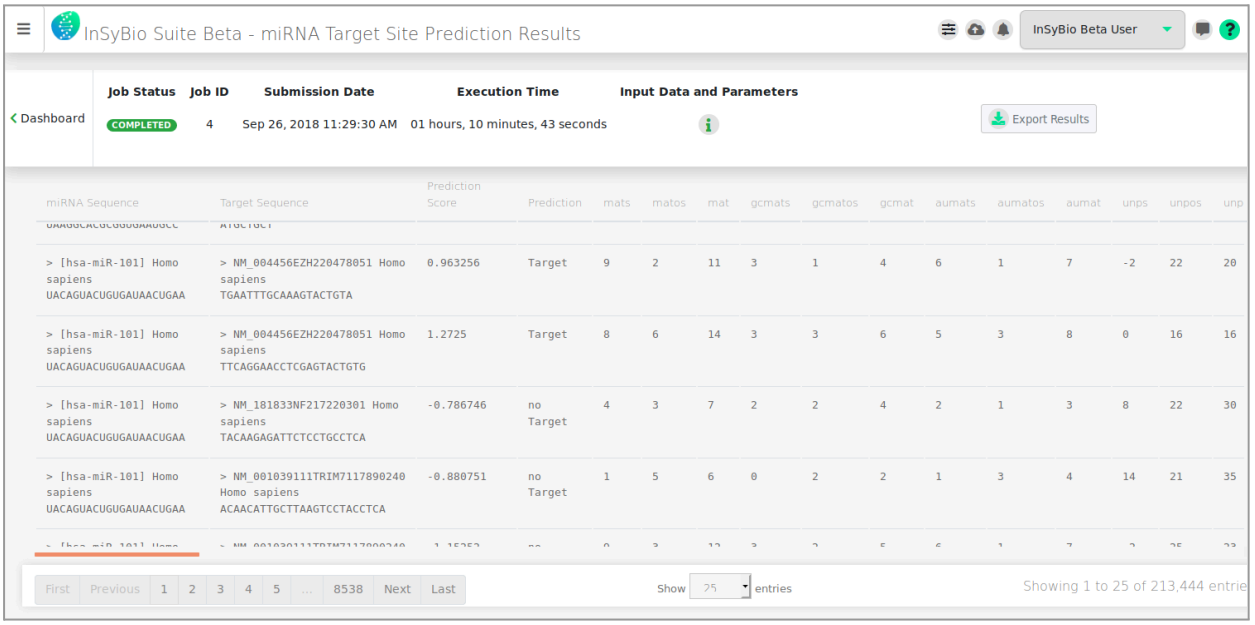
Batch predictions of many miRNA:mRNA pairs with a single run are allowed. Just put the candidate mRNA target binding sites sequences in the first file and miRNA sequences in the second file in fasta format.



Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	11	mRNAs: test, miRNAs: test	11/30/18 9:52 AM	11/30/18 9:52 AM	11/30/18 10:23 AM	View Results
Completed	9	mRNAs: targetshsa-miR-324-5pTCL1B-001.fa, miRNAs: miRNAshsa-miR-324-5pTCL1B-001.fa	11/15/18 9:01 PM	11/15/18 9:01 PM	11/15/18 9:02 PM	View Results
Completed	8	mRNAs: mrnas462, miRNAs: mirnas462	11/8/18 1:45 PM	11/8/18 1:45 PM	11/8/18 5:51 PM	View Results
Completed	3	mRNAs: mrnas462, miRNAs: mirnas462	9/26/18 11:21 AM	9/26/18 11:21 AM	9/26/18 12:00 PM	View Results
Completed	1	mRNAs: genes_5_S_0_shuffled_targets, miRNAs: genes_5_S_0_miRNAs	8/17/18 7:13 AM	8/17/18 7:13 AM	8/17/18 7:33 AM	View Results
Pending	13	mRNAs: mirnas462, miRNAs: mrnas462	11/11/19 11:51 AM	-	-	View Details

To view the results:

By starting a calculation the “miRNA Target Site Prediction” dashboard is updated with the new job, where you can view the status of your current and previous miRNA Target Site Prediction. At completion of the calculation, you can select the View Results in the Actions column and view the predictions and calculated features.

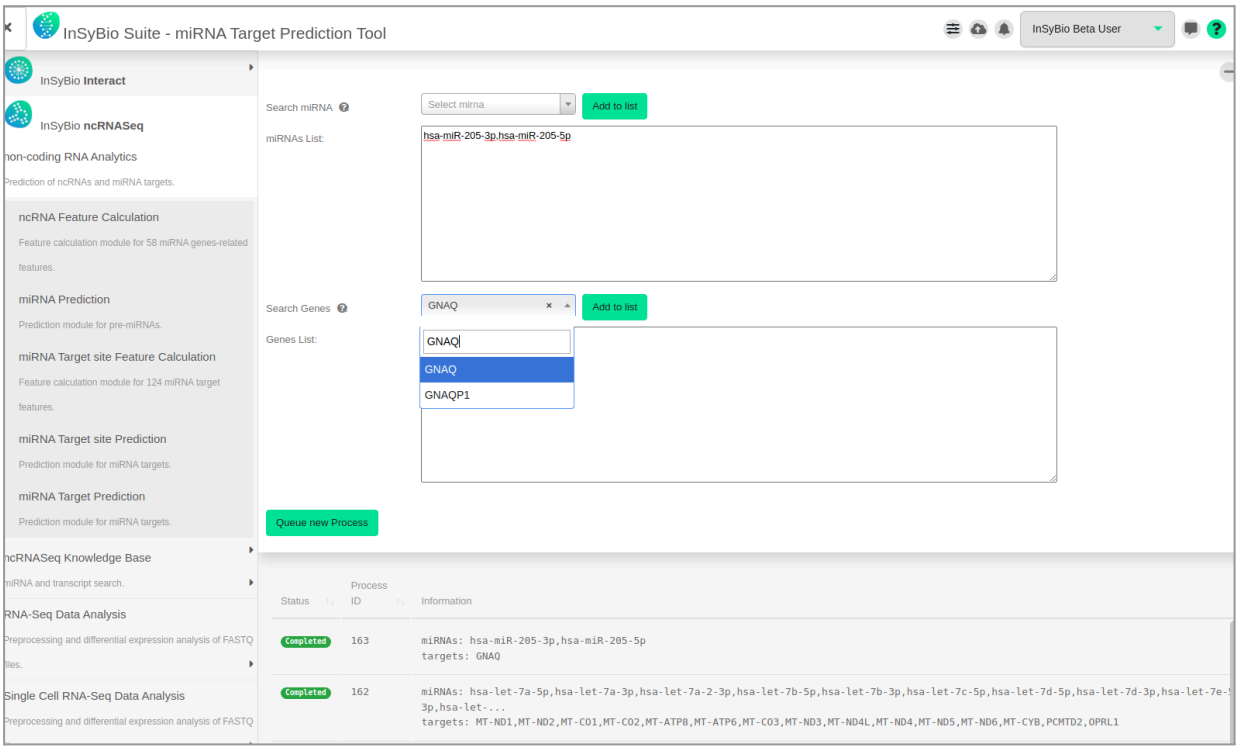


The results are presented on your screen in a browseable table or you can download them as a TAB delimited txt file.

For each miRNA:mRNA pair, the miRNA sequence, the mRNA binding site sequence, whether the miRNA:mRNA pairs share a targeting relation or not, the confidence score of the prediction and all 124 miRNA::mRNA are presented.

miRNA Target Prediction

You can computationally predict potential miRNA targets at given Genes or Transcripts and given miRNAs. BLAST is performed to find potential target sites, and then the computational intelligent technique, which was applied for the prediction of miRNAs (hybrid combination of Genetic Algorithms and epsilon-SVRs), and 124 informative features are used to calculate a prediction score.



To start the prediction:

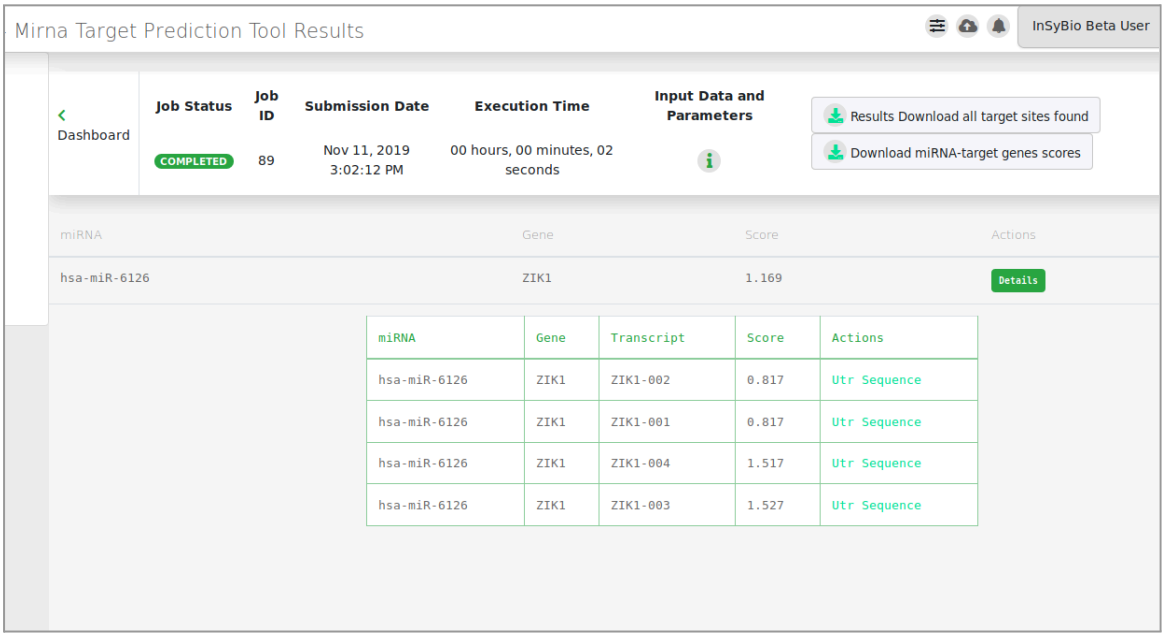
Select from the menu “InSyBio ncRNASeq” → “non-coding RNA Analytics” → “miRNA Target Prediction” field and then:

- Select the miRNAs and the Genes you want to search for potential targets by searching in our Database and adding them to the miRNA List and Genes List or add them manually to their Lists and separating them with commas.

Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	89	miRNAs: hsa-miR-6126 targets: ZIK1	11/11/19 3:02 PM	11/11/19 3:02 PM	11/11/19 3:02 PM	View Results
Completed	88	miRNAs: mmu-miR-3072-3p,mmu-miR-7051-3p,mmu-miR-3968,mmu-miR-8106,mmu-miR-99a-3p,mmu-miR-21a-5p,mmu-miR-3110-5p,mmu-miR-505-3p,mmu-miR-7091-5p,mmu-miR-337-5p,mmu-miR-18a-3p,mmu-miR-1949,mm... targets: ZIK1	2/11/19 12:11 PM	6/6/19 11:21 AM	6/6/19 3:39 PM	View Results
Completed	87	miRNAs: hsa-miR-576-3p,hsa-miR-140-5p,hsa-miR-522-5p,hsa-miR-1298-5p,hsa-miR-133a-3p,hsa-miR-4743-3p,hsa-miR-557,hsa-miR-548ao-3p,hsa-miR-5088-5p,hsa-miR-4649-5p,hsa-miR-665,hsa-miR-3622b-... targets: NELL2,SERPINI1,SMOC1,FGF2,MMRN2,PRSS3,VEGFB,ADAM21,ADAMTSL4,C1QTNF4,CCL3L3,COL4A2,LAMB1	11/29/18 3:40 PM	11/29/18 3:40 PM	11/29/18 3:52 PM	View Results
Completed	86	miRNAs: hsa-miR-6126, hsa-miR-1200, hsa-let-7a-2-3p, hsa-miR-106b-3p targets: ZIK1, A1BG-AS1, FGGY	11/29/18 3:39 PM	11/29/18 3:39 PM	11/29/18 3:39 PM	View Results
Completed	85	miRNAs: hsa-miR-6126 targets: ZIK1	11/29/18 3:09 PM	11/29/18 3:09 PM	11/29/18 3:09 PM	View Results
Error	84	miRNAs: targets: ZIK1	11/29/18 3:08 PM	11/29/18 3:08 PM	11/29/18 3:08 PM	View Details

To view the results:

By starting a calculation the “miRNA target Prediction” dashboard is updated with the new job’s information, you can view the status of your current and previous miRNA Target Predictions. After the calculation, you can select the View Results in the Actions column and view the results.



Mirna Target Prediction Tool Results — InSyBio Beta User

Dashboard

Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters
COMPLETED	89	Nov 11, 2019 3:02:12 PM	00 hours, 00 minutes, 02 seconds	

Results Download all target sites found

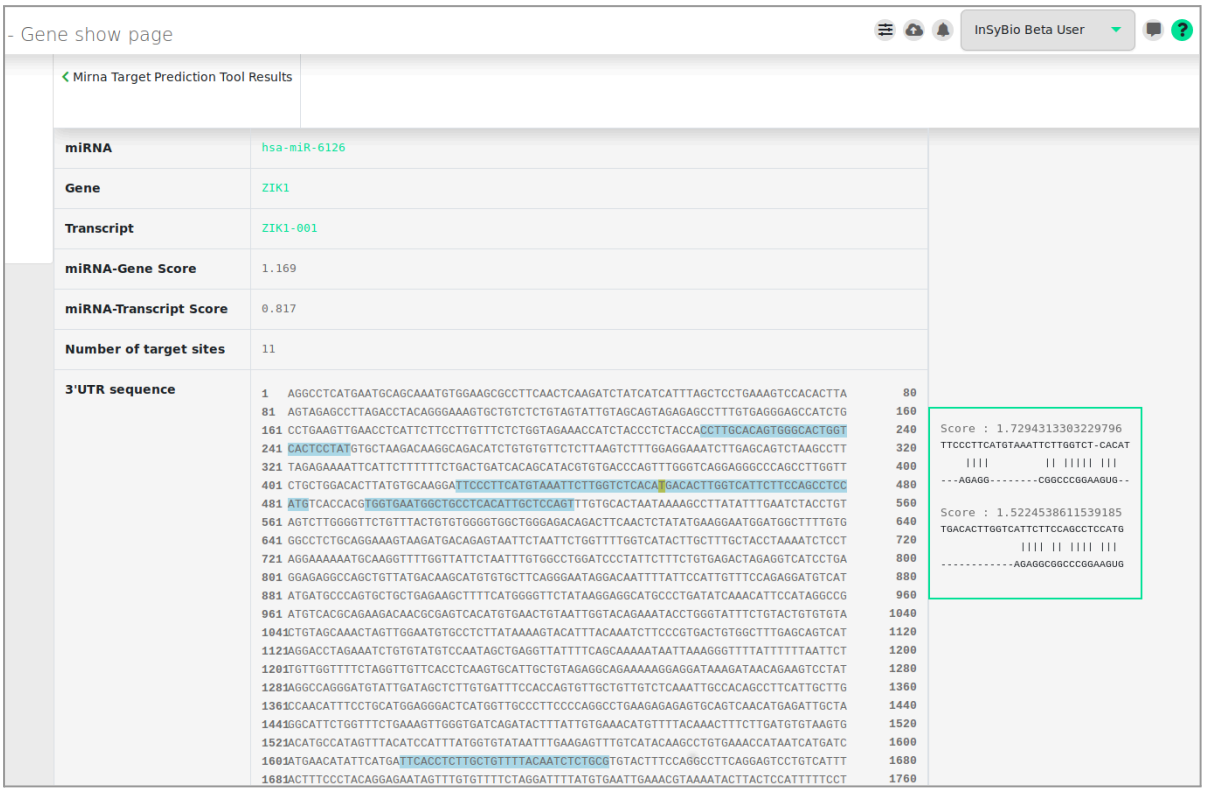
Download miRNA-target genes scores

miRNA	Gene	Score	Actions
hsa-miR-6126	ZIK1	1.169	Details

miRNA	Gene	Transcript	Score	Actions
hsa-miR-6126	ZIK1	ZIK1-002	0.817	Utr Sequence
hsa-miR-6126	ZIK1	ZIK1-001	0.817	Utr Sequence
hsa-miR-6126	ZIK1	ZIK1-004	1.517	Utr Sequence
hsa-miR-6126	ZIK1	ZIK1-003	1.527	Utr Sequence

The results are presented on your screen in a browseable table, with each miRNA and gene pair in a row with their confidence score. By pressing Details at the Actions Column the specific scores between the miRNA and the gene’s transcripts can be

viewed. If no target sites are found “No targets found!” is presented at the score column. If one or more target sites are found you can view its UTR sequence, with the target sites of the miRNA highlighted. Multiple target sites are marked with green color and unique target sites are marked with light blue.



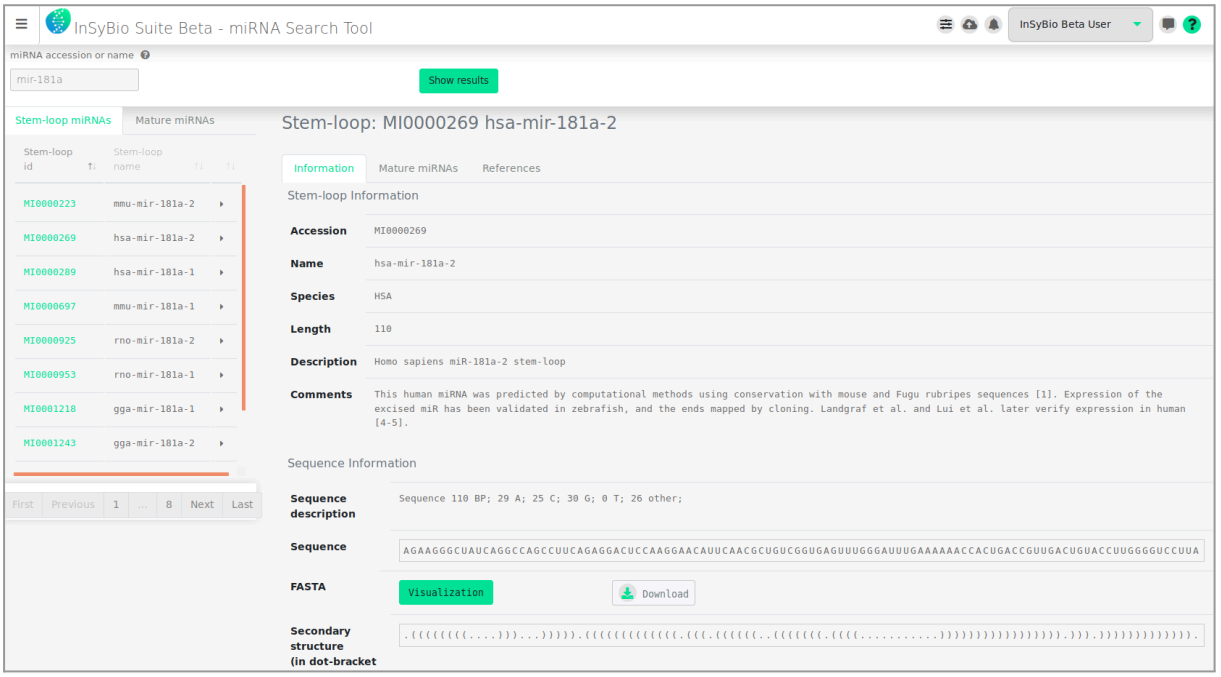
You can download all target sites found as a txt file.

ncRNASeq Knowledge Base

miRNA Search

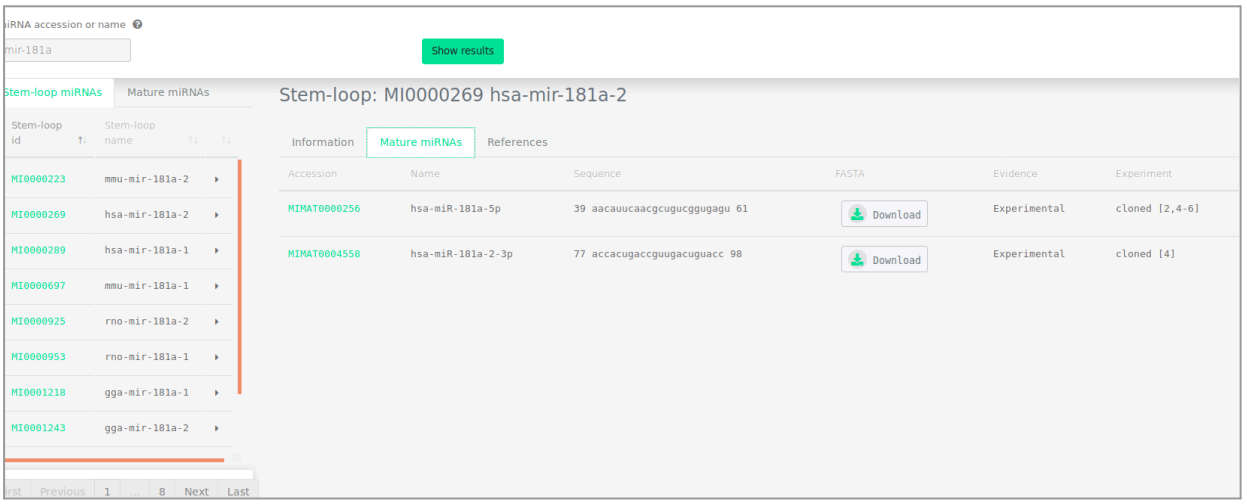
You can search stem-loop and mature miRNAs giving a miRNA accession or name or part of them. Choosing the stem-loop or mature miRNA of those returned, its show page is shown.

Stem-loop information



For the stem-loop you can view its accession, name, species, length, description and comments. Concerning its sequence, you can download the fasta format and view the sequence description, the sequence and the secondary structure in dot-bracket notation. You can view the visualization of the miRNA by clicking the “Visualization” button, this visualization of the secondary structure is performed with FornaContainer. It is the Minimum Free Energy (MFE) structure.

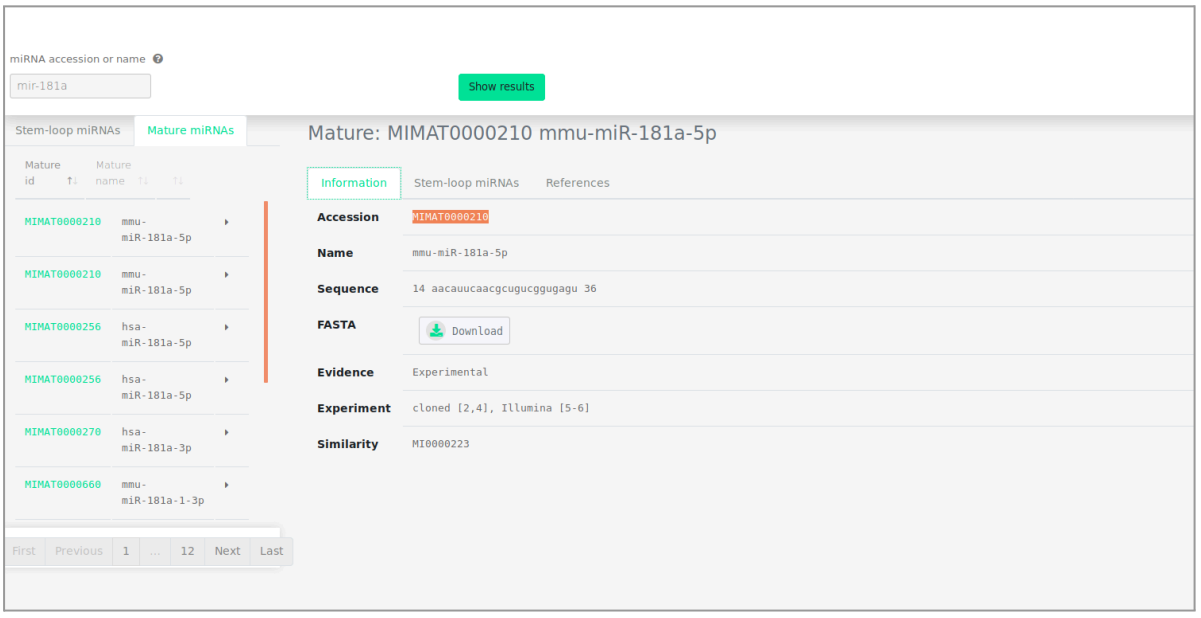
Mature miRNAs and references



For the mature miRNAs related to the stem-loop of interest you can view their accession, name and sequence. Concerning the sequence, you can download the fasta format. You can also view the evidence of each mature miRNA, which can be experimental, or by the similarity of the related stem-loop to another stem-loop or found in the literature.

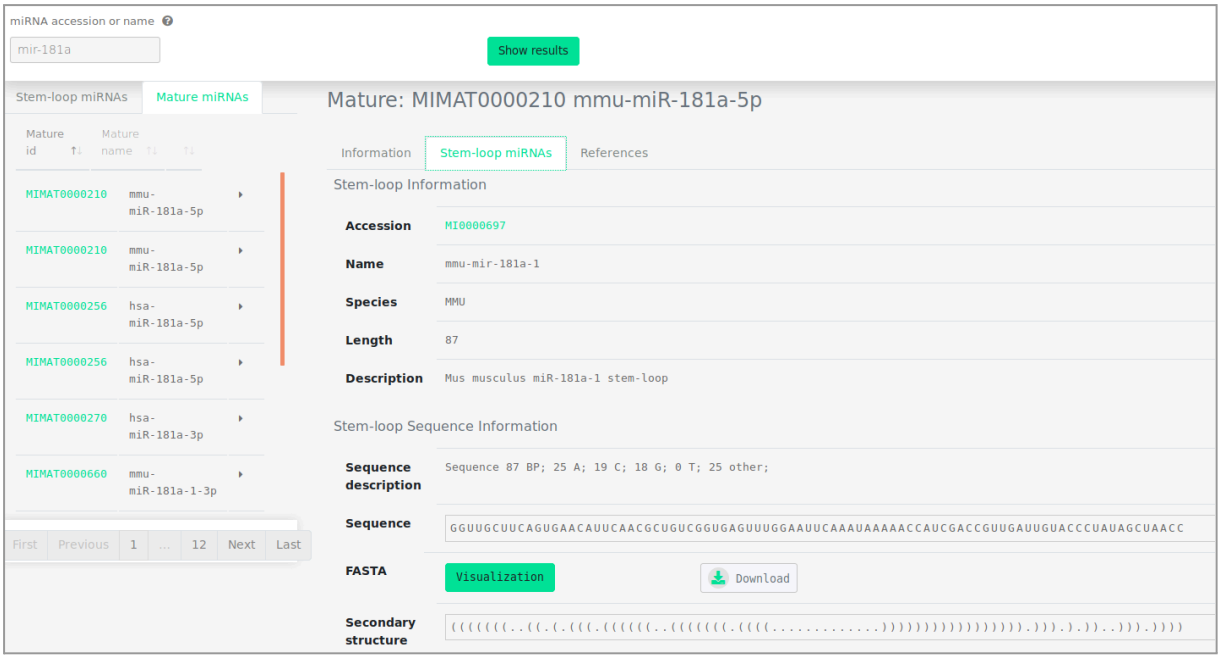
You can also view references for the miRNA of interest. There are external links to other databases (MIRBASE, ENTEZGENE, HGNC, RFAM, MGI, and WORMABASE) and publications.

Mature miRNA information

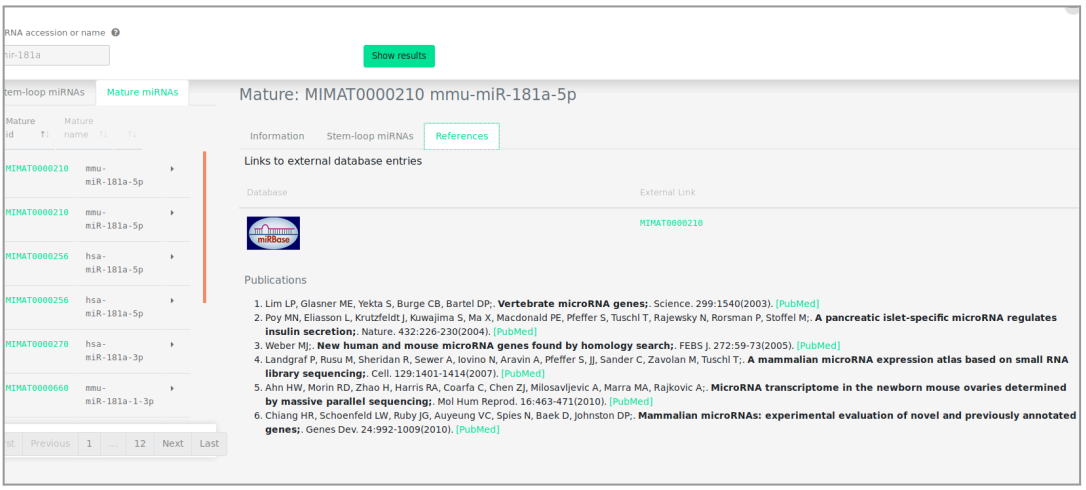


For the Mature miRNA you can view their accession, name and sequence. Concerning the sequence, you can download the fasta format. You can also view the

evidence of each mature miRNA, which can be experimental, or by similarity of the related stem-loop to another stem-loop or found in the literature.



For the stem-loop related to the mature mi-RNA of interest you can view its accession, name, species, length, description and comments. Concerning its sequence, you can download the fasta format and view the sequence description, the sequence and the secondary structure in dot-bracket notation. You can view the visualization of the secondary structure by clicking the “Visualization” button, this visualization is performed with FornaContainer. It is the Minimum Free Energy (MFE) structure.

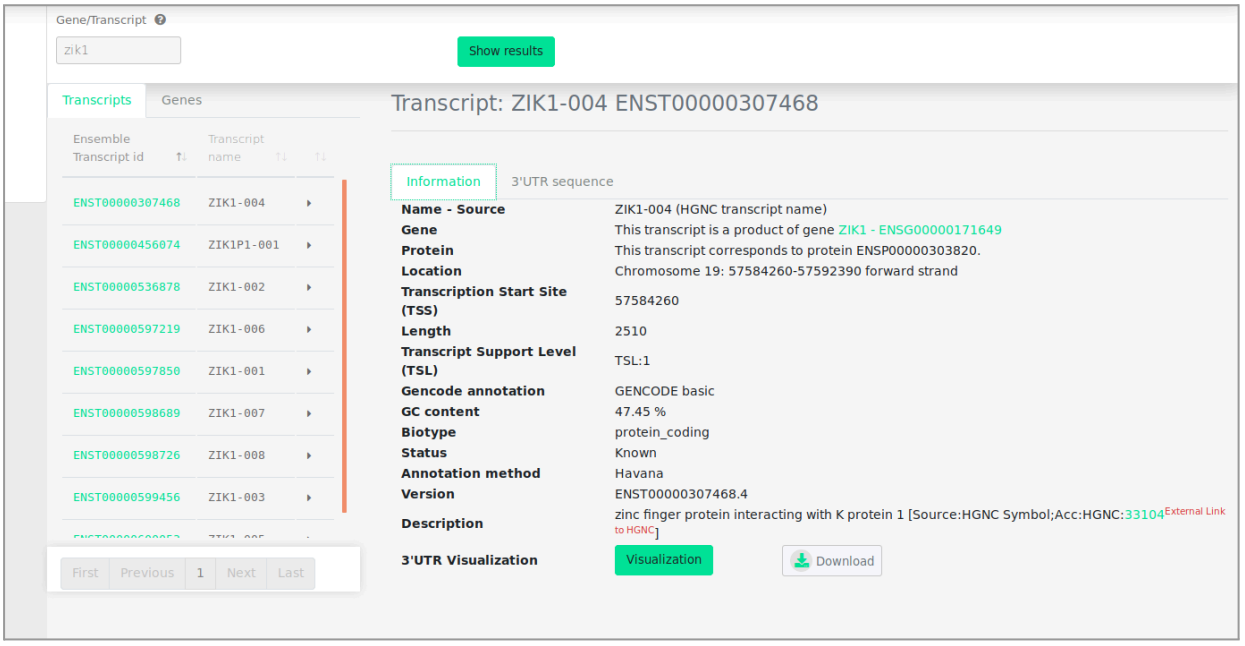


You can also view references for the mature miRNA of interest. There are external links to other databases (MIRBASE, ENTEZGENE, HGNC, RFAM, MGI, and WORMABASE) and publications.

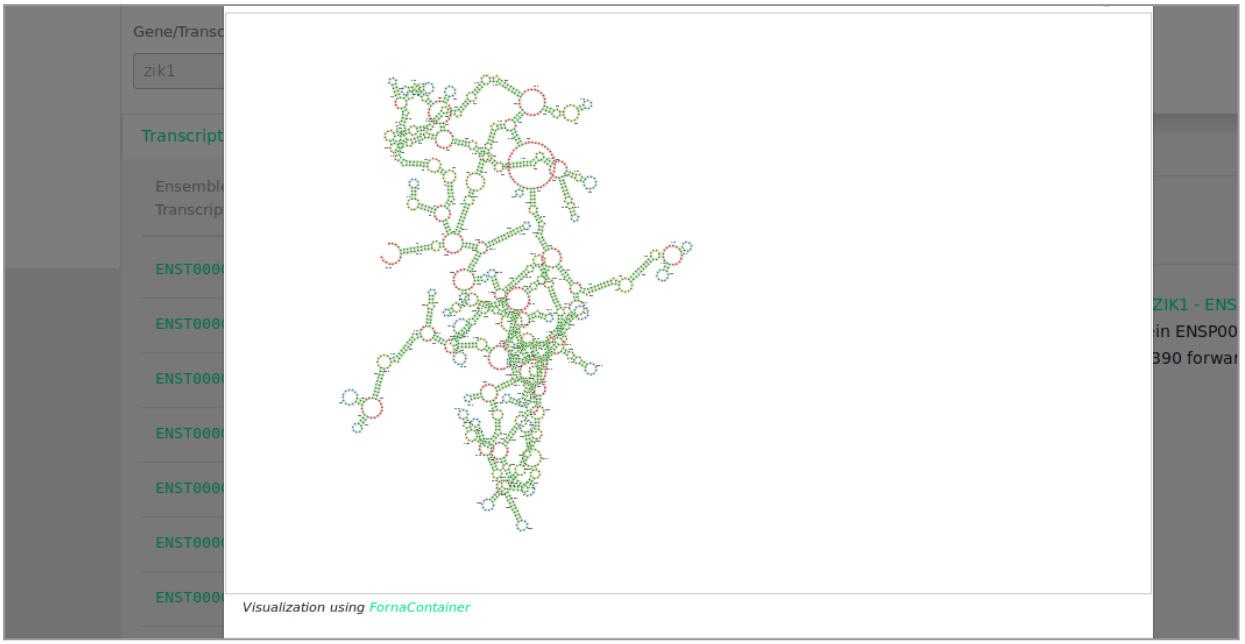
Transcript Search

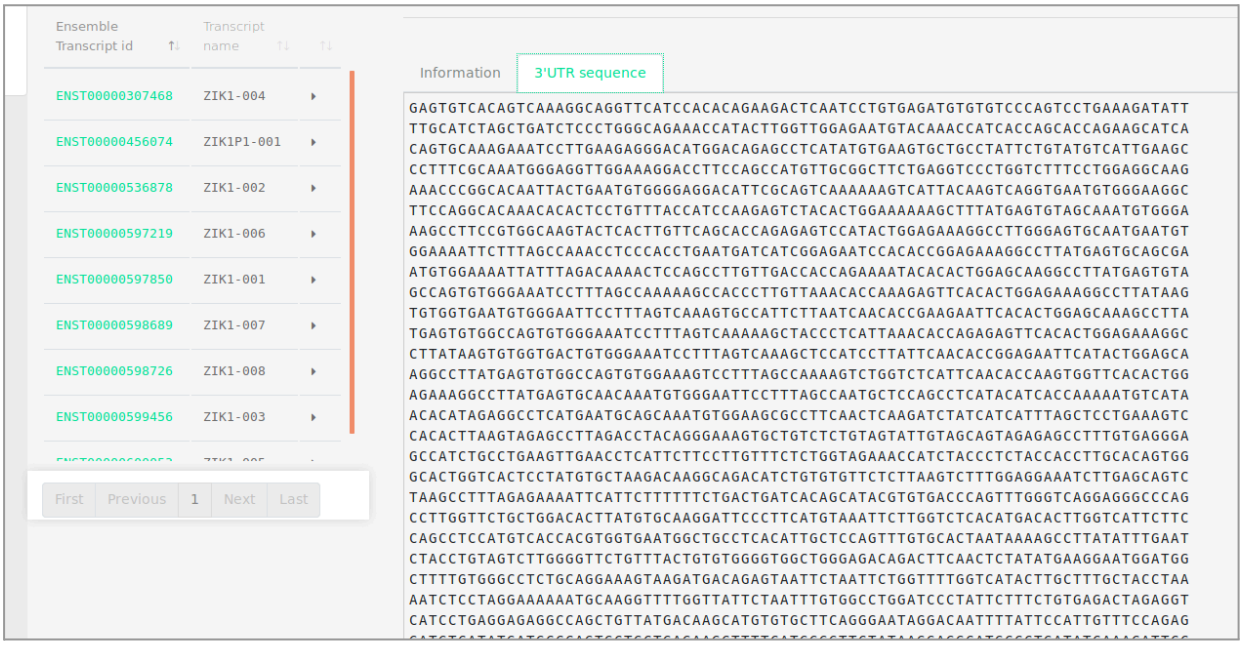
You can search transcripts and genes by giving a transcript accession or name or part of them. Choosing the transcript or gene of those returned, its show page is shown.

Transcripts information



For the Transcript you can view its name-source, gene, protein, location, transcription start site (TSS), length, transcription support level (TSL), Gencode annotation, GC content, biotype, status, annotation method and version description. Concerning its 3'UTR sequence, you can download the fasta format and view the sequence description, the sequence and the secondary structure in dot-bracket notation. You can view the visualization of the secondary structure by clicking the "Visualization" button, this visualization of the secondary structure is performed with FornaContainer. It is the Minimum Free Energy (MFE) structure.


Gene/Transc
zik1
Transcript
Visualization using FornaContainer


Ensemble Transcript id
Transcript name
ENST00000307468 ZIK1-004
ENST00000456074 ZIK1P1-001
ENST00000536878 ZIK1-002
ENST00000597219 ZIK1-006
ENST00000597850 ZIK1-001
ENST00000598689 ZIK1-007
ENST00000598726 ZIK1-008
ENST00000599456 ZIK1-003
First Previous 1 Next Last
Information
3'UTR sequence
GAGTGTCACAGTCAAAGGCAGGTTCATCCACACAGAAGACTCAATCCTGTGAGATGTGTGTCCCAGTCCTGAAAGATATT
TTGCATCTAGCTGATCTCCCTGGGCAGAAACCATACTTGGTTGGAGAATGTACAAACCATCACCAGCACCAGAAGCATCA
CAGTGCAAAGAAATCCTTGAAGAGGGACATGGACAGAGCCTCATATGTGAAGTGCTGCCTATTCTGTATGTCATTGAAGC
CCTTTCGCAAATGGGAGGTTGGAAAGGACCTTCCAGCCATGTTGCGGCTTCTGAGGTCCCTGGTCTTTCCTGGAGGCAAG
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GGAAAATTCTTTAGCCAAACCTCCCACCTGAATGATCATCGGAGAATCCACACCGGAGAAAGGCCTTATGAGTGCAGCGA
ATGTGGAAAATTATTTAGACAAAACTCCAGCCTTGTTGACCACCAGAAAATACACACTGGAGCAAGGCCTTATGAGTGTA
GCCAGTGTGGGAAATCCTTTAGCCAAAAAGCCACCCTTGTTAAACACCAAAGAGTTCACACTGGAGAAAGGCCTTATAAG
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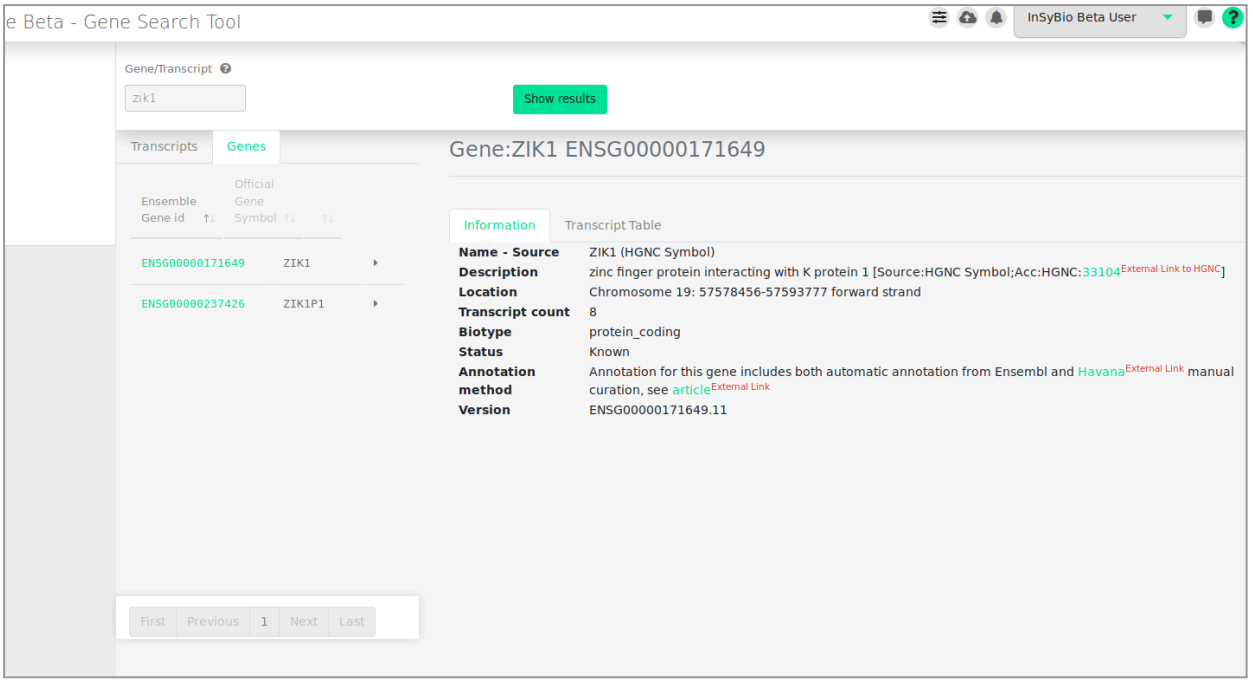
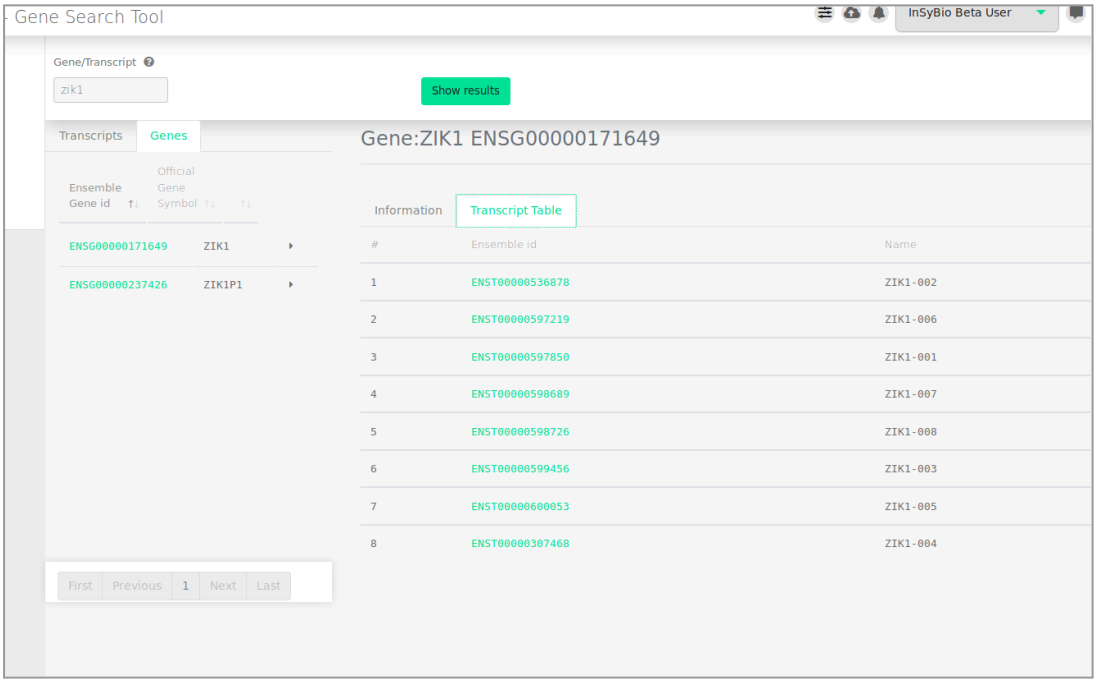
TGAGTGTGGCCAGTGTGGGAAATCCTTTAGTCAAAAAGCTACCCTCATTAAACACCAGAGAGTTCACACTGGAGAAAGGC
CTTATAAGTGTGGTGACTGTGGGAAATCCTTTAGTCAAAGCTCCATCCTTATTCAACACCGGAGAATTCATACTGGAGCA
AGGCCTTATGAGTGTGGCCAGTGTGGAAAGTCCTTTAGCCAAAAGTCTGGTCTCATTCAACACCAAGTGGTTCACACTGG
AGAAAGGCCTTATGAGTGCAACAAATGTGGGAATTCCTTTAGCCAATGCTCCAGCCTCATACATCACCAAAAATGTCATA
ACACATAGAGGCCTCATGAATGCAGCAAATGTGGAAGCGCCTTCAACTCAAGATCTATCATCATTTAGCTCCTGAAAGTC
CACACTTAAGTAGAGCCTTAGACCTACAGGGAAAGTGCTGTCTCTGTAGTATTGTAGCAGTAGAGAGCCTTTGTGAGGGA
GCCATCTGCCTGAAGTTGAACCTCATTCTTCCTTGTTTCTCTGGTAGAAACCATCTACCCTCTACCACCTTGCACAGTGG
GCACTGGTCACTCCTATGTGCTAAGACAAGGCAGACATCTGTGTGTTCTCTTAAGTCTTTGGAGGAAATCTTGAGCAGTC
TAAGCCTTTAGAGAAAATTCATTCTTTTTTCTGACTGATCACAGCATACGTGTGACCCAGTTTGGGTCAGGAGGGCCCAG
CCTTGGTTCTGCTGGACACTTATGTGCAAGGATTCCCTTCATGTAAATTCTTGGTCTCACATGACACTTGGTCATTCTTC
CAGCCTCCATGTCACCACGTGGTGAATGGCTGCCTCACATTGCTCCAGTTTGTGCACTAATAAAAGCCTTATATTTGAAT
CTACCTGTAGTCTTGGGGTTCTGTTTACTGTGTGGGGTGGCTGGGAGACAGACTTCAACTCTATATGAAGGAATGGATGG
CTTTTGTGGGCCTCTGCAGGAAAGTAAGATGACAGAGTAATTCTAATTCTGGTTTTGGTCATACTTGCTTTGCTACCTAA
AATCTCCTAGGAAAAAATGCAAGGTTTTGGTTATTCTAATTTGTGGCCTGGATCCCTATTCTTTCTGTGAGACTAGAGGT
CATCCTGAGGAGAGGCCAGCTGTTATGACAAGCATGTGTGCTTCAGGGAATAGGACAATTTTATTCCATTGTTTCCAGAG

Genes information



For the Genes you can view its name-source, description, location, transcript count, biotype, status, annotation method and version. Also, a Transcript Table is provided with the genes associated transcripts and links to their information.



RNA-Seq Data Analysis

Rna-Seq Differential Expression Pipeline

You can calculate the differential expression between two RNA-Seq experiments. It uses FastQC and Trimmomatic for Quality Control, HISAT2 for Alignment, FeatureCounts for Quantification and DESeq2 for Differential Expression analysis. The Rna-Seq Differential Expression we have implemented consists of 4 steps:

A. Quality Control using FastQC and Filtering using Trimmomatic (Optional step).
B. Alignment using HISAT2, and sorting with Samtools.
C. Quantification using FeatureCounts.
D. Differential Expression using Deseq2.

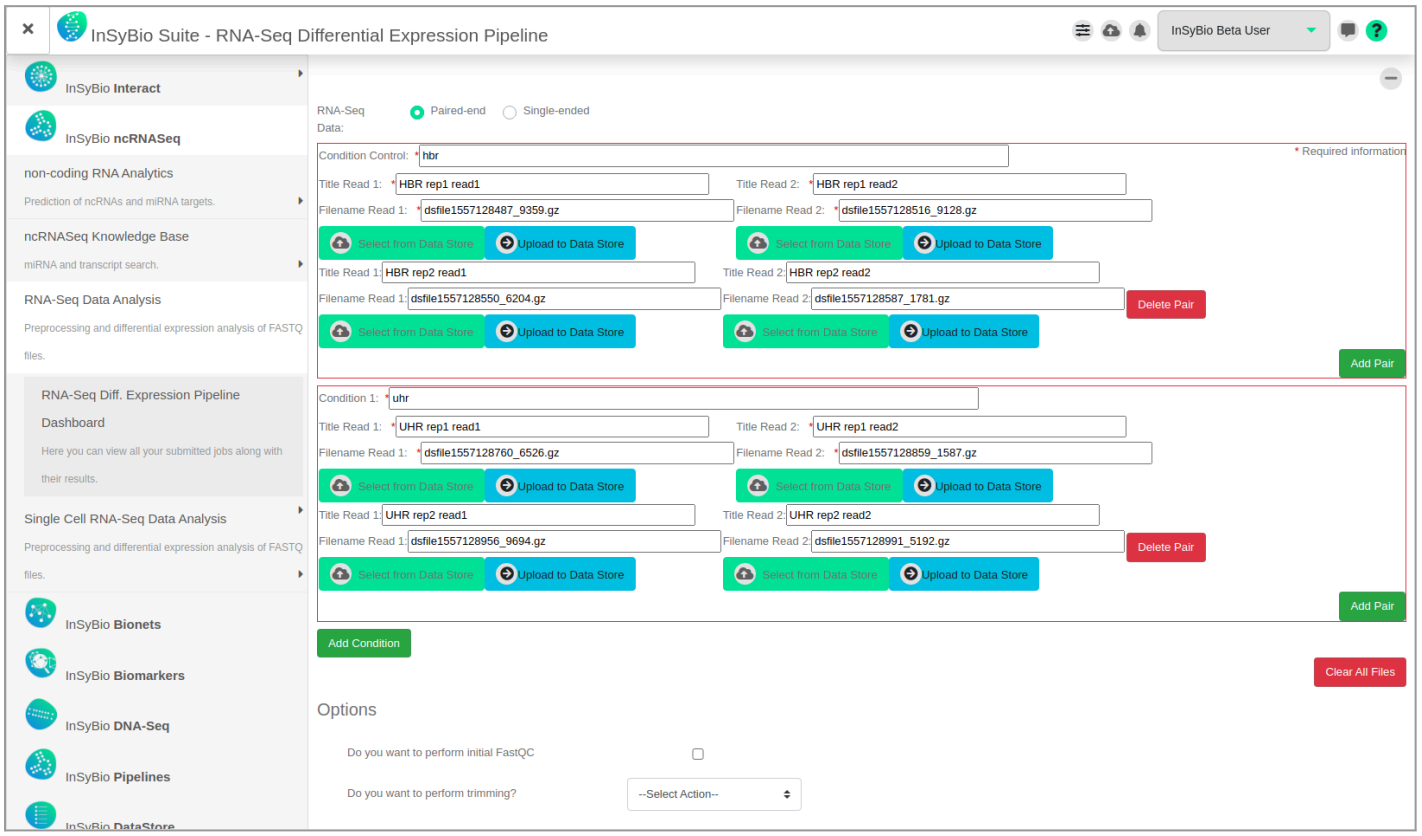
Firstly, the Pipeline uses Fastqc to create a report with the sequence quality, then trim the sequences accordingly using Trimmomatic and create new reports with Fastqc. Then using HISAT2 it creates the alignment SAM files, we sort them using SAMtools and transform them to BAM files. The BAM files are used as input for FeatureCounts, which creates text files with the quantity of each gene. In the end, DESeq2 performs Differential Expression Analysis for all the pairs of conditions using R.

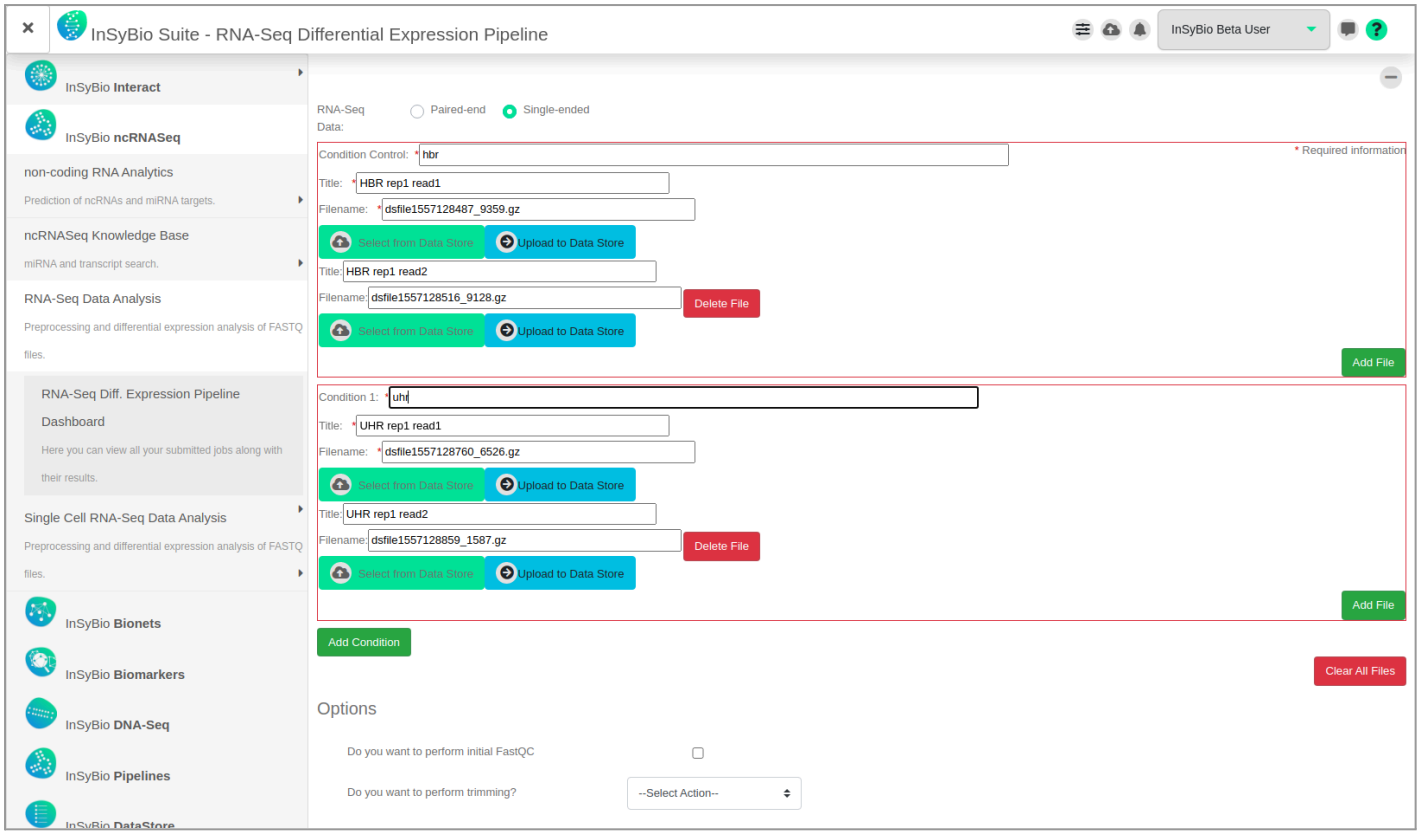
We also offer a modification to the above pipeline, called ncRNA-Seq Differential Expression Pipeline, where the unaligned reads from the Alignment step are used to enhance the quantification files with known or predicted ncRNAs. This is done by finding all the contigs of the unaligned reads files using the AbySS Assembler, and then checking if these contigs are known ncRNAs (from a list of 6 ncRNA types: miRNA, pre-miRNA, tRNA, rRNA, snoRNA and tRf) or use our novel method of an EnsembleGASVR Classifier to predict if the contigs are possible ncRNAs. Then the quantity of the known and predicted ncRNAs is used to enhance the quantification files produced by featureCounts and continue the pipeline as described above.

To start the differential expression:

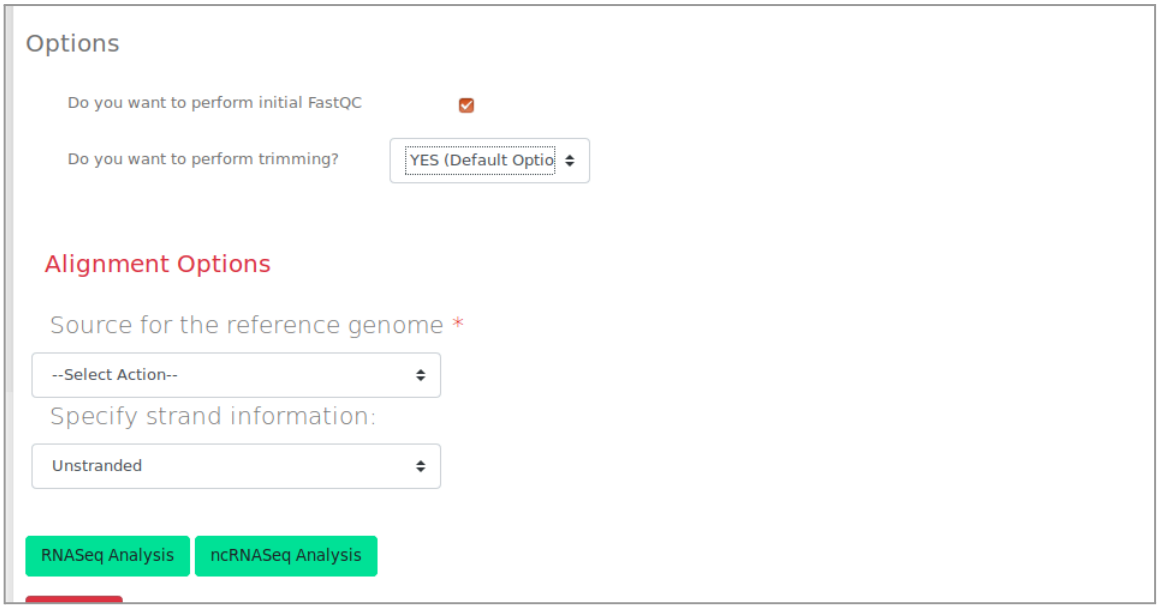
Click in the menu “InSyBio ncRNASeq” → “RNA-Seq Data Analysis” → “RNA-Seq Diff. Expression Pipeline Dashboard”, select the “Add new job” button and then:

- Select if you have Paired or Single Ended data.

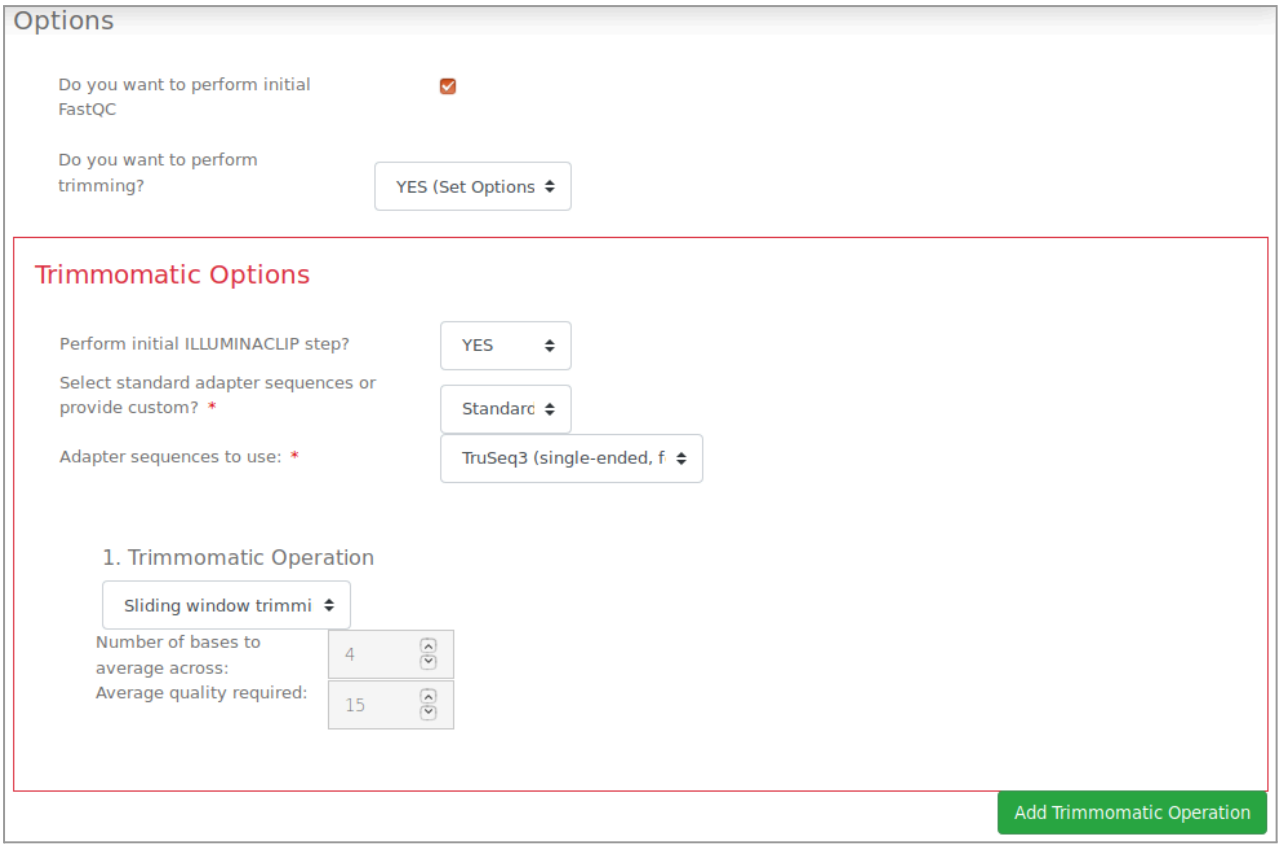




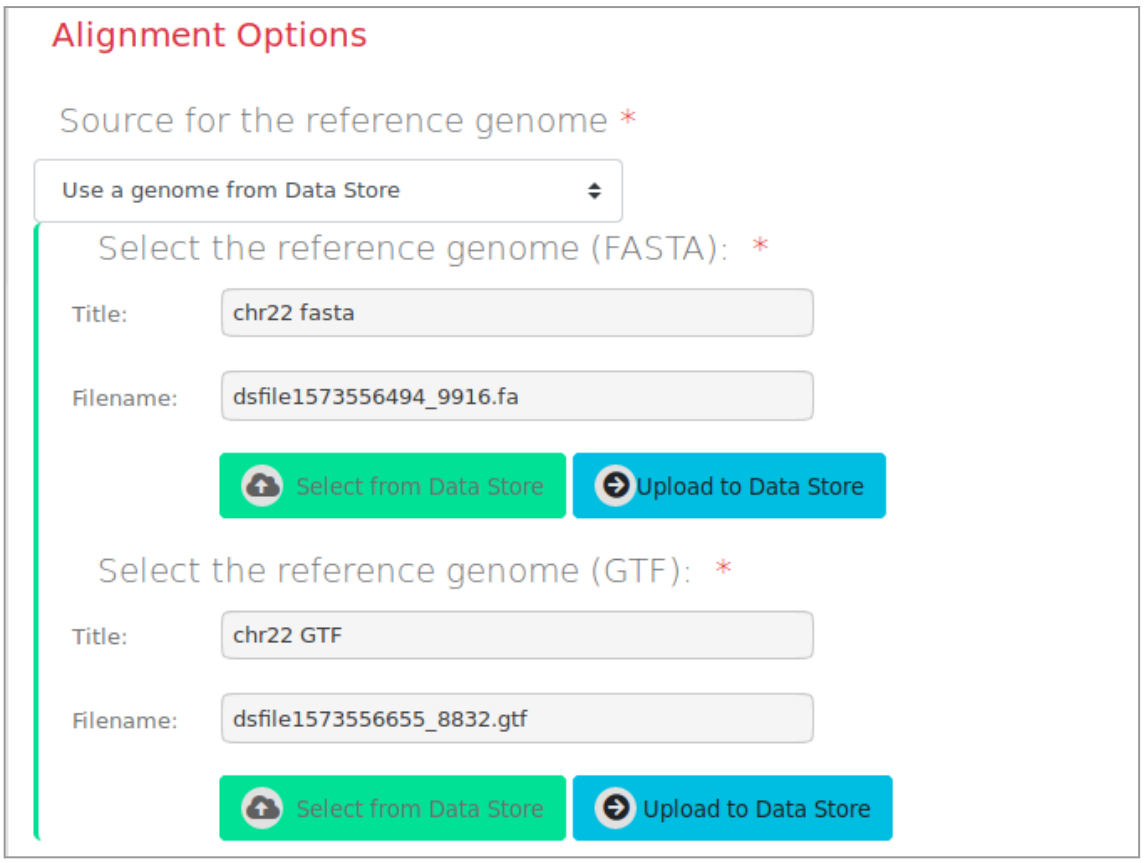
- Name Conditions/Group of files you want to compare.
- For each condition add single or paired files by:
    - Uploading a new file of Rna-Seq Experiments in fastq format. You are redirected to the Data Store where step-by-step instructions guide you for both files uploading.
    - Or Selecting a file of Rna-Seq Experiments in fastq format from the Data Store. There you can find your previously uploaded files or InSyBio pre-uploaded sample datasets.
- Select if you want to perform FastQC Quality Control on the initial Data.

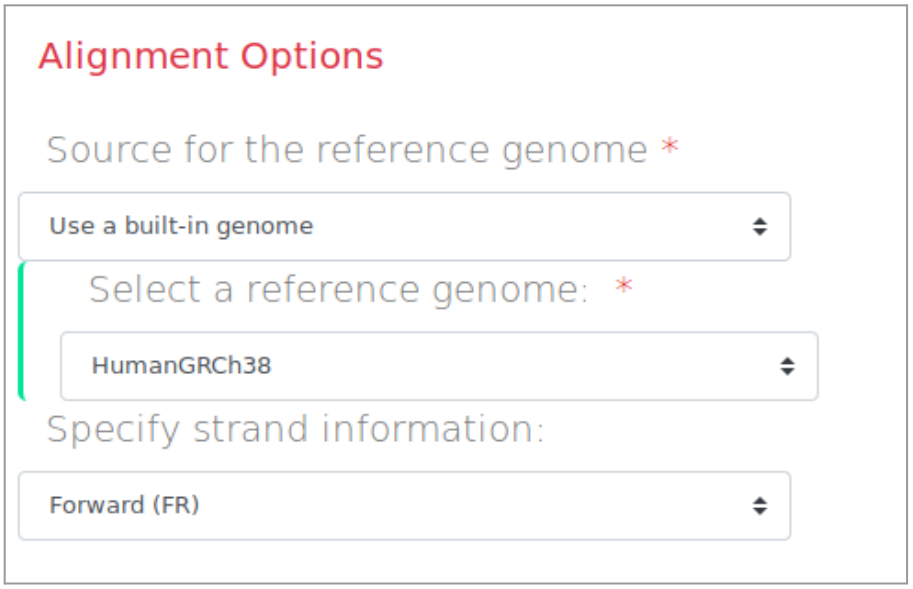


- Select if you want to perform trimming of the data with Trimmomatic, either with our Default Options or add your own (If trimming is selected FastQC will be performed to the trimmed data). Possible manual options are to:
    - Perform initial ILLUMINACLIP step
        - With Standard adapters (TrueSeq2, TrueSeq3 or Nextera for paired or single-ended)
        - Or With Custom adapters in fasta format
    - Perform sliding window trimming
    - Drop reads below a specific length
    - Cut bases off the start of a read, if below a threshold quality
    - Cut bases off the end of a read, if below a threshold quality
    - Cut the read to a specified length
    - Cut the specified number of bases from the start of the read
    - Drop the read if the average quality is below a specified value
    - Trim reads adaptively, balancing read length and error rate to maximise the value of each read



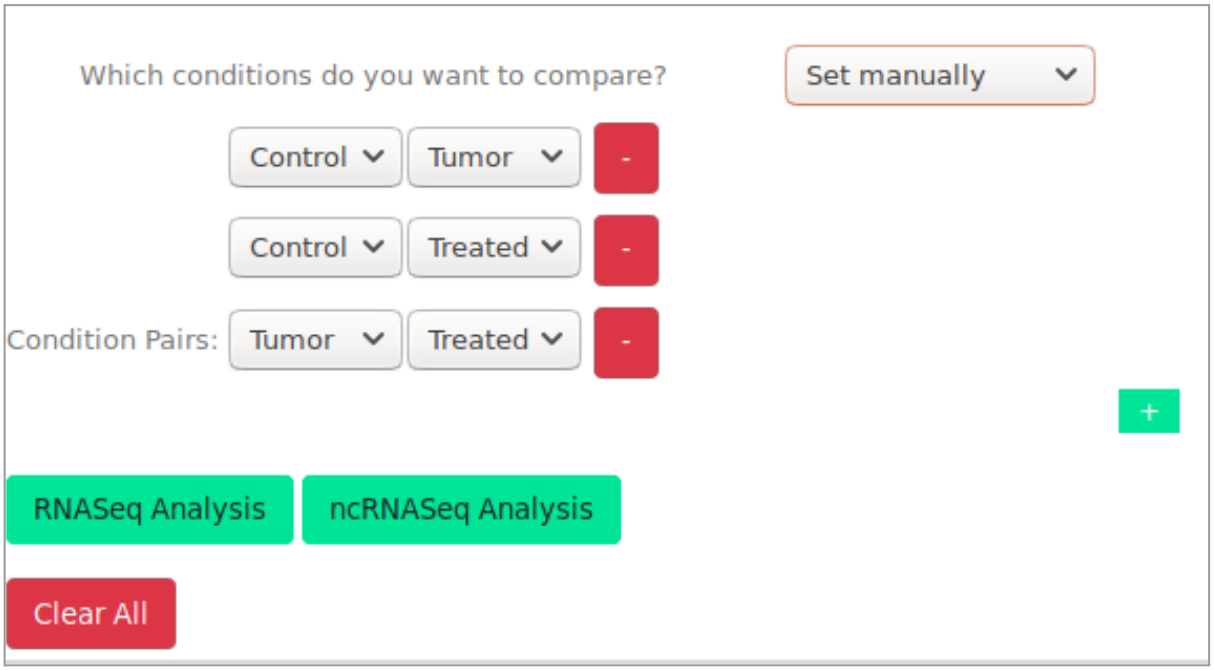
- Select the Genome the input files belong, either from our 4 built-in options (HumanGRCh37, HumanGRCh38, MouseGRCm38 and ZebrafishGRCz11), or
    - Upload new reference Genome files in fasta and gtf format. You are redirected to the Data Store where step-by-step instructions guide you for both files uploading.
    - Or Select two reference Genome files one in fasta and one in gtf format from the Data Store. There you can find your previously uploaded files or InSyBio pre-uploaded sample datasets.





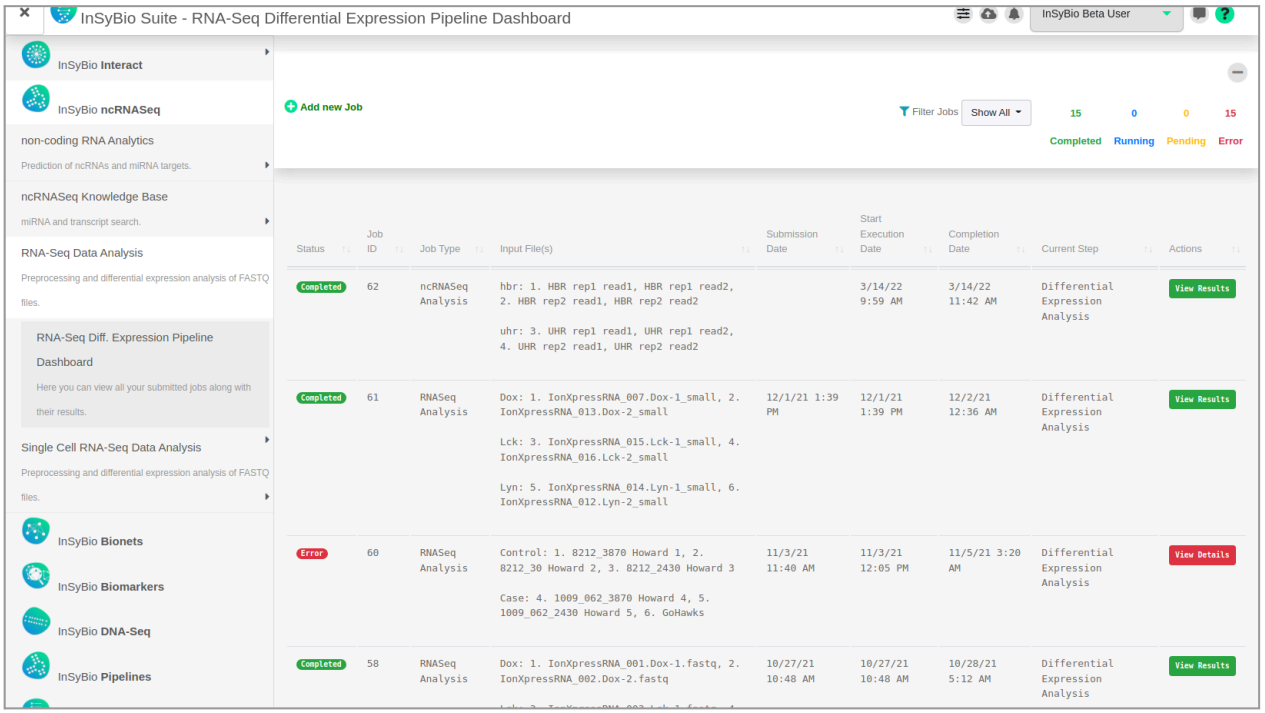
- Select the strandness of your input files, Unstranded, Forward or Reverse.
- If more than 2 Conditions are selected, you can select which pairs of conditions to Differentially Express (all versus Control, all versus all or assign manually).

- Last but not least select either to perform the regular RNASeq Differential Expression Pipeline or the enhanced ncRNASeq Differential Expression Pipeline.

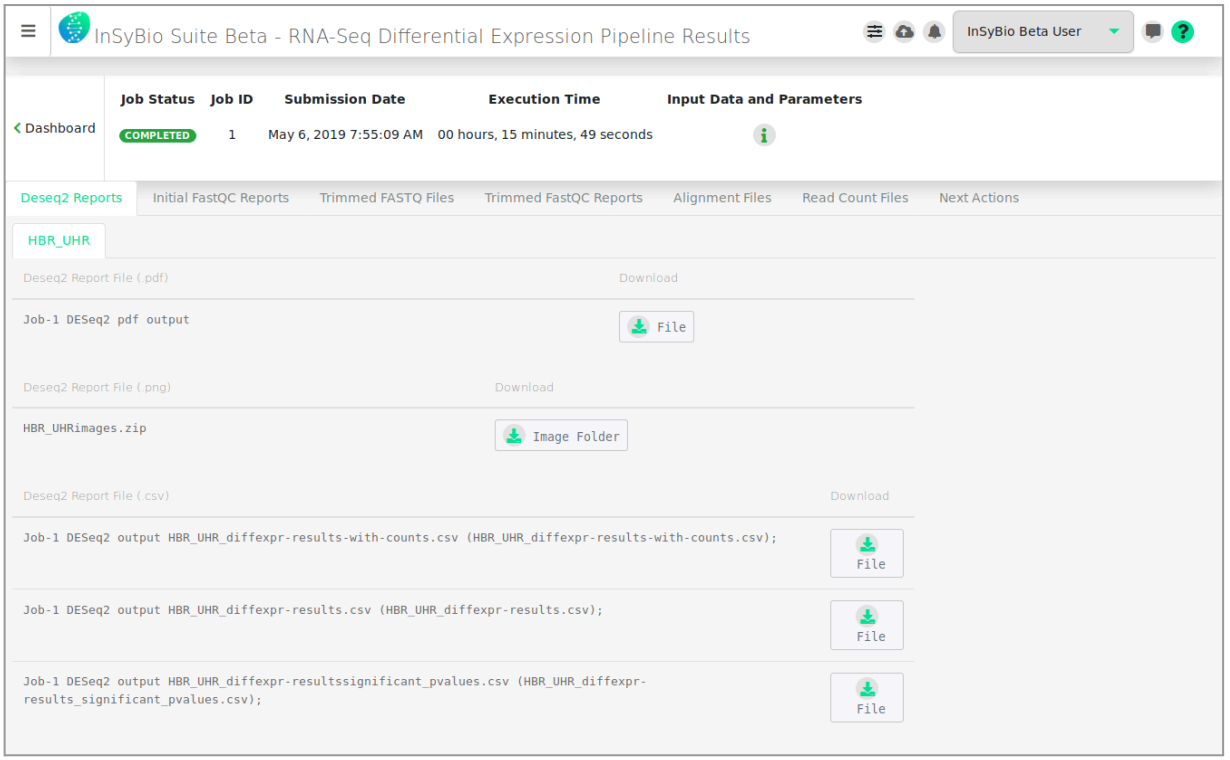


To view the results:

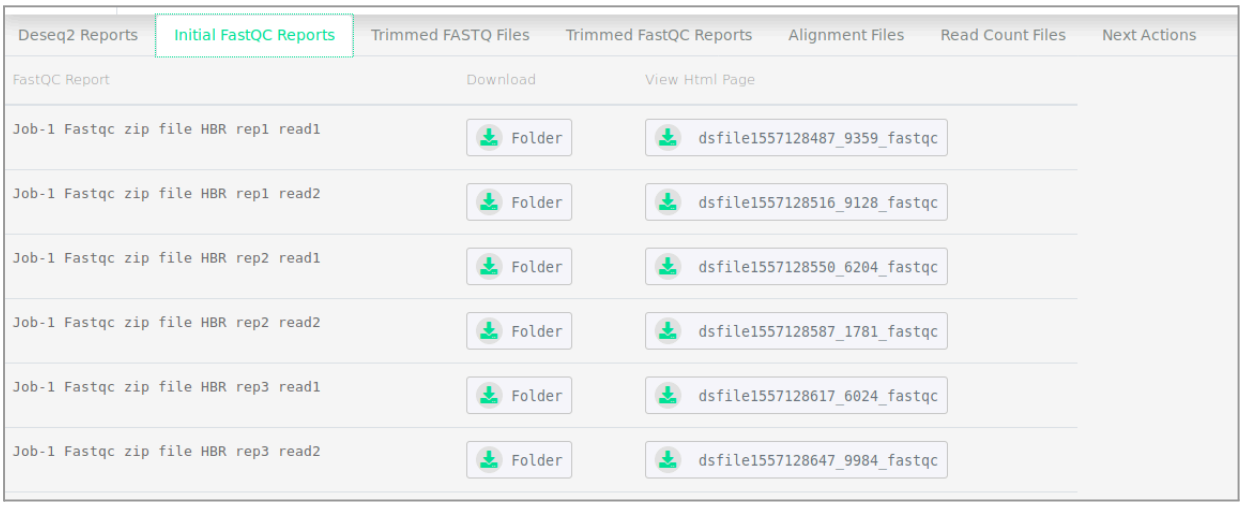
By starting a calculation you are informed if it was submitted successfully. Then you can move to the Rna-Seq Differential Expression Pipeline and view the Dashboard, where you can view the status of your current and previous Rna-Seq Differential Expression jobs.



After the analysis, you can select the View Results at the Actions column and view the produced files, that are separated according to the step they were produced.

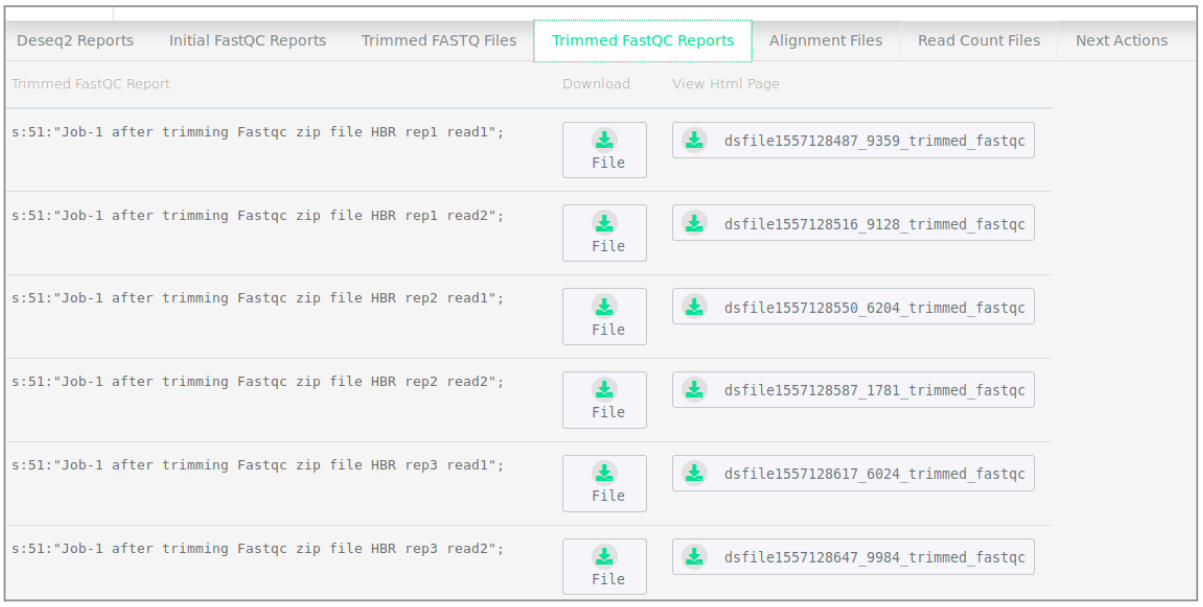


In Deseq2 reports tab you can download visual information and the Differential Expression calculated values for each pair compared.

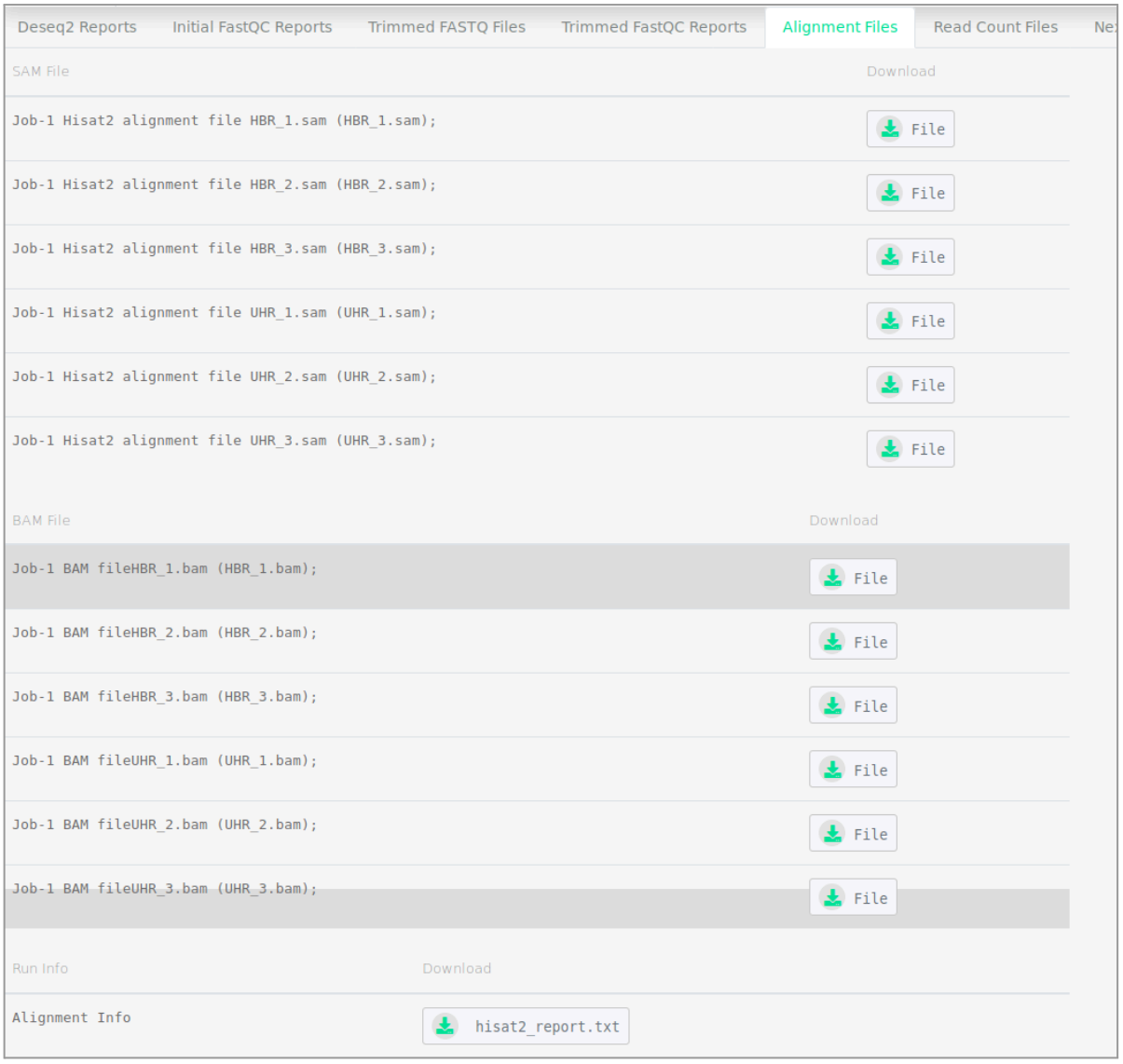


In the Initial FastQC reports the FastQC reports of the input files can be downloaded.

In the Trimmed FASTQ Files, the output Fastq files after trimming can be downloaded.

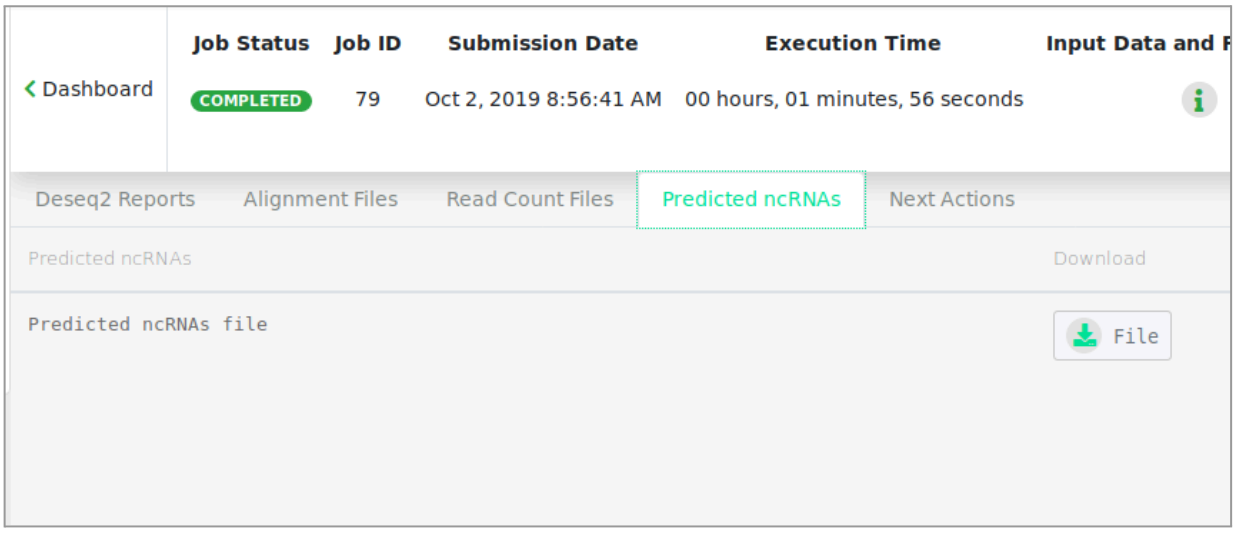


In the Trimmed FastQC reports the FastQC reports of the trimmed files can be downloaded.

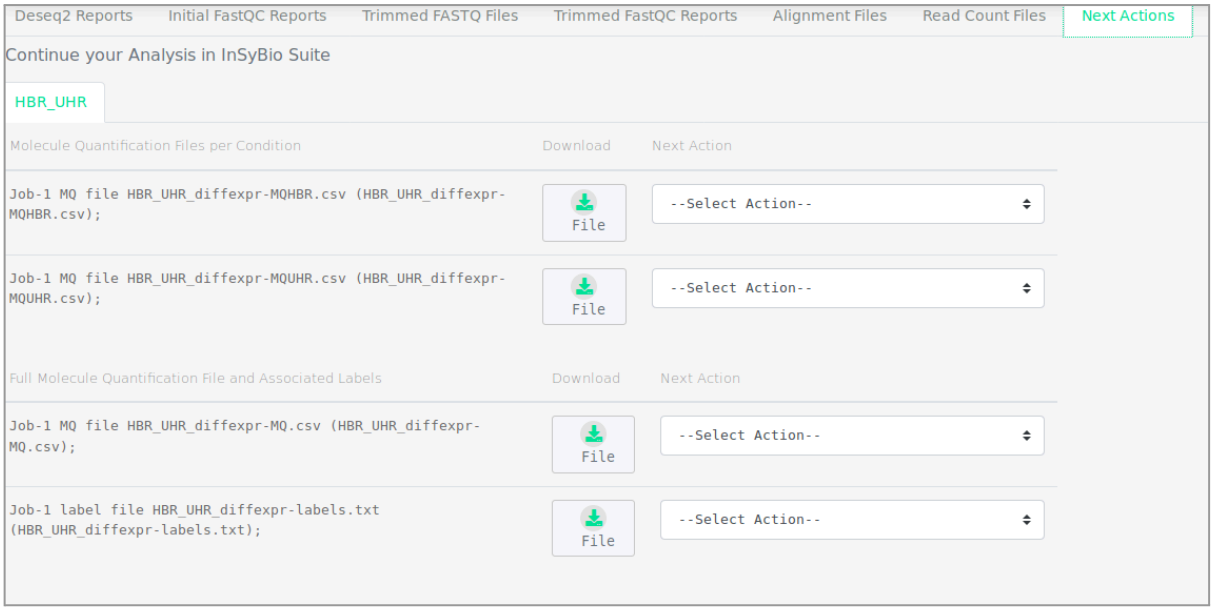


In the Alignment files tab, the HISAT2 alignment sam and bam files can be downloaded.

In the Read Count Files tab the Count files for each sample can be downloaded.



If ncRNASeq Analysis is selected in the Predicted ncRNAs tab a tsv file with the found ncRNAs in the unaligned file is provided, with its name and predicted labels can be downloaded.



In the Next Action tab, Molecule Quantifications files, with the 10% most significant genes, for each comparison are provided. They can be downloaded or used as input in **InSyBio Bionets,** to construct gene correlation networks with the gene expressions of the genes found as statistically significantly differential expressed, and in **InSyBio Biomarkers,** to perform additional statistical analysis and build a classification model able to predict to which of the two conditions a potential new sample belongs.

single - cell RNA-Seq Data Analysis

single-cell RNA-Seq Differential Expression Pipeline

You can analyze single-cell RNA-Seq experiments. Alignment, read counts computation and additional secondary analysis are all performed in one job. Depending on the selected workflow, the single-cell RNA-Seq Differential Expression pipeline consists of the following 2 or 3 steps:

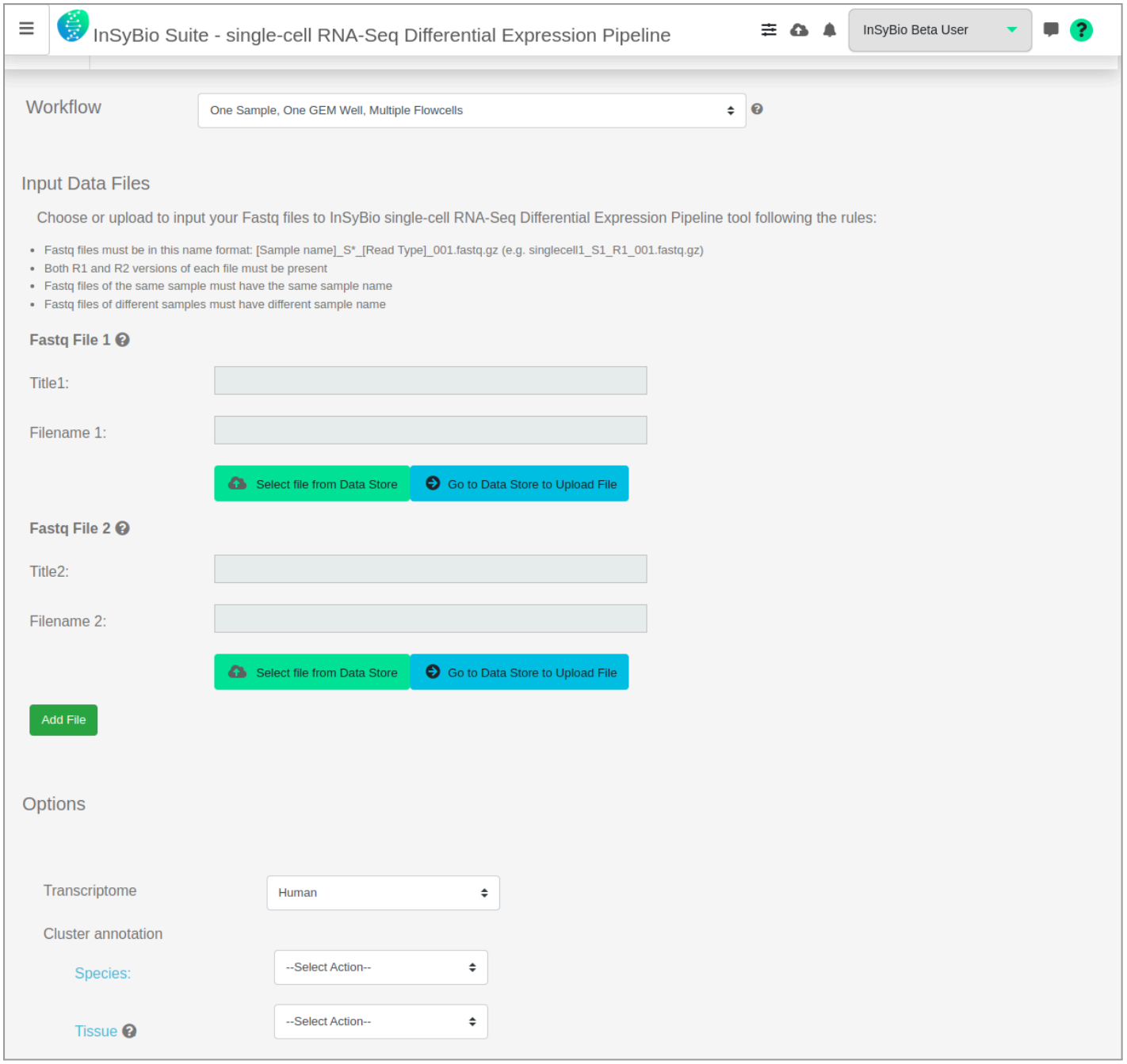
- Workflow 0 or 1:
  - Alignment and read counts computation using Cellranger count.
  - Further analysis using our single-cell Analysis.
- Workflow 2 or 3:
  - Alignment and read counts computation using Cellranger count pipeline for each different sample or different GEM well.
  - Aggregation of the Cellranger count runs using the Cellranger aggr pipeline.
  - Further analysis using our single-cell Analysis.

Firstly, the Pipeline uses the Cellranger count pipeline to perform the alignment and the read counts computation of the input fastq files. If the input fastq files are generated from different samples or different GEM wells, an extra step is performed. Specifically, the Cellranger aggr pipeline is used to aggregate the cellranger count runs for the creation of a single feature-barcode matrix and analysis. At the end, our single-cell Analysis script is used to perform additional secondary differential expression analysis.

To start the single-cell differential expression:

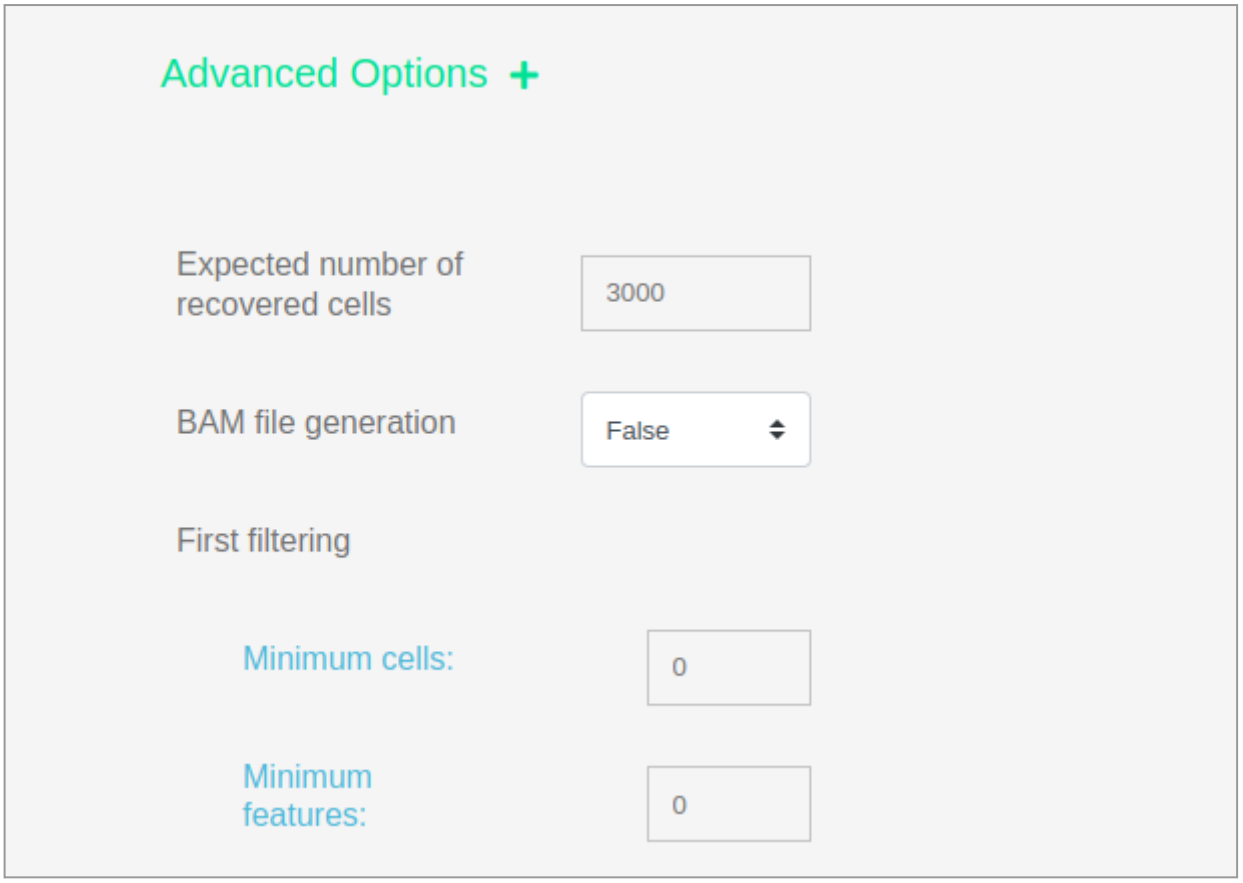
Click in the menu “InSyBio ncRNASeq” → “single-cell RNA-Seq Data Analysis” → “single-cell RNA-Seq Pipeline Dashboard”, select the “Add new job” button and then:

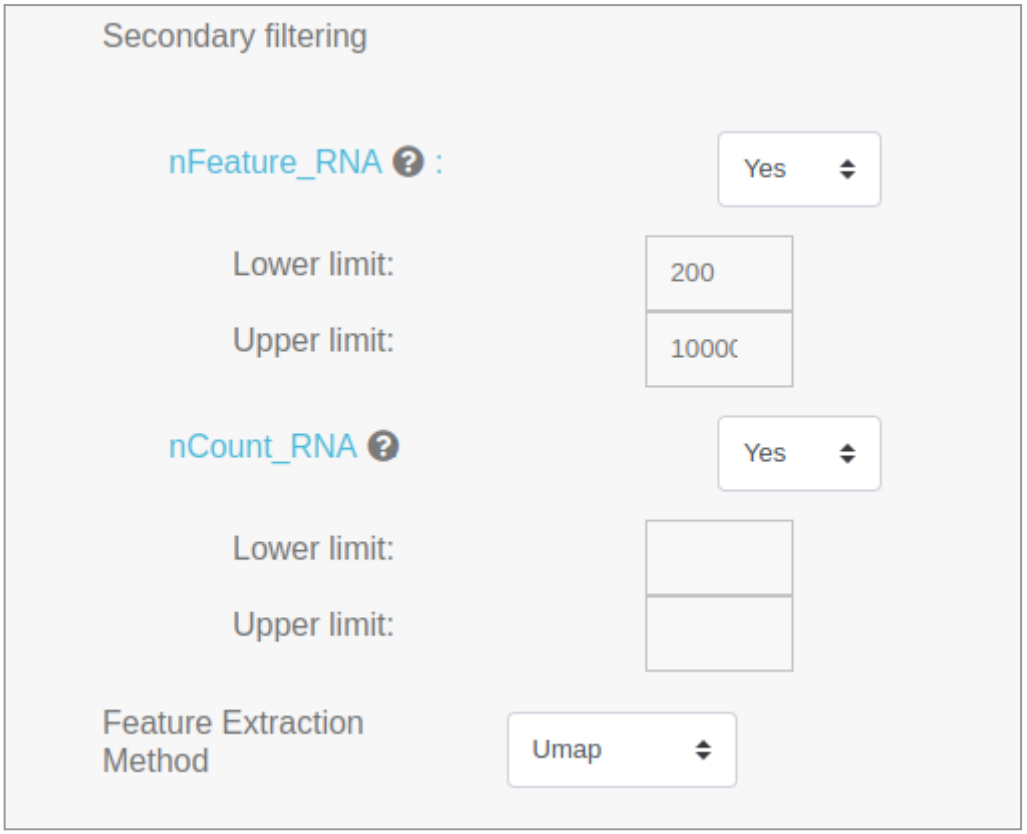
- Select your workflow.

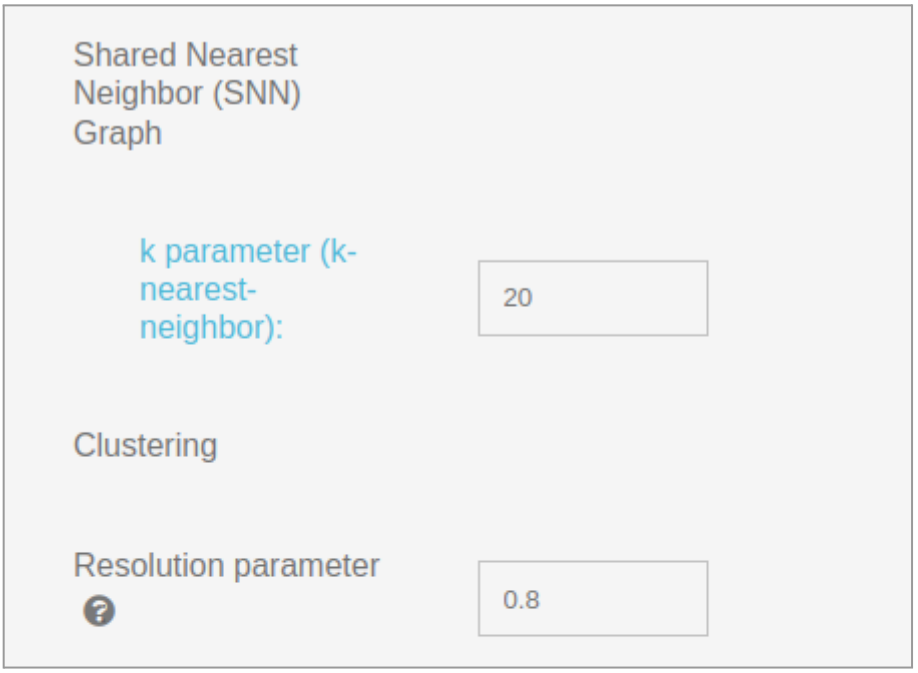


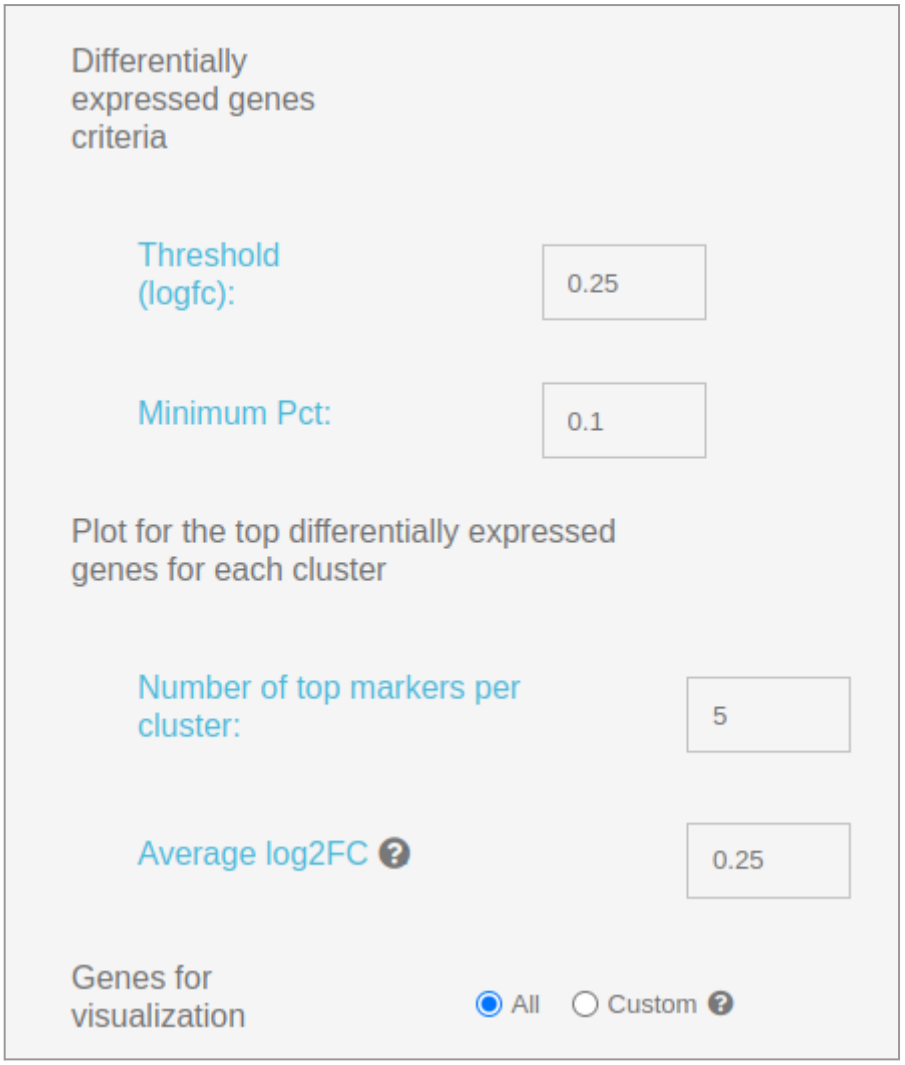
- Upload your files of single-cell RNA-Seq Experiments in the following format:
  - Fastq files must be in this name format: [Sample name]_S*_[Read Type]_001.fastq.gz
  - Fastq files of the same sample must have the same sample name
  - Fastq files of different samples must have different sample name
- Select the transcriptome the input files belong to from our 3 built-in options (Human, Mouse, Human-mouse mixture).

- Select the species and tissue type of your sample for cluster annotation to be performed.
- Select if you want to manually configure the parameters of the pipeline. If you don't, our Default Options will be applied. Possible manual options are:
    - Expected number of recovered cells
    - BAM file generation
    - First filtering:
        - Minimum cells
        - Minimum features
    - Secondary filtering:
        - nFeature_RNA with lower and upper limits
        - nCount_RNA with lower and upper limits
    - Feature Extraction Method
    - Shared Nearest Neighbor (SNN) Graph
    - Clustering
    - Differentially expressed genes criteria
    - Plot for the top differentially expressed genes for each cluster
    - Genes for visualization


Advanced Options +
Expected number of recovered cells
3000
BAM file generation
False
First filtering
Minimum cells:
0
Minimum features:
0


Secondary filtering
nFeature_RNA :
Yes
Lower limit:
200
Upper limit:
10000
nCount_RNA
Yes
Lower limit:
Upper limit:
Feature Extraction Method
Umap

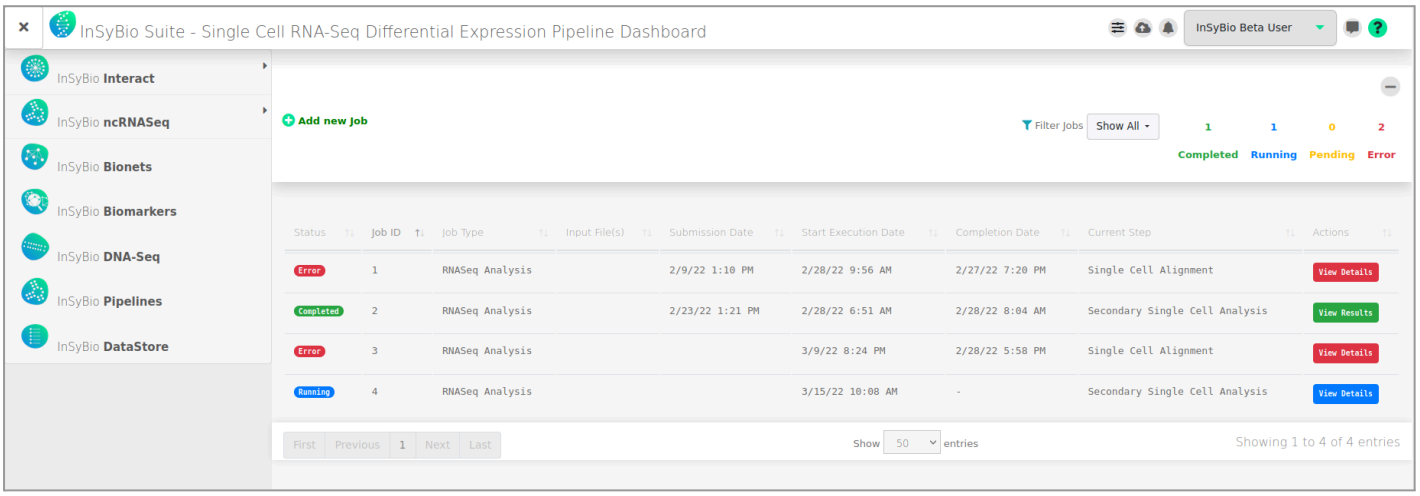

Shared Nearest Neighbor (SNN) Graph
k parameter (k-nearest-neighbor):
20
Clustering
Resolution parameter
0.8


Differentially expressed genes criteria
Threshold (logfc):
0.25
Minimum Pct:
0.1
Plot for the top differentially expressed genes for each cluster
Number of top markers per cluster:
5
Average log2FC
0.25
Genes for visualization
All
Custom

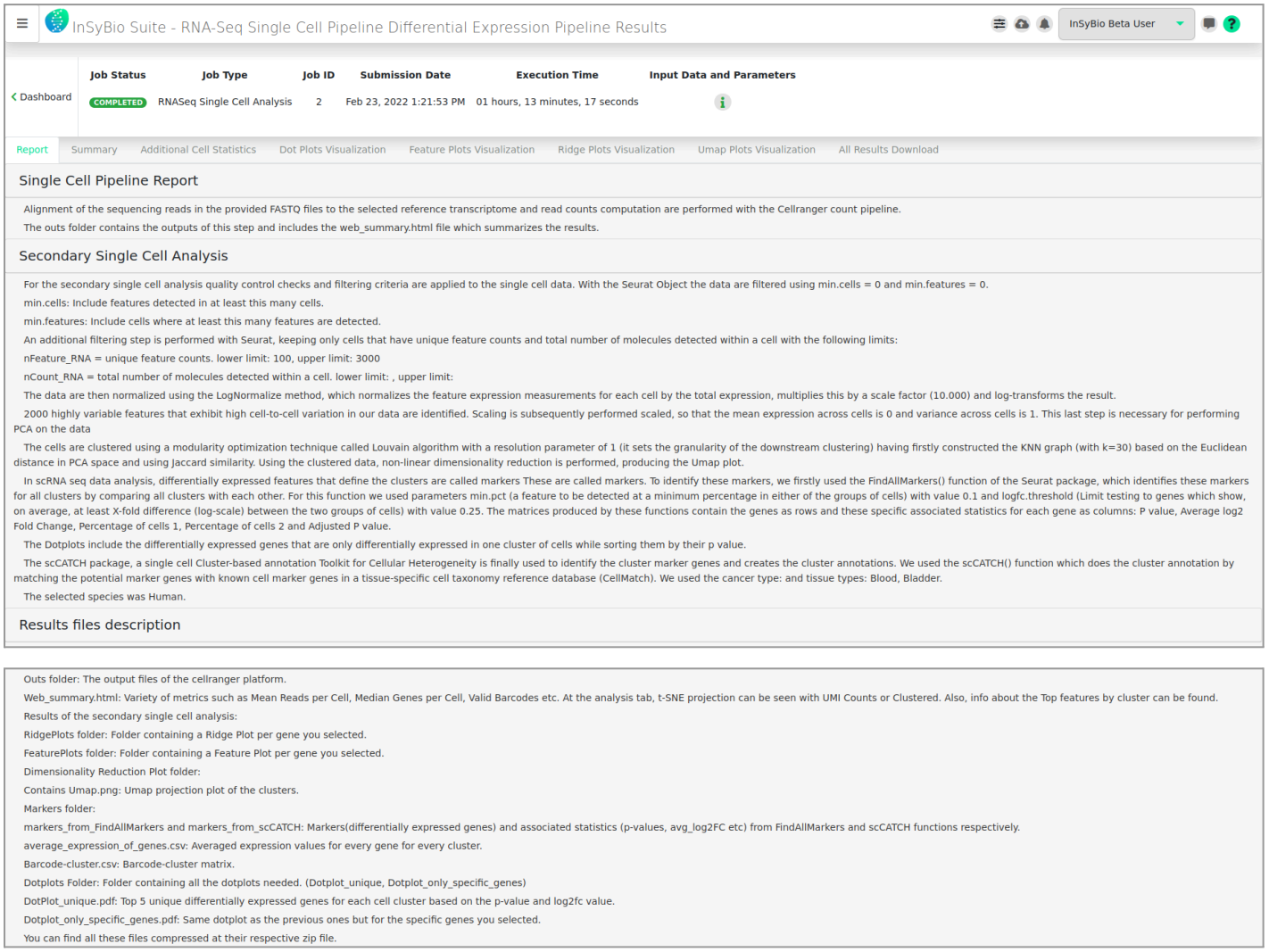
- Submit your job pressing the respective button.

To view the results:

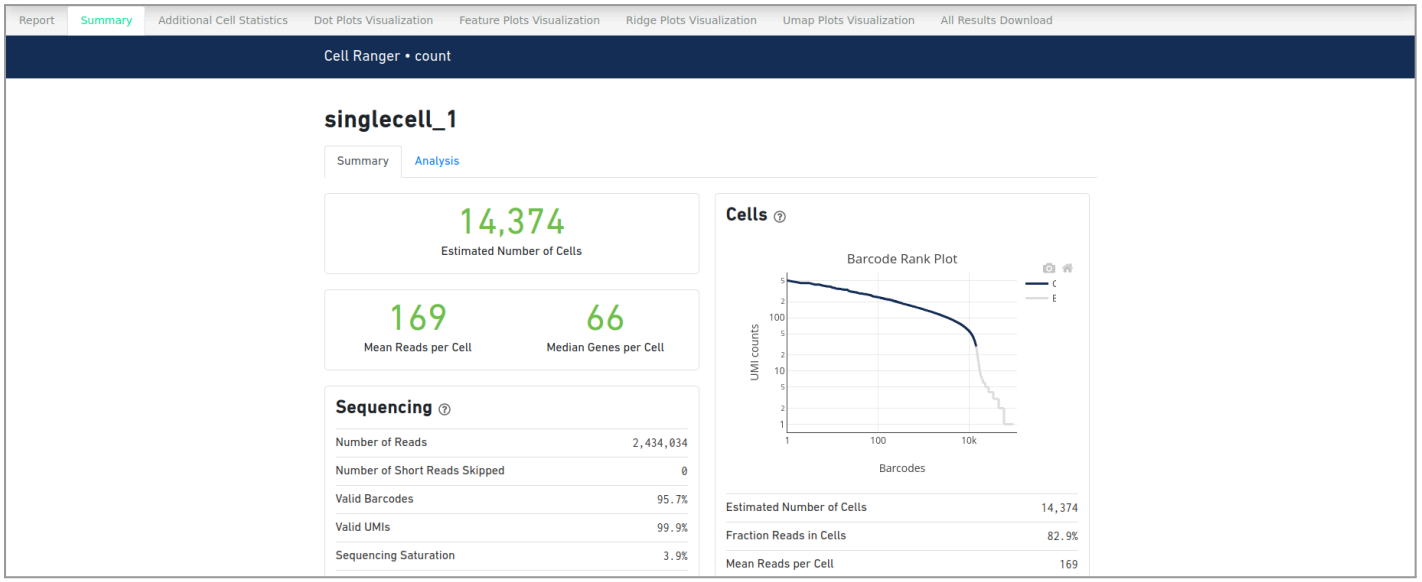
By starting a calculation you are informed if it was submitted successfully. Then you can move to the single-cell RNA-Seq Differential Expression Pipeline and view the Dashboard, where you can view the status of your current and previous single-cell RNA-Seq Differential Expression jobs.

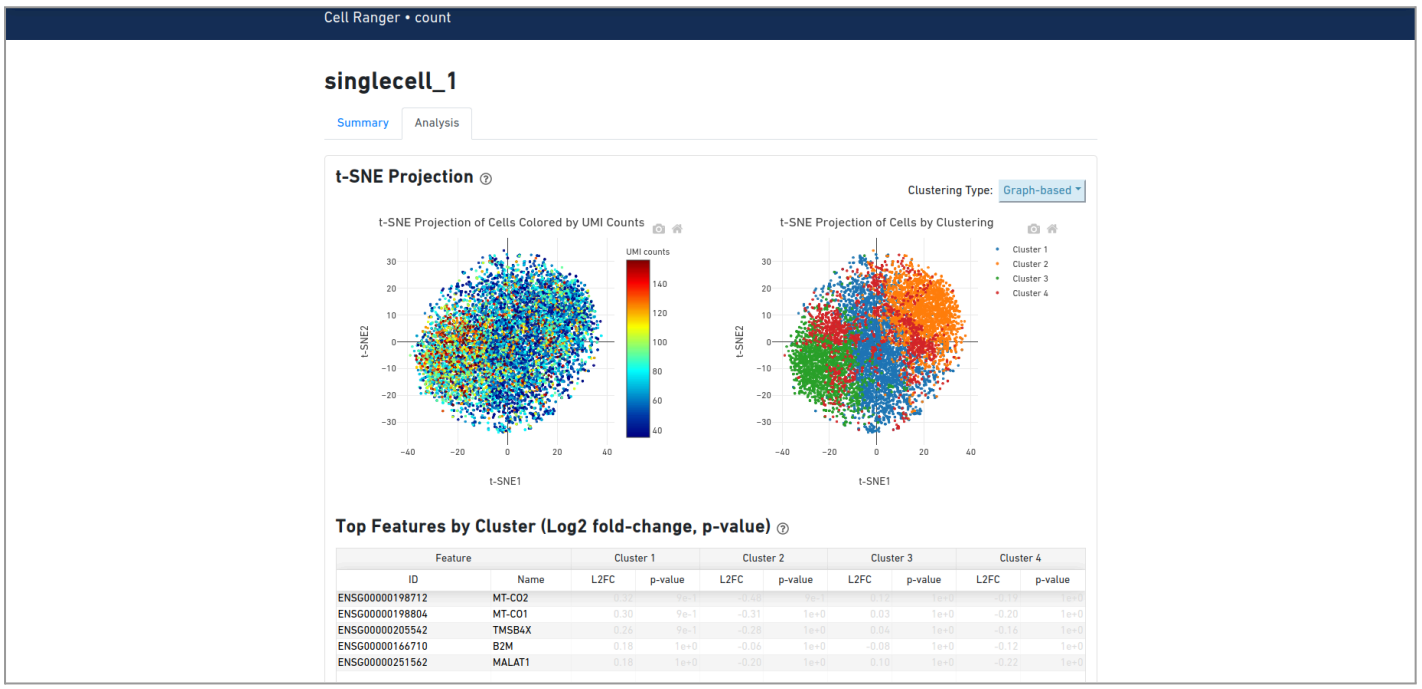


After the analysis, you can select the View Results at the Actions column and view the produced files, that are separated according to the step that they were produced.

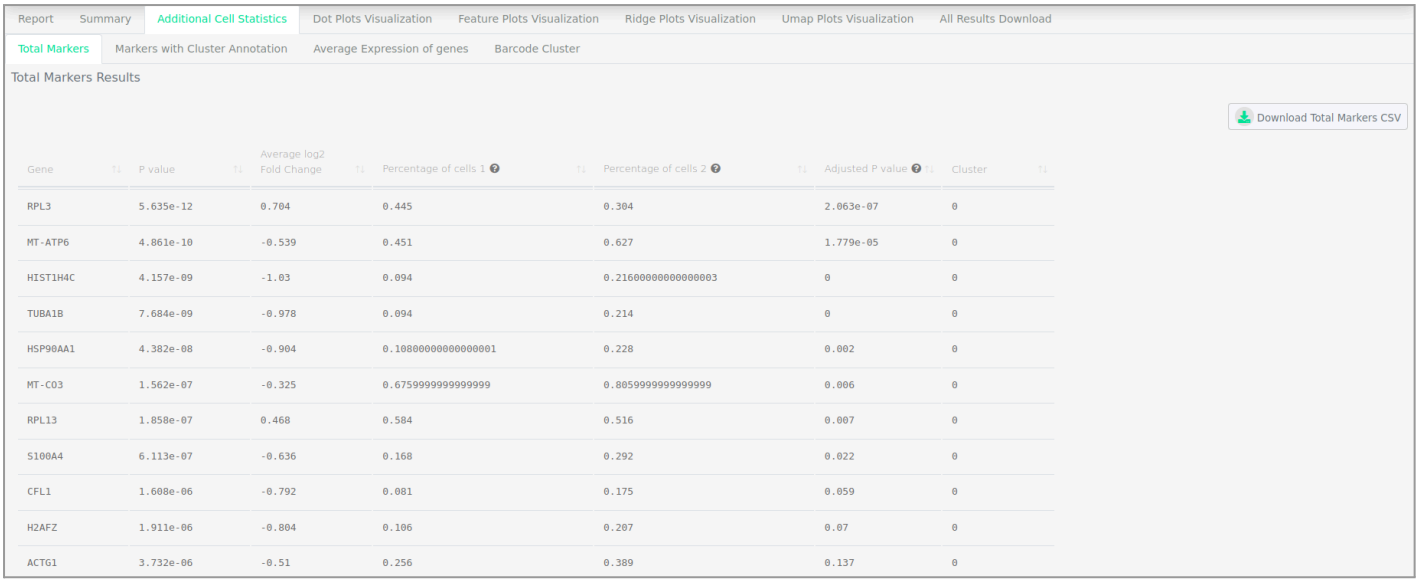


In the Report tab you can see a generated report that includes descriptions for every step and every parameter of the single-cell RNA-Seq Differential Expression Pipeline for your job.



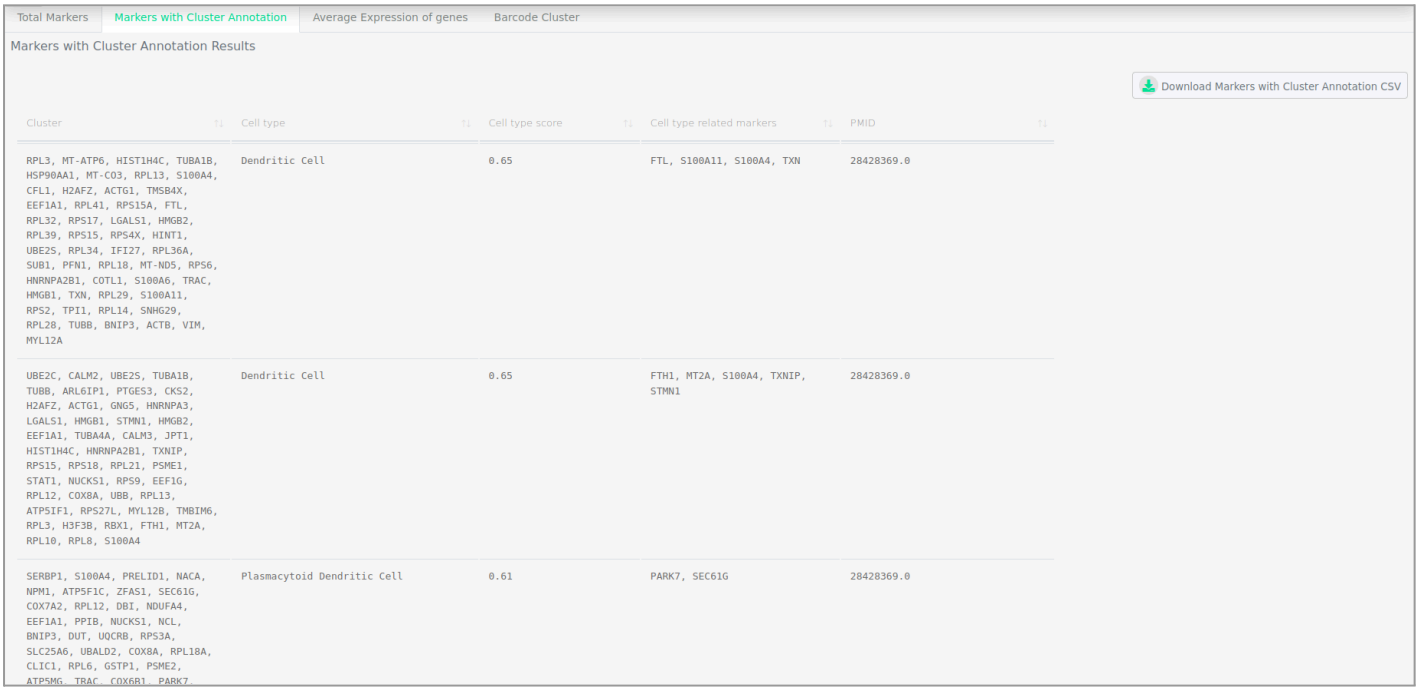


In the Summary tab you can see a summary of a variety of metrics from the first step of the single-cell RNA-Seq Differential Expression Analysis and some T-SNE plots and information about the Top features by Cluster.



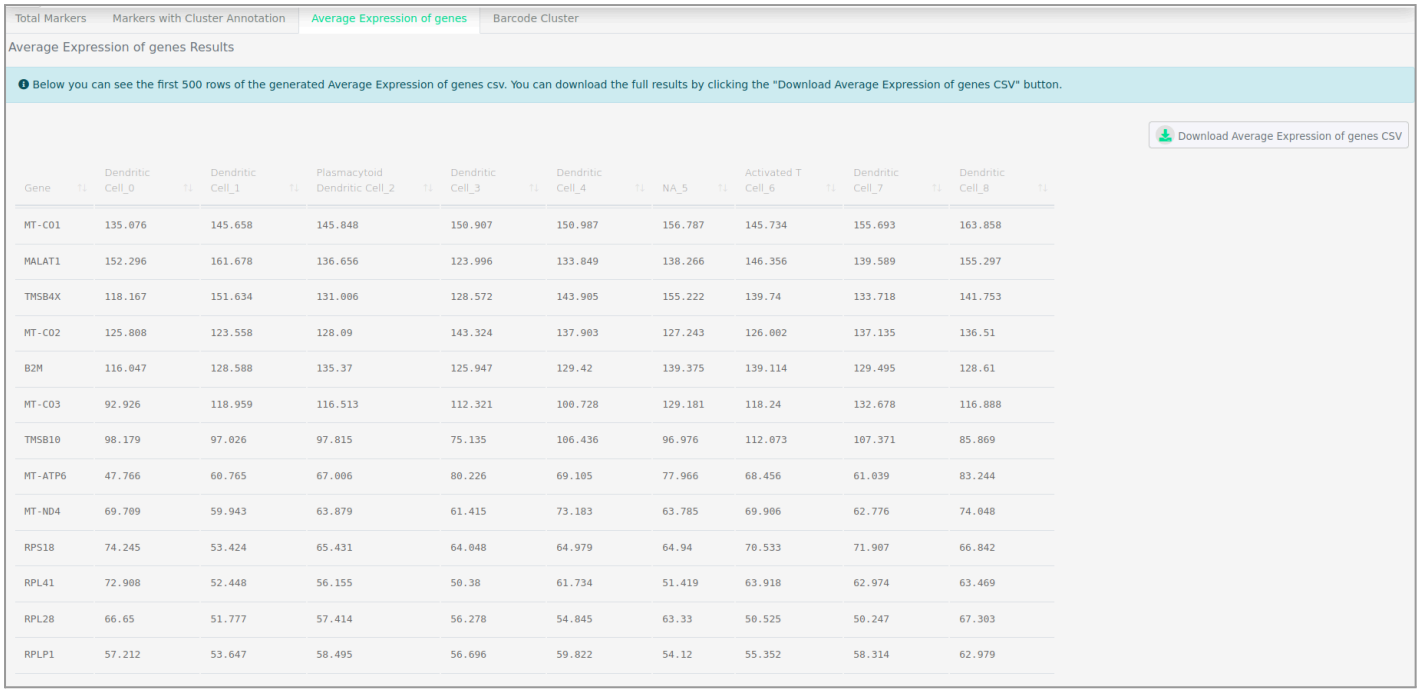
Gene	P value	Average log2 Fold Change	Percentage of cells 1	Percentage of cells 2	Adjusted P value	Cluster
RPL3	5.635e-12	0.704	0.445	0.304	2.063e-07	0
MT-ATP6	4.861e-10	-0.539	0.451	0.627	1.779e-05	0
HIST1H4C	4.157e-09	-1.03	0.094	0.21600000000000003	0	0
TUBA1B	7.684e-09	-0.978	0.094	0.214	0	0
HSP90AA1	4.382e-08	-0.904	0.10800000000000001	0.228	0.002	0
MT-CO3	1.562e-07	-0.325	0.6759999999999999	0.8059999999999999	0.006	0
RPL13	1.858e-07	0.468	0.584	0.516	0.007	0
S100A4	6.113e-07	-0.636	0.168	0.292	0.022	0
CFL1	1.608e-06	-0.792	0.081	0.175	0.059	0
H2AFZ	1.911e-06	-0.804	0.106	0.207	0.07	0
ACTG1	3.732e-06	-0.51	0.256	0.389	0.137	0

In the Additional Cell Statistics tab the user can view four different tabs that represent different information for the genes of the input files. The results for these four different tabs can be downloaded at the respective tab. At the Total Markers tab, markers (differentially expressed genes) and associated statistics (p-values, average log2 Fold change etc) can be found.



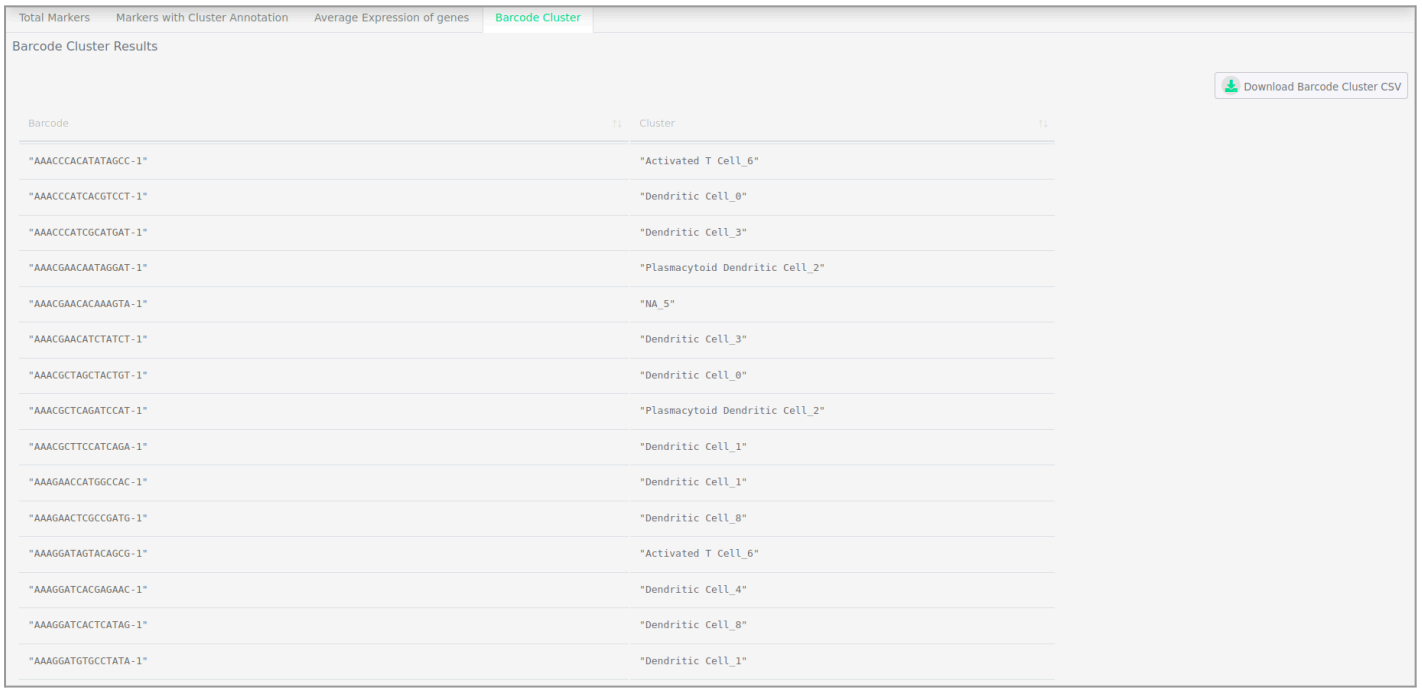
Cluster	Cell type	Cell type score	Cell type related markers	PMID
RPL3, MT-ATP6, HIST1H4C, TUBA1B, HSP90AA1, MT-CO3, RPL13, S100A4, CFL1, H2AFZ, ACTG1, TMSB4X, EEF1A1, RPL41, RPS15A, FTL, RPL32, RPS17, LGALS1, HMGB2, RPL39, RPS15, RPS4X, HINT1, UBE2S, RPL34, IFI27, RPL36A, SUB1, PFN1, RPL18, MT-ND5, RPS6, HNRNPA2B1, COTL1, S100A6, TRAC, HMGB1, TXN, RPL29, S100A11, RPS2, TPI1, RPL14, SNHG29, RPL28, TUBB, BNIP3, ACTB, VIM, MYL12A	Dendritic Cell	0.65	FTL, S100A11, S100A4, TXN	28428369.0
UBE2C, CALM2, UBE2S, TUBA1B, TUBB, ARL6IP1, PTGES3, CKS2, H2AFZ, ACTG1, GNG5, HNRNPA3, LGALS1, HMGB1, STMN1, HMGB2, EEF1A1, TUBA4A, CALM3, JPT1, HIST1H4C, HNRNPA2B1, TXNIP, RPS15, RPS18, RPL21, PSME1, STAT1, NUCKS1, RPS9, EEF1G, RPL12, COX8A, UBB, RPL13, ATP5IF1, RPS27L, MYL12B, TMBIM6, RPL3, H3F3B, RBX1, FTH1, MT2A, RPL10, RPL8, S100A4	Dendritic Cell	0.65	FTH1, MT2A, S100A4, TXNIP, STMN1	28428369.0
SERBP1, S100A4, PRELID1, NACA, NPM1, ATP5F1C, ZFAS1, SEC61G, COX7A2, RPL12, DBI, NDUFA4, EEF1A1, PPIB, NUCKS1, NCL, BNIP3, DUT, UQCRB, RPS3A, SLC25A6, UBALD2, COX8A, RPL18A, CLIC1, RPL6, GSTP1, PSME2, ATP5MG, TRAC, COX6B1, PARK7	Plasmacytoid Dendritic Cell	0.61	PARK7, SEC61G	28428369.0

At the Markers with Cluster Annotation tab, the results of the Cluster Annotation step can be found.



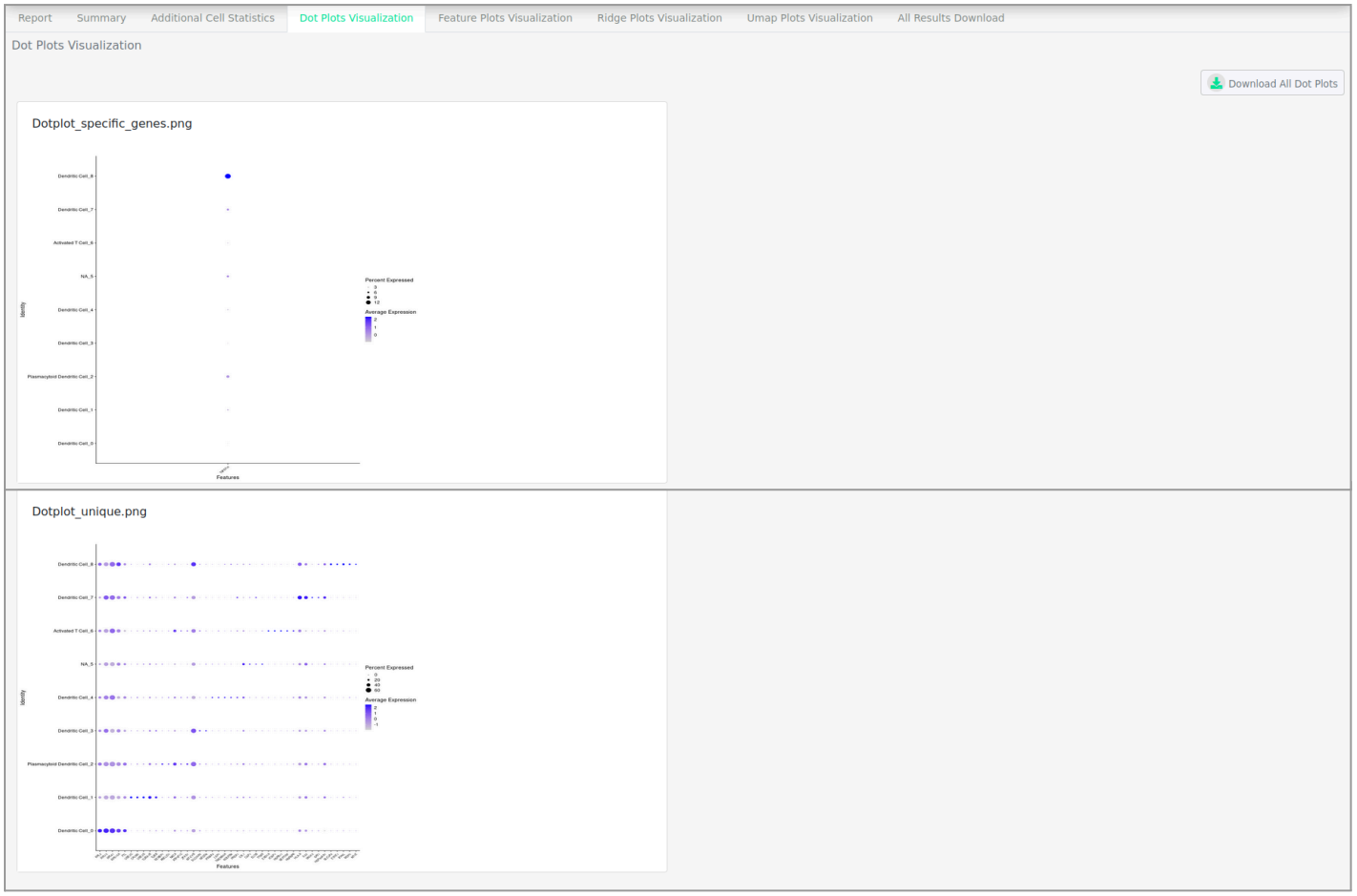
Gene	Dendritic Cell_0	Dendritic Cell_1	Plasmacytoid Dendritic Cell_2	Dendritic Cell_3	Dendritic Cell_4	NA_5	Activated T Cell_6	Dendritic Cell_7	Dendritic Cell_8
MT-CO1	135.076	145.658	145.848	150.907	150.987	156.787	145.734	155.693	163.858
MALAT1	152.296	161.678	136.656	123.996	133.849	138.266	146.356	139.589	155.297
TMSB4X	118.167	151.634	131.006	128.572	143.905	155.222	139.74	133.718	141.753
MT-CO2	125.808	123.558	128.09	143.324	137.903	127.243	126.002	137.135	136.51
B2M	116.047	128.588	135.37	125.947	129.42	139.375	139.114	129.495	128.61
MT-CO3	92.926	118.959	116.513	112.321	100.728	129.181	118.24	132.678	116.888
TMSB10	98.179	97.026	97.815	75.135	106.436	96.976	112.073	107.371	85.869
MT-ATP6	47.766	60.765	67.006	80.226	69.105	77.966	68.456	61.039	83.244
MT-ND4	69.709	59.943	63.879	61.415	73.183	63.785	69.906	62.776	74.048
RPS18	74.245	53.424	65.431	64.048	64.979	64.94	70.533	71.907	66.842
RPL41	72.908	52.448	56.155	50.38	61.734	51.419	63.918	62.974	63.469
RPL28	66.65	51.777	57.414	56.278	54.845	63.33	50.525	50.247	67.303
RPLP1	57.212	53.647	58.495	56.696	59.822	54.12	55.352	58.314	62.979

At the Average Expression of genes Results tab the first 500 rows of the generated Average Expression of genes file can be found and it contains the expression levels of every gene for every cluster.



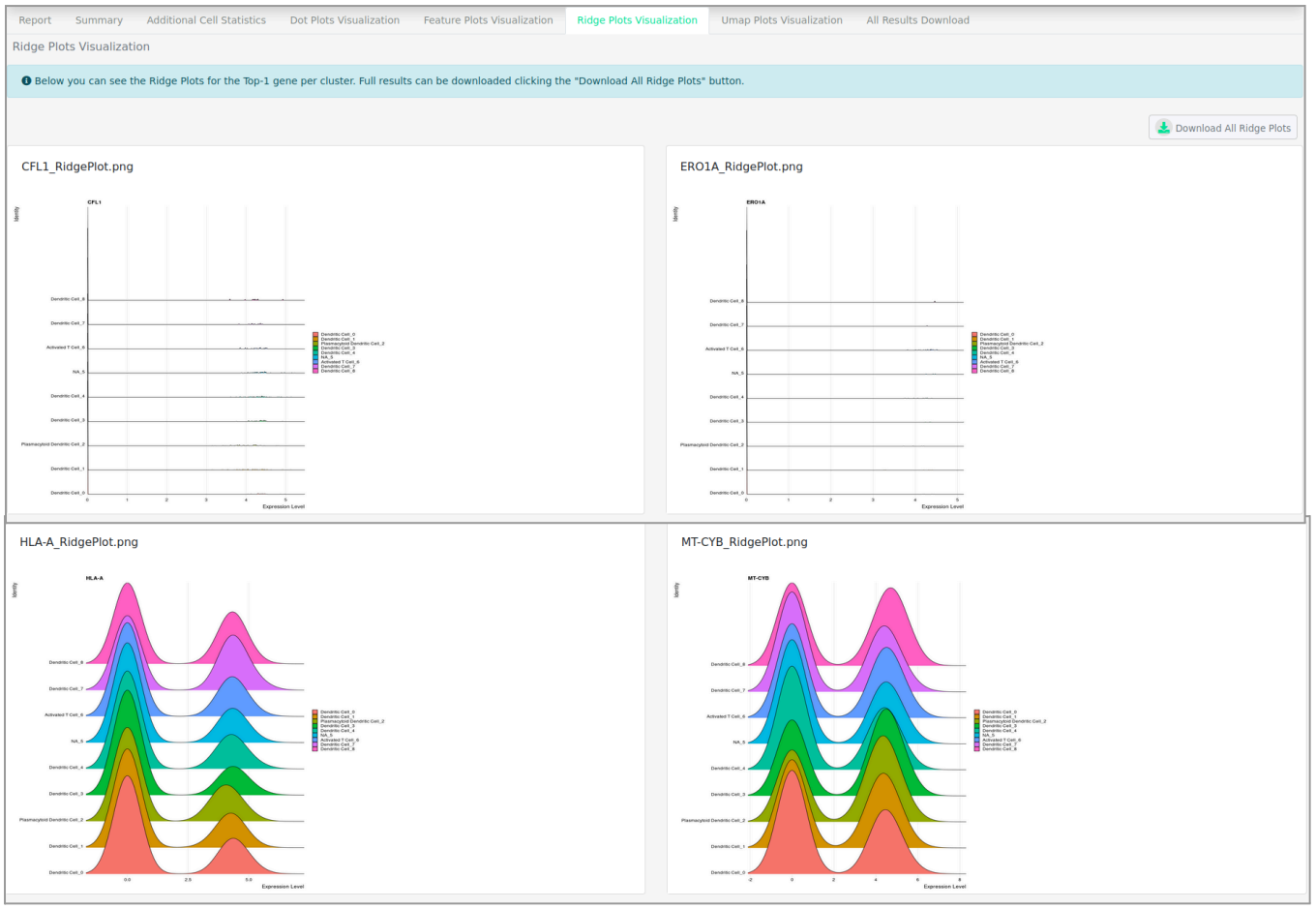
Barcode	Cluster
"AAACCCACATATAGCC-1"	"Activated T Cell_6"
"AAACCCATCACGTCCT-1"	"Dendritic Cell_0"
"AAACCCATCGCATGAT-1"	"Dendritic Cell_3"
"AAACGAACAATAGGAT-1"	"Plasmacytoid Dendritic Cell_2"
"AAACGAACACAAAGTA-1"	"NA_5"
"AAACGAACATCTATCT-1"	"Dendritic Cell_3"
"AAACGCTAGCTACTGT-1"	"Dendritic Cell_0"
"AAACGCTCAGATCCAT-1"	"Plasmacytoid Dendritic Cell_2"
"AAACGCTTCCATCAGA-1"	"Dendritic Cell_1"
"AAAGAACCATGGCCAC-1"	"Dendritic Cell_1"
"AAAGAACTCGCCGATG-1"	"Dendritic Cell_8"
"AAAGGATAGTACAGCG-1"	"Activated T Cell_6"
"AAAGGATCACGAGAAC-1"	"Dendritic Cell_4"
"AAAGGATCACTCATAG-1"	"Dendritic Cell_8"
"AAAGGATGTGCCTATA-1"	"Dendritic Cell_1"

At the Barcode Cluster tab, the Barcode-Cluster matrix can be found.

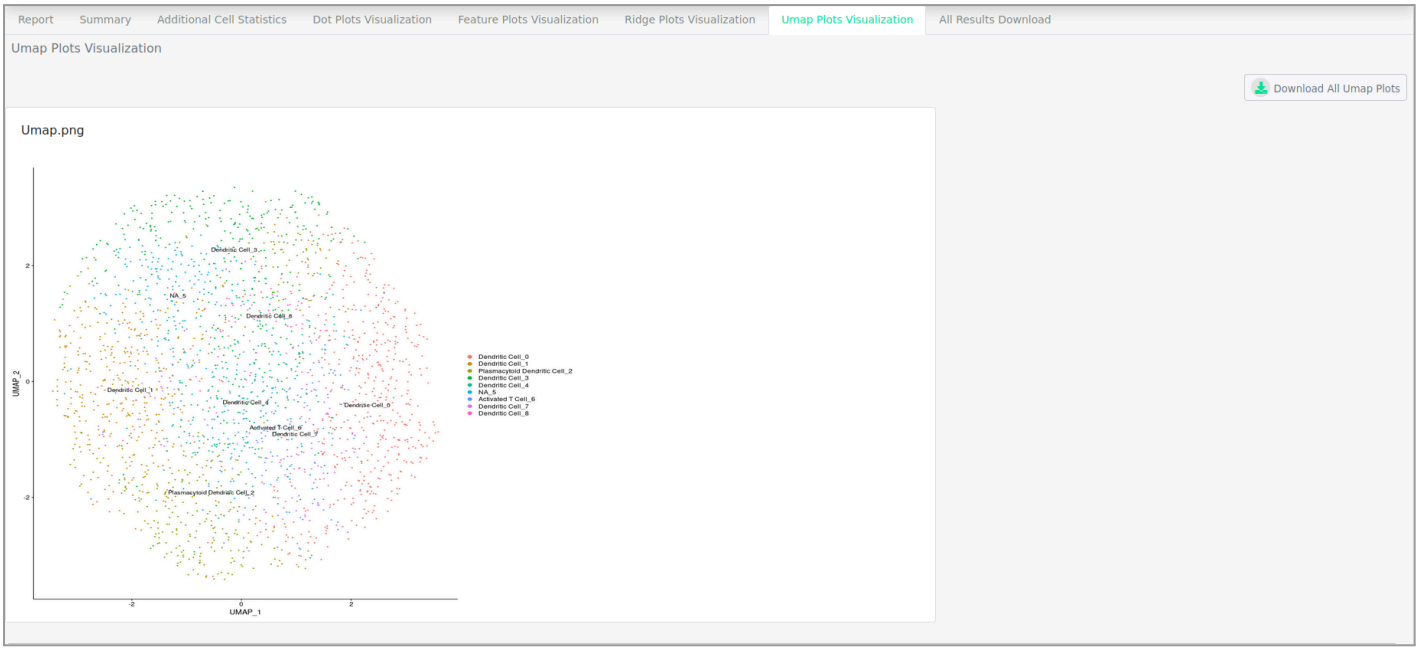


At the Dot Plots Visualization tab you can see the two Dot plots that are created. The first one is a Dot Plot with only the genes you specified at the manual parameters and the second one is a Dot Plot that shows the Top 5 unique differentially expressed genes for each cell cluster based on the p-value and log2 fold change value. These plots can be downloaded.

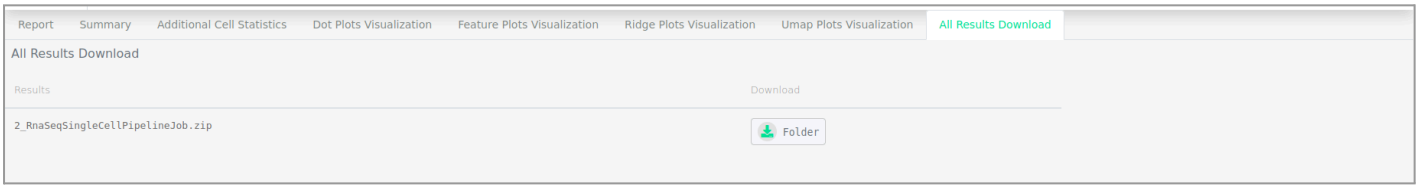
At the Feature Plots Visualization tab the Feature Plots for the Top-1 gene per cluster can be found. The Feature Plots of all the genes can be downloaded.



At the Ridge Plots Visualization tab the Ridge Plots for the Top-1 gene per cluster can be found. The Ridge Plots of all the genes can be downloaded.



At the Umap Plots Visualization tab the Umap Plots can be found. The Umap Plot can be downloaded.



At the All Results Download tab, all the results of your job can be downloaded.

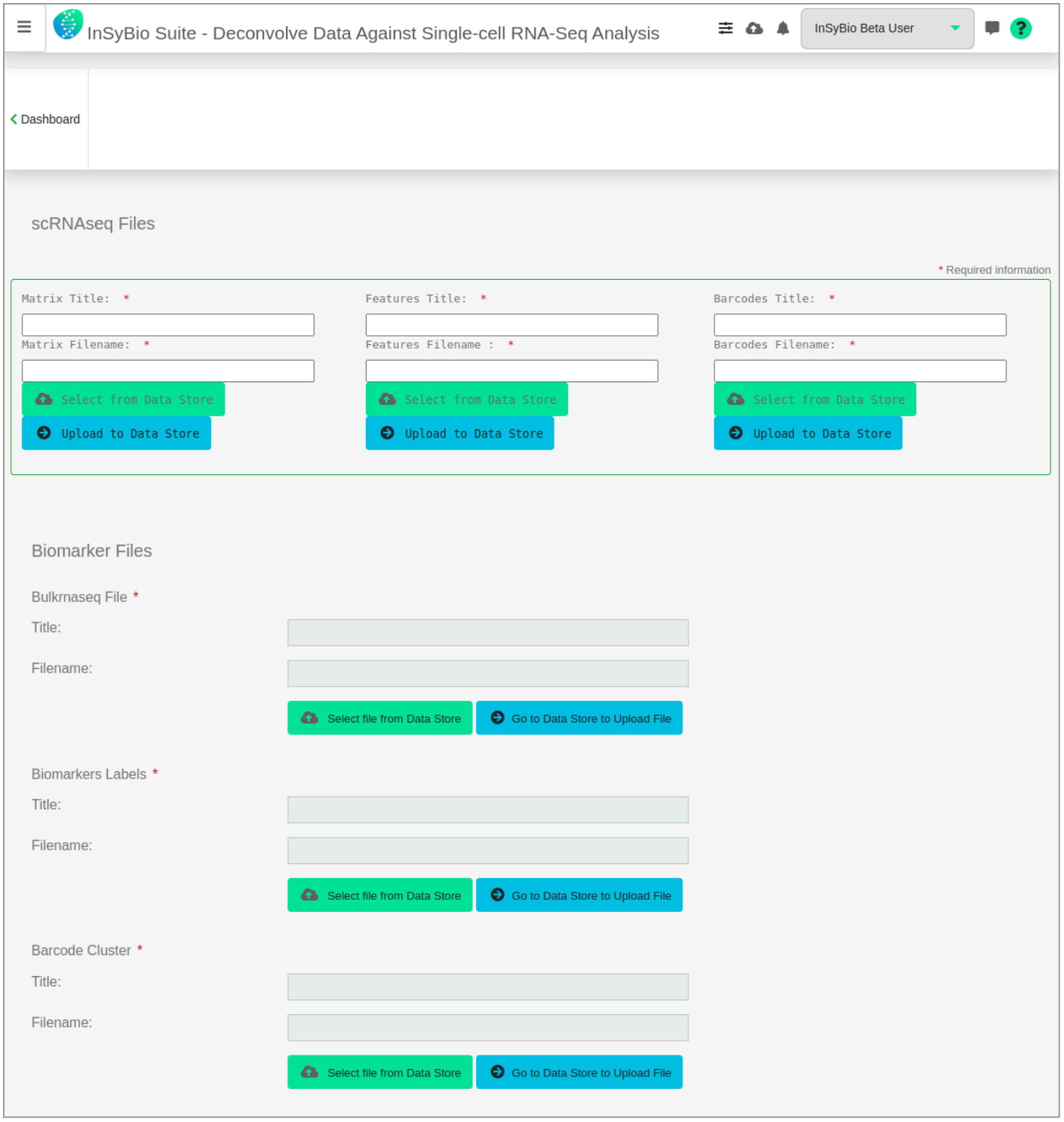
Deconvolve Data against single-cell RNA-seq Analysis

You can deconvolve data against a single-cell RNA-Seq dataset. Firstly, it is required to import the single-cell RNA-Seq 10x datasets, the Matrix, the Feature and the Barcodes datasets. Secondly, you must import the biomarker files, a BulkRnaSeq file, the Biomarkers Labels and the Barcode-Cluster file. This Pipeline uses the SCDC method (Bulk Gene Expression Deconvolution by Multiple Single-Cell RNA Sequencing Referencing) to perform the deconvolution.

To start the deconvolution pipeline:

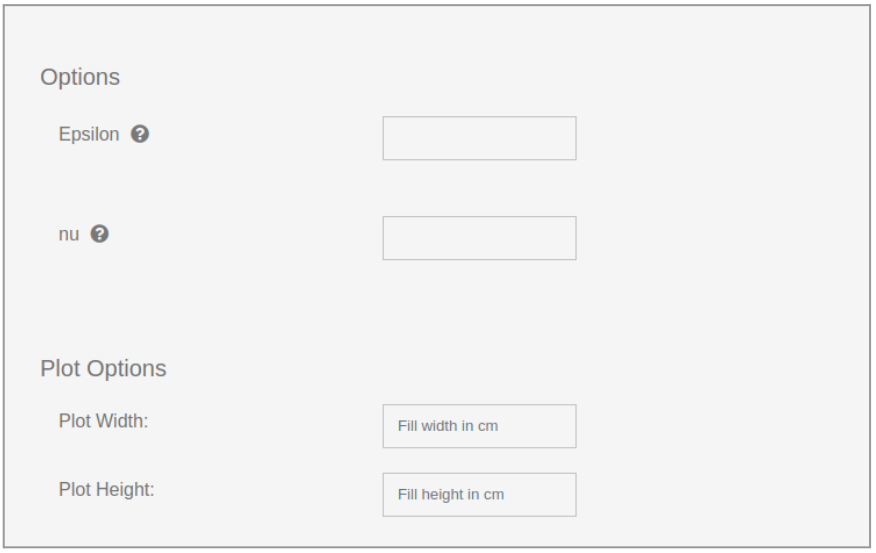
Click in the menu “InSyBio ncRNASeq” → “single-cell RNA-Seq Data Analysis” → “single-cell RNA-Seq Pipeline Dashboard”, select the “Add new job” button and then choose the “Deconvolve Data against single-cell RNA-seq Analysis” option. Then do the following steps:

- Upload your files of single-cell RNA-Seq Experiments Matrix, Features and Barcodes datasets.
- Upload your fastq or Read Count Biomarker files.



- Fill in the Epsilon integer, a small constant number used for convergence criteria.

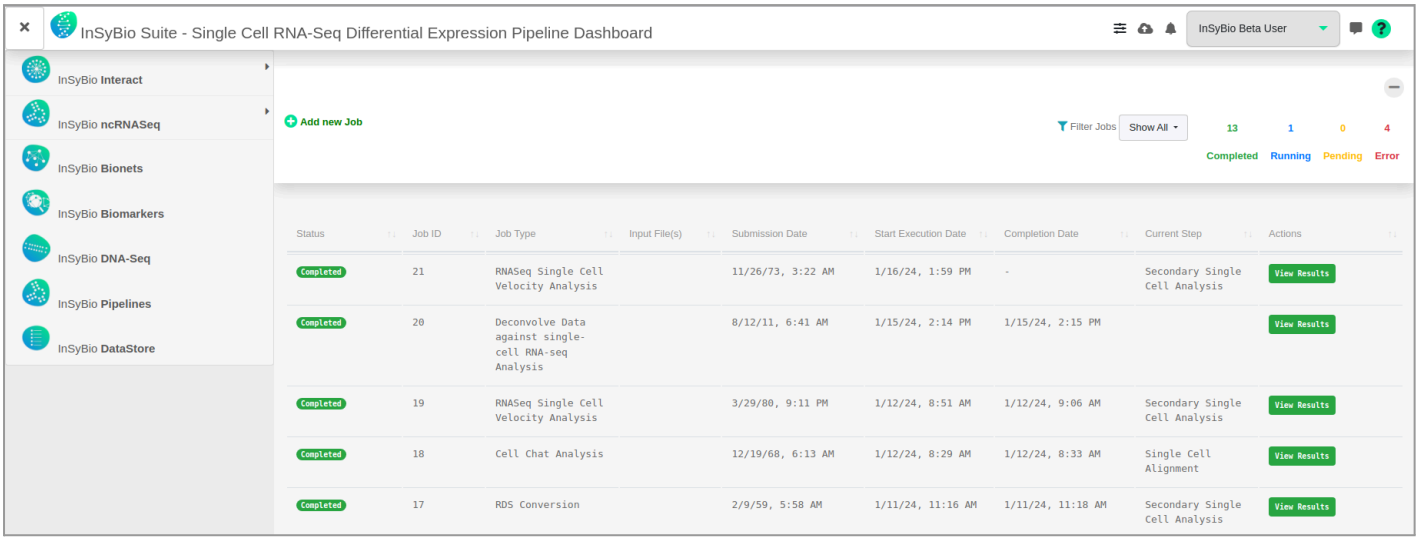
- Fill in the nu integer, a small constant number to facilitate the calculation of variance.
- Fill in the Plot options
    - Plot width
    - Plot height



- Submit your job pressing the respective button.

To view the results:

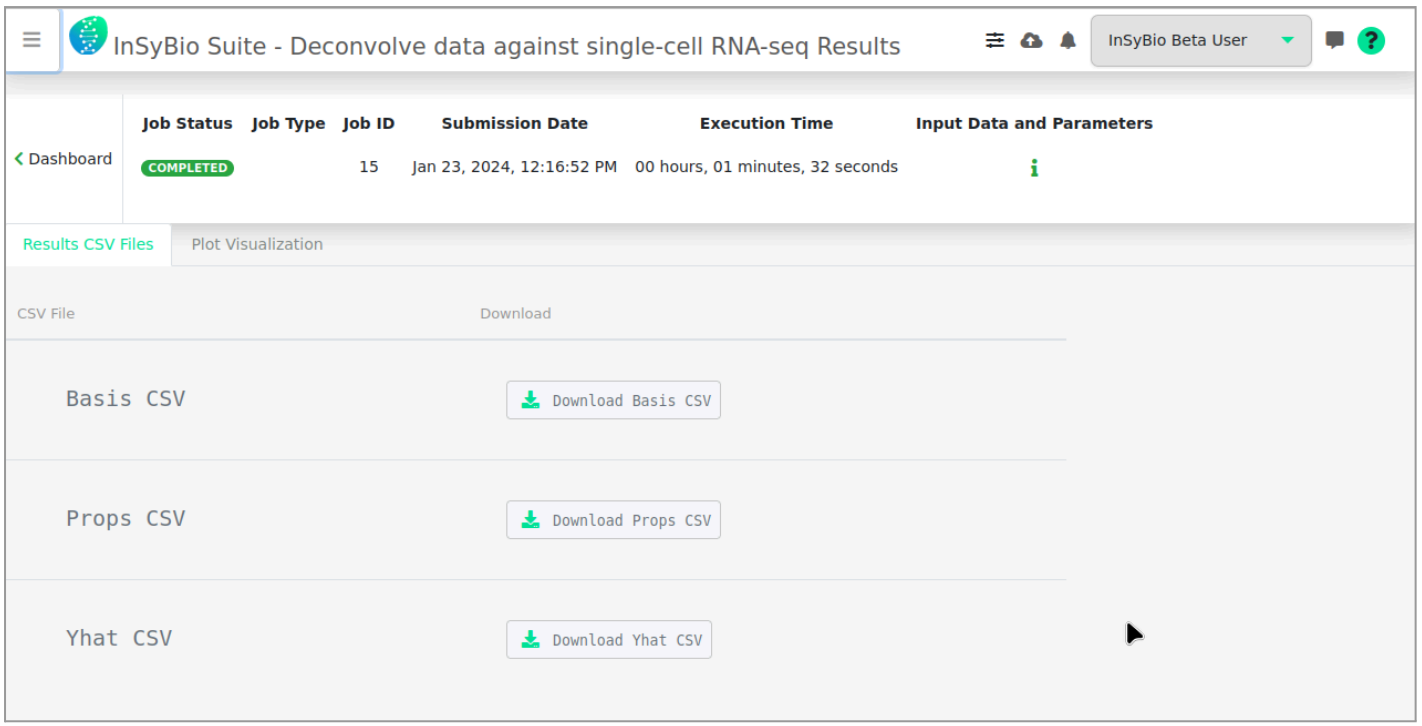
By starting a calculation you are informed if it was submitted successfully. Then you can move to the single-cell RNA-Seq Differential Expression Pipeline Dashboard, where you can view the status of your current and previous single-cell RNA-Seq Differential Expression jobs.

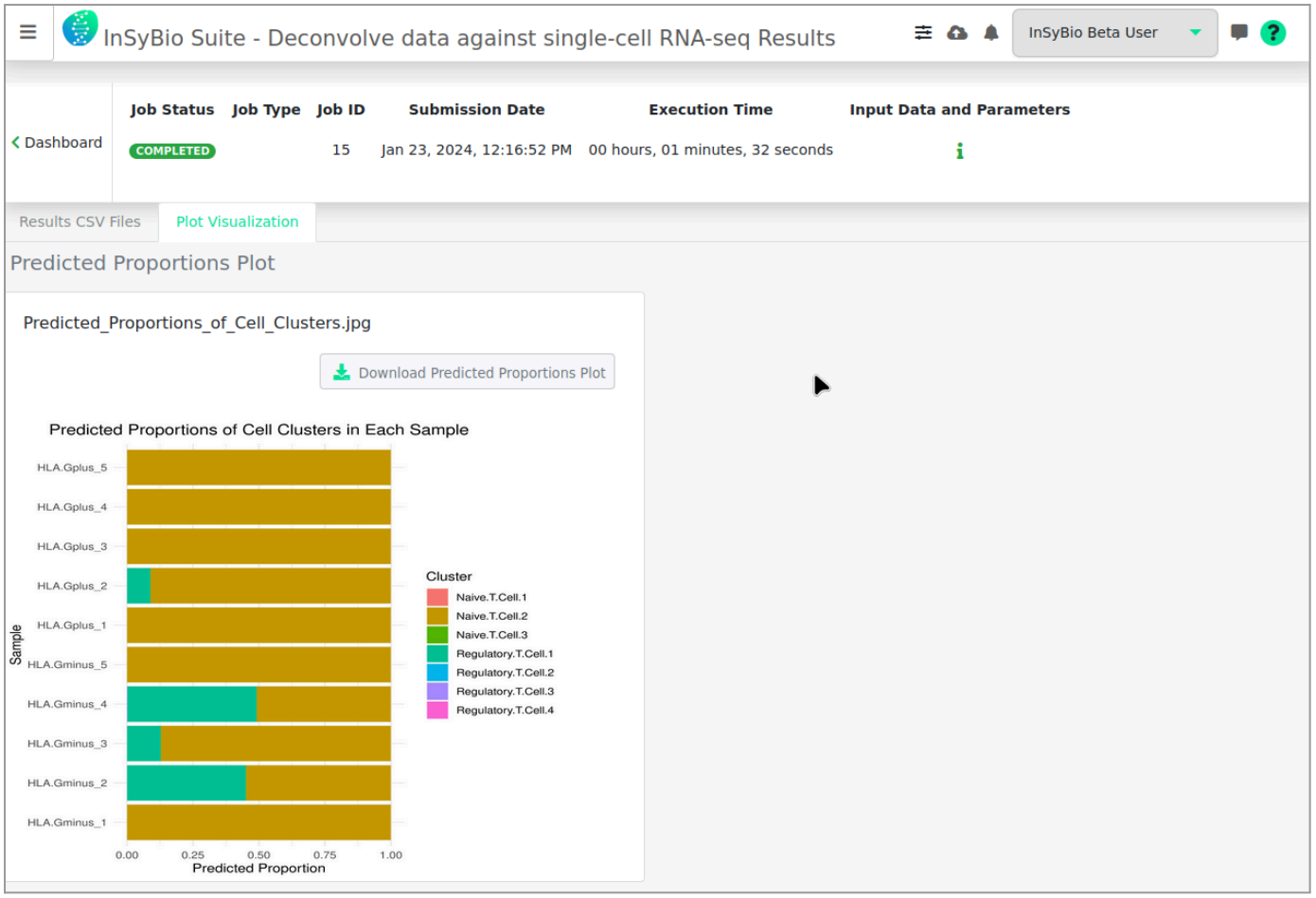


At completion of the Analysis you can select the View Results at the Actions column and view the produced files, that are separated according to the step that they were produced.

In the Results CSV tab, you can see the three generated csv files, basic.csv, yhat.csv and props1.csv. Props1 shows the predicted proportions of cell clusters in every sample, Yhat shows the predicted proportions of cell clusters in every gene and Basis represents the basis matrix.

At the Plots Visualization tab you can see the plot that is created. This plot represents the predicted proportions of cell clusters in every sample. This plot can be downloaded.


InSyBio Suite - Deconvolve data against single-cell RNA-seq Results
InSyBio Beta User
Dashboard
Job Status
Job Type
Job ID
Submission Date
Execution Time
Input Data and Parameters
COMPLETED
15
Jan 23, 2024, 12:16:52 PM
00 hours, 01 minutes, 32 seconds
Results CSV Files
Plot Visualization
CSV File
Download
Basis CSV
Download Basis CSV
Props CSV
Download Props CSV
Yhat CSV
Download Yhat CSV



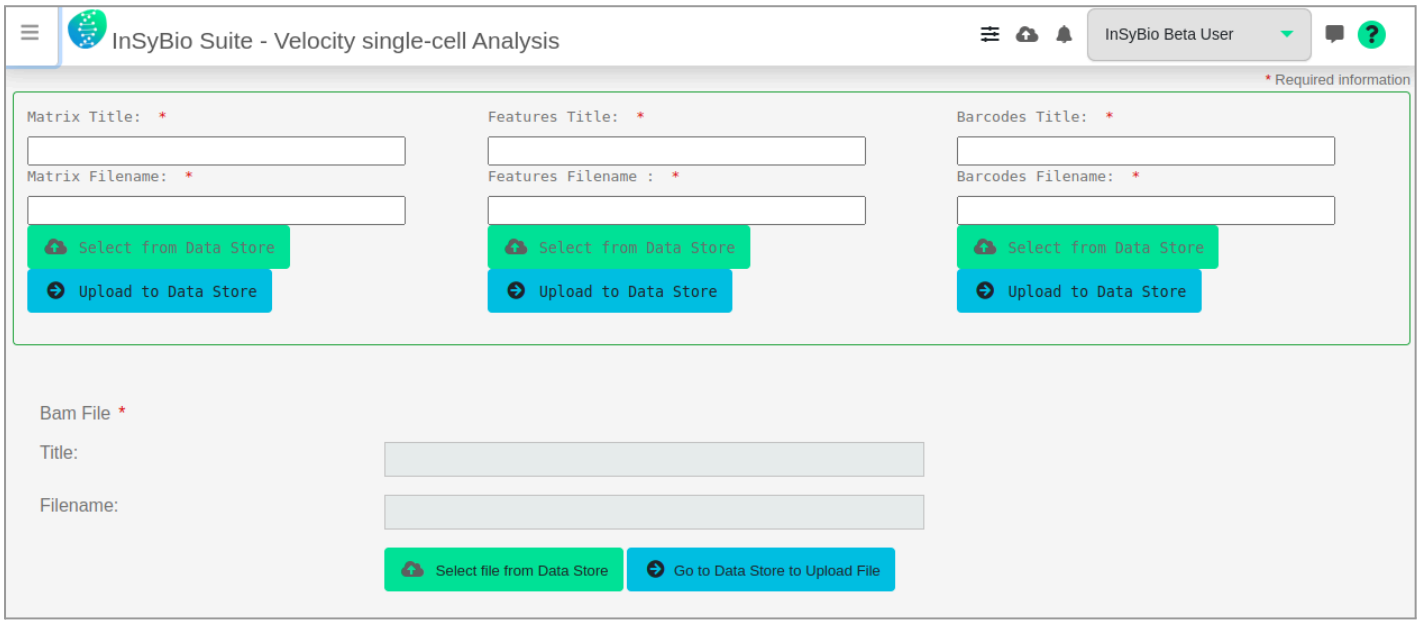
Velocity single-cell Analysis

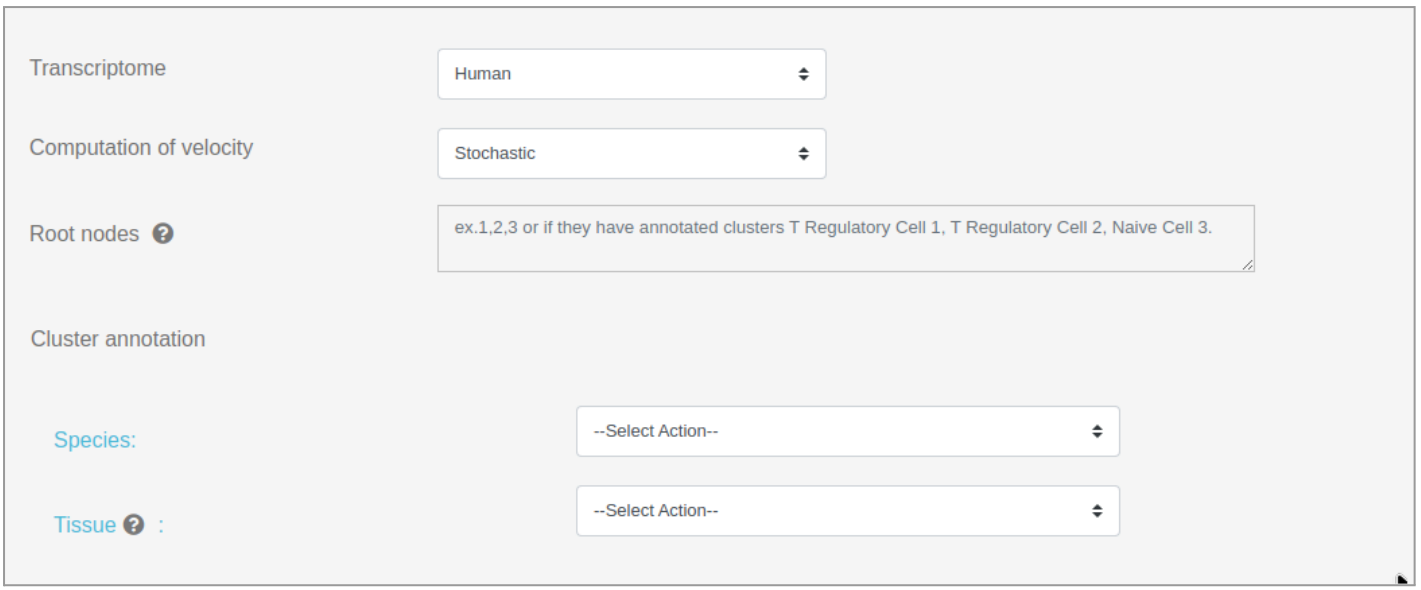
You can do the Velocity single-cell Analysis. Firstly, it is required to import the single-cell RNA-Seq 10X datasets, the Matrix, the Feature and the Barcodes datasets. This Pipeline uses the velocyto tool to estimate the RNA velocities of single-cells and the monocle3 and scvelo packages to identify trajectories and further analyse the estimated velocities.

To start the Velocity single-cell Analysis:

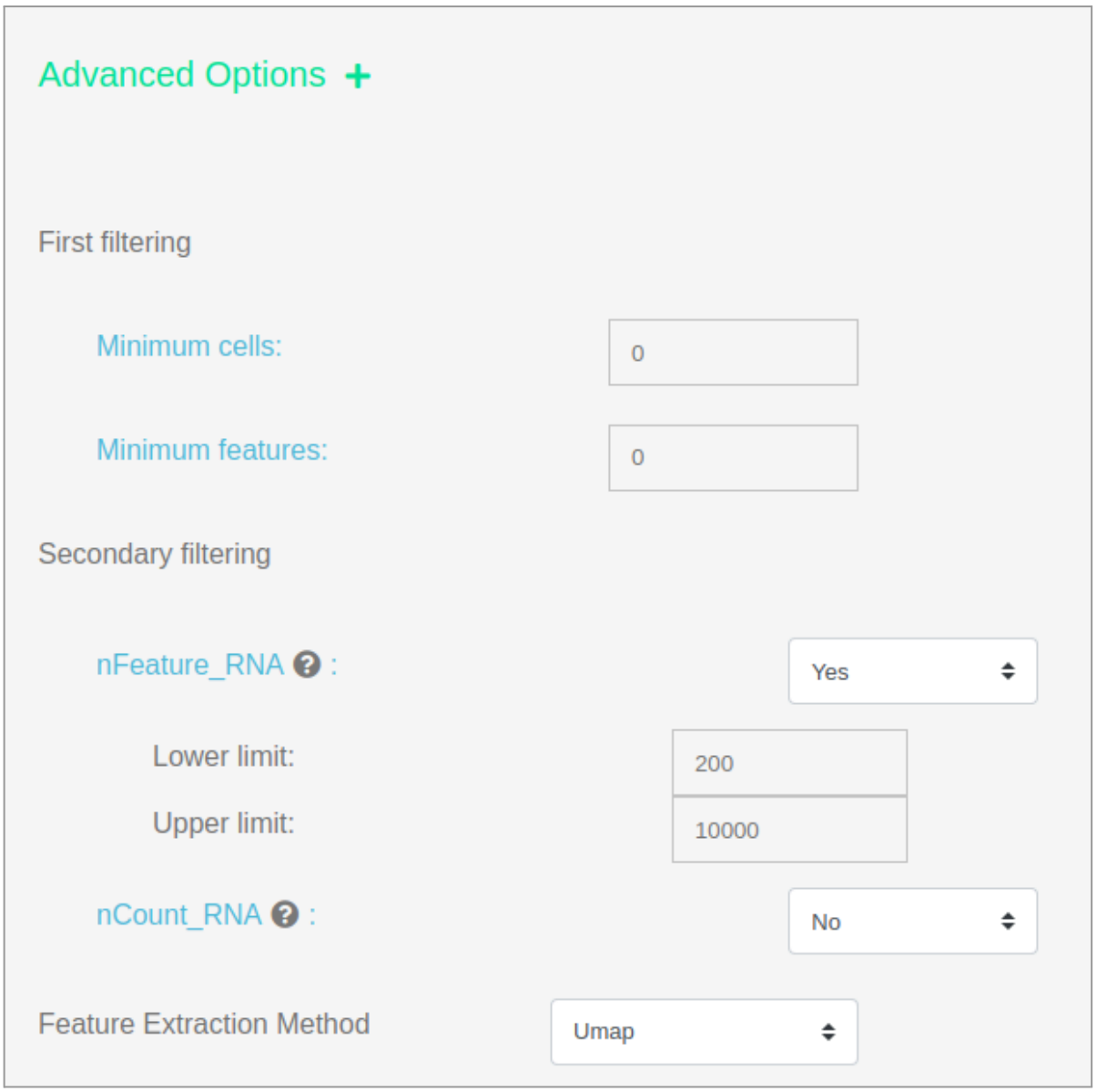
Click in the menu “InSyBio ncRNASeq” → “single-cell RNA-Seq Data Analysis” → “single-cell RNA-Seq Pipeline Dashboard”, select the “Add new job” button and then choose the “Velocity single-cell Analysis” option. Then do the following steps:

- Upload your files of single-cell RNA-Seq Experiments 10X Matrix, Features and Barcodes datasets.
- Select the transcriptome the input files belong to from our 3 built-in options (Human, Mouse, Human-mouse mixture).
- Select the computation type of velocity.
- Fill in the root nodes, because you need to specify the start of the trajectory, meaning the group (cluster) of cells which is undifferentiated at the beginning of the analysis.





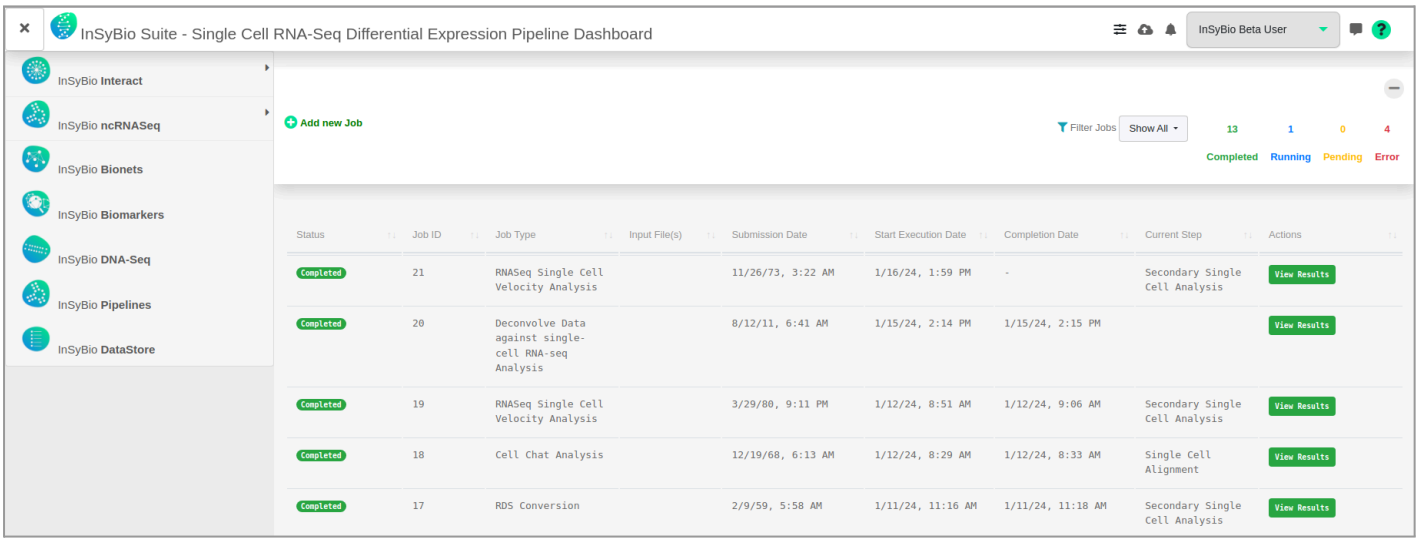
- Select if you want to manually configure other parameters of the job. If you don't, our Default Options will be applied. Possible manual options are:
    - First filtering:
        - Minimum cells
        - Minimum features
    - Secondary filtering:
        - nFeature_RNA with lower and upper limits
        - nCount_RNA with lower and upper limits
    - Feature Extraction Method
    - Shared Nearest Neighbor (SNN) Graph
        - K parameter (k-nearest- neighbor)
    - Clustering
        - Resolution parameter
        - Threshold (logfc)
        - Minimum Pct


Advanced Options +
First filtering
Minimum cells:
0
Minimum features:
0
Secondary filtering
nFeature_RNA :
Yes
Lower limit:
200
Upper limit:
10000
nCount_RNA :
No
Feature Extraction Method
Umap

- Submit your job pressing the respective button.

To view the results:

By starting a calculation you are informed if it was submitted successfully. Then you can move to the single-cell RNA-Seq Differential Expression Pipeline Dashboard, where you can view the status of your current and previous single-cell RNA-Seq Differential Expression jobs.

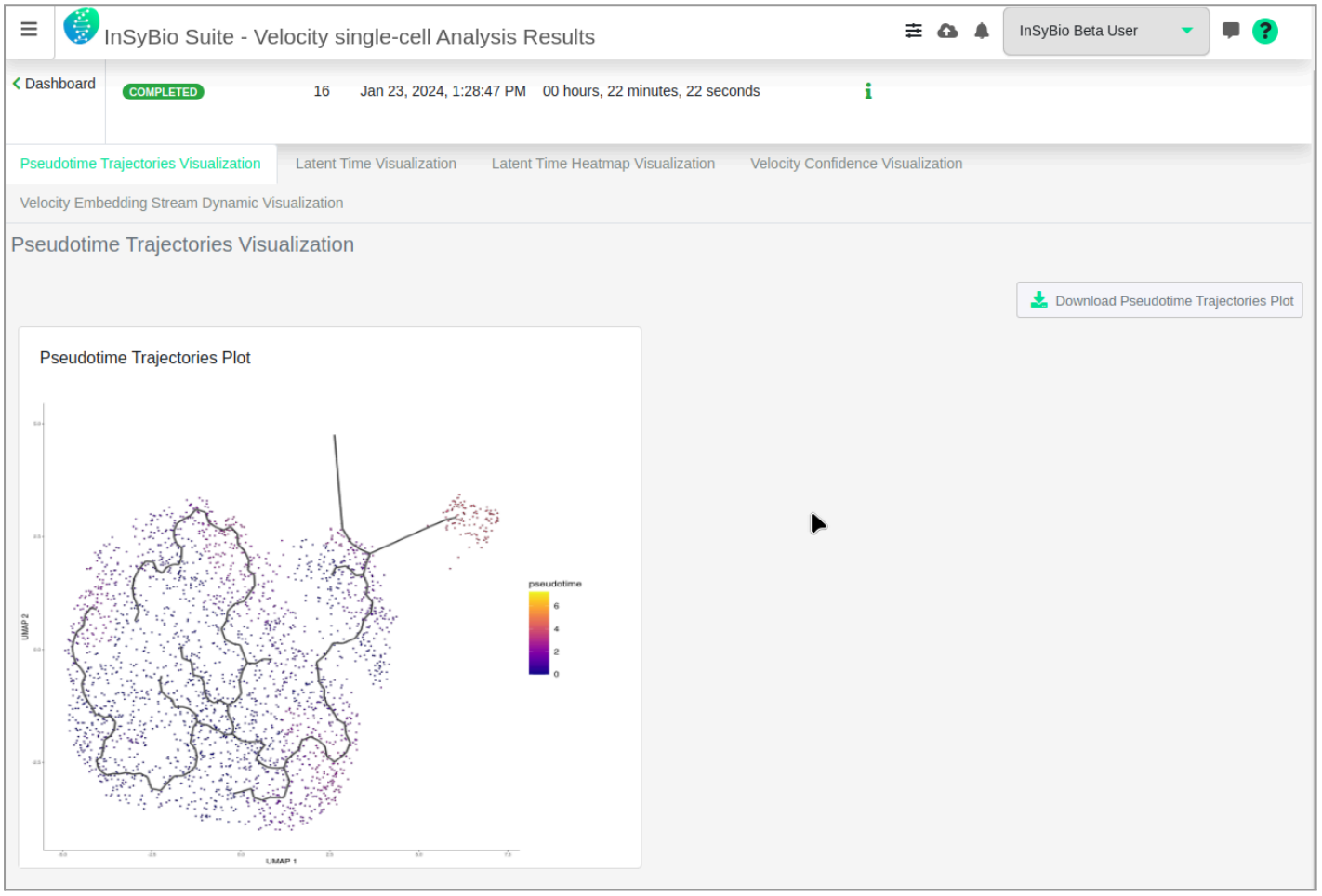


At completion of the Analysis you can select the View Results at the Actions column and view the produced files, that are separated according to the step that they were produced.

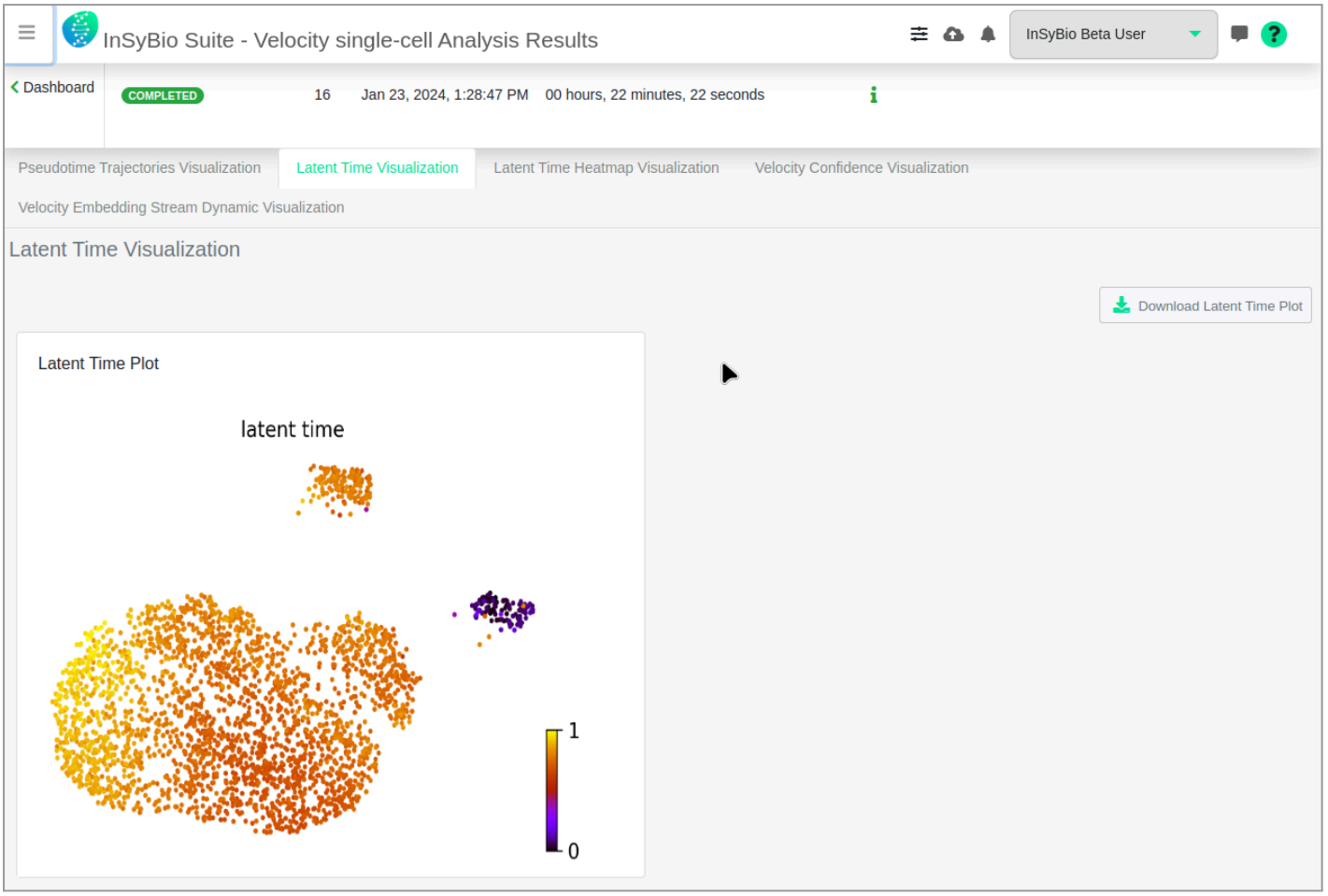
Depending on the computation type of velocity you selected, different tabs will appear.

For dynamic analysis of velocity, five different tabs are present, each one representing a different step in the analysis and a produced plot.

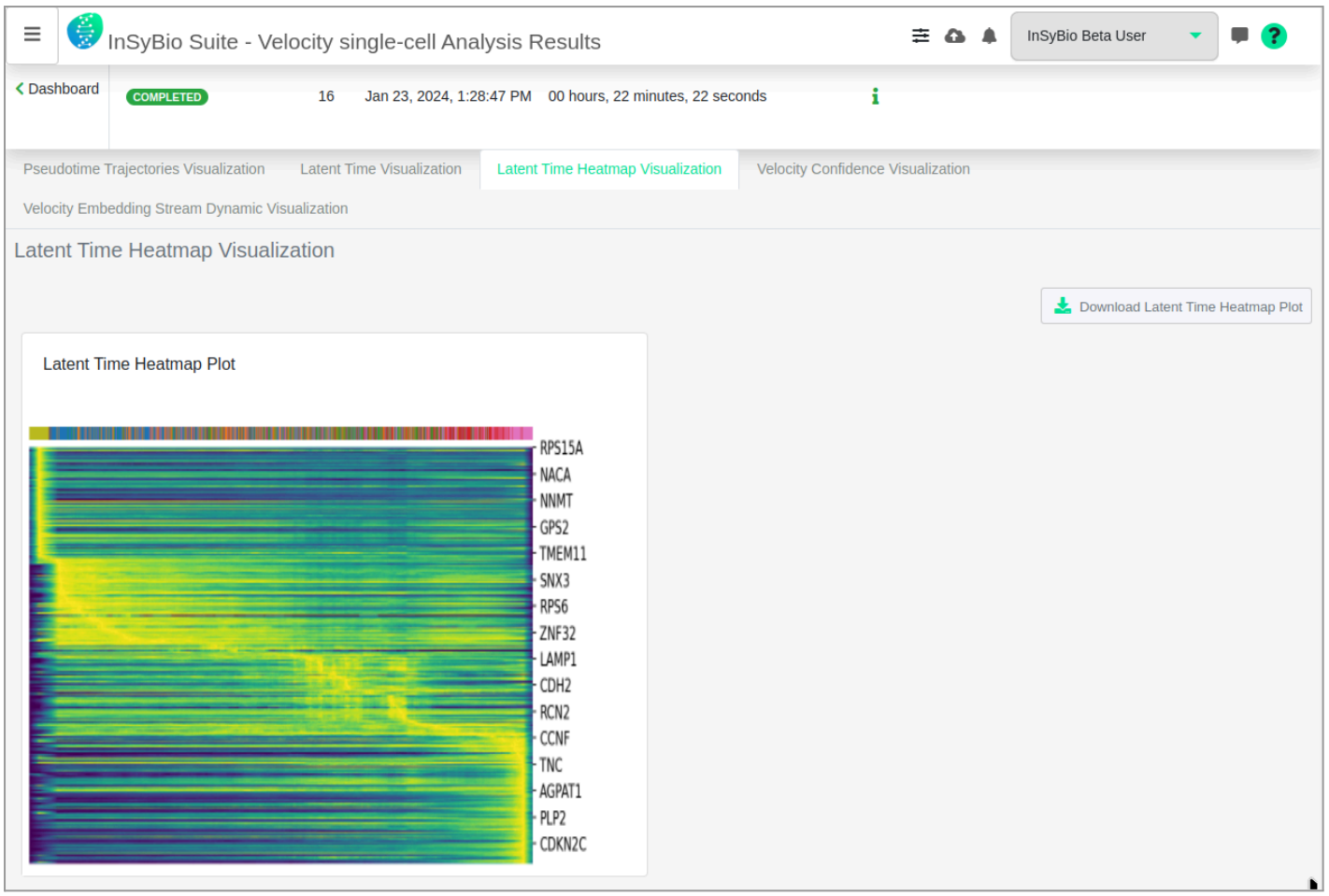
In the Pseudotime Trajectories Visualization tab, the plot visualizes the pseudotime trajectories calculated by monocle3.



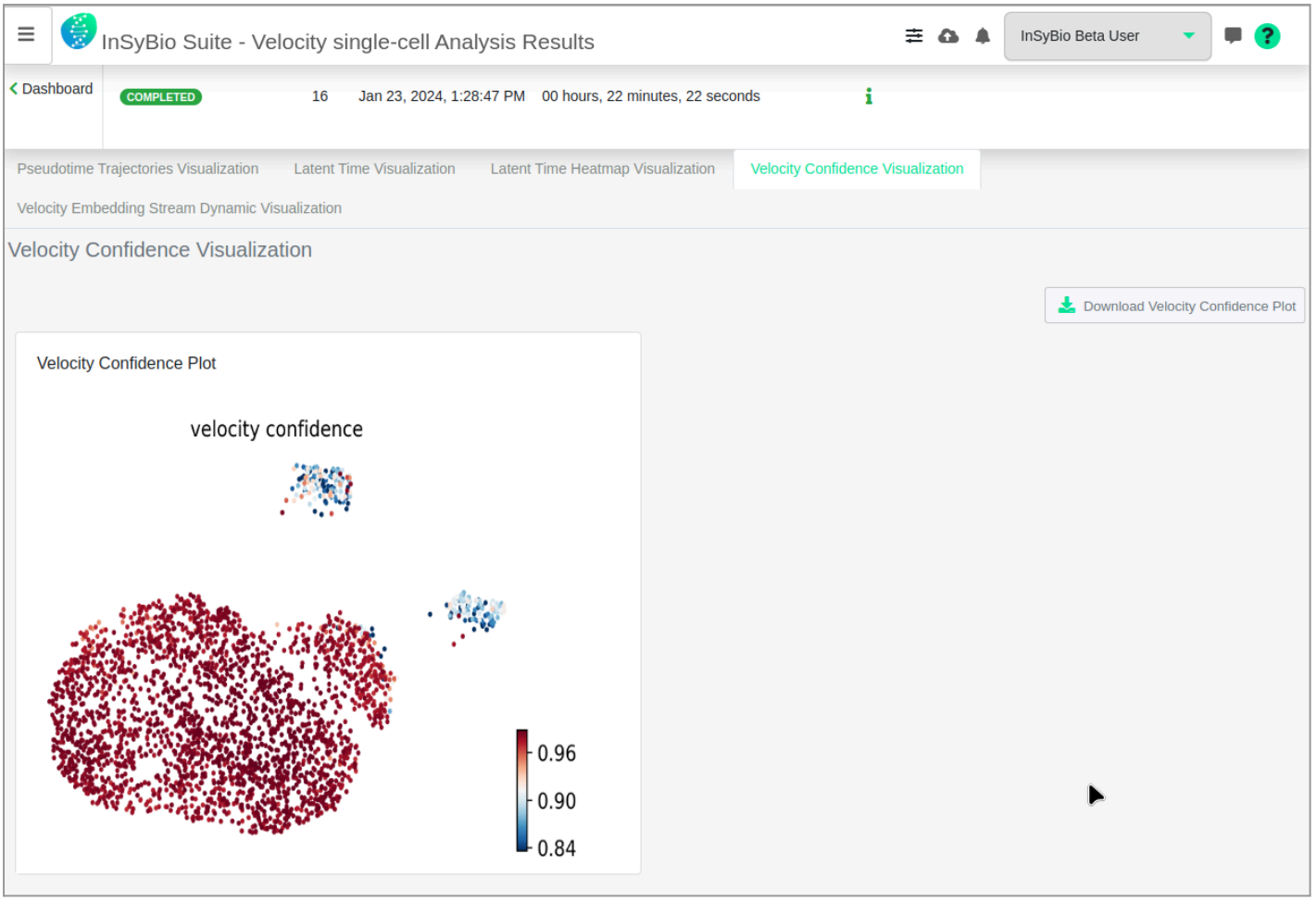
In the Latent time VIsualization tab, the plot represents the latent time of the underlying cellular processes, an approximation of the real time experienced by cells as they differentiate.



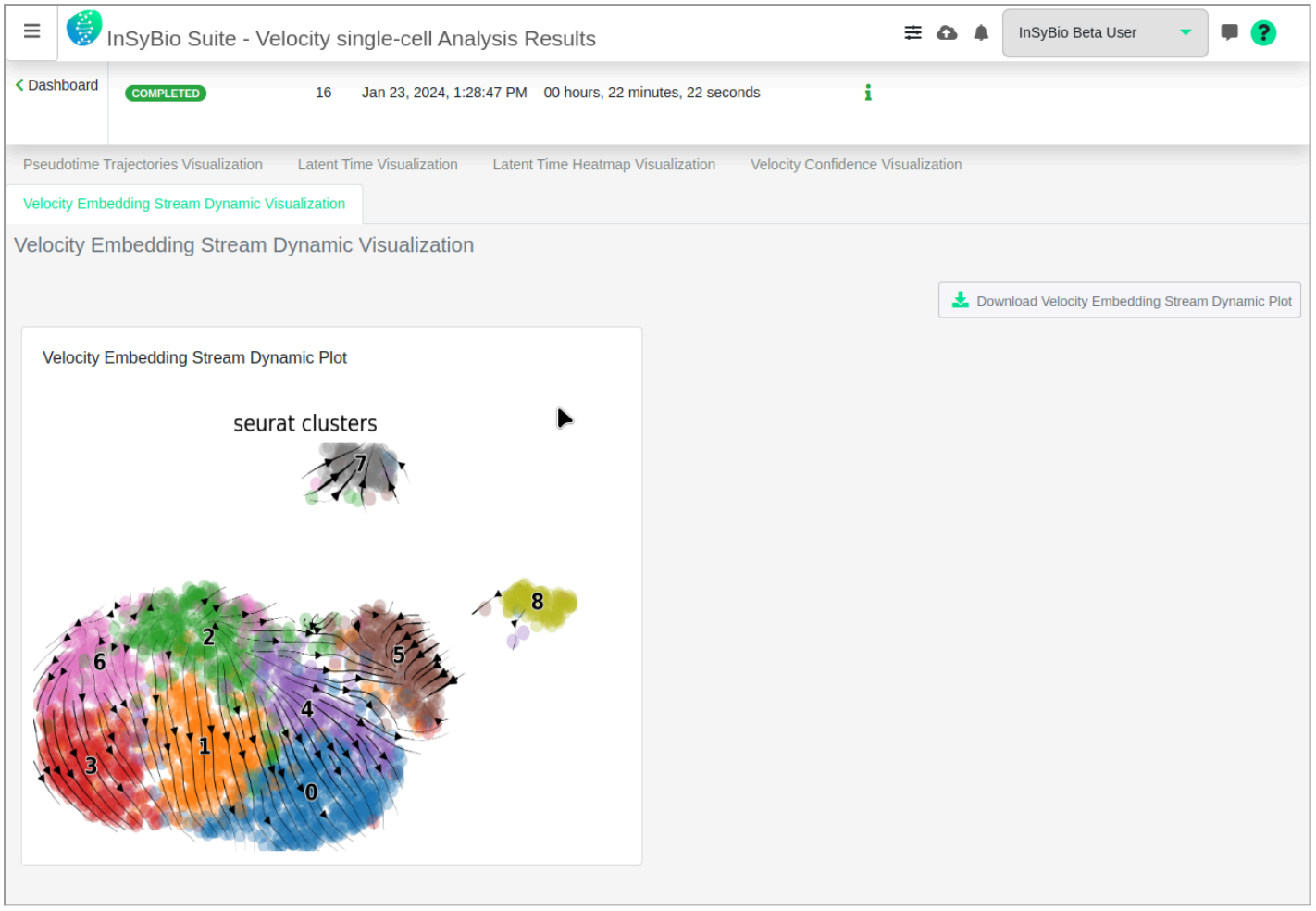
In the Latent time heatmap Visualization tab, the plot represents the latent time heatmap of the top genes.



In the Velocity confidence Visualization tab, the plot represents the computation confidences of velocities.



In the Velocity embedding stream dynamic Visualization tab, the plot visualizes the dynamic stream of velocities.



These plots can also be downloaded individually.

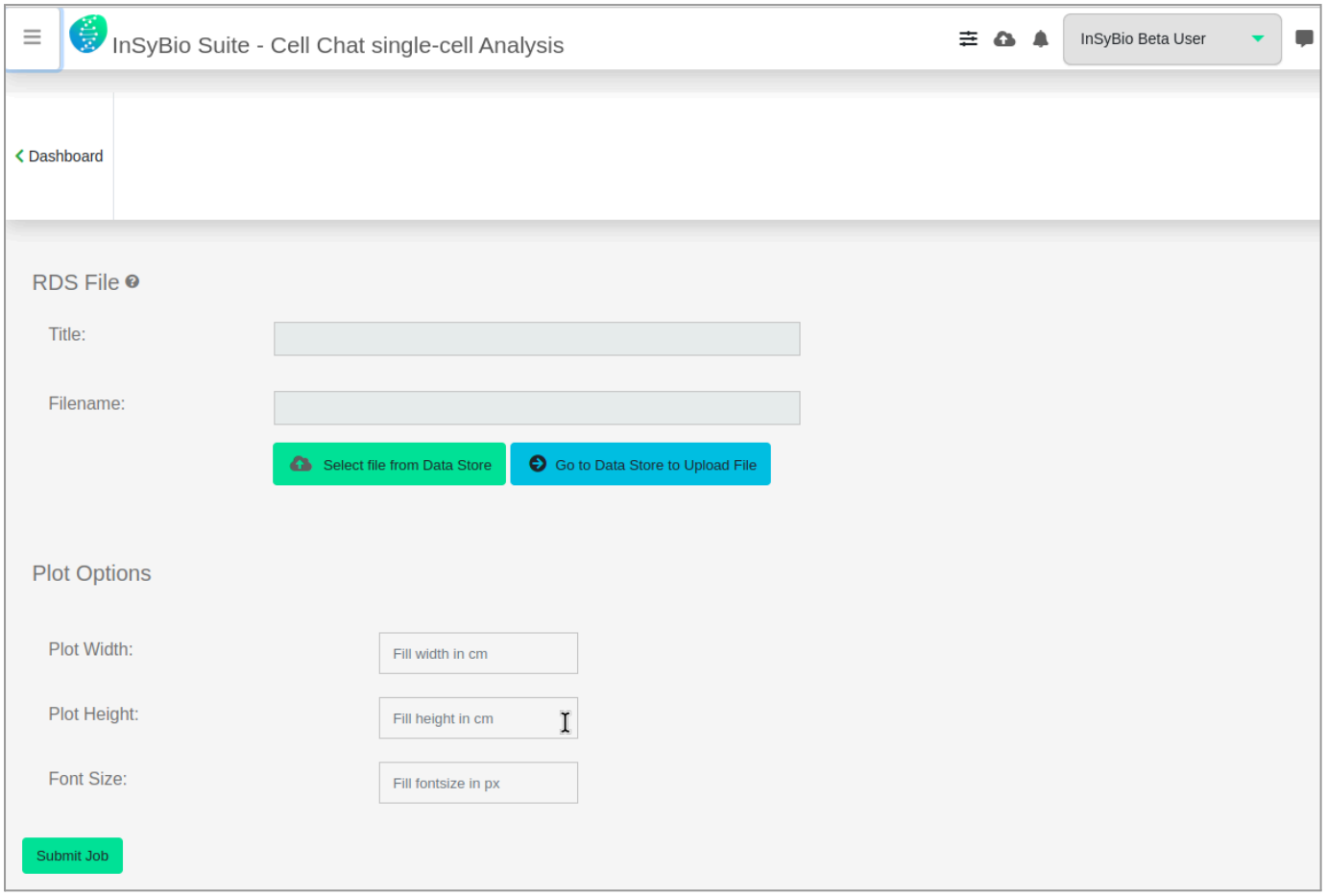
Cell Chat single-cell Analysis

You can do the Cell Chat single-cell Analysis. Firstly, it is required to import the single-cell seurat rds dataset. This pipeline uses the CellChat R toolkit to visualize cell-cell communication from single-cell data.

To start the Cell Chat single-cell pipeline:

Click in the menu “InSyBio ncRNASeq” → “single-cell RNA-Seq Data Analysis” → “ single-cell RNA-Seq Pipeline Dashboard” , select the “Add new job” button and then choose the “Cell Chat single-cell Analysis” option. Then do the following steps :

- Upload your seurat object file (.rds format) file, which should already have annotated clusters. These annotations should be accessible by reading the output of the levels function on this object.
- Select if you want to manually configure the plot parameters of the job. If you don’t, our Default Options will be applied. Possible manual options are:
    - Plot width
    - Plot height
    - Plot fontsize



- Submit your job pressing the respective button.

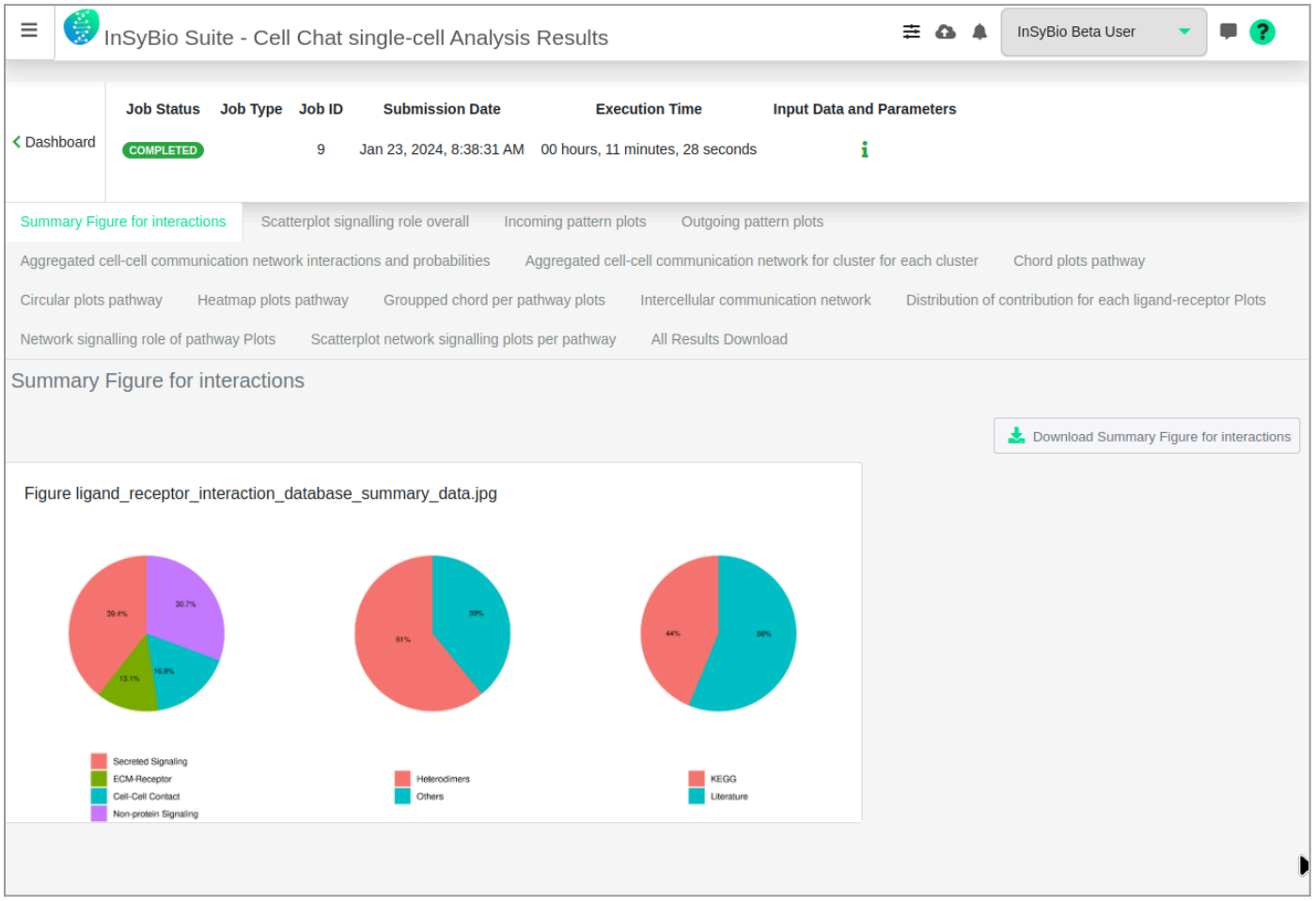
To view the results:

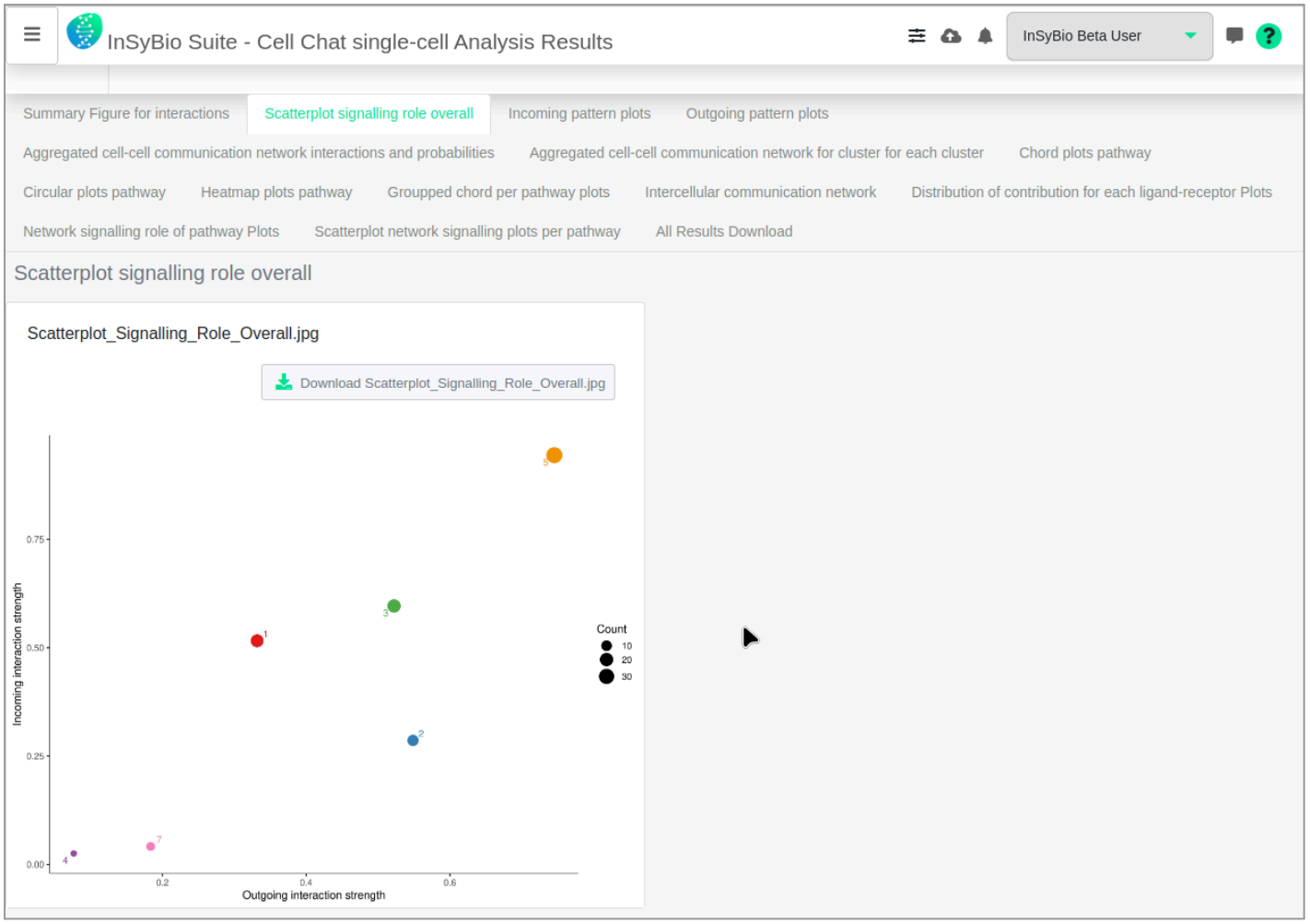
By starting a calculation you are informed if it was submitted successfully. Then you can move to the single-cell RNA-Seq Differential Expression Pipeline Dashboard, where you can view the status of your current and previous single-cell RNA-Seq Differential Expression jobs.

After the analysis, you can select the View Results in the Actions column and view the produced files, that are separated according to the step that they were produced.

Fourteen different result tabs are present, each of which represents a different analysis performed on the Seurat object. Below a representative example of each tab will be shown.

At the All Results Download tab, all the results of your job can be downloaded.




InSyBio Suite - Cell Chat single-cell Analysis Results
InSyBio Beta User
Summary Figure for interactions
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Incoming pattern plots
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Aggregated cell-cell communication network interactions and probabilities
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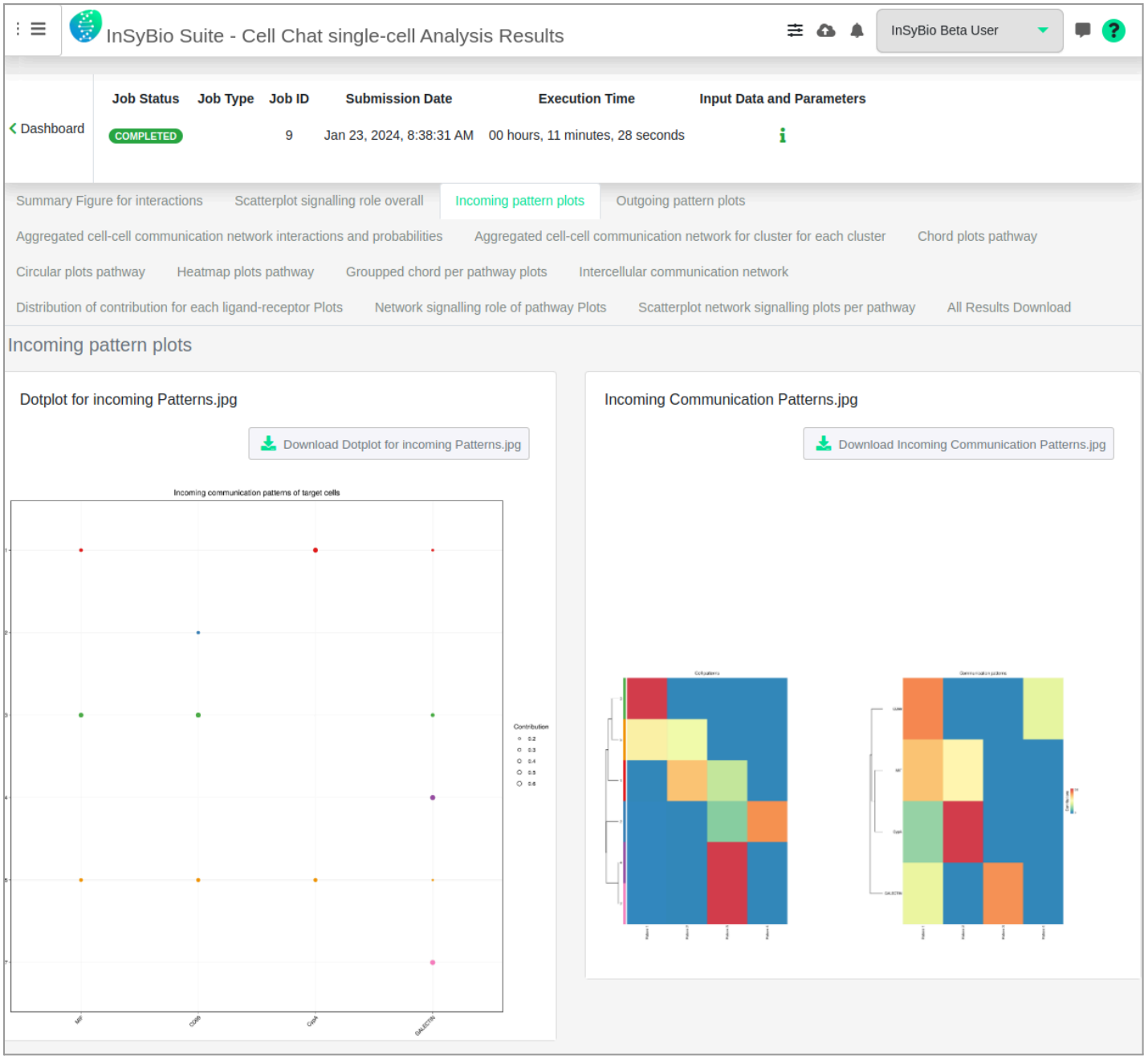
Circular plots pathway
Heatmap plots pathway
Groupped chord per pathway plots
Intercellular communication network
Distribution of contribution for each ligand-receptor Plots
Network signalling role of pathway Plots
Scatterplot network signalling plots per pathway
All Results Download
Scatterplot signalling role overall
Scatterplot_Signalling_Role_Overall.jpg
Download Scatterplot_Signalling_Role_Overall.jpg
Incoming interaction strength
Outgoing interaction strength
Count
10
20
30


InSyBio Suite - Cell Chat single-cell Analysis Results
InSyBio Beta User
Dashboard
Job Status
Job Type
Job ID
Submission Date
Execution Time
Input Data and Parameters
COMPLETED
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Jan 23, 2024, 8:38:31 AM
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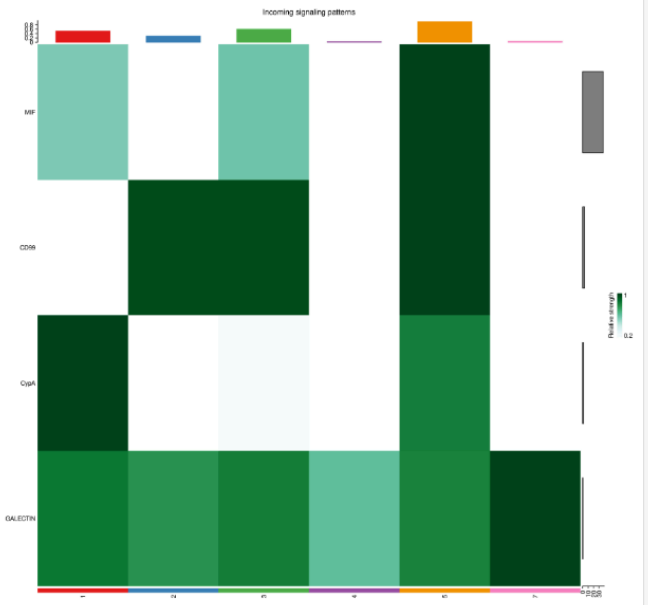
Scatterplot network signalling plots per pathway
All Results Download
Incoming pattern plots
Dotplot for incoming Patterns.jpg
Download Dotplot for incoming Patterns.jpg
Incoming communication patterns of target cells
Contribution
Incoming Communication Patterns.jpg
Download Incoming Communication Patterns.jpg

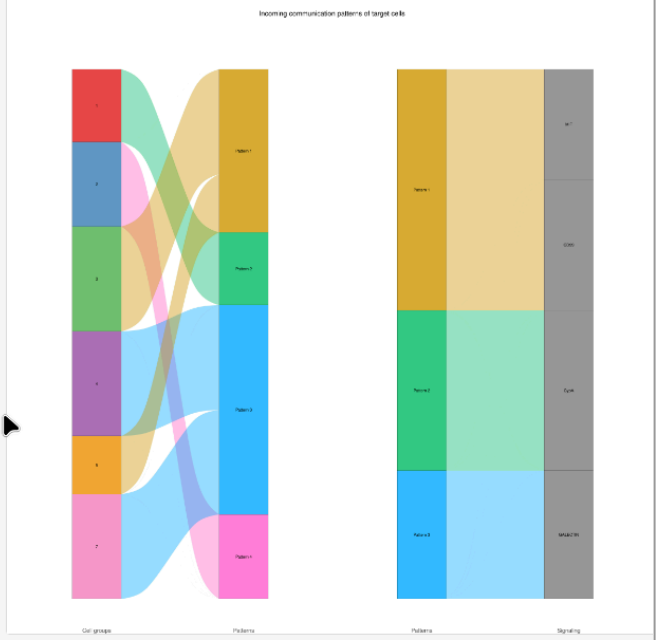
Incoming_signal_patterns.jpg

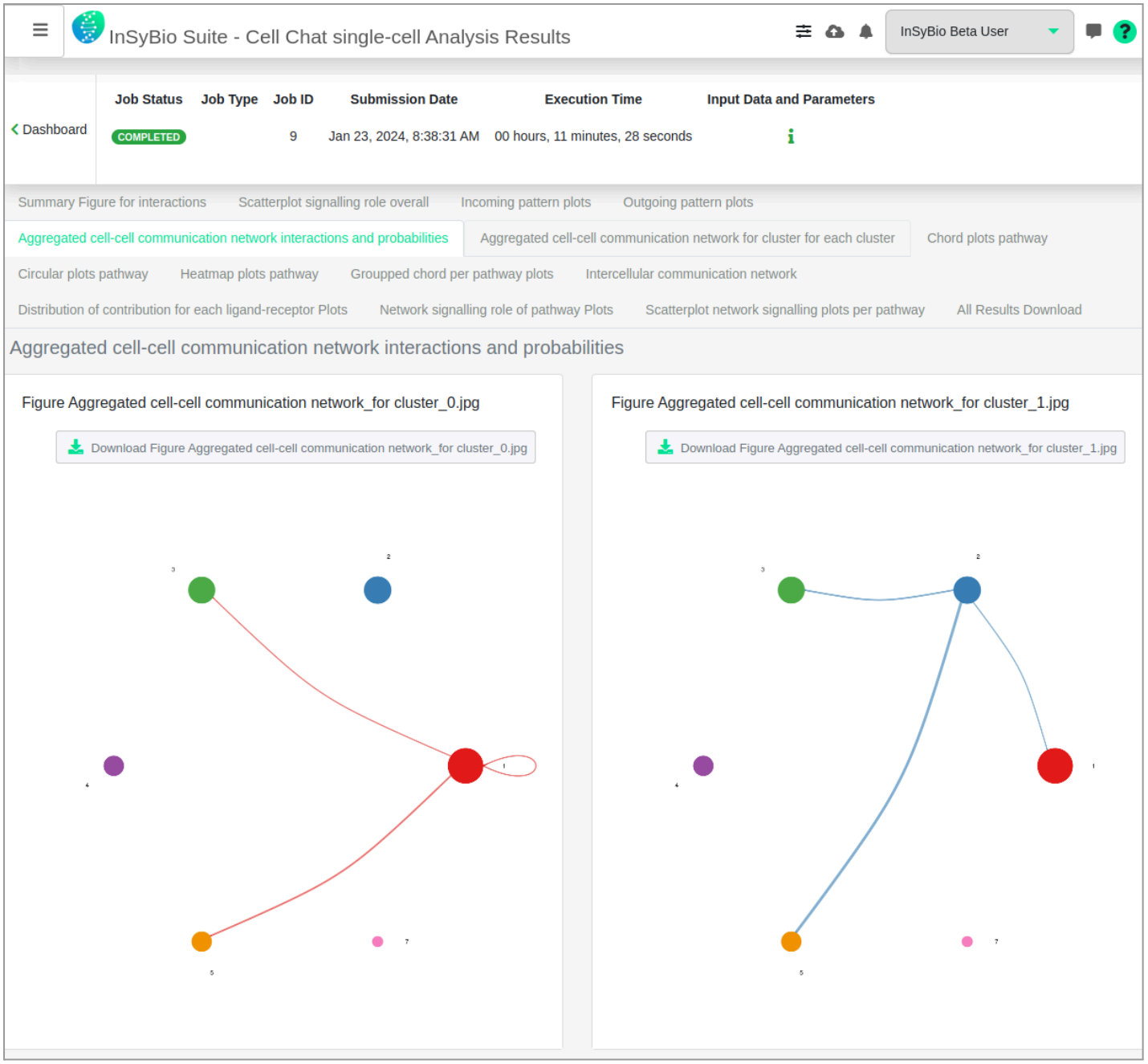
Download Incoming_signal_patterns.jpg



RiverPlot for Incoming Patterns.jpg

Download RiverPlot for Incoming Patterns.jpg




InSyBio Suite - Cell Chat single-cell Analysis Results
InSyBio Beta User
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Input Data and Parameters
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Intercellular communication network
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Network signalling role of pathway Plots
Scatterplot network signalling plots per pathway
All Results Download
Aggregated cell-cell communication network interactions and probabilities
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Figure Aggregated cell-cell communication network_for cluster_1.jpg
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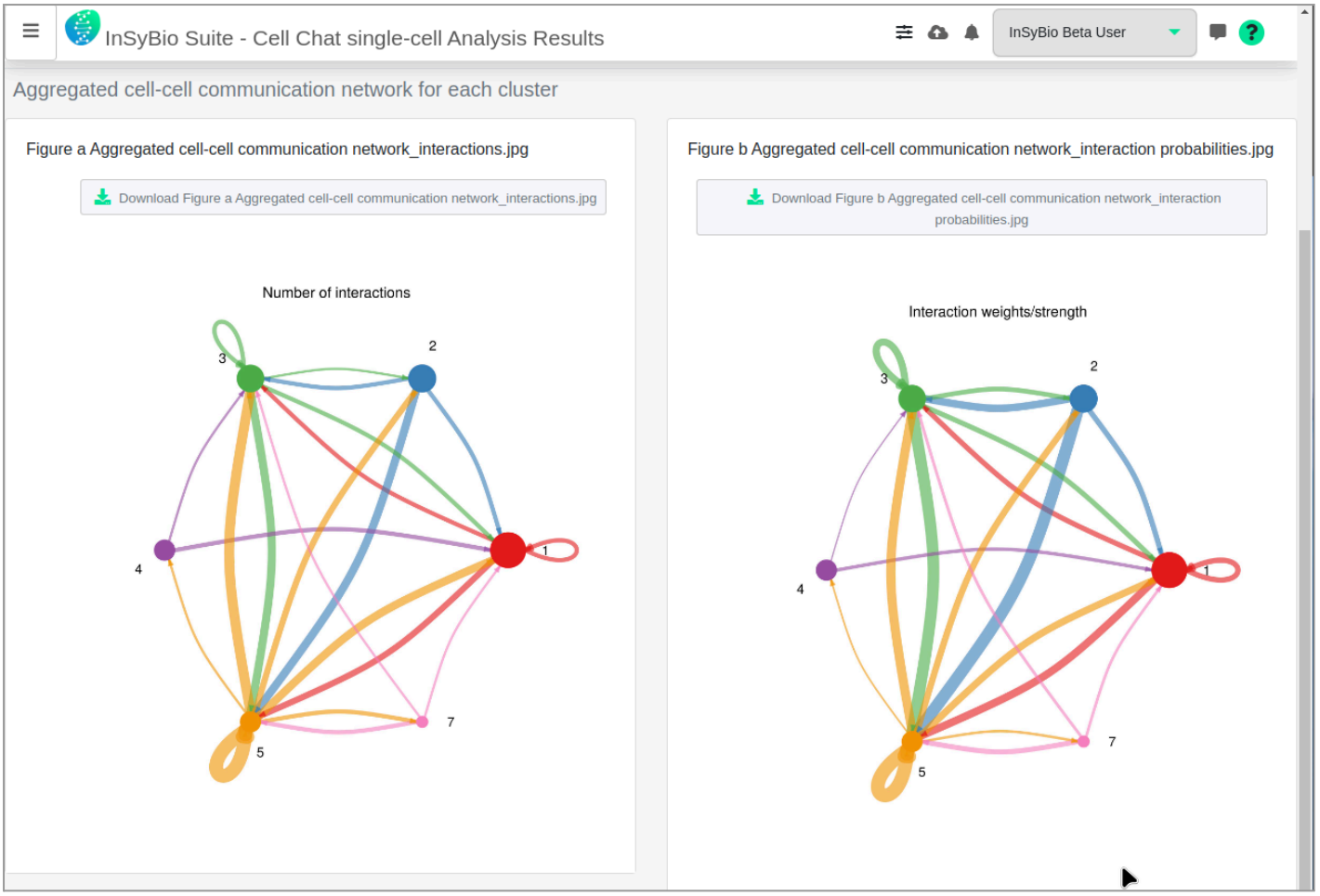
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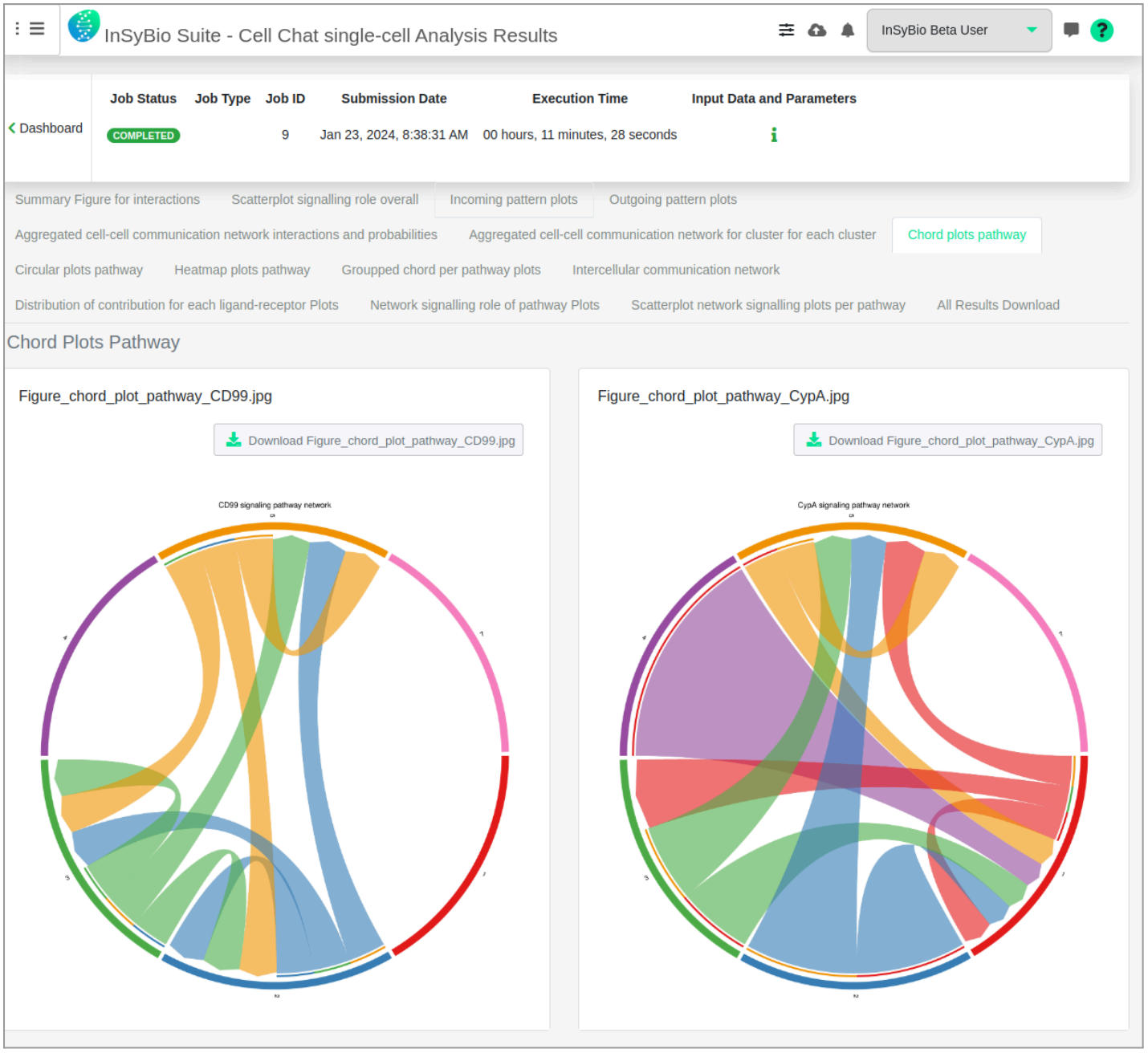
InSyBio Beta User
Aggregated cell-cell communication network for each cluster
Figure a Aggregated cell-cell communication network_interactions.jpg
Download Figure a Aggregated cell-cell communication network_interactions.jpg
Number of interactions
Figure b Aggregated cell-cell communication network_interaction probabilities.jpg
Download Figure b Aggregated cell-cell communication network_interaction probabilities.jpg
Interaction weights/strength


InSyBio Suite - Cell Chat single-cell Analysis Results
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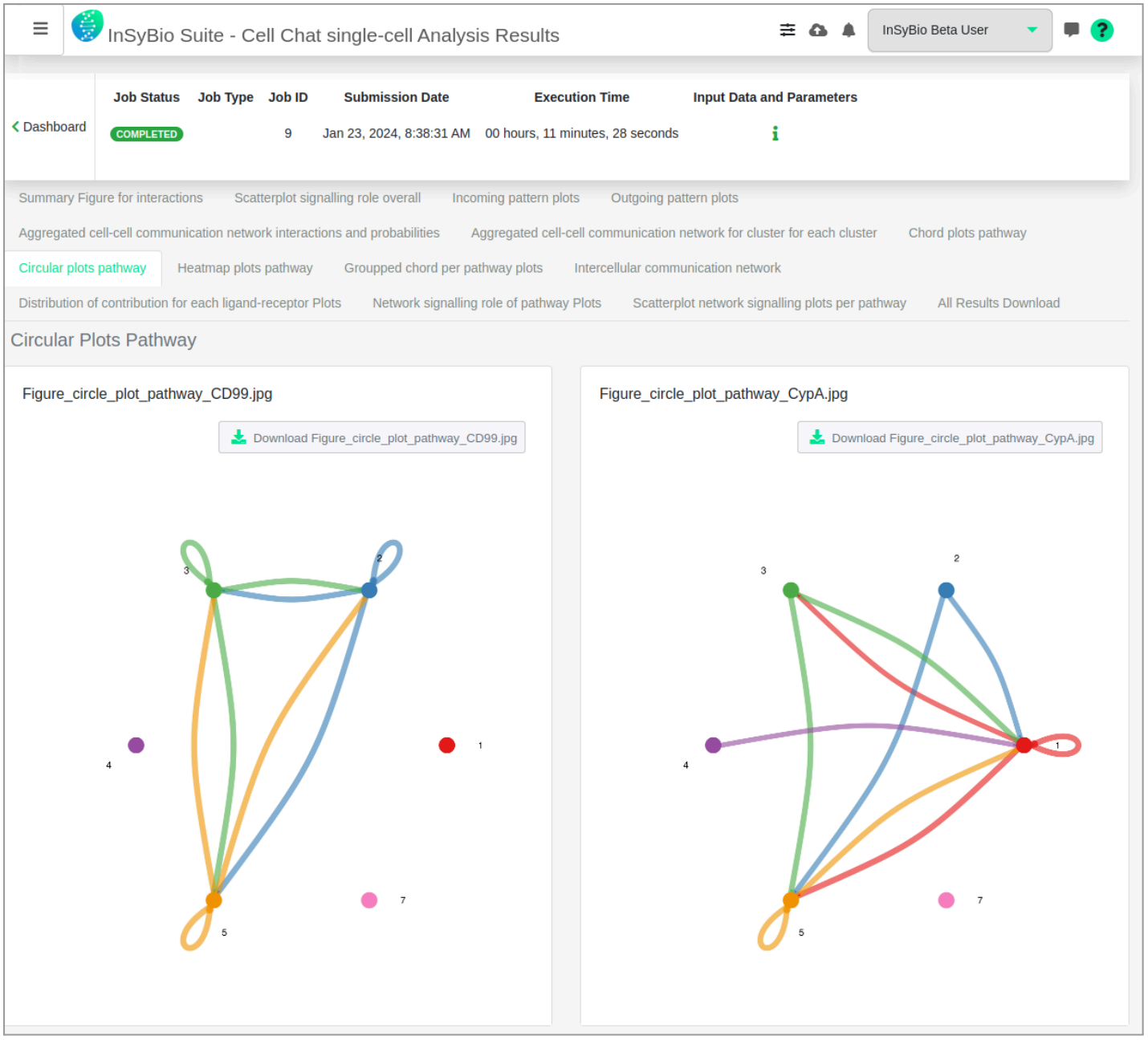
Chord Plots Pathway
Figure_chord_plot_pathway_CD99.jpg
Download Figure_chord_plot_pathway_CD99.jpg
CD99 signaling pathway network
Figure_chord_plot_pathway_CypA.jpg
Download Figure_chord_plot_pathway_CypA.jpg
CypA signaling pathway network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Circular Plots Pathway
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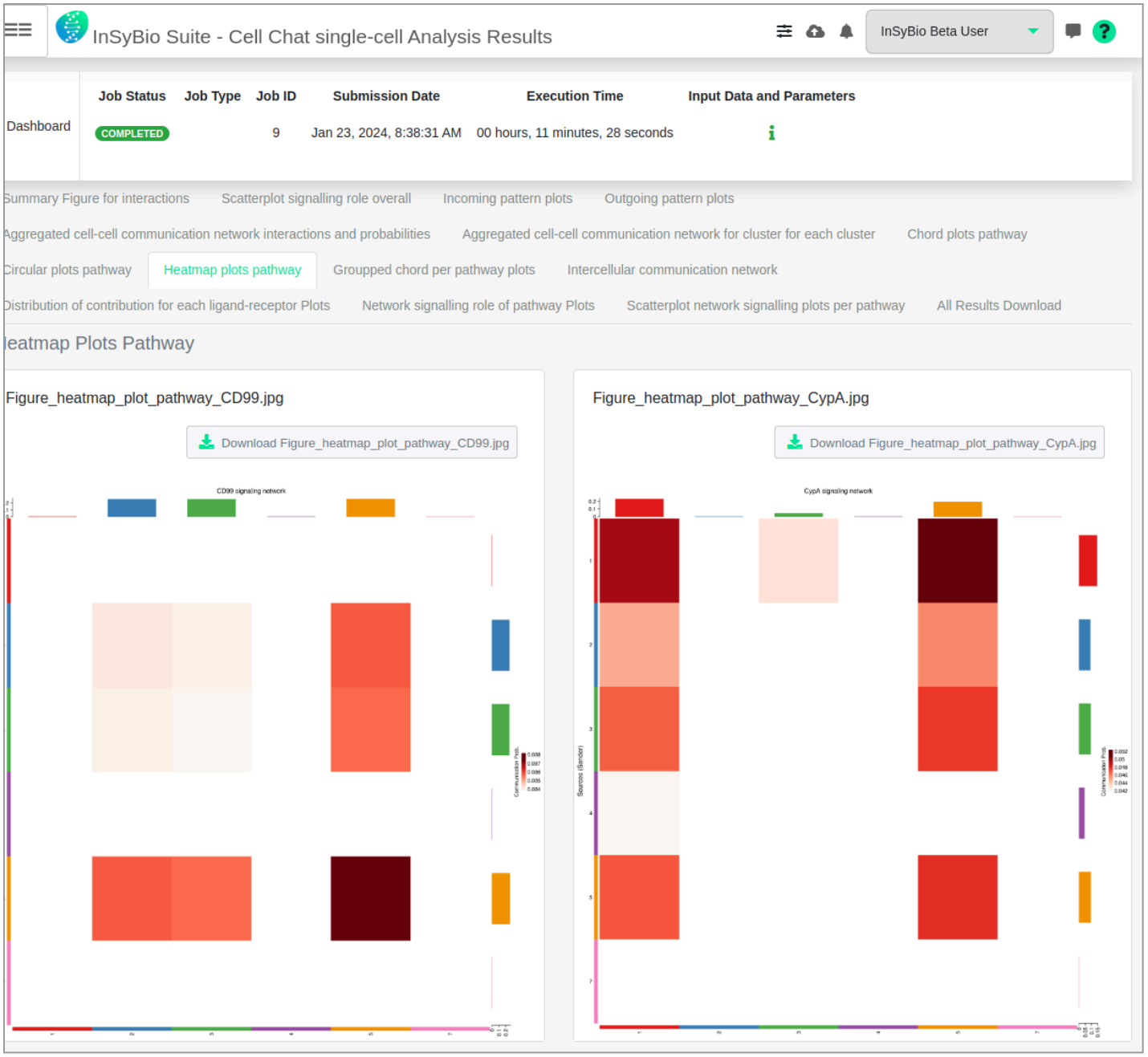
Figure_circle_plot_pathway_CypA.jpg
Download Figure_circle_plot_pathway_CypA.jpg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CD99 signaling network
Figure_heatmap_plot_pathway_CypA.jpg
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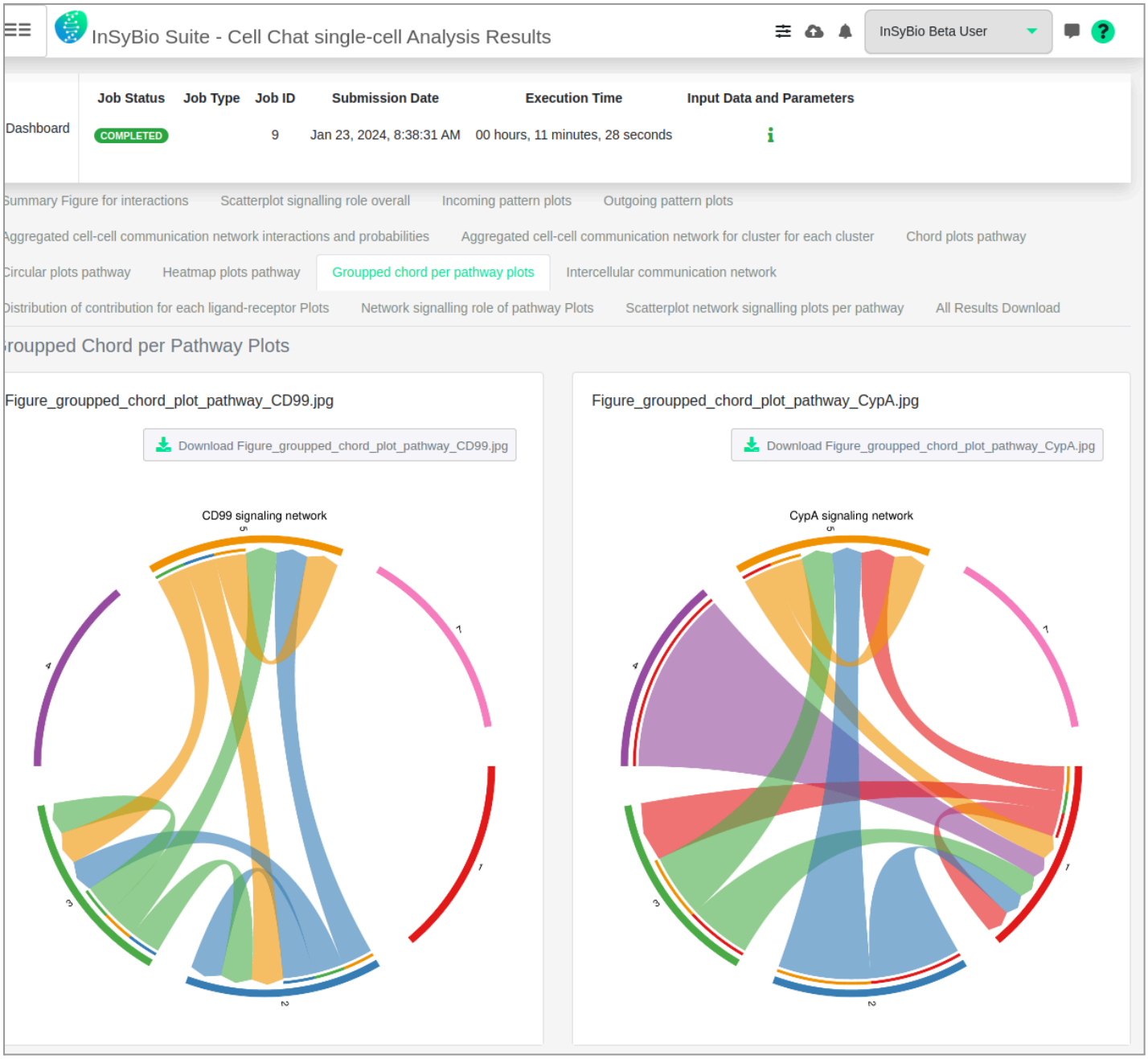
CypA signaling network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CD99 signaling network
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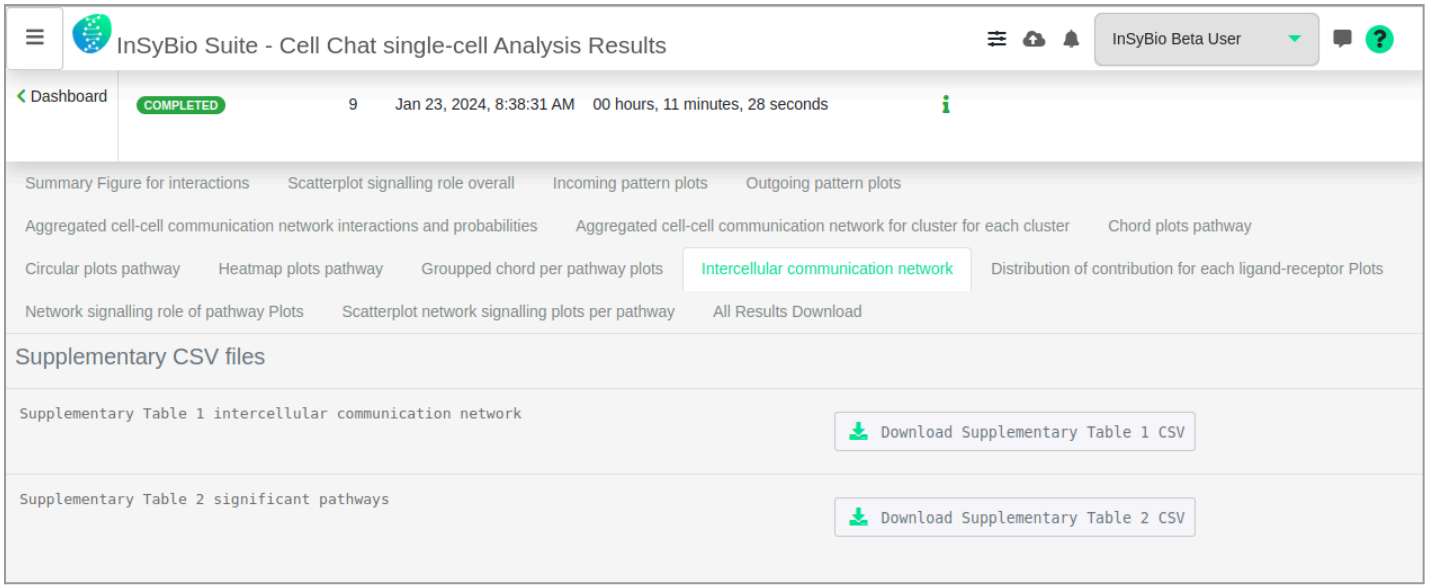
CypA signaling network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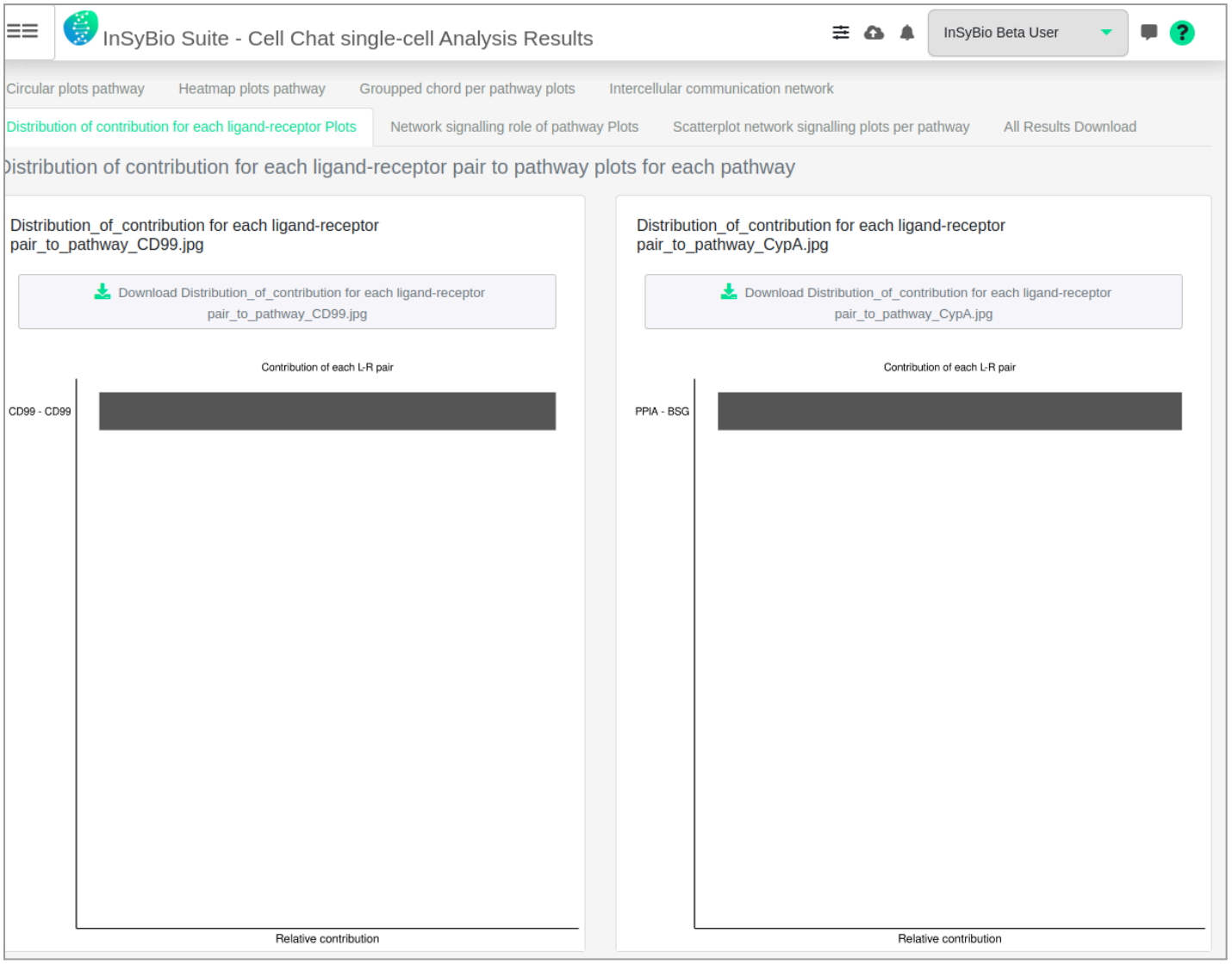
Scatterplot network signalling plots per pathway
All Results Download
Supplementary CSV files
Supplementary Table 1 intercellular communication network
Download Supplementary Table 1 CSV
Supplementary Table 2 significant pathways
Download Supplementary Table 2 CSV


InSyBio Suite - Cell Chat single-cell Analysis Results
InSyBio Beta User
Circular plots pathway
Heatmap plots pathway
Groupped chord per pathway plots
Intercellular communication network
Distribution of contribution for each ligand-receptor Plots
Network signalling role of pathway Plots
Scatterplot network signalling plots per pathway
All Results Download
Distribution of contribution for each ligand-receptor pair to pathway plots for each pathway
Distribution_of_contribution for each ligand-receptor pair_to_pathway_CD99.jpg
Download Distribution_of_contribution for each ligand-receptor pair_to_pathway_CD99.jpg
Contribution of each L-R pair
CD99 - CD99
Relative contribution
Distribution_of_contribution for each ligand-receptor pair_to_pathway_CypA.jpg
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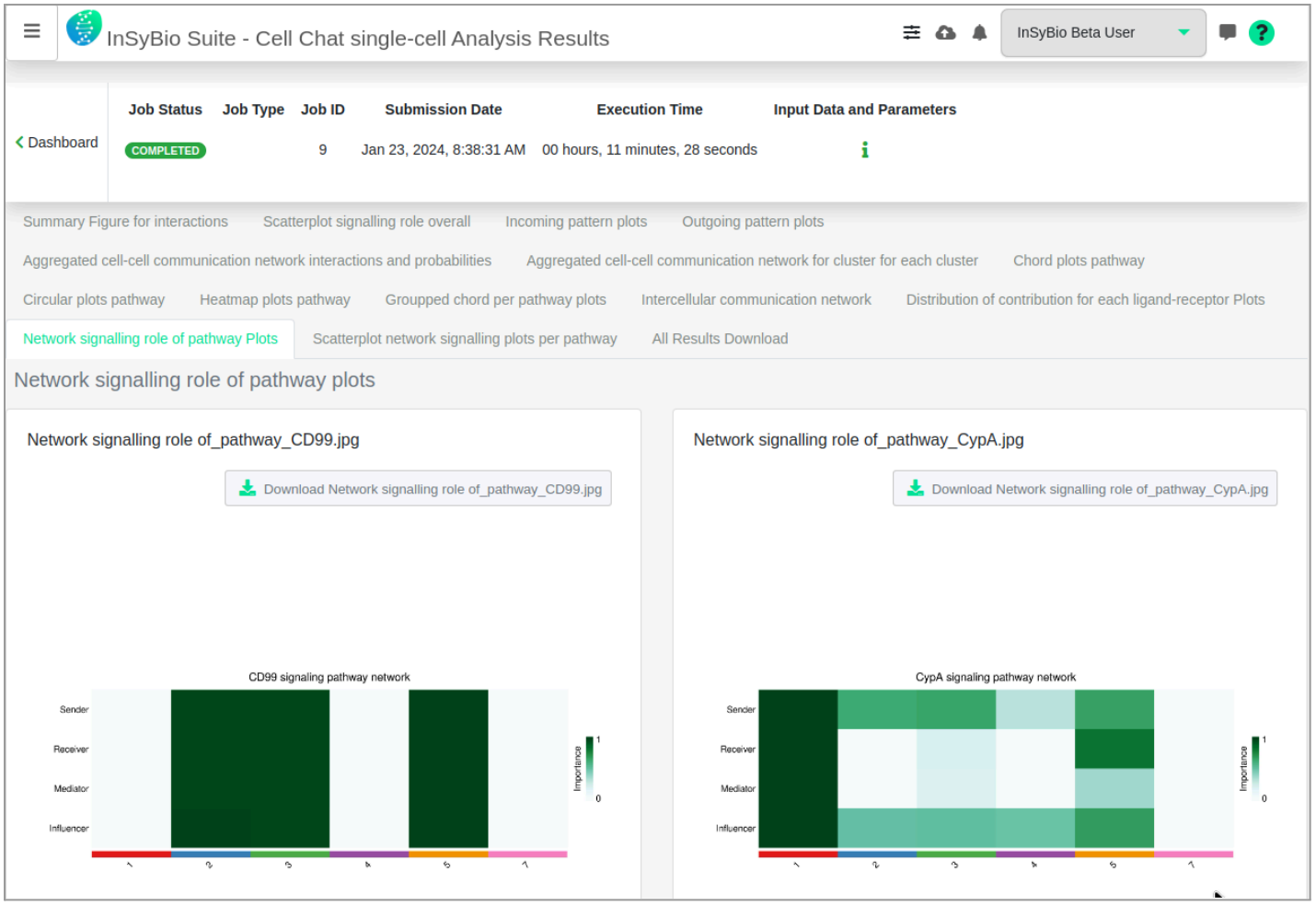
Contribution of each L-R pair
PPIA - BSG
Relative contribution


InSyBio Suite - Cell Chat single-cell Analysis Results
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Network signalling role of pathway plots
Network signalling role of_pathway_CD99.jpg
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CD99 signaling pathway network
Sender
Receiver
Mediator
Influencer
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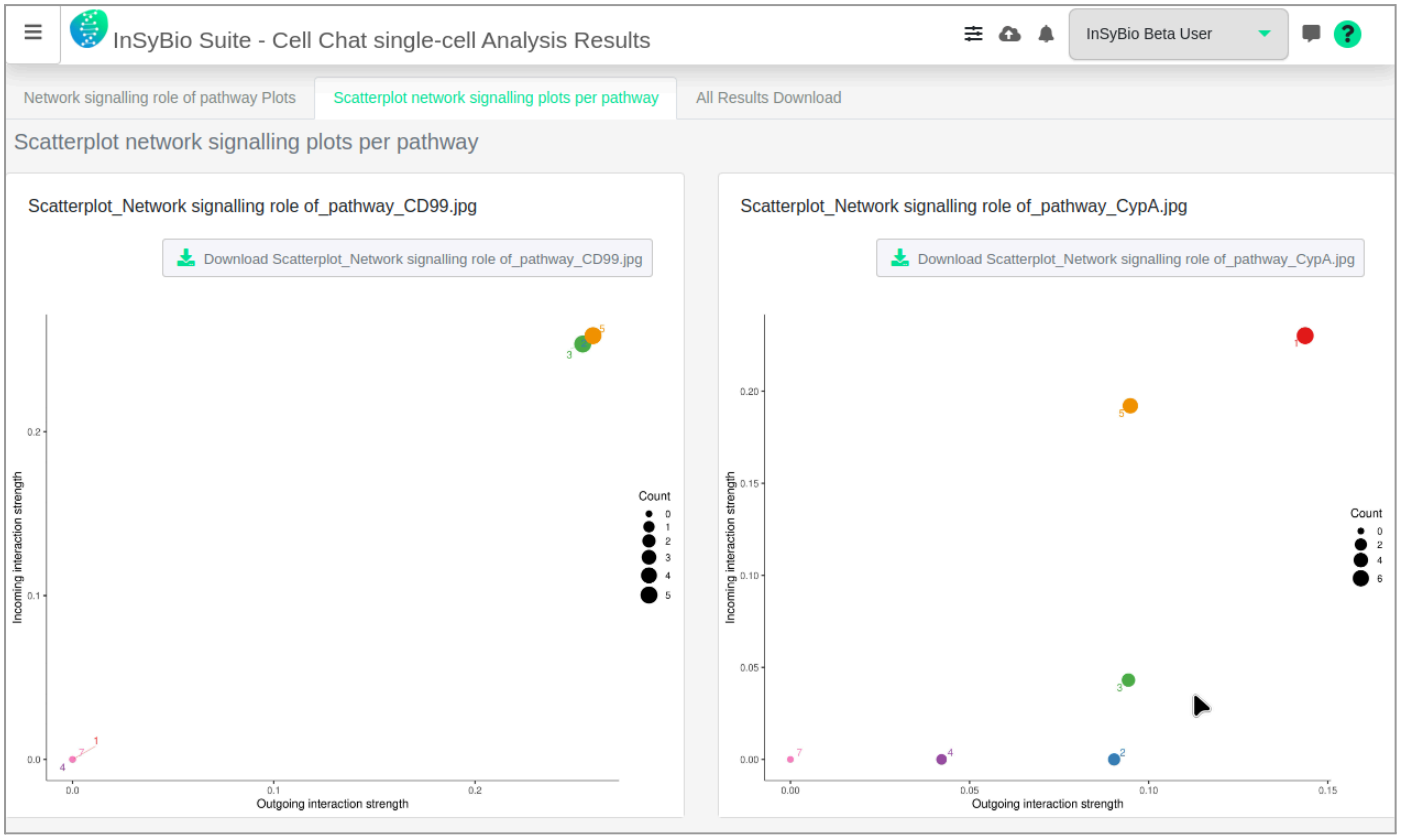
CypA signaling pathway network


InSyBio Suite - Cell Chat single-cell Analysis Results
InSyBio Beta User
Network signalling role of pathway Plots
Scatterplot network signalling plots per pathway
All Results Download
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Download Scatterplot_Network signalling role of_pathway_CD99.jpg
Incoming interaction strength
Outgoing interaction strength
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Scatterplot_Network signalling role of_pathway_CypA.jpg
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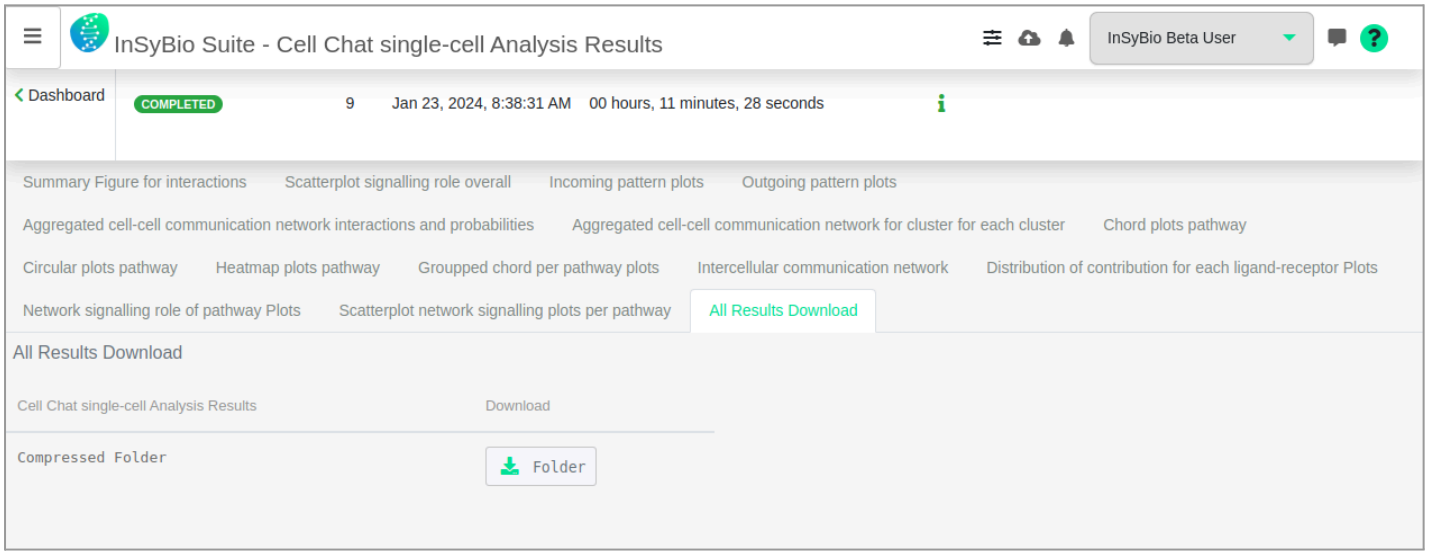
Incoming interaction strength
Outgoing interaction strength
Count


InSyBio Suite - Cell Chat single-cell Analysis Results
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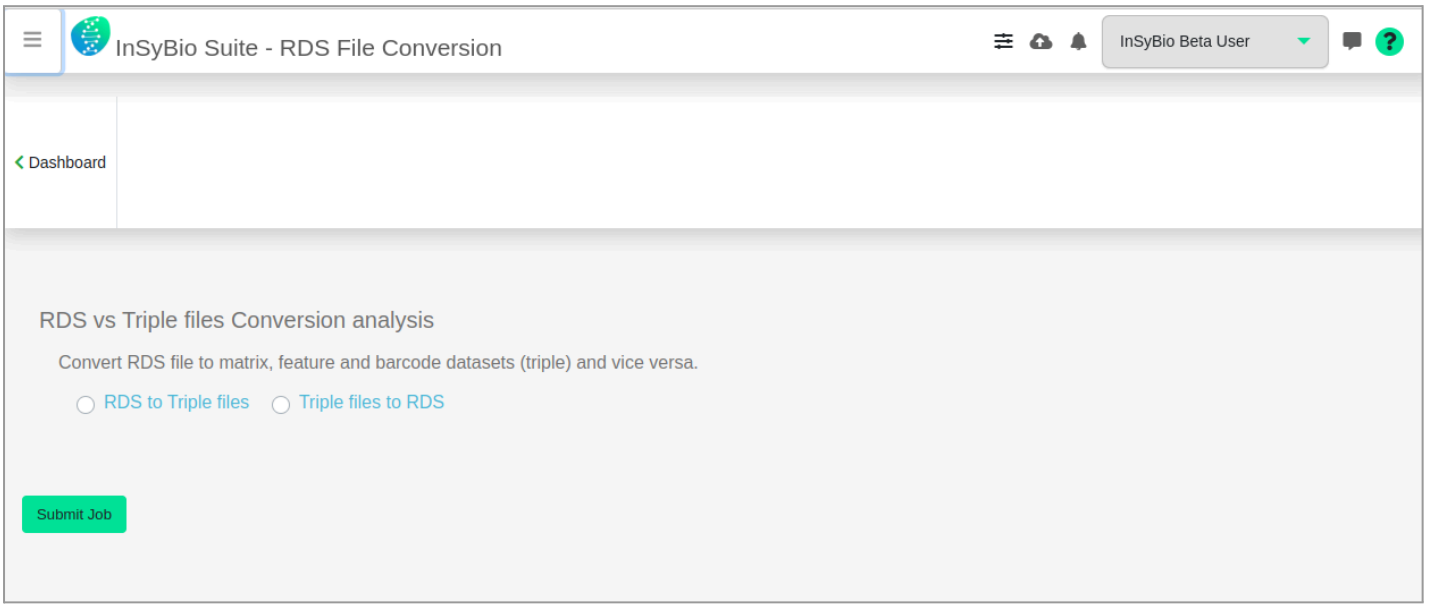
RDS File Conversion

You can convert a Seurat object file (.rds format) file to 10X Matrix, Features and Barcodes datasets (triple) and vice versa. Depending on the selected option,

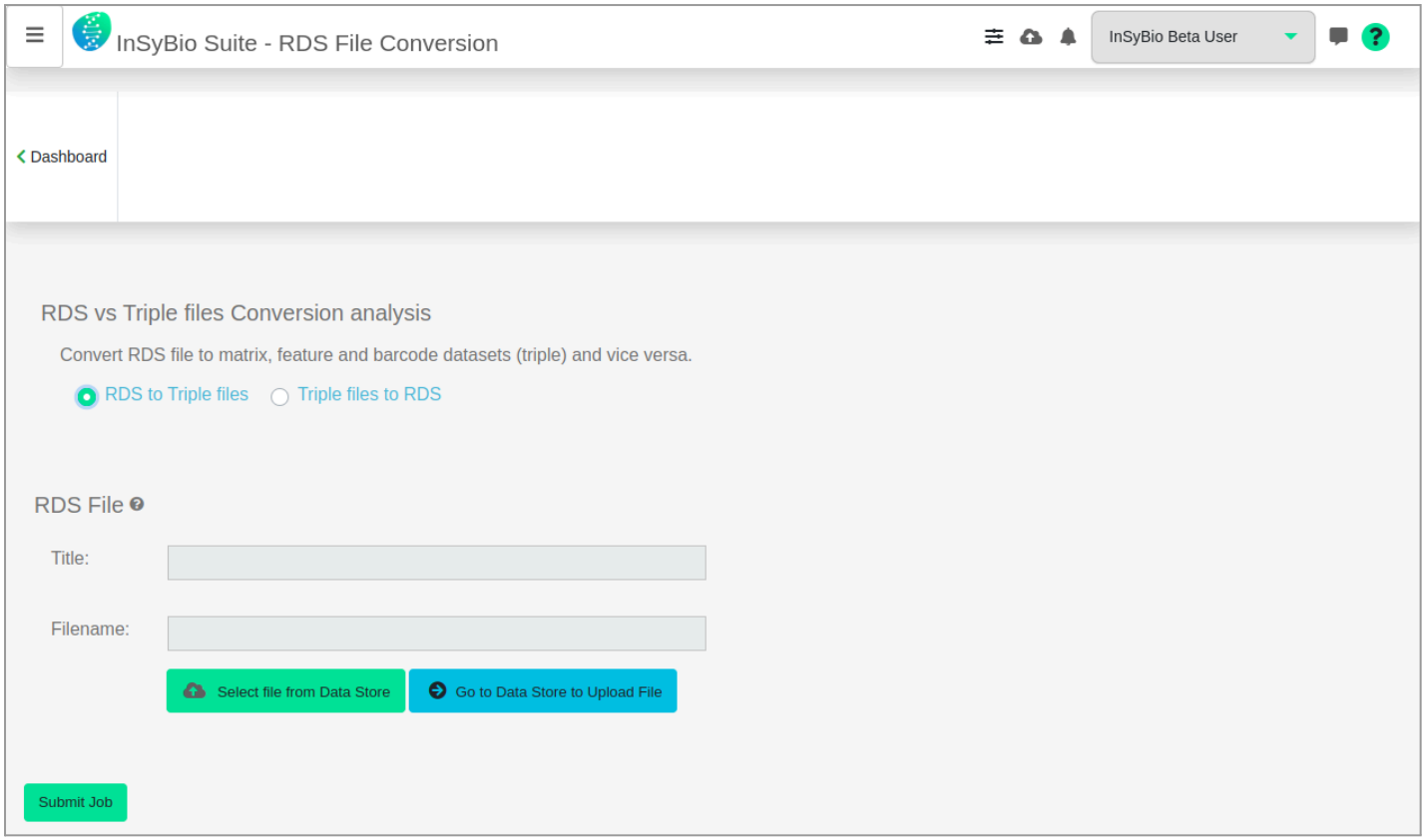
- RDS to triple
- Triple to RDS



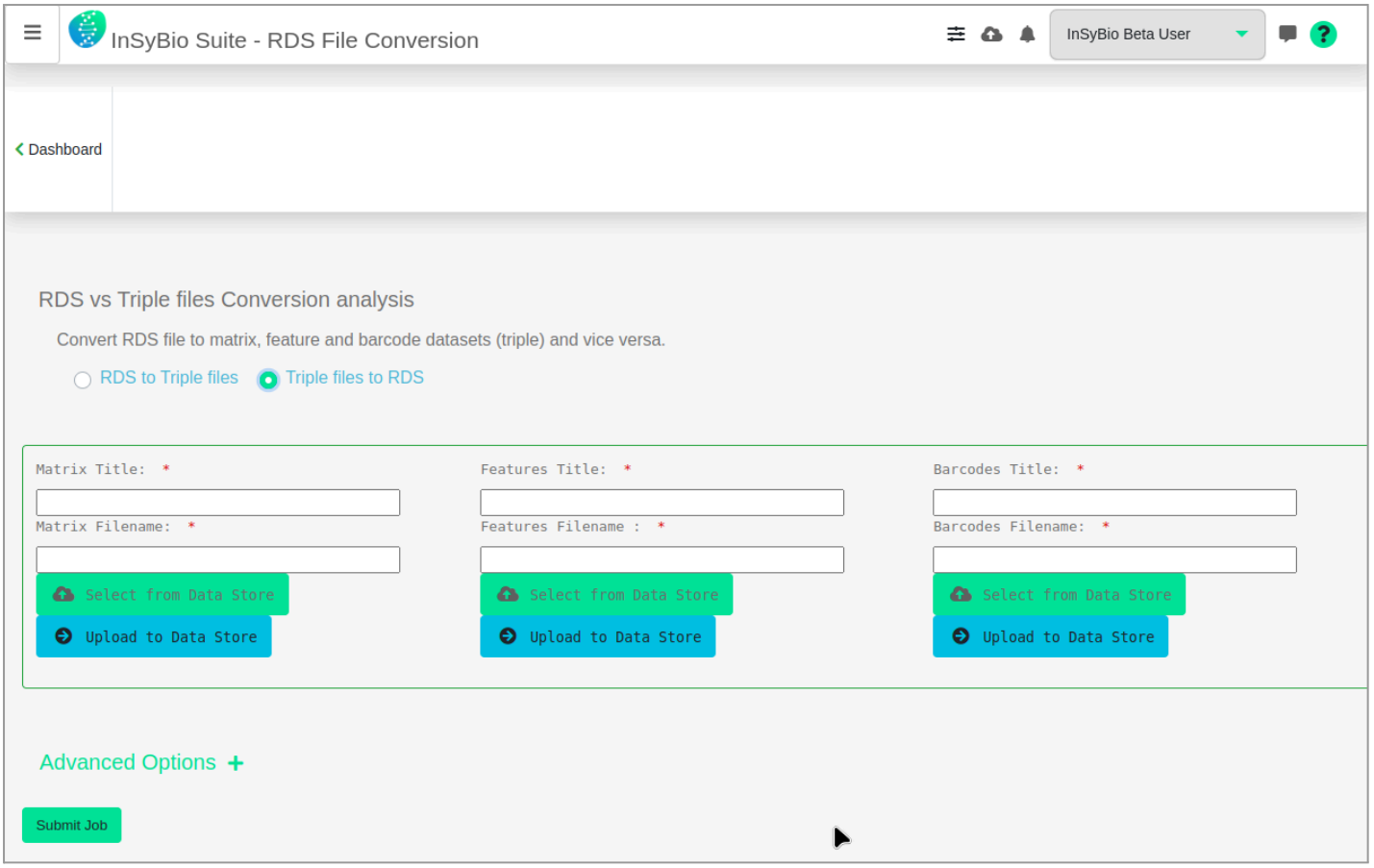
To start the RDS File Conversion:

Click in the menu “InSyBio ncRNASeq” → “single-cell RNA-Seq Data Analysis” → “single-cell RNA-Seq Pipeline Dashboard”, select the “Add new job” button and then choose the “RDS file conversion” option. Then depending on the selected option do the following steps:

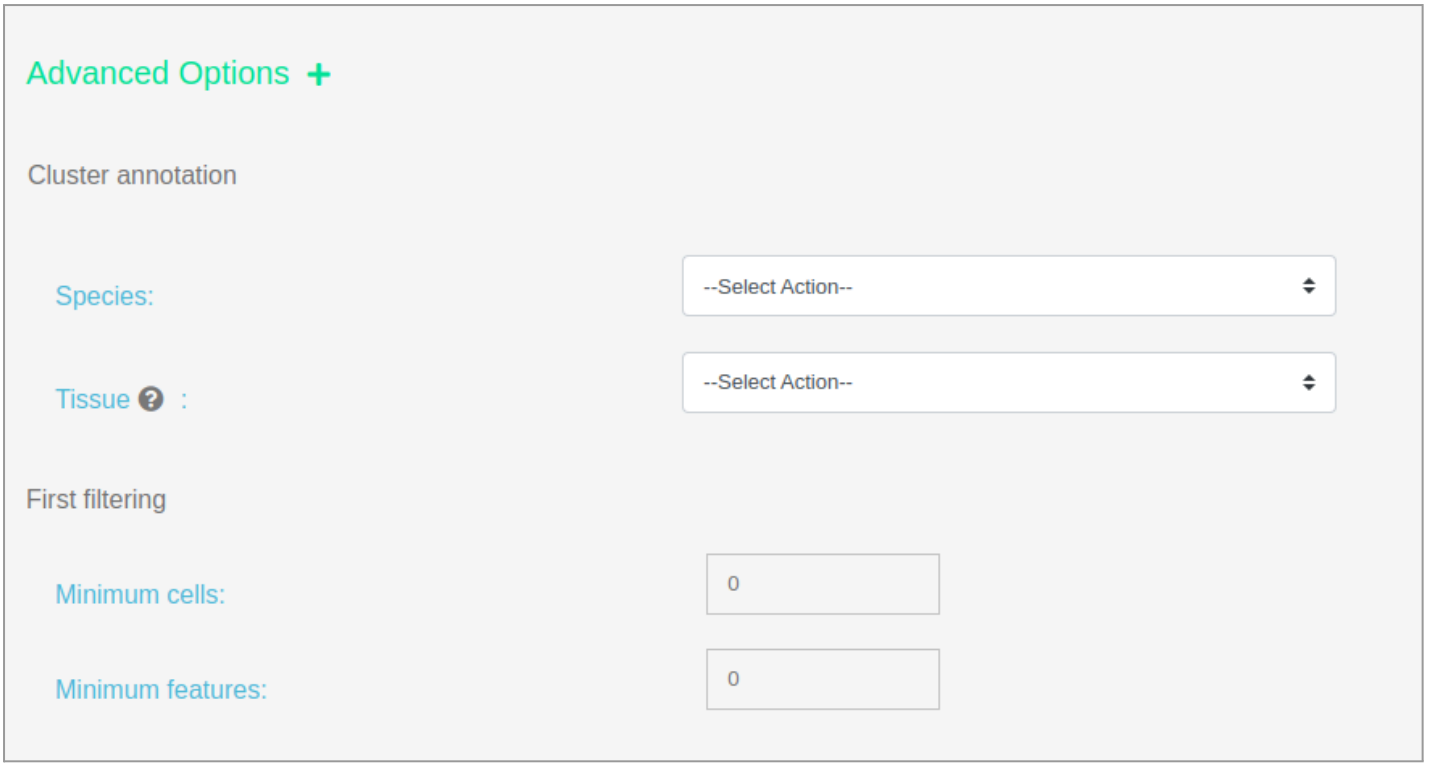
- RDS to triple:
    - Select or upload a Seurat object and the algorithm will convert it to matrix, features and barcode datasets.

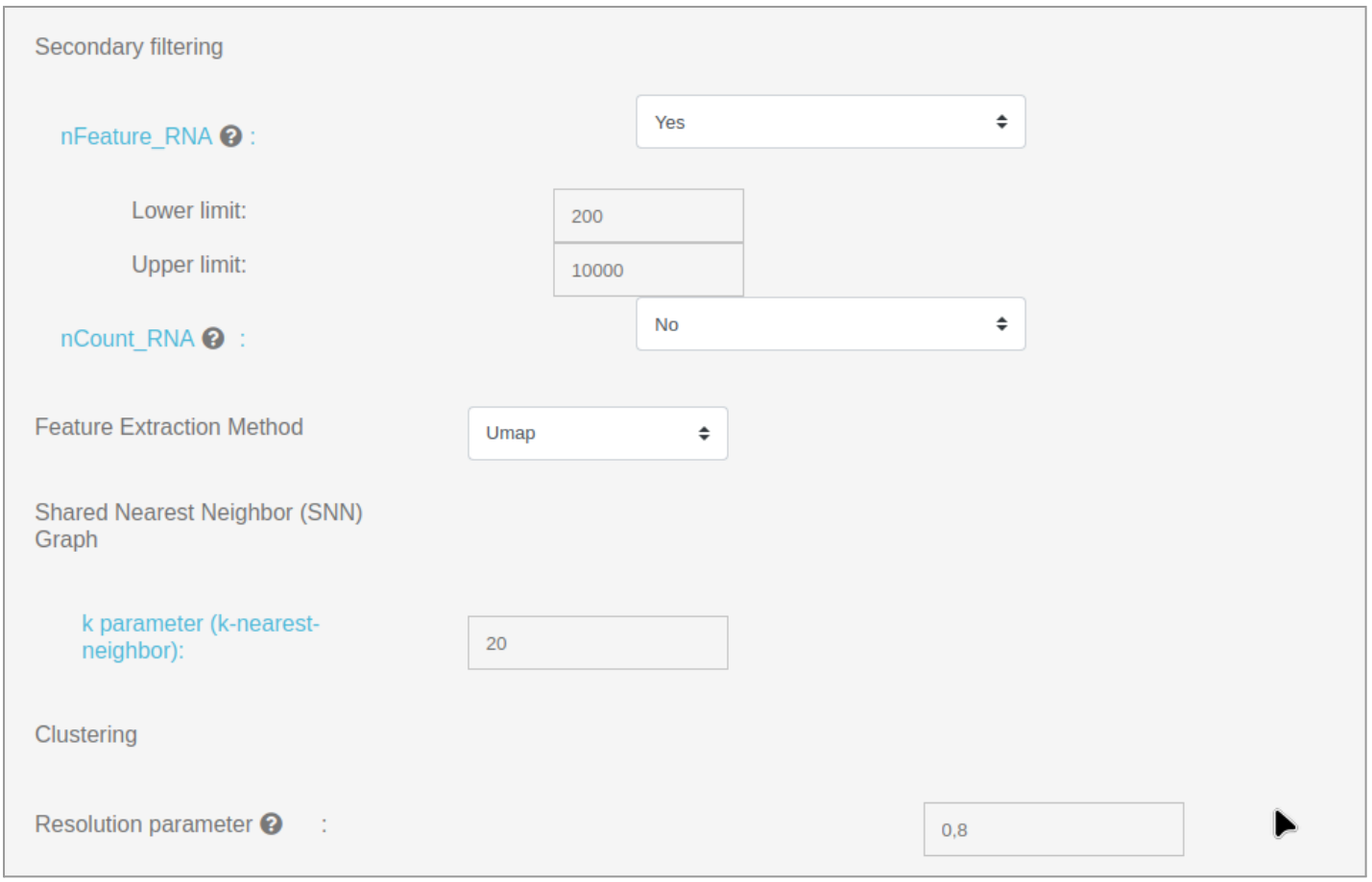


- Triple to RDS:
    - Select or upload the three matrix, features and barcodes files and the algorithm will convert it to a Seurat object file.



- Select if you want to manually configure other parameters of the job. If you don't, our Default Options will be applied. Possible manual options are:
    - First filtering:
        - Minimum cells
        - Minimum features
    - Secondary filtering:
        - nFeature_RNA with lower and upper limits
        - nCount_RNA with lower and upper limits
    - Feature Extraction Method
    - Shared Nearest Neighbor (SNN) Graph
        - K parameter (k-nearest- neighbor)
    - Clustering
        - Resolution parameter

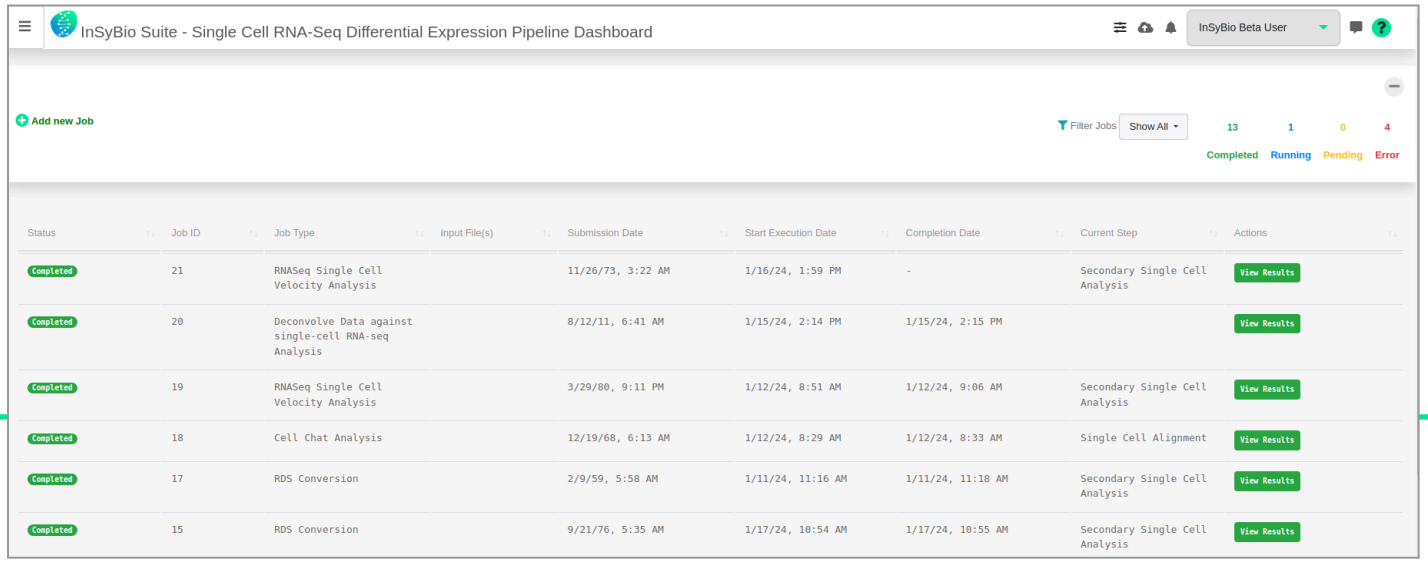

Advanced Options +
Cluster annotation
Species:
--Select Action--
Tissue :
--Select Action--
First filtering
Minimum cells:
0
Minimum features:
0



- Submit your job pressing the respective button.

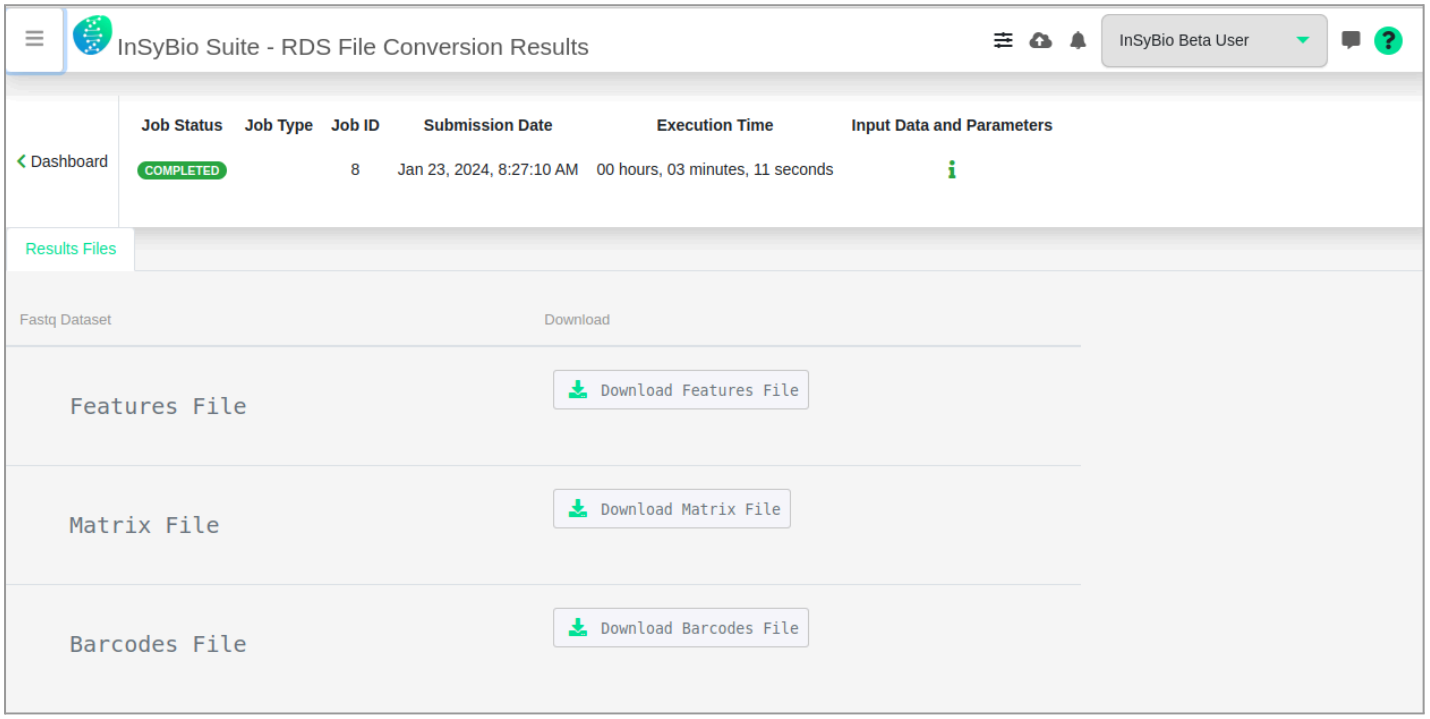
To view the results:

By starting a calculation you are informed if it was submitted successfully. Then you can move to the single-cell RNA-Seq Differential Expression Pipeline Dashboard, where you can view the status of your current and previous single-cell RNA-Seq Differential Expression Pipeline jobs.

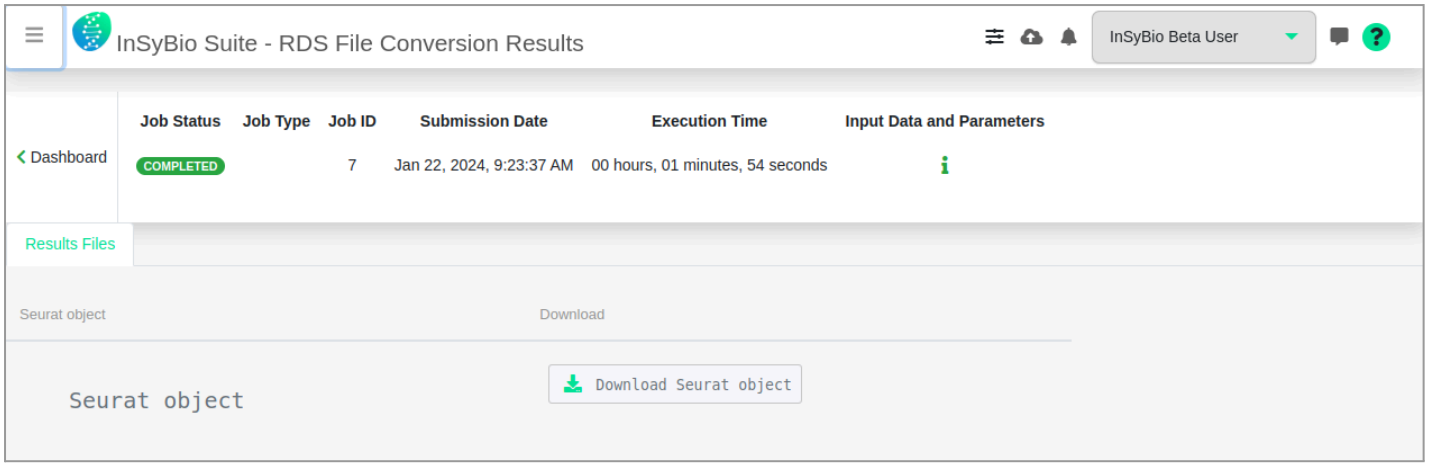


After the analysis, you can select the View Results in the Actions column and view the produced files, that are separated according to the step that they were produced.

- RDS to triple: The 10X triple files, matrix, barcodes and features files are produced and ready to be downloaded from the Results Files tab.



- Triple to RDS: The produced Seurat object can be downloaded from the Results Files tab.



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InSyBio Ltd is a bioinformatics pioneer company (www.insybio.com) in personalized healthcare, that focuses on developing computational frameworks and tools for the analysis of complex life-science and biological data in order to develop predictive integrated biomarkers (biomarkers of various categories) with increased prognostic and diagnostic aspects for the personalized Healthcare Industry.

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