



InSyBio

Intelligent Systems Biology

User Manual

www.insybio.com

Analyze coding & non-coding RNAs with InSyBio ncRNASeq

October 2025

Insybio Suite v4.0

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.

The screenshot shows the InSyBio ncRNASeq web interface. On the left is a sidebar with navigation options: InSyBio Interact, InSyBio ncRNASeq, non-coding RNA Analytics, ncRNA Feature Calculation, miRNA Prediction, miRNA Target site Feature Calculation, miRNA Target site Prediction, miRNA Target Prediction, ncRNASeq Knowledge Base, and RNA-Seq Data Analysis. The main area displays the 'ncRNA Feature Calculation' module. It includes a 'Sequences' field with the value 'ncrna15_12_', a 'Filename' field with 'dsfile1639562377_7291.txt', and two buttons: 'Select file from Data Store' and 'Go to Data Store to Upload File'. A 'Start calculation' button is also present. Below this is a table showing the status of various calculations.

Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	35	ncrna15_12_	3/16/22 3:22 PM	3/16/22 3:22 PM	3/16/22 3:22 PM	View Results
Completed	34	ncrna15_12_	12/15/21 10:00 AM	12/15/21 10:00 AM	12/15/21 10:00 AM	View Results
Completed	33	ncrna 15_12	12/15/21 9:48 AM	12/15/21 9:48 AM	12/15/21 9:48 AM	View Results
Completed	32	ncrna14_12	12/14/21 10:01 AM	12/14/21 10:01 AM	12/14/21 10:01 AM	View Results
Completed	31	test	6/4/21 8:11 AM	6/4/21 8:11 AM	6/4/21 8:11 AM	View Results
Completed	29	75 sequences including pre-miRNAs, random cds and snoRNAs	3/4/21 4:43 PM	3/4/21 4:43 PM	3/4/21 4:43 PM	View Results
Completed	28	75 sequences including pre-miRNAs, random cds and snoRNAs	3/1/21 10:17 PM	3/1/21 10:17 PM	3/1/21 10:17 PM	View Results
Completed	27	75 sequences including pre-miRNAs, random cds and snoRNAs	1/4/21 6:06 PM	1/4/21 6:06 PM	1/4/21 6:06 PM	View Results

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.

InSyBio Suite Beta - ncRNA Feature Calculation Results
InSyBio Beta UI

	Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters	
< Dashboard	COMPLETED	1	Aug 17, 2018 7:04:12 AM	00 hours, 02 minutes, 35 seconds		Export Results

Sequence	G+C	AU	AA	AC	AG	AU	CA	CC	CG	CU
> hsa-mir-26a-1 MI0000083 GUGGCUCGUUCAAGUAUCCAGGAUAGGCCUGUGCAGGUCCA AUGGGCCUAUUCUUGGUUACUUGCACGGGGACGC	55.844	44.156	3.947	3.947	5.263	5.263	6.579	6.579	3.947	6.579
> random_sequence_from_cds_1 GAGGGCAGGGGGCACAGUC CAUCUCCAGGCUUGUAGCUGGCCUGGGUGGCCCGCCGGCAGCGGCAGACUGUGUCUGUGUGGCCCGUGCACA	69.072	38.928	1.042	4.167	8.333	0	10.417	9.375	4.167	6.25
> snoRNA_1 AAGAUGAGUGAUGAAGUUCUGUGGCAUUAUUAUUAUUUUGAUUA AACCCUUAACUCUGAAGUCC	32.857	67.143	14.493	2.899	5.797	11.594	2.899	4.348	0	5.797
> hsa-mir-32 MI0000090 GGAGAUUUGCACAUUUAAGUUGCAGGCUACGGCCUA AUGCAUUAUUGUGUGUGAUUUUUUC	38.571	61.429	4.348	4.348	4.348	11.594	8.696	1.449	1.449	2.899
> hsa-mir-199a-1 MI0000242 GCCAACCCAGUGUUCAGACUACUGUUCAGGAGGCUCUCAAUGUGUAGUACAGUUGUCACAUUGGUUAGGC	59.784	49.296	2.857	7.143	10	2.857	11.429	5.714	0	7.143
> hsa-mir-148a MI0000253 GAGGCAAGUGUUCGAGACACUCCGACUCUGAGUAGUAGUAGUGCAGUCACUACAGAACUUUGUCUC	45.588	54.412	5.97	8.955	11.94	2.985	7.463	1.493	1.493	10.448

First Previous 1 2 3 4 5 ... 8 Next Last
Show entries Showing

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_snoRNAs222	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

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 $X,Y \in \Sigma[A,C,G,U]$	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 = $dG/\text{number_of_stems}$, where each stem is at least 3 continuous base pairs in the structure	MFE2
MFE Index 3 = $dG/\text{number_of_loops}$, where number_of_loops is the number of the loops in the secondary structure	MFE3
MFE Index 4 = $dG/\text{total_bases}$	MFE4
MFE Index 5 = $dG/\%(A+U)$ ratio	MFE5
Adjusted Minimum Free Energy of folding $dG = \text{MFE}/L$, where MFE is the minimum free energy of the structure as calculated by the Vienna fold routine	dG
Adjusted base pairing propensity $dP = \text{total_bases}/L$, 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.

The screenshot displays the InSyBio ncRNASeq web application. On the left is a sidebar with navigation links. The main content area is titled 'miRNA Prediction' and shows a form for uploading a file named 'ncrna15_12_'. Below the form is a table listing completed prediction processes.

Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	36	ncrna15_12_	3/16/22 3:26 PM	3/16/22 3:26 PM	3/16/22 3:26 PM	View Results
Completed	30	75 sequences including pre-miRNAs, random cds and snoRNAs	3/4/21 4:49 PM	3/4/21 4:50 PM	3/4/21 4:50 PM	View Results
Completed	14	75 sequences including pre-miRNAs, random cds and snoRNAs	11/11/19 11:36 AM	11/11/19 11:36 AM	11/11/19 11:36 AM	View Results
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

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.

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.

The screenshot shows the InSyBio Interact web interface. On the left is a sidebar menu with options like 'InSyBio ncRNASeq', 'non-coding RNA Analytics', 'ncRNA Feature Calculation', 'miRNA Prediction', 'miRNA Target site Feature Calculation', 'miRNA Target site Prediction', 'miRNA Target Prediction', 'ncRNASeq Knowledge Base', 'RNA-Seq Data Analysis', and 'Single Cell RNA-Seq Data Analysis'. The main panel is titled 'miRNA Target site Feature Calculation' and contains input fields for 'mRNA Target Sequences' (with value 'mrmnas462') and 'miRNA Sequences' (with value 'mirnas462'). Below these are buttons for 'Select file from Data Store' and 'Go to Data Store to Upload File'. A 'Start calculation' button is at the bottom right. Below the input fields is a table of completed processes.

Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	16	mRNAs: mrmnas462, miRNAs: mirnas462	3/16/22 3:28 PM	3/16/22 3:28 PM	3/16/22 3:32 PM	View Results
Completed	14	mRNAs: mrmnas462, miRNAs: mirnas462	3/4/21 5:22 PM	3/4/21 5:22 PM	3/4/21 5:44 PM	View Results
Completed	13	mRNAs: mirnas462, miRNAs: mrmnas462	11/11/19 11:51 AM	11/11/19 11:51 AM	11/11/19 12:36 PM	View Results
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:	11/15/18 9:01	11/15/18 9:01	11/15/18 9:02	View Results

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: targetsha-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_5_0_shuffled_targets, miRNAs: genes_5_5_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.

InSyBio Suite Beta - miRNA Target Site Features Calculation Results																
Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters												
COMPLETED	3	Sep 26, 2018 11:21:20 AM	00 hours, 39 minutes, 03 seconds	Export Results												
miRNA Sequence	Target Sequence	mats	matos	mat	gcmats	gcmatos	gcmat	aumats	aumatos	aumat	unps	unpos	unp	gus	guos	gu
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUAAACUGAA	> NM_004456EZH220478051 Homo sapiens TGAATTTGCAAGTACTGTA	9	2	11	3	1	4	6	1	7	-2	22	20	0	0	-2
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUAAACUGAA	> NM_004456EZH220478051 Homo sapiens TTCAGGAACCTCGACTACTGTG	8	6	14	3	3	6	5	3	8	0	16	16	2	2	-2
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUAAACUGAA	> NM_101833NF217220301 Homo sapiens TACAAGAGATTCTCGCCTCA	4	3	7	2	2	4	2	1	3	8	22	30	0	0	8
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUAAACUGAA	> NM_001039111TRIM7117890240 Homo sapiens ACAACATTGCTTAAGTCCTACCTCA	1	5	6	0	2	2	1	3	4	14	21	35	0	2	14
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUAAACUGAA	> NM_001039111TRIM7117890240 Homo sapiens ACAACATTGCTTAAGTCCTACCTCA	9	3	12	3	2	5	6	1	7	-2	25	23	0	0	-2
Showing 1 to 25 of 213,444 entries																

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= $(-1 * \text{free energy of the target sequence}) / \log(\text{length of target} * \text{length of miRNA})$	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.

InSyBio Interact

InSyBio ncRNASeq

non-coding RNA Analytics
Prediction of ncRNAs and miRNA targets.

- ncRNA Feature Calculation
Feature calculation module for 58 miRNA genes-related features.
- miRNA Prediction
Prediction module for pre-miRNAs.
- miRNA Target site Feature Calculation
Feature calculation module for 124 miRNA target features.
- miRNA Target site Prediction
Prediction module for miRNA targets.
- miRNA Target Prediction
Prediction module for miRNA targets.

ncRNASeq Knowledge Base
miRNA and transcript search.

RNA-Seq Data Analysis
Preprocessing and differential expression analysis of FASTQ files.

Single Cell RNA-Seq Data Analysis

mRNA Target Sequences:
Filename:
[Select file from Data Store](#)
[Go to Data Store to Upload File](#)

miRNA Sequences:
Filename:
[Select file from Data Store](#)
[Go to Data Store to Upload File](#)

[Start calculation](#)

Status	Process ID	Information	Submission Date	Start Execution Date	Completion Date	Actions
Completed	17	mRNAs: mrnas462, miRNAs: mirnas462	3/16/22 4:18 PM	3/16/22 4:19 PM	3/16/22 4:22 PM	View Results
Completed	15	mRNAs: mrnas462, miRNAs: mirnas462	3/4/21 5:24 PM	3/4/21 5:24 PM	3/4/21 5:47 PM	View Results
Completed	12	mRNAs: , miRNAs: test	11/30/18 9:54 AM	11/30/18 9:54 AM	11/30/18 9:56 AM	View Results
Completed	10	mRNAs: test, miRNAs: test	11/15/18 9:29 PM	11/15/18 9:29 PM	11/16/18 12:00 AM	View Results
Error	7	mRNAs: , miRNAs: pseudomi1848	9/27/18 9:36 AM	9/27/18 9:36 AM	11/30/18 10:11 AM	View Details

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.

InSyBio Suite Beta - miRNA Target Site Prediction Results																
Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters												
COMPLETED	4	Sep 26, 2018 11:29:30 AM	01 hours, 10 minutes, 43 seconds	Export Results												
miRNA Sequence	Target Sequence	Prediction Score	Prediction	mats	matos	mat	gcmats	gcmatos	gcmat	aumats	aumatos	aumat	unps	unpos	unp	
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUACUGAA	> NM_004456EZ220478051 Homo sapiens TGAATTTCGCAAGTACTGTA	0.963256	Target	9	2	11	3	1	4	6	1	7	-2	22	28	
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUACUGAA	> NM_004456EZ220478051 Homo sapiens TTCAGGAACCTCGAGTACTGTG	1.2725	Target	8	6	14	3	3	6	5	3	8	0	16	16	
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUACUGAA	> NM_181833NF217220301 Homo sapiens TACAAGAGATTCTCCTGCCTCA	-0.786746	no Target	4	3	7	2	2	4	2	1	3	8	22	30	
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUACUGAA	> NM_001039111TRIM7117890240 Homo sapiens ACAACTTGTCTTAAGTCTACCTCA	-0.880751	no Target	1	5	6	0	2	2	1	3	4	14	21	35	
> [hsa-miR-101] Homo sapiens UACAGUACUGUGAUACUGAA	> NM_001039111TRIM7117890240 Homo sapiens ACAACTTGTCTTAAGTCTACCTCA	-0.880751	no Target	1	5	6	0	2	2	1	3	4	14	21	35	
Showing 1 to 25 of 213,444 entries																

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.

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, SMOG1, 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.

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.

- Gene show page

← Mirna Target Prediction Tool Results

miRNA	hsa-miR-6126
Gene	ZIK1
Transcript	ZIK1-001
miRNA-Gene Score	1.169
miRNA-Transcript Score	0.817
Number of target sites	11
3'UTR sequence	<pre> 1 AGGCCTCATGAATGCAGCAATGTGGAAGCGCCTCAACTCAAGATCTATCATCTTTAGCTCCTGAAAGTCCACACTTA 80 81 AGTAGAGCCTTAGACCTACAGGAAAGTGCTGCTCTGCTAGTATTGTAGCAGTAGAGAGCCTTTGTGAGGAGCCATCTG 160 161 CTTGAAGTTGAACCTCATTCTCTGTTGTTCTGCTGGTAGAAACCATCTACCCTCTACCACTTGCACAGTGGGCACTGGT 240 241 CACTCCTATGTGCTAAGACAAGGCAGACATCTGTGTCTCTTAAGTCTTTGGAGGAAATCTTGAGCAGTCTAAGCCTT 320 321 TAGAGAAAATTCATTCTTTTCTGACTGATCACAGCATACGTGTGCCCAAGTTTGGGTCAAGAGGCGCCAGCCTTGTT 400 401 CTGCTGGACACTTATGTGCAAGGATTCCTTCATGTAAATCTCTGGTCTCACAAGACACTTGGTCACTTCTTCCAGCCTCC 480 481 ATGTCAACACGTGGTGAATGGCTGCCTCAGATTGCTCCAGTTGTGCACATAAAGAGCCTTATATTGAATCTACCTGT 560 561 AGTCTTGGGGTTCTGTTTACTGTGTGGGGTGGCTGGGAGACAGACTTCAACTCTATATGAAGGAATGGATGGCTTTTGTG 640 641 GGCCTCTGAGGAAAGTAAGATGACAGAGTAATTCTAATTCTGGTTTGGTCTACTTGTCTTGTCTACCTAAATCTCCT 720 721 AGGAAAAAATGCAAGGTTTGGTTATTCTAATTGTGGCCTGGATCCCTATTCTTCTGTGAGACTAGAGGTCATCCTGA 800 801 GGAGAGGCCAGCTGTTATGACAAGCATGTGTCTTCAAGGGAATAGGACAATTTATTCCATTGTTCCAGAGGATGTCAT 880 881 ATGATGCCAGTGCTGCTGAGAAGCTTTTCATGGGGTCTATAAGGAGGCATGCCCTGATCAAACTCCATAGGCCG 960 961 ATGTCACGCAGAGACAACCGGAGTCACATGTGAAGTGTAAATGGTACAGAAATACCTGGGTATTCTGTACTGTGTGTA 1040 1041 CTGTAGCAAACTAGTTGGAATGTGCCTCTTATAAAGTACATTTACAAATCTTCCCGTACTGTGGCTTTGAGCAGTCAT 1120 1121 AGGACCTAGAAATCTGTGTATGTCCAAAGCTGAGGTATTTTTCAGCAAAAATTAATAAGGGTTTTATTTTTTAATCT 1200 1201 TGTGTGTTTCTAGGTTGTTCACCTCAAGTGCAATGTGCTAGAGGCAGAAAAAGGAGGATAAGATAACAGAGTCCTAT 1280 1281 AGGCCAGGGATGATTGATAGCTCTTGTGATTTCCACCACTGTTGCTGTTGTCTCAAAATGCCCACAGCCTTCATTGCTT 1360 1361 CACACATTTCTGTCATGAGAGGACTCATGTTGCCCTTCCCGAGGCCTGAAGAGAGAGTGCAGTCAACATGAGATTGCTA 1440 1441 GGCATTCTGGTTTCTGAAAGTTGGGTGATCAGATACTTTATTGTGAACATGTTTACAACTTCTTGATGTGTAAGT 1520 1521 ACATGCCATAGTTTACATCCATTTATGGTGTATAATTTGAAGAGTTTGTACACAAGCCTGTGAAACCAATATCATGATC 1600 1601 ATGAACATATTTCATGATTCACCTCTTGGTGTTTTACAACTCTGCGGTGTACTTCCAGGCCTTCAGGAGTCTGTCATT 1680 1681 ACTTTCCCTACAGGAGAATAGTTTGTGTTTCTAGGATTTTATGTGAATTGAACGTAAATACTTACTCCATTTTCCCT 1760 </pre>

Score : 1.7294313303229796

TTCCCTTCATGTAATTTCTTGGTCT-CACAT

||||| || ||||| |||

...AGAGG...-CGGCCCGGAAGUG...

Score : 1.5224538611539185

TGACACTTGGTCACTTCTCCAGCCTCCATG

||||| || ||||| |||

.....AGAGCGGCCCGGAAGUG

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

The screenshot displays the InSyBio Suite Beta - miRNA Search Tool interface. The search bar at the top contains 'mir-181a' and a 'Show results' button. Below the search bar, there are two tabs: 'Stem-loop miRNAs' and 'Mature miRNAs'. The 'Stem-loop miRNAs' tab is active, showing a list of results. The first result is highlighted: 'MI0000269 hsa-mir-181a-2'. To the right of this list, the 'Stem-loop: MI0000269 hsa-mir-181a-2' page is displayed. This page has three tabs: 'Information', 'Mature miRNAs', and 'References'. The 'Information' tab is active, showing details about the stem-loop. The details include: Accession (MI0000269), Name (hsa-mir-181a-2), Species (HSA), Length (110), and Description (Homo sapiens miR-181a-2 stem-loop). There is also a 'Comments' section with text about the prediction and validation of the miRNA. Below the information, there is a 'Sequence Information' section with a 'Sequence description' (Sequence 110 BP; 29 A; 25 C; 30 G; 0 T; 26 other;), a 'Sequence' field containing the nucleotide sequence, a 'FASTA' button, a 'Download' button, and a 'Secondary structure (in dot-bracket)' field containing the secondary structure notation.

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

miRNA accession or name [Show results](#)

Stem-loop miRNAs Mature miRNAs

Stem-loop: MI0000269 hsa-mir-181a-2

Accession	Name	Sequence	FASTA	Evidence	Experiment
MI0000223	mmu-mir-181a-2				
MI0000269	hsa-mir-181a-2				
MI0000289	hsa-mir-181a-1				
MI0000697	mmu-mir-181a-1				
MI0000925	rno-mir-181a-2				
MI0000953	rno-mir-181a-1				
MI0001218	gga-mir-181a-1				
MI0001243	gga-mir-181a-2				
MI0000256	hsa-mir-181a-5p	39 aacauucaacgcugucgugagugu 61	Download	Experimental	cloned [2,4-6]
MI0000458	hsa-mir-181a-2-3p	77 accacugaccguugacugauacc 98	Download	Experimental	cloned [4]

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.

miRNA accession or name 

mir-181a Show results

Stem-loop miRNAs Mature miRNAs **Stem-loop: MI0000269 hsa-mir-181a-2**

Stem-loop id	Stem-loop name	Stem-loop	Stem-loop
id	name	T1	T2
MI0000223	mmu-mir-181a-2		
MI0000269	hsa-mir-181a-2		
MI0000289	hsa-mir-181a-1		
MI0000697	mmu-mir-181a-1		
MI0000925	rno-mir-181a-2		
MI0000953	rno-mir-181a-1		
MI0001218	gga-mir-181a-1		
MI0001243	gga-mir-181a-2		

Information Mature miRNAs **References**

Links to external database entries

Database	External Link
	MI0000269
	mir-181
	MIR181A2
	MIR181A2

Publications

1. Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP; **Vertebrate microRNA genes**; Science. 299:1540(2003). [\[PubMed\]](#)
2. Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G; **Numerous microRNPs in neuronal cells containing novel microRNAs**; RNA. 9:180-186(2003). [\[PubMed\]](#)
3. Weber MJ; **New human and mouse microRNA genes found by homology search**; FEBS J. 272:59-73(2005). [\[PubMed\]](#)
4. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, J, Sander C, Zavolan M, Tuschl T; **A mammalian microRNA expression atlas based on small RNA library sequencing**; Cell. 129:1401-1414(2007). [\[PubMed\]](#)
5. Lui WO, Pourmand N, Patterson BK, Fire A; **Patterns of known and novel small RNAs in human cervical cancer**; Cancer Res. 67:6031-6043(2007). [\[PubMed\]](#)
6. Marton S, Garcia MR, Robello C, Persson H, Trajtenberg F, Pritsch O, Rovira C, Naya H, Dighiero G, Cayota A; **Small RNAs analysis in CLL reveals a deregulation of miRNA**

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

miRNA accession or name 

mir-181a Show results

Stem-loop miRNAs **Mature miRNAs** **Mature: MIMAT0000210 mmu-miR-181a-5p**

Mature id	Mature name	Mature	Mature
id	name	T1	T2
MIMAT0000210	mmu-miR-181a-5p		
MIMAT0000210	mmu-miR-181a-5p		
MIMAT0000256	hsa-miR-181a-5p		
MIMAT0000256	hsa-miR-181a-5p		
MIMAT0000270	hsa-miR-181a-3p		
MIMAT0000660	mmu-miR-181a-1-3p		

Information Stem-loop miRNAs References

Accession MIMAT0000210

Name mmu-miR-181a-5p

Sequence 14 aacauucaacgcugucggugagu 36

FASTA Download

Evidence Experimental

Experiment cloned [2,4], Illumina [5-6]

Similarity MI0000223

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

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.

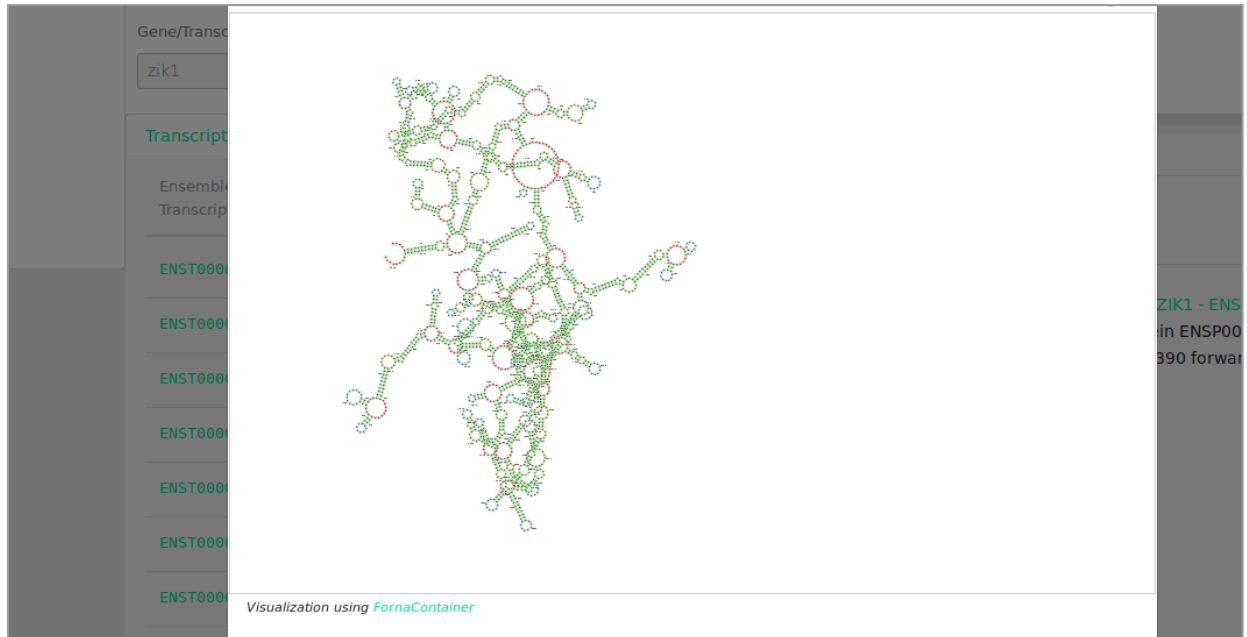
Transcript Search

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

The screenshot shows the InSyBio ncRNASeq interface. At the top, there is a search bar with 'zik1' entered and a 'Show results' button. Below the search bar, there are two tabs: 'Transcripts' (selected) and 'Genes'. The 'Transcripts' tab displays a list of transcripts with columns for 'Ensemble Transcript id', 'Transcript name', and 'T1'. The list includes transcripts like ENST00000307468 (ZIK1-004), ENST00000456074 (ZIK1P1-001), and others. Below the list are navigation buttons: 'First', 'Previous', '1', 'Next', 'Last'. To the right of the list, the 'Transcript: ZIK1-004 ENST00000307468' page is displayed. This page has an 'Information' tab selected, showing details about the transcript. The details include: Name - Source (ZIK1-004 (HGNC transcript name)), Gene (This transcript is a product of gene ZIK1 - ENSG00000171649), Protein (This transcript corresponds to protein ENSP00000303820), Location (Chromosome 19: 57584260-57592390 forward strand), Transcription Start Site (TSS) (57584260), Length (2510), Transcript Support Level (TSL) (TSL:1), Gencode annotation (GENCODE basic), GC content (47.45 %), Biotype (protein_coding), Status (Known), Annotation method (Havana), Version (ENST00000307468.4), and Description (zinc finger protein interacting with K protein 1 [Source:HGNC Symbol;Acc:HGNC:33104 External Link to HGNC]). There is also a '3'UTR sequence' section and a '3'UTR Visualization' button. A 'Download' button is also present.

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.



Ensemble Transcript id	Transcript name	Information
ENST00000307468	ZIK1-004	3'UTR sequence
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

GAGTGTACAGTCAAAGGCAGGTTTCACACAGAAAGACTCAATCCTGTGAGATGTGTGTCCAGTCTGAAAGATATT
TTGCATCTAGCTGATCTCCCTGGGCAGAAACCATACTTGGTTGGAGAATGTACAAACCATCACAGCACCAGAAAGCATCA
CAGTGCAAGAAATCCTTGAAGAGGACATGGACAGAGCCTCATATGTGAAGTGTCTGCTATTCTGTATGTCAATTGAAAGC
CCTTTCGCAAAATGGAGGTTGGAAAGGACCTCCAGCCATGTTGCGGCTTCTGAGGTCCCTGGTCTTTCTGGAGGCAAG
AAACCCGGCACAATTACTGAATGTGGGAGGACATTGCGAGTCAAAAAAGTCATTACAAGTCAGGTGAATGTGGGAAGGC
TTCCAGGCACAAACACACTCTGTTTACCATCCAAGAGTCTACACTGGAAAAAGCTTTATGAGTGTAGCAAAATGTGGGA
AAGCCTTCCGTGGCAAGTACTCACTTGTTCAGCACCAGAGAGTCCATACTGGAGAAAGGCTTGGGAGTGCATGAATGT
GGAAATTTTATGCAAAACCTCCACCTGAATGATCATCGGAGAATCCACACCGGAGAAAGGCTTATGAGTGCAGCGA
ATGTGGAAATTTATGACAAAACTCCAGCCTTGTGACCACGAAAAATACACTGGAGCAAGGCTTATGAGTGTGA
GCCAGTGTGGGAATCCTTTAGCCAAAAAGCCACCTTGTAAACACCAAGAGTTACACTGGAGAAAGGCTTATAAG
TGTGTTGAATGTGGGAATTCCTTTAGTCAAAGTGCCATTCTTAATCAACACCGAAGAATTCACACTGGAGCAAAAGCCTTA
TGAGTGTGGCAGTGTGGGAATCCTTTAGTCAAAAAGCTACCTCATTAAACACGAGAGATTCACACTGGAGAAAGGC
CTTATAAGTGTGTTGACTGTGGGAATCCTTTAGTCAAAGCTCCATCCTTATTCAACACCGGAGAAATTCATCTGGAGCA
AGGCTTATGAGTGTGGCAGTGTGGAAAGTCTTTAGCAGAAAGTCTGCTCTATTCAACACCAAGTGGTTCACACTGG
AGAAAGGCTTATGAGTGCAACAAATGTGGGAATTCCTTTAGCAATGCTCCAGCTCATACATCAACAAAAATGTGATA
ACACATAGAGGCTCATGAATGCAGCAAAATGTGGAGGCGCTTCAACTCAAGATCTATCATCTTTAGTCTCTGAAAGTC
CACACTAAGTAGAGCCTTAGACCTACAGGAAAGTGTGTCTCTGTAGTATTGTAGCAGTAGAGAGCCTTTGTGAGGGA
GCCATCTGCTGAAGTTGAACCTCATTCTTCTTGTCTCTGTTGAGAAACCATCTACCTCTACCACTTGCACAGTGG
GCACTGGTCACTCCTATGTGCTAAGACAAGGACAGACATCTGTGTCTCTTAAGTCTTTGGAGGAAATCTTGAGCAGTC
TAAGCCTTTAGAGAAATTCATTCTTTTTCTGACTGATCACAGCATACGTGTGACCCAGTTTGGGTGAGGAGGCCCCAG
CCTTGGTTCTGTGGACACTTATGTGCAAGGATTCCTTCATGTAAATCTTGGTCTCACATGACACTTGGTCAATCTTC
CAGCCTCATGTACACAGTGGTGAATGGTGGCTCACATTGCTCCAGTTTGTGCACTAATAAAAGCCTTATATTTGAAT
CTACCTGTAGTCTTGGGTTCTGTTTACTGTGTGGGTGGCTGGGAGACAGACTTCAACTCTATATGAAGGAATGGATGG
CTTTTGTGGGCTCTGAGGAAAGTAAGATGACAGAGTAATCTAATCTGTTTGGTCTACACTTGGCTTGTACCTAA
AATCTCCTAGGAAAAATGCAAGGTTTGGTTATTCTAATTTGTGGCTGGATCCCTATTCTTCTGTGAGACTAGAGGT
CATCTGAGGAGAGGCGAGCTGTATGACAAGCATGTGTGCTTCAGGGAATAGGACAATTTATTCATTGTTTCCAGAG

Genes information

The screenshot shows the 'Gene Search Tool' interface. A search bar contains 'zik1' and a 'Show results' button is visible. The 'Genes' tab is selected, displaying a table of results:

Ensemble Gene id	Official Gene Symbol
ENSG00000171649	ZIK1
ENSG00000237426	ZIK1P1

Below the table, a detailed view for 'Gene:ZIK1 ENSG00000171649' is shown. It includes an 'Information' tab with the following details:

- Name - Source:** ZIK1 (HGNC Symbol)
- Description:** zinc finger protein interacting with K protein 1 [Source:HGNC Symbol;Acc:HGNC:33104 [External Link to HGNC](#)]
- Location:** Chromosome 19: 57578456-57593777 forward strand
- Transcript count:** 8
- Biotype:** protein_coding
- Status:** Known
- Annotation method:** Annotation for this gene includes both automatic annotation from Ensembl and [Havana](#) [External Link](#) manual curation, see [article](#) [External Link](#)
- Version:** ENSG00000171649.11

A 'Transcript Table' tab is also available, which is shown in the next screenshot.

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.

This screenshot shows the 'Transcript Table' for the gene ZIK1. The 'Transcript Table' tab is selected, displaying a table of transcripts:

#	Ensemble id	Name
1	ENST00000536878	ZIK1-002
2	ENST00000597219	ZIK1-006
3	ENST00000597850	ZIK1-001
4	ENST00000598689	ZIK1-007
5	ENST00000598726	ZIK1-008
6	ENST00000599456	ZIK1-003
7	ENST00000600053	ZIK1-005
8	ENST00000607468	ZIK1-004

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.

InSyBio Suite - RNA-Seq Differential Expression Pipeline

RNA-Seq Data: ☒ Paired-end ☐ Single-ended

Condition Control: * Required information

Title Read 1: Title Read 2:

Filename Read 1: Filename Read 2:

Title Read 1: Title Read 2:

Filename Read 1: Filename Read 2:

Condition 1:

Title Read 1: Title Read 2:

Filename Read 1: Filename Read 2:

Title Read 1: Title Read 2:

Filename Read 1: Filename Read 2:

Options

Do you want to perform initial FastQC? ☐

Do you want to perform trimming?

InSyBio Suite - RNA-Seq Differential Expression Pipeline

RNA-Seq ☐ Paired-end ☒ Single-ended

Data:

Condition Control: * Required information

Title:

Filename:

Title:

Filename:

Condition 1:

Title:

Filename:

Title:

Filename:

Options

Do you want to perform initial FastQC? ☐

Do you want to perform trimming?

- 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.

Options

Do you want to perform initial FastQC ☒

Do you want to perform trimming? YES (Default Options) ▾

Alignment Options

Source for the reference genome *

--Select Action-- ▾

Specify strand information:

Unstranded ▾

RNASeq Analysis **ncRNASeq Analysis**

- 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

Options

Do you want to perform initial FastQC ☒

Do you want to perform trimming? YES (Set Options ▾)

Trimmomatic Options

Perform initial ILLUMINACLIP step? YES ▾

Select standard adapter sequences or provide custom? * Standard ▾

Adapter sequences to use: * TruSeq3 (single-ended, f ▾)

1. Trimmomatic Operation

Sliding window trimmi ▾

Number of bases to average across:	4	⬆️⬇️⬆️
Average quality required:	15	⬆️⬇️⬆️

Add Trimmomatic Operation

- 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.

Alignment Options

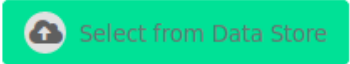
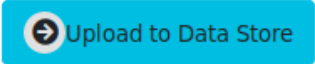
Source for the reference genome *

Use a genome from Data Store

Select the reference genome (FASTA): *

Title: chr22 fasta

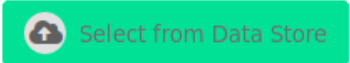
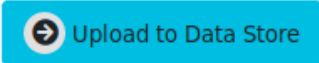
Filename: dsfile1573556494_9916.fa

Select the reference genome (GTF): *

Title: chr22 GTF

Filename: dsfile1573556655_8832.gtf

Alignment Options

Source for the reference genome *

Use a built-in genome

Select a reference genome: *

HumanGRCh38

Specify strand information:

Forward (FR)

- 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.

Which conditions do you want to compare?

Control ▾

Tumor ▾

-

Control ▾

Treated ▾

-

Condition Pairs:

Tumor ▾

Treated ▾

-

+

RNASeq Analysis

ncRNASeq Analysis

Clear All

The screenshot shows the InSyBio Suite Beta interface for RNA-Seq Differential Expression Pipeline Results. The top navigation bar includes a menu icon, the suite name, and user information. The main content area displays job details for a completed job (Job ID 1, Submission Date May 6, 2019 7:55:09 AM, Execution Time 00 hours, 15 minutes, 49 seconds). The 'Deseq2 Reports' tab is active, showing a list of downloadable files and folders. The files include Deseq2 Report File (.pdf), Job-1 DESeq2 pdf output, Deseq2 Report File (.png), HBR_UHRimages.zip, and several Deseq2 Report File (.csv) files. Each file has a 'Download' button with a file icon.

Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters
COMPLETED	1	May 6, 2019 7:55:09 AM	00 hours, 15 minutes, 49 seconds	

Initial FastQC Reports	Trimmed FASTQ Files	Trimmed FastQC Reports	Alignment Files	Read Count Files	Next Actions
Deseq2 Reports					
HBR_UHR					
Deseq2 Report File (.pdf) Download					
Job-1 DESeq2 pdf output File					
Deseq2 Report File (.png) Download					
HBR_UHRimages.zip Image Folder					
Deseq2 Report File (.csv) Download					
Job-1 DESeq2 output HBR_UHR_diffexpr-results-with-counts.csv (HBR_UHR_diffexpr-results-with-counts.csv); File					
Job-1 DESeq2 output HBR_UHR_diffexpr-results.csv (HBR_UHR_diffexpr-results.csv); File					
Job-1 DESeq2 output HBR_UHR_diffexpr-resultssignificant_pvalues.csv (HBR_UHR_diffexpr-results_significant_pvalues.csv); File					

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

The screenshot shows the 'Initial FastQC Reports' tab in the InSyBio Suite Beta interface. The tab is highlighted with a green border. It displays a list of FastQC reports for various input files. Each report has a 'Download' button with a folder icon and a 'View Html Page' button with a document icon. The reports are organized by job and read pair.

Deseq2 Reports	Initial FastQC Reports	Trimmed FASTQ Files	Trimmed FastQC Reports	Alignment Files	Read Count Files	Next Actions
FastQC Report Download View Html Page						
Job-1 Fastqc zip file HBR rep1 read1 Folder dsfile1557128487_9359_fastqc						
Job-1 Fastqc zip file HBR rep1 read2 Folder dsfile1557128516_9128_fastqc						
Job-1 Fastqc zip file HBR rep2 read1 Folder dsfile1557128550_6204_fastqc						
Job-1 Fastqc zip file HBR rep2 read2 Folder dsfile1557128587_1781_fastqc						
Job-1 Fastqc zip file HBR rep3 read1 Folder dsfile1557128617_6024_fastqc						
Job-1 Fastqc zip file HBR rep3 read2 Folder dsfile1557128647_9984_fastqc						

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

Deseq2 Reports	Initial FastQC Reports	Trimmed FASTQ Files	Trimmed FastQC Reports	Alignment Files	Read Count Files	Next Actions
Trimmed FASTQ File			Download			
Job-1 trimmend paired file of HBR rep1 read1 (dsfile1557128487_9359_trimmed.gz);			 File			
Job-1 trimmend paired file of HBR rep1 read2 (dsfile1557128516_9128_trimmed.gz);			 File			
Job-1 trimmend paired file of HBR rep2 read1 (dsfile1557128550_6204_trimmed.gz);			 File			
Job-1 trimmend paired file of HBR rep2 read2 (dsfile1557128587_1781_trimmed.gz);			 File			
Job-1 trimmend paired file of HBR rep3 read1 (dsfile1557128617_6024_trimmed.gz);			 File			
Job-1 trimmend paired file of HBR rep3 read2 (dsfile1557128647_9984_trimmed.gz);			 File			
Job-1 trimmend paired file of UHR rep1 read1 (dsfile1557128760_6526_trimmed.gz);			 File			

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

Deseq2 Reports	Initial FastQC Reports	Trimmed FASTQ Files	Trimmed FastQC Reports	Alignment Files	Read Count Files	Next Actions
Trimmed FastQC Report			Download	View Html Page		
s:51:"Job-1 after trimming Fastqc zip file HBR rep1 read1";			 File	 dsfile1557128487_9359_trimmed_fastqc		
s:51:"Job-1 after trimming Fastqc zip file HBR rep1 read2";			 File	 dsfile1557128516_9128_trimmed_fastqc		
s:51:"Job-1 after trimming Fastqc zip file HBR rep2 read1";			 File	 dsfile1557128550_6204_trimmed_fastqc		
s:51:"Job-1 after trimming Fastqc zip file HBR rep2 read2";			 File	 dsfile1557128587_1781_trimmed_fastqc		
s:51:"Job-1 after trimming Fastqc zip file HBR rep3 read1";			 File	 dsfile1557128617_6024_trimmed_fastqc		
s:51:"Job-1 after trimming Fastqc zip file HBR rep3 read2";			 File	 dsfile1557128647_9984_trimmed_fastqc		

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

Deseq2 Reports	Initial FastQC Reports	Trimmed FASTQ Files	Trimmed FastQC Reports	Alignment Files	Read Count Files	Ne
----------------	------------------------	---------------------	------------------------	-----------------	------------------	----

SAM File

Download

Job-1 Hisat2 alignment file HBR_1.sam (HBR_1.sam);

 File

Job-1 Hisat2 alignment file HBR_2.sam (HBR_2.sam);

 File

Job-1 Hisat2 alignment file HBR_3.sam (HBR_3.sam);

 File

Job-1 Hisat2 alignment file UHR_1.sam (UHR_1.sam);

 File

Job-1 Hisat2 alignment file UHR_2.sam (UHR_2.sam);

 File

Job-1 Hisat2 alignment file UHR_3.sam (UHR_3.sam);

 File

BAM File

Download

Job-1 BAM fileHBR_1.bam (HBR_1.bam);

 File

Job-1 BAM fileHBR_2.bam (HBR_2.bam);

 File

Job-1 BAM fileHBR_3.bam (HBR_3.bam);

 File

Job-1 BAM fileUHR_1.bam (UHR_1.bam);

 File

Job-1 BAM fileUHR_2.bam (UHR_2.bam);

 File

Job-1 BAM fileUHR_3.bam (UHR_3.bam);

 File

Run Info

Download

Alignment Info

 hisat2_report.txt

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

Deseq2 Reports	Initial FastQC Reports	Trimmed FASTQ Files	Trimmed FastQC Reports	Alignment Files	Read Count Files	Next A
Read Count File		Download		Download Run Info File		
Job-1 Feature counts file (HBR_1.counts);		 HBR_1.counts		 HBR_1.features.summary		
Job-1 Feature counts file (HBR_2.counts);		 HBR_2.counts		 HBR_1.features.summary		
Job-1 Feature counts file (HBR_3.counts);		 HBR_3.counts		 HBR_1.features.summary		
Job-1 Feature counts file (UHR_1.counts);		 UHR_1.counts		 HBR_1.features.summary		
Job-1 Feature counts file (UHR_2.counts);		 UHR_2.counts		 HBR_1.features.summary		
Job-1 Feature counts file (UHR_3.counts);		 UHR_3.counts		 HBR_1.features.summary		

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

	Job Status	Job ID	Submission Date	Execution Time	Input Data and F
< Dashboard	COMPLETED	79	Oct 2, 2019 8:56:41 AM	00 hours, 01 minutes, 56 seconds	
Deseq2 Reports	Alignment Files	Read Count Files	Predicted ncRNAs	Next Actions	
Predicted ncRNAs					Download
Predicted ncRNAs file					 File

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.

Deseq2 Reports

Initial FastQC Reports

Trimmed FASTQ Files

Trimmed FastQC Reports

Alignment Files

Read Count Files

Next Actions

Continue your Analysis in InSyBio Suite

HBR_UHR

Molecule Quantification Files per Condition	Download	Next Action
Job-1 MQ file HBR_UHR_diffexpr-MQHBR.csv (HBR_UHR_diffexpr-MQHBR.csv);	 File	--Select Action--
Job-1 MQ file HBR_UHR_diffexpr-MQUHR.csv (HBR_UHR_diffexpr-MQUHR.csv);	 File	--Select Action--
Full Molecule Quantification File and Associated Labels	Download	Next Action
Job-1 MQ file HBR_UHR_diffexpr-MQ.csv (HBR_UHR_diffexpr-MQ.csv);	 File	--Select Action--
Job-1 label file HBR_UHR_diffexpr-labels.txt (HBR_UHR_diffexpr-labels.txt);	 File	--Select Action--

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.

 InSyBio Suite - single-cell RNA-Seq Differential Expression Pipeline

 InSyBio Beta User 

Workflow One Sample, One GEM Well, Multiple Flowcells

Input Data Files

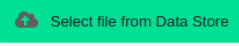
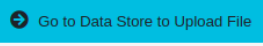
Choose or upload to input your Fastq files to InSyBio single-cell RNA-Seq Differential Expression Pipeline tool following the rules:

- Fastq files must be in this name format: [Sample name]_S*_[Read Type]_001.fastq.gz (e.g. singlecell1_S1_R1_001.fastq.gz)
- Both R1 and R2 versions of each file must be present
- Fastq files of the same sample must have the same sample name
- Fastq files of different samples must have different sample name

Fastq File 1

Title1:

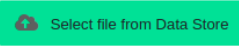
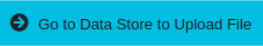
Filename 1:

Fastq File 2

Title2:

Filename 2:



Options

Transcriptome

Human

Cluster annotation

Species:

--Select Action--

Tissue 

--Select Action--

- 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

BAM file generation



First filtering

Minimum cells:

Minimum
features:

Secondary filtering

nFeature_RNA ? :

Lower limit: 200

Upper limit: 10000

nCount_RNA ?

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.

Status	Job ID	Job Type	Input File(s)	Submission Date	Start Execution Date	Completion Date	Current Step	Actions
Error	1	RNASeq Analysis		2/9/22 1:10 PM	2/28/22 9:56 AM	2/27/22 7:20 PM	Single Cell Alignment	View Details
Completed	2	RNASeq Analysis		2/23/22 1:21 PM	2/28/22 6:51 AM	2/28/22 8:04 AM	Secondary Single Cell Analysis	View Results
Error	3	RNASeq Analysis			3/9/22 8:24 PM	2/28/22 5:58 PM	Single Cell Alignment	View Details
Running	4	RNASeq Analysis			3/15/22 10:08 AM	-	Secondary Single Cell Analysis	View Details

Showing 1 to 4 of 4 entries

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.

InSyBio Suite - RNA-Seq Single Cell Pipeline Differential Expression Pipeline Results

InSyBio Beta User

	Job Status	Job Type	Job ID	Submission Date	Execution Time	Input Data and Parameters
Dashboard	COMPLETED	RNASeq Single Cell Analysis	2	Feb 23, 2022 1:21:53 PM	01 hours, 13 minutes, 17 seconds	

Report
Summary
Additional Cell Statistics
Dot Plots Visualization
Feature Plots Visualization
Ridge Plots Visualization
Umap Plots Visualization
All Results Download

Single Cell Pipeline Report

Alignment of the sequencing reads in the provided FASTQ files to the selected reference transcriptome and read counts computation are performed with the Cellranger count pipeline. The outs folder contains the outputs of this step and includes the web_summary.html file which summarizes the results.

Secondary Single Cell Analysis

For the secondary single cell analysis quality control checks and filtering criteria are applied to the single cell data. With the Seurat Object the data are filtered using min.cells = 0 and min.features = 0.

min.cells: Include features detected in at least this many cells.

min.features: Include cells where at least this many features are detected.

An additional filtering step is performed with Seurat, keeping only cells that have unique feature counts and total number of molecules detected within a cell with the following limits:

nFeature_RNA = unique feature counts. lower limit: 100, upper limit: 3000

nCount_RNA = total number of molecules detected within a cell. lower limit: , upper limit:

The data are then normalized using the LogNormalize method, which normalizes the feature expression measurements for each cell by the total expression, multiplies this by a scale factor (10.000) and log-transforms the result.

2000 highly variable features that exhibit high cell-to-cell variation in our data are identified. Scaling is subsequently performed scaled, so that the mean expression across cells is 0 and variance across cells is 1. This last step is necessary for performing PCA on the data

The cells are clustered using a modularity optimization technique called Louvain algorithm with a resolution parameter of 1 (it sets the granularity of the downstream clustering) having firstly constructed the KNN graph (with k=30) based on the Euclidean distance in PCA space and using Jaccard similarity. Using the clustered data, non-linear dimensionality reduction is performed, producing the Umap plot.

In scRNA seq data analysis, differentially expressed features that define the clusters are called markers. These are called markers. To identify these markers, we firstly used the FindAllMarkers() function of the Seurat package, which identifies these markers for all clusters by comparing all clusters with each other. For this function we used parameters min.pct (a feature to be detected at a minimum percentage in either of the groups of cells) with value 0.1 and logfc.threshold (Limit testing to genes which show, on average, at least X-fold difference (log-scale) between the two groups of cells) with value 0.25. The matrices produced by these functions contain the genes as rows and these specific associated statistics for each gene as columns: P value, Average log2 Fold Change, Percentage of cells 1, Percentage of cells 2 and Adjusted P value.

The Dotplots include the differentially expressed genes that are only differentially expressed in one cluster of cells while sorting them by their p value.

The scCATCH package, a single cell Cluster-based annotation Toolkit for Cellular Heterogeneity is finally used to identify the cluster marker genes and creates the cluster annotations. We used the scCATCH() function which does the cluster annotation by matching the potential marker genes with known cell marker genes in a tissue-specific cell taxonomy reference database (CellMatch). We used the cancer type: and tissue types: Blood, Bladder.

The selected species was Human.

Results files description

Outs folder: The output files of the cellranger platform.

Web_summary.html: Variety of metrics such as Mean Reads per Cell, Median Genes per Cell, Valid Barcodes etc. At the analysis tab, t-SNE projection can be seen with UMI Counts or Clustered. Also, info about the Top features by cluster can be found.

Results of the secondary single cell analysis:

RidgePlots folder: Folder containing a Ridge Plot per gene you selected.

FeaturePlots folder: Folder containing a Feature Plot per gene you selected.

Dimensionality Reduction Plot folder:

Contains Umap.png: Umap projection plot of the clusters.

Markers folder:

markers_from_FindAllMarkers and markers_from_scCATCH: Markers(differentially expressed genes) and associated statistics (p-values, avg_log2FC etc) from FindAllMarkers and scCATCH functions respectively.

average_expression_of_genes.csv: Averaged expression values for every gene for every cluster.

Barcode-cluster.csv: Barcode-cluster matrix.

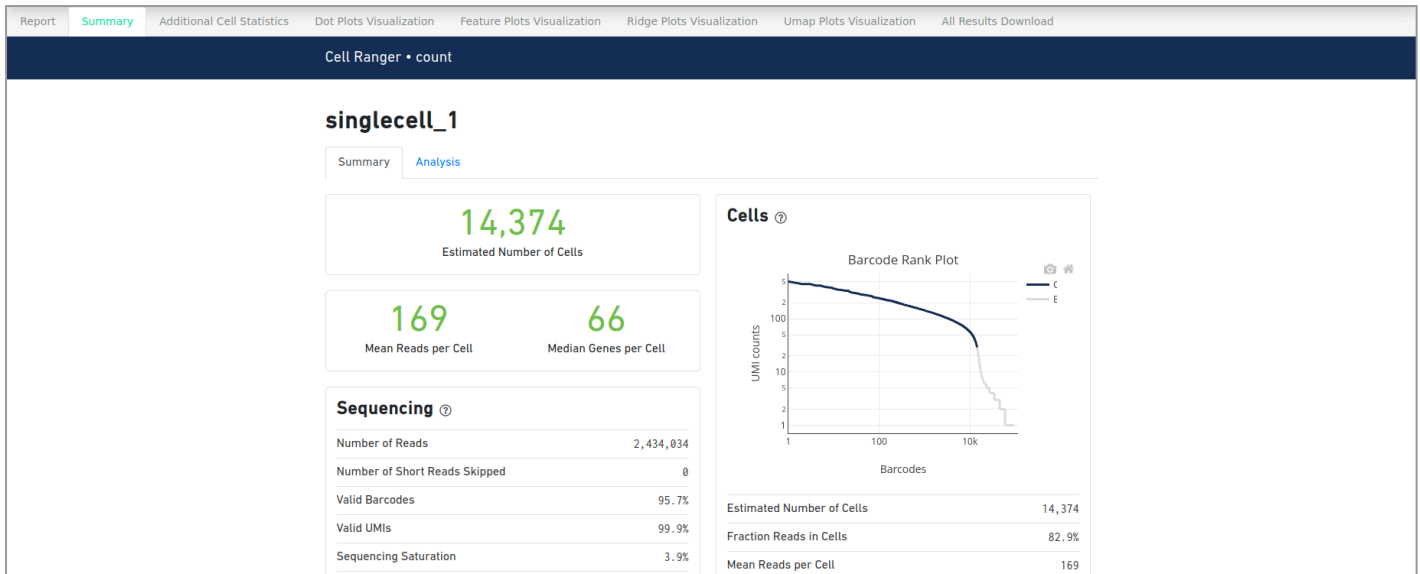
Dotplots Folder: Folder containing all the dotplots needed. (Dotplot_unique, Dotplot_only_specific_genes)

DotPlot_unique.pdf: Top 5 unique differentially expressed genes for each cell cluster based on the p-value and log2fc value.

Dotplot_only_specific_genes.pdf: Same dotplot as the previous ones but for the specific genes you selected.

You can find all these files compressed at their respective zip file.

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.

Report

Summary

Additional Cell Statistics

Dot Plots Visualization

Feature Plots Visualization

Ridge Plots Visualization

Umap Plots Visualization

All Results Download

Total Markers

Markers with Cluster Annotation

Average Expression of genes

Barcode Cluster

Total Markers Results

Download Total Markers CSV

Gene	T1	P value	T1	Average log2 Fold Change	T1	Percentage of cells 1 ?	T1	Percentage of cells 2 ?	T1	Adjusted P value ? ?	Cluster	T1
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.

Total MarkersMarkers with Cluster AnnotationAverage Expression of genesBarcode Cluster

Markers with Cluster Annotation Results

Download Markers with Cluster Annotation CSV

Cluster	T1	Cell type	T1	Cell type score	T1	Cell type related markers	T1	PMID	T1
RPL3, MT-ATP6, HIST1H4C, TUBA1B, HSP90AA1, MT-CO3, RPL13, S100A4, CFL1, H2AF2, 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, H2AF2, 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, TM6SF6, 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, PP1B, NUCKS1, NCL, BNIP3, DUT, UQCRCB, 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.

Total Markers

Markers with Cluster Annotation

Average Expression of genes

Barcode Cluster

Average Expression of genes Results

Below you can see the first 500 rows of the generated Average Expression of genes csv. You can download the full results by clicking the "Download Average Expression of genes CSV" button.

Download Average Expression of genes CSV

Gene	T1	Dendritic Cell_0	T1	Dendritic Cell_1	T1	Plasmacytoid Dendritic Cell_2	T1	Dendritic Cell_3	T1	Dendritic Cell_4	T1	NA_5	T1	Activated T Cell_6	T1	Dendritic Cell_7	T1	Dendritic Cell_8	T1
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.

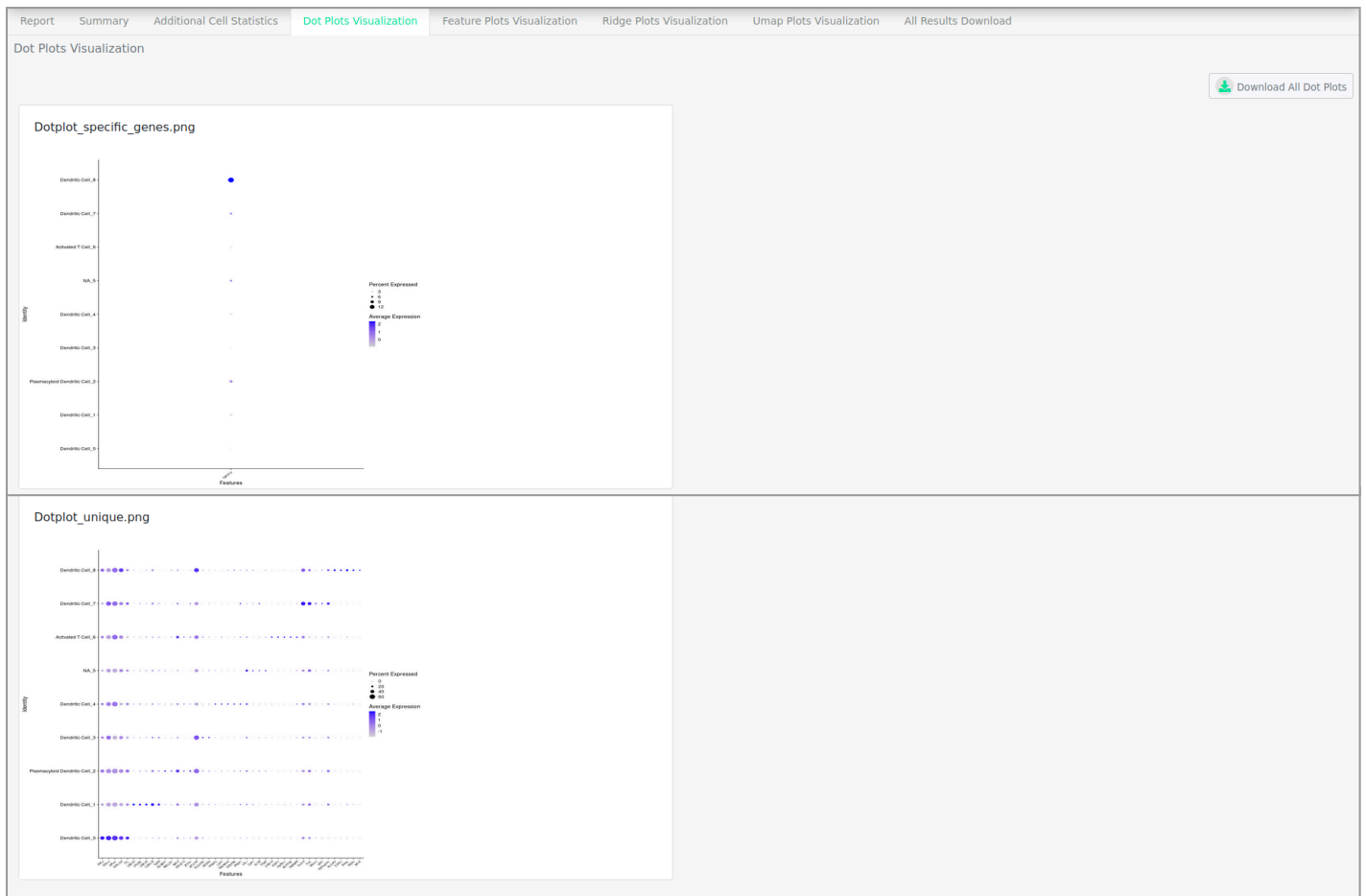
Total MarkersMarkers with Cluster AnnotationAverage Expression of genesBarcode Cluster

Barcode Cluster Results

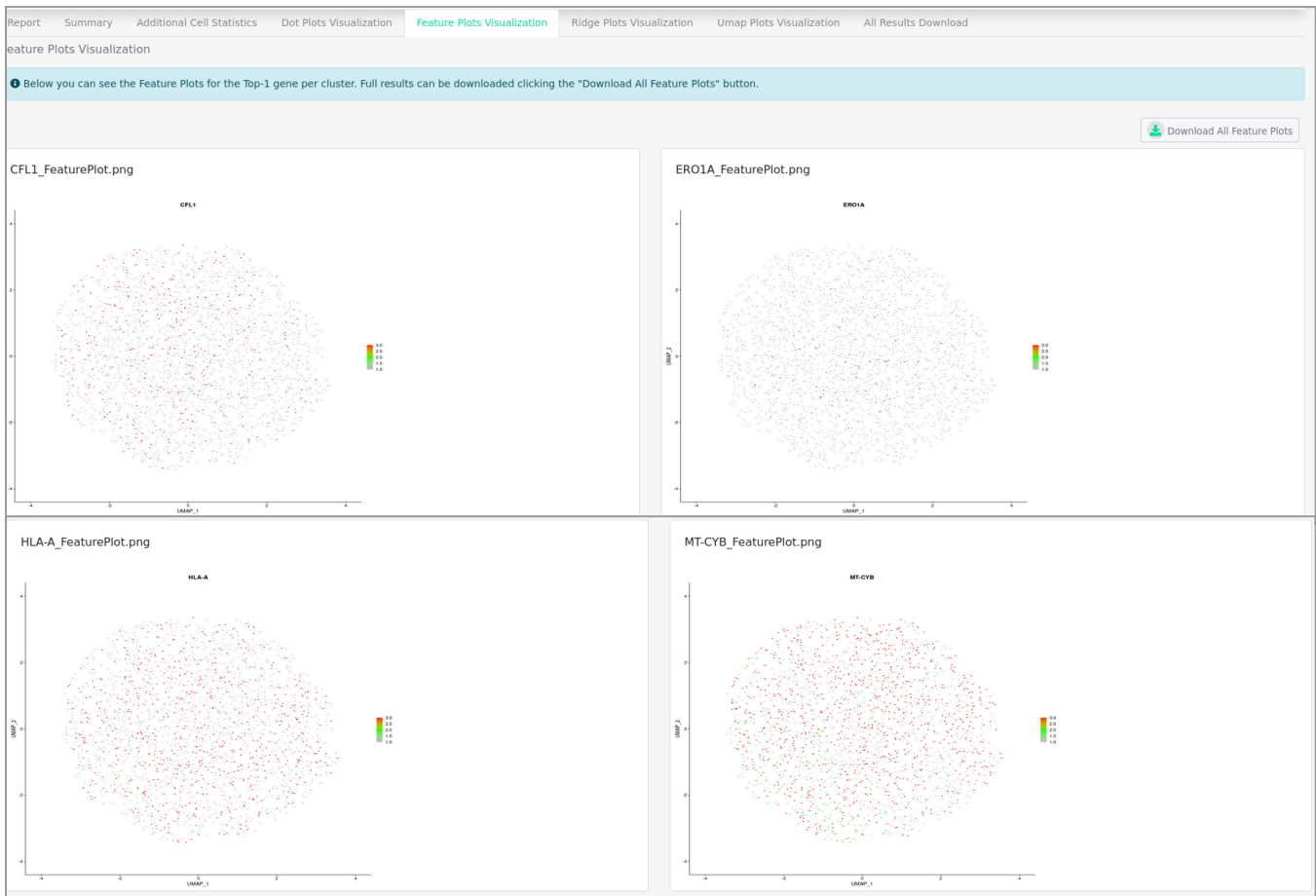
Download Barcode Cluster CSV

Barcode	Cluster
"AAACCCACATATAGCC-1"	"Activated T Cell_6"
"AAACCCATCAGTCCT-1"	"Dendritic Cell_0"
"AAACCCATCGCATGAT-1"	"Dendritic Cell_3"
"AAACGAACAATAGGAT-1"	"Plasmacytoid Dendritic Cell_2"
"AAACGAACACAAGTA-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"
"AAAGGATCAGGAAAC-1"	"Dendritic Cell_4"
"AAAGGATCACTCATAG-1"	"Dendritic Cell_8"
"AAAGGATGTGCTATA-1"	"Dendritic Cell_1"

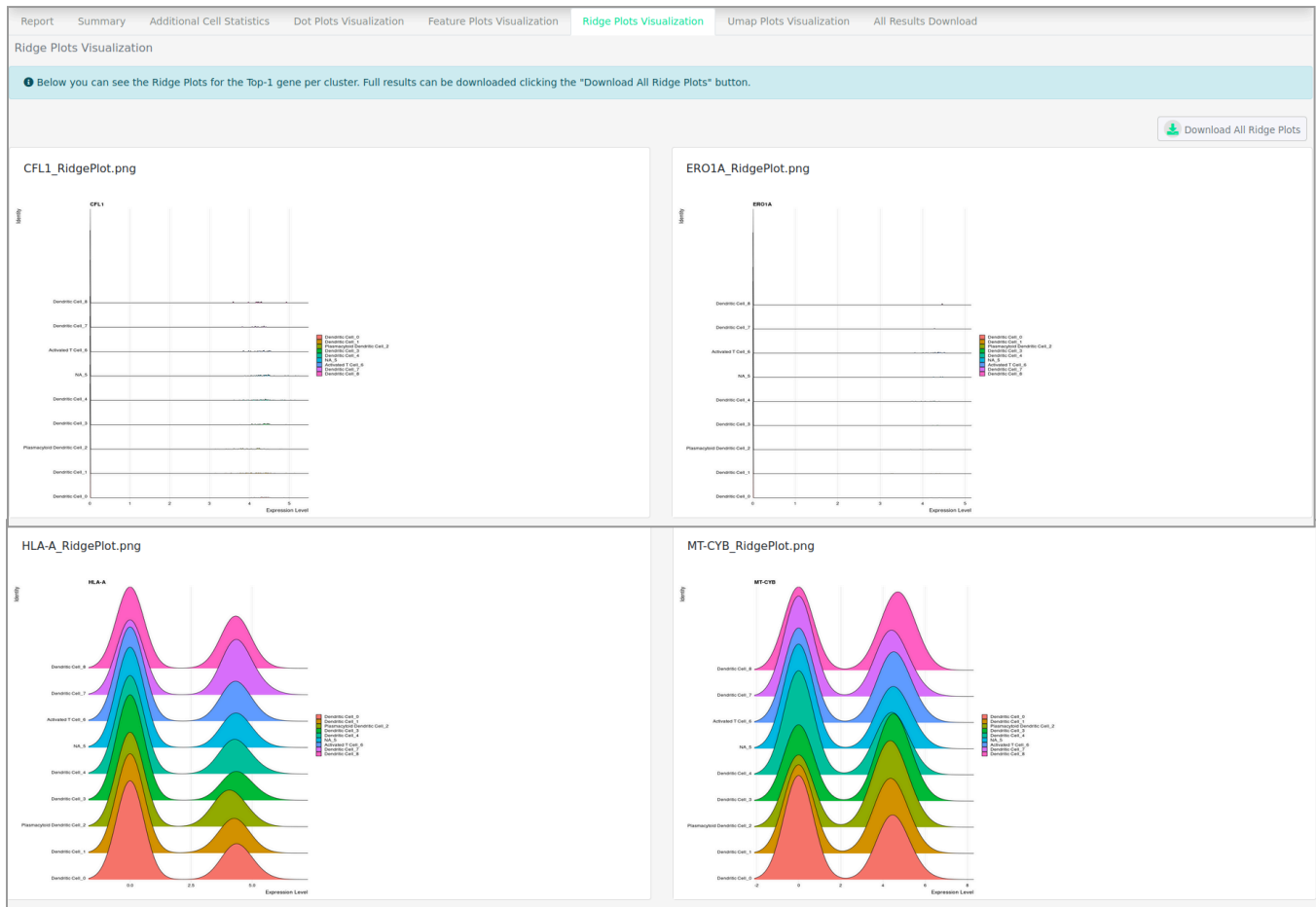
At the Barcode Cluster tab, the Barcode-Cluster matrix can be found.



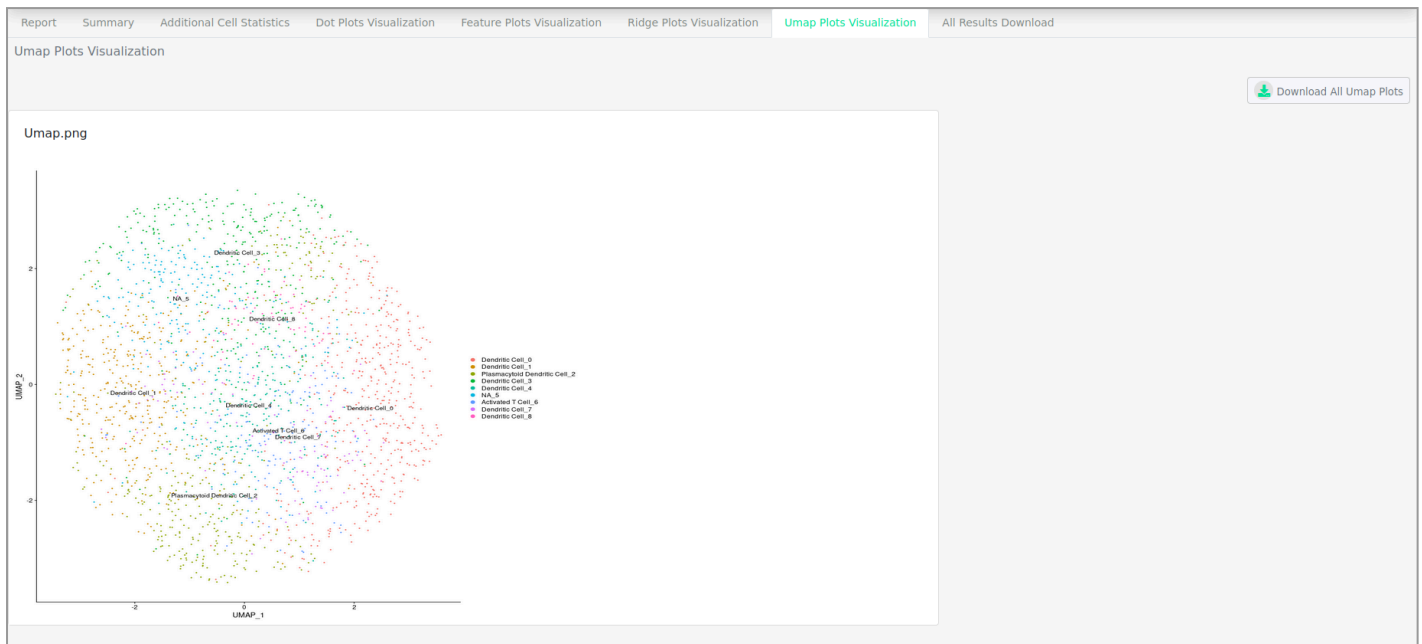
At the Dot Plots Visualization tab you can see the two Dot plots that are created. The first one is a Dot Plot with only the genes you specified at the manual parameters and the second one is a Dot Plot that shows the Top 5 unique differentially expressed genes for each cell cluster based on the p-value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



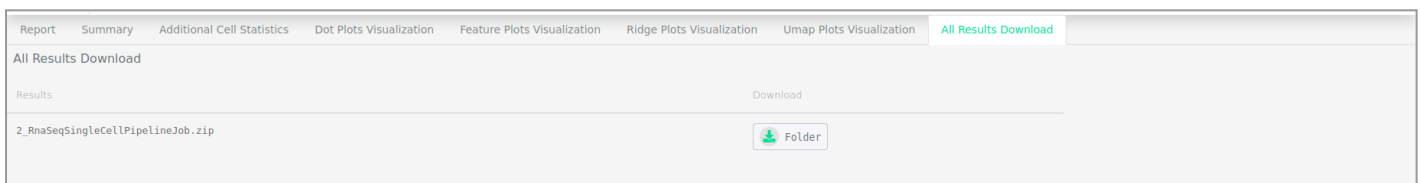
At the Feature Plots Visualization tab the Feature Plots for the Top-1 gene per cluster can be found. The Feature Plots of all the genes can be downloaded.



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.


InSyBio Suite - Deconvolve Data Against Single-cell RNA-Seq Analysis



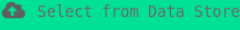
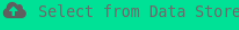
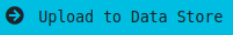
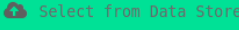
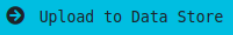

InSyBio Beta User




[Dashboard](#)

scRNAseq Files

* Required information

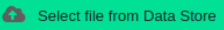
Matrix Title: * Matrix Filename: *  	Features Title: * Features Filename: *  	Barcodes Title: * Barcodes Filename: *  
---	---	---

Biomarker Files

BulkRNAseq File *

Title:

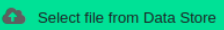
Filename:




Biomarkers Labels *

Title:

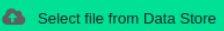
Filename:




Barcode Cluster *

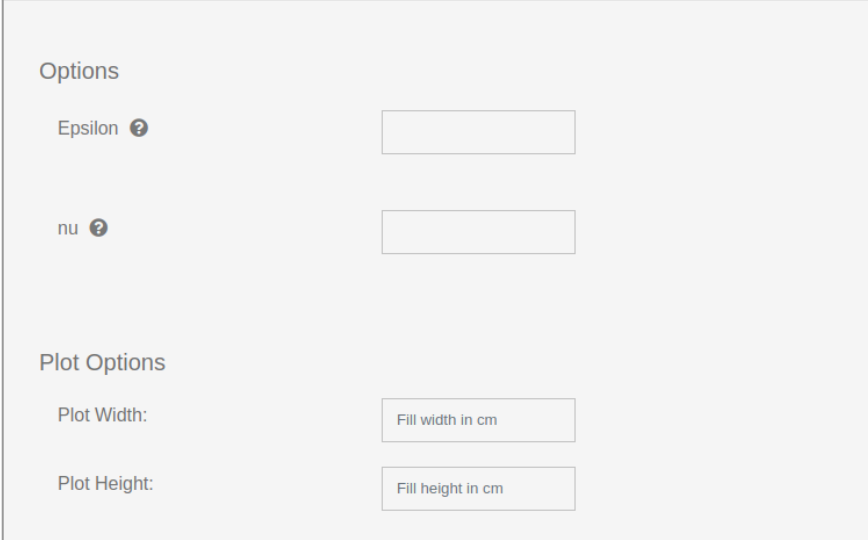
Title:

Filename:




- 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



The screenshot shows a light gray rectangular form with a thin black border. Inside the form, the text 'Options' is at the top left. Below it, 'Epsilon' is followed by a question mark icon and a text input field. Below that, 'nu' is followed by a question mark icon and another text input field. Further down, the text 'Plot Options' is displayed. Under 'Plot Options', 'Plot Width:' is followed by a text input field containing the placeholder text 'Fill width in cm'. Below that, 'Plot Height:' is followed by a text input field containing the placeholder text 'Fill height in cm'.

- 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.

Status	Job ID	Job Type	Input File(s)	Submission Date	Start Execution Date	Completion Date	Current Step	Actions
Completed	21	RNASeq Single Cell Velocity Analysis		11/26/73, 3:22 AM	1/16/24, 1:59 PM	-	Secondary Single Cell Analysis	View Results
Completed	20	Deconvolve Data against single-cell RNA-seq Analysis		8/12/11, 6:41 AM	1/15/24, 2:14 PM	1/15/24, 2:15 PM		View Results
Completed	19	RNASeq Single Cell Velocity Analysis		3/29/80, 9:11 PM	1/12/24, 8:51 AM	1/12/24, 9:06 AM	Secondary Single Cell Analysis	View Results
Completed	18	Cell Chat Analysis		12/19/68, 6:13 AM	1/12/24, 8:29 AM	1/12/24, 8:33 AM	Single Cell Alignment	View Results
Completed	17	RDS Conversion		2/9/59, 5:58 AM	1/11/24, 11:16 AM	1/11/24, 11:18 AM	Secondary Single Cell Analysis	View Results

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 StatusJob TypeJob IDSubmission DateExecution TimeInput Data and Parameters

COMPLETED

15

Jan 23, 2024, 12:16:52 PM

00 hours, 01 minutes, 32 seconds

i

Results CSV Files

Plot Visualization

CSV File

Download

Basis CSV

Download Basis CSV

Props CSV

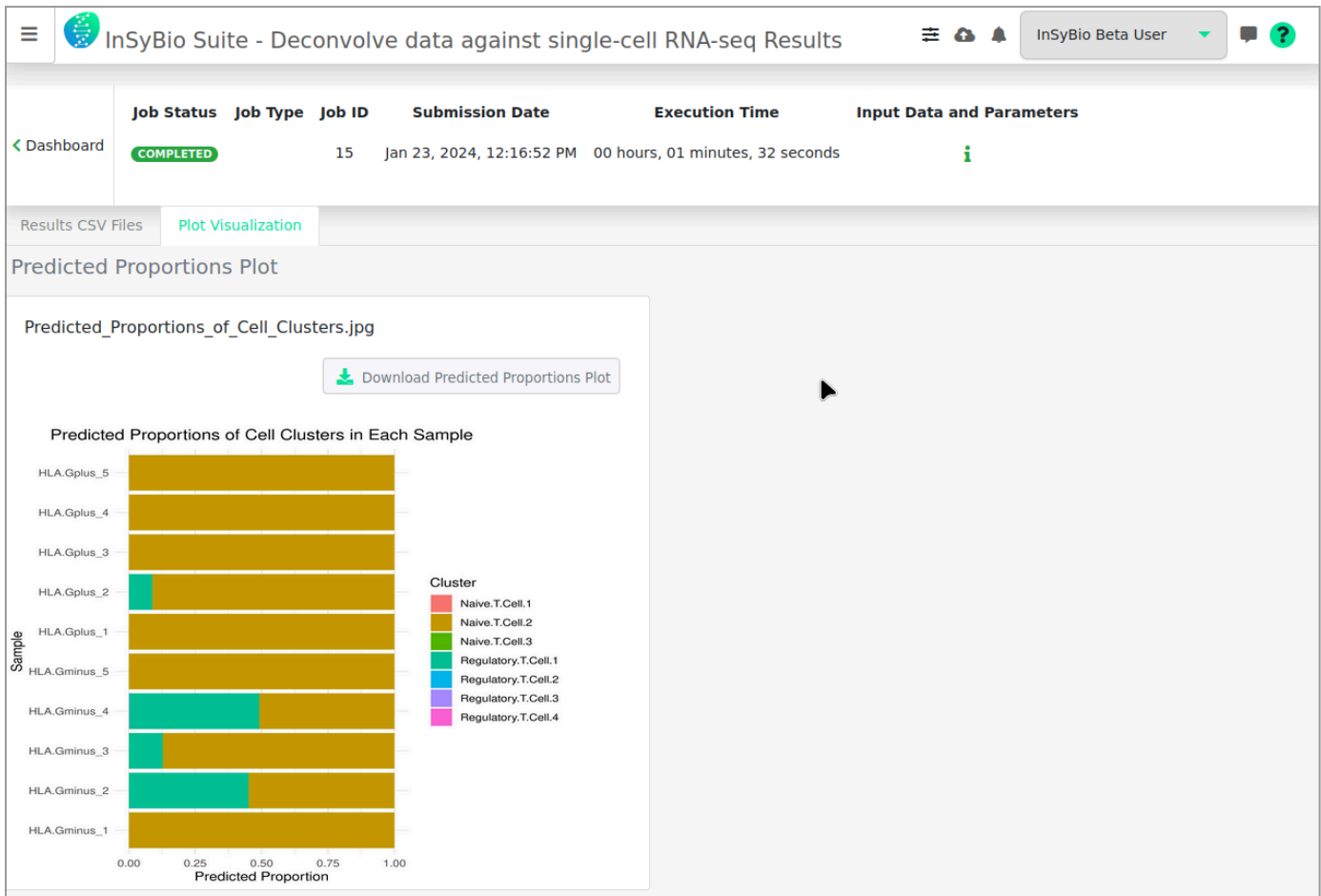
Download Props CSV

Yhat CSV

Download Yhat CSV

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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 velocity tool to estimate the RNA velocities of single-cells and the monocle3 and scvelo packages to identify trajectories and further analyse the estimated velocities.

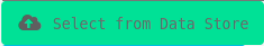
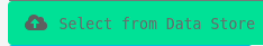
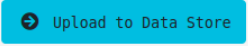
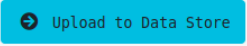
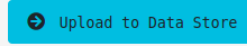
To start the Velocity single-cell Analysis:

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.

 InSyBio Suite - Velocity single-cell Analysis ☰ 📁 🔔 InSyBio Beta User ▼ 💬 ?

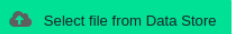
* Required information

Matrix Title: *	Features Title: *	Barcodes Title: *
Matrix Filename: *	Features Filename: *	Barcodes Filename: *
		
		

Bam File *

Title:

Filename:

Transcriptome: Human

Computation of velocity: Stochastic

Root nodes ? : ex.1,2,3 or if they have annotated clusters T Regulatory Cell 1, T Regulatory Cell 2, Naive Cell 3.

Cluster annotation: --Select Action--

Species: --Select Action--

Tissue ? : --Select Action--

- 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

Shared Nearest Neighbor (SNN) Graph

k parameter (k-nearest-neighbor):

Clustering

Resolution parameter ? :

- 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.

InSyBio Suite - Single Cell RNA-Seq Differential Expression Pipeline Dashboard

InSyBio Interact

InSyBio ncRNASeq

InSyBio Bionets

InSyBio Biomarkers

InSyBio DNA-Seq

InSyBio Pipelines

InSyBio DataStore

+ Add new Job

Filter Jobs

Show All

13

1

0

4

Completed

Running

Pending

Error

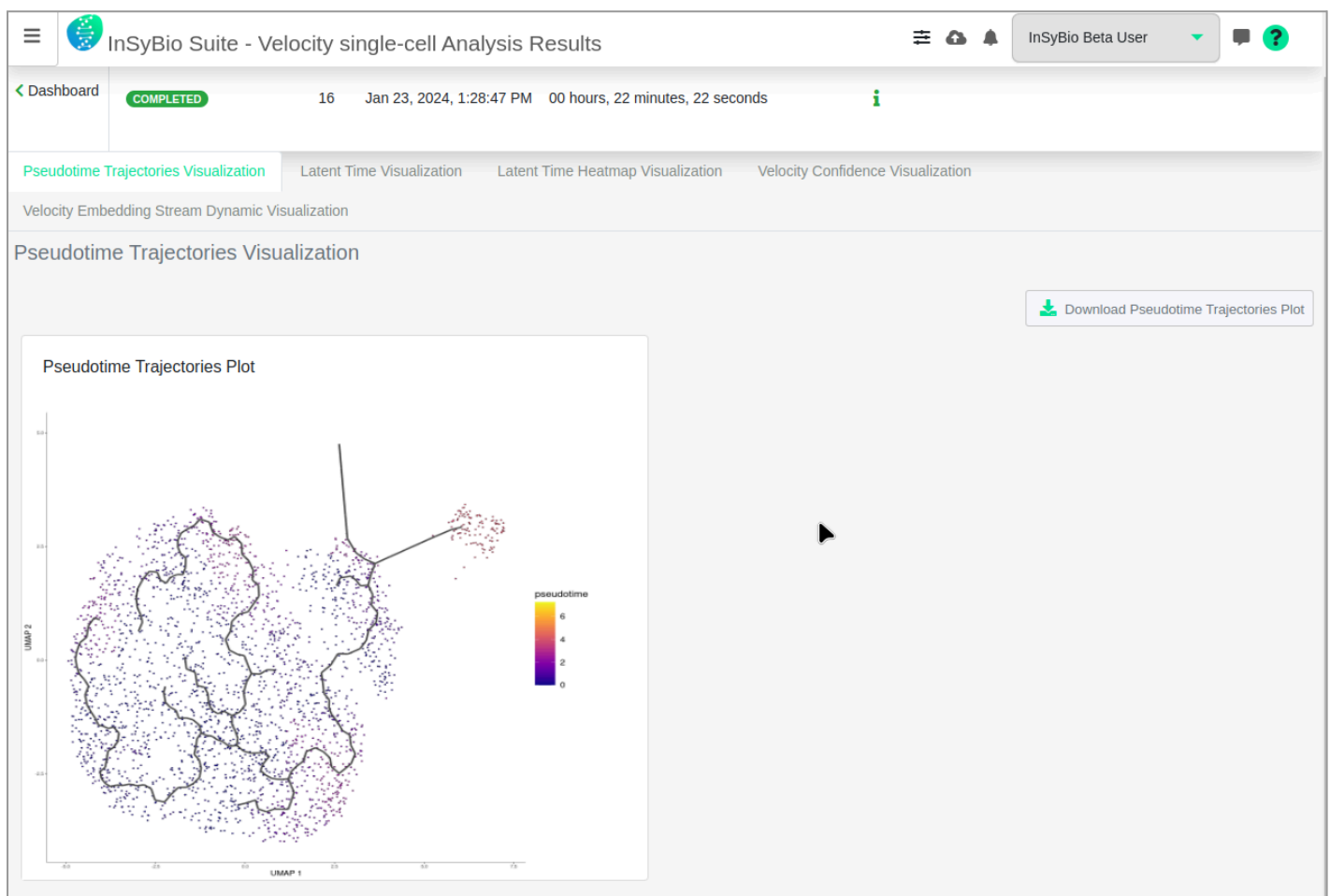
Status	Job ID	Job Type	Input File(s)	Submission Date	Start Execution Date	Completion Date	Current Step	Actions
Completed	21	RNASeq Single Cell Velocity Analysis		11/26/73, 3:22 AM	1/16/24, 1:59 PM	-	Secondary Single Cell Analysis	View Results
Completed	20	Deconvolve Data against single-cell RNA-seq Analysis		8/12/11, 6:41 AM	1/15/24, 2:14 PM	1/15/24, 2:15 PM		View Results
Completed	19	RNASeq Single Cell Velocity Analysis		3/29/80, 9:11 PM	1/12/24, 8:51 AM	1/12/24, 9:06 AM	Secondary Single Cell Analysis	View Results
Completed	18	Cell Chat Analysis		12/19/68, 6:13 AM	1/12/24, 8:29 AM	1/12/24, 8:33 AM	Single Cell Alignment	View Results
Completed	17	RDS Conversion		2/9/59, 5:58 AM	1/11/24, 11:16 AM	1/11/24, 11:18 AM	Secondary Single Cell Analysis	View Results

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.

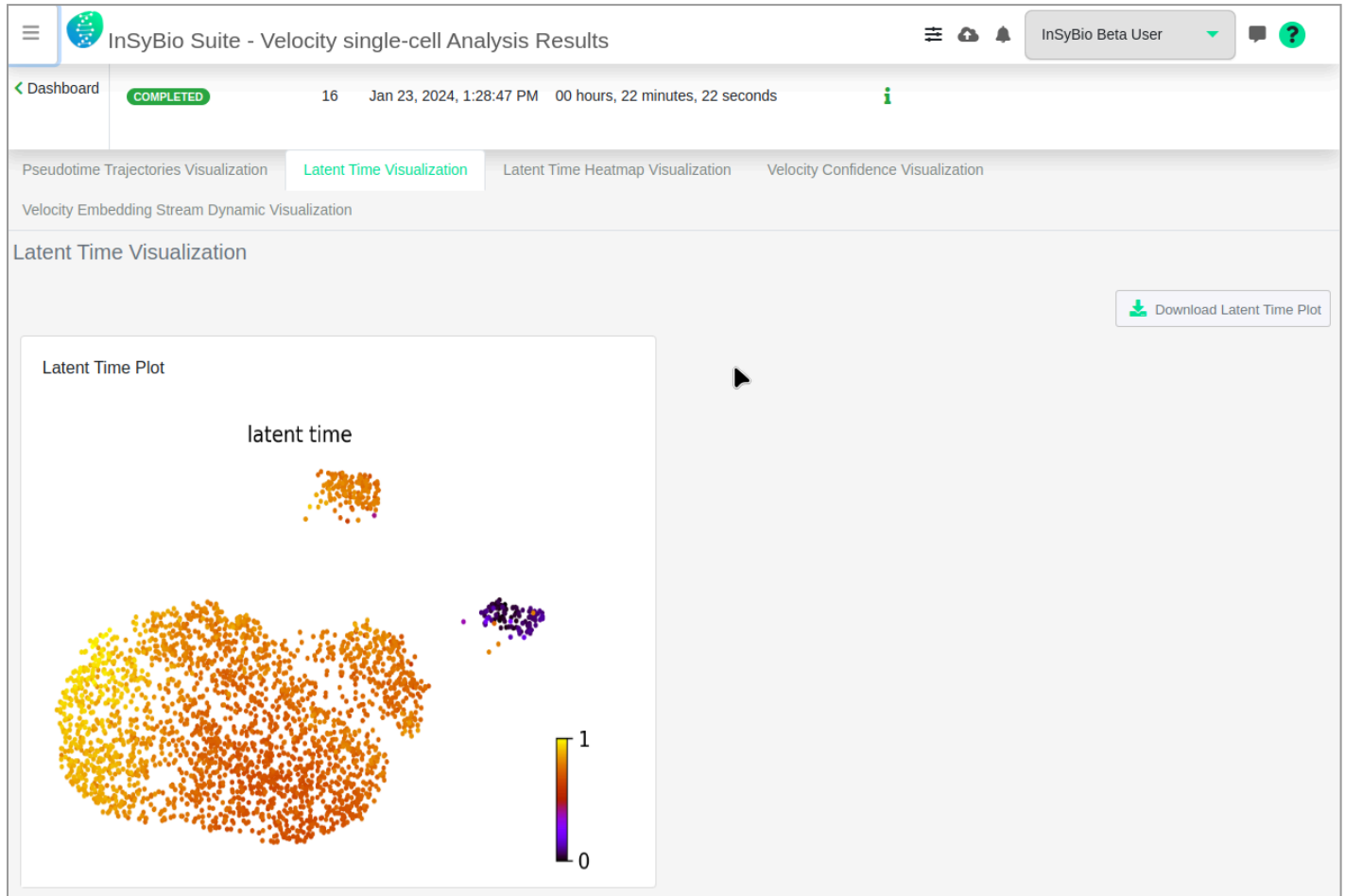
Depending on the computation type of velocity you selected, different tabs will appear.

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

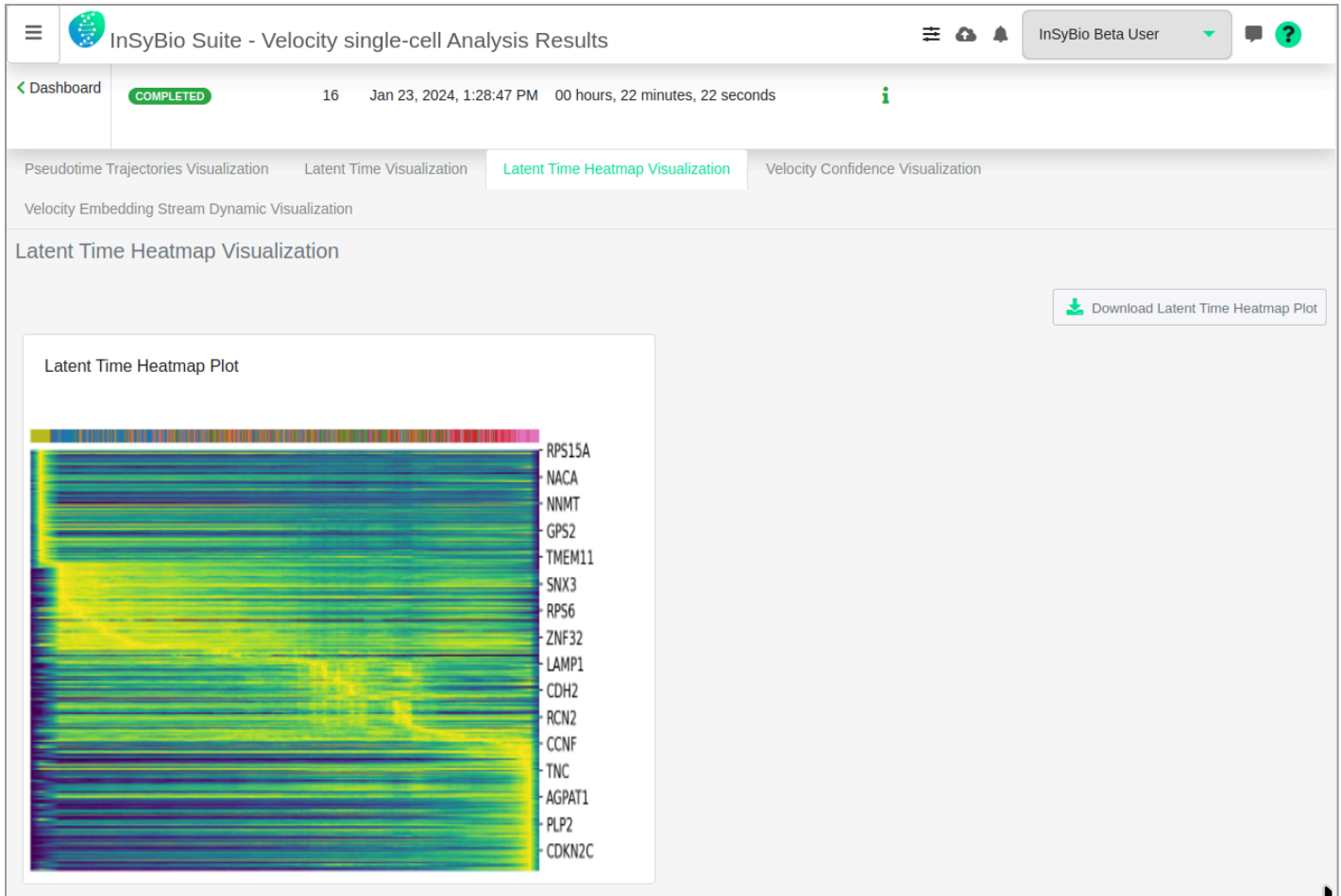
In the Pseudotime Trajectories Visualization tab, the plot visualizes the pseudotime trajectories calculated by monocle3.



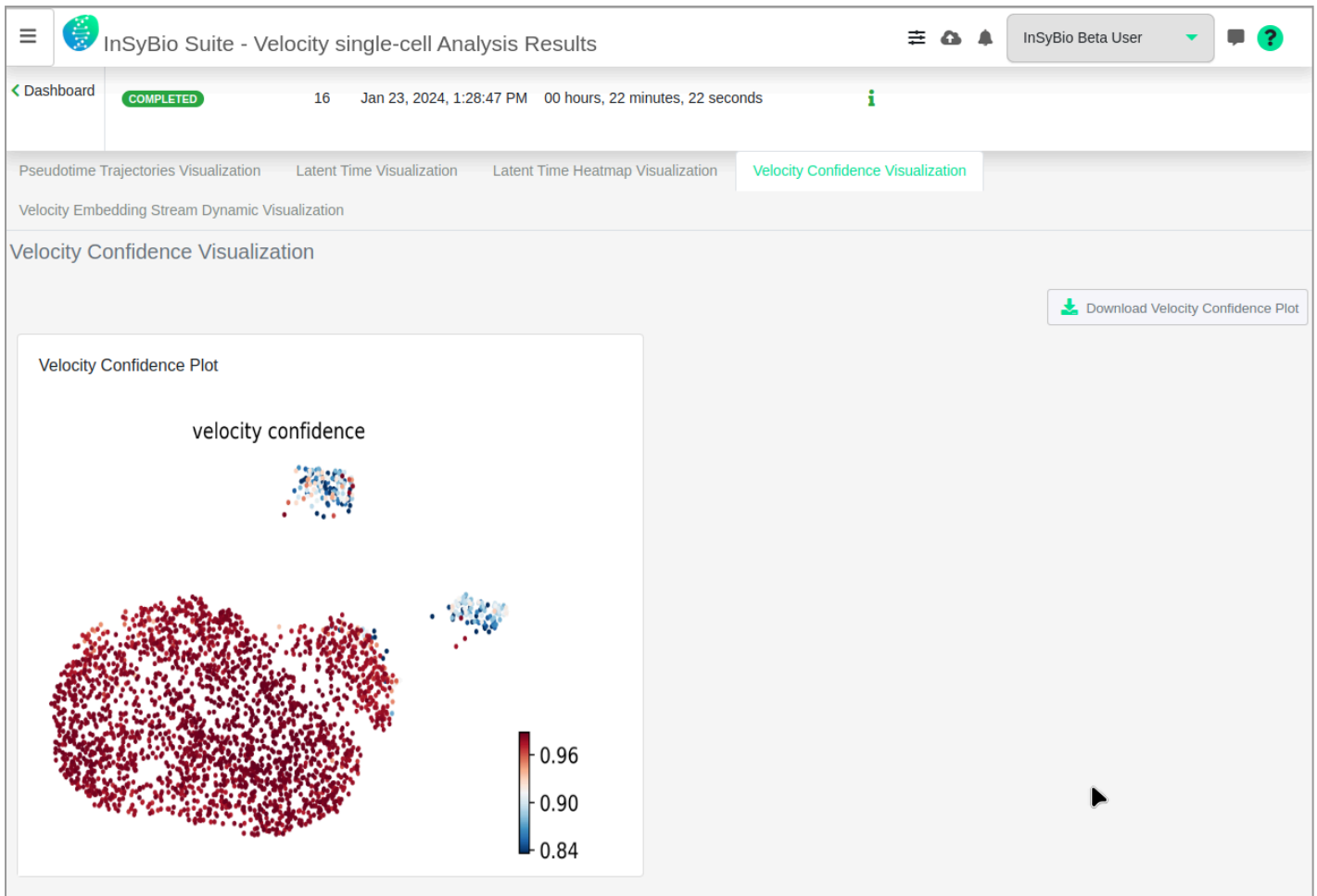
In the Latent time Visualization tab, the plot represents the latent time of the underlying cellular processes, an approximation of the real time experienced by cells as they differentiate.



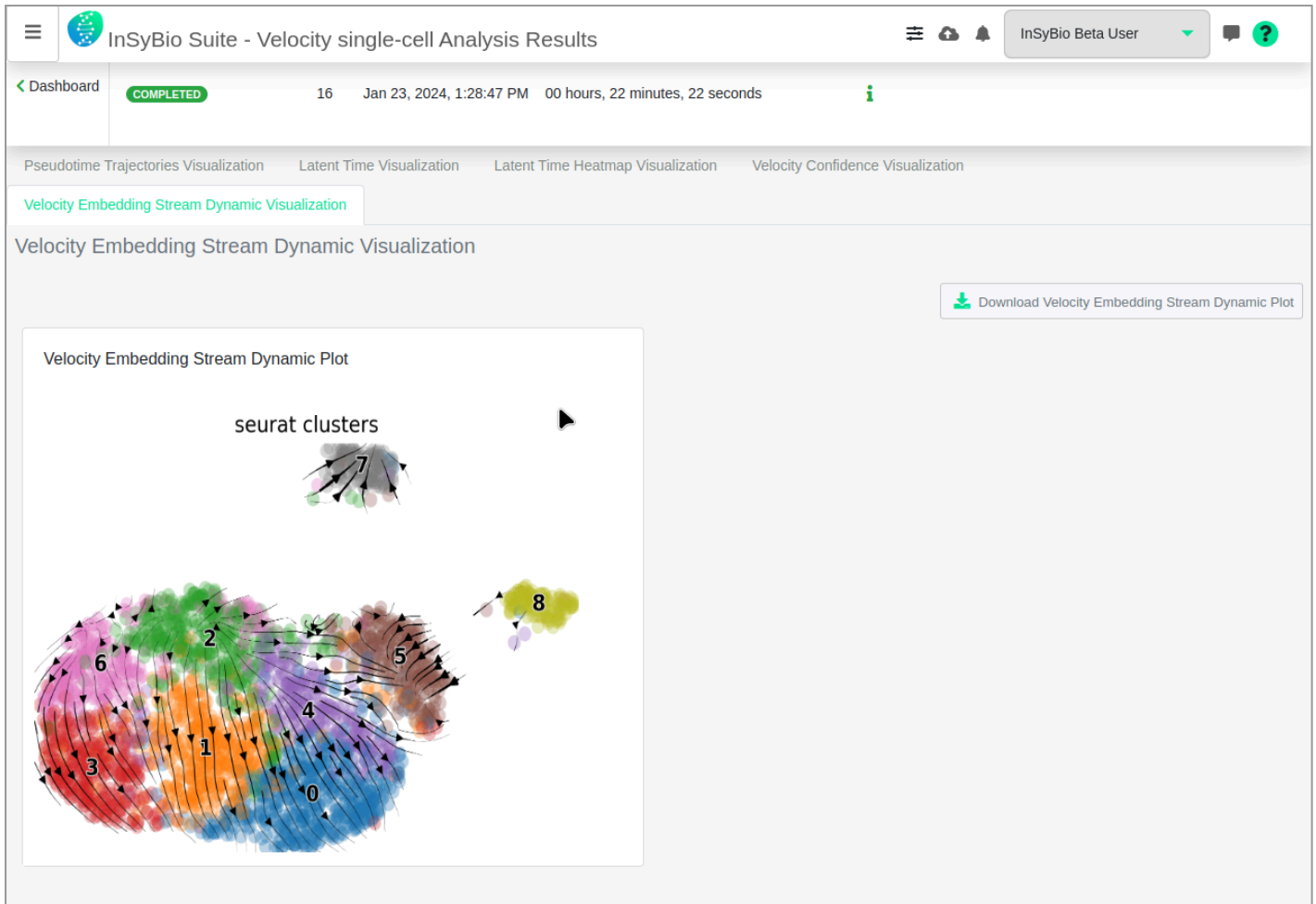
In the Latent time heatmap Visualization tab, the plot represents the latent time heatmap of the top genes.



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

InSyBio Suite - Cell Chat single-cell Analysis

Dashboard

RDS File ⓘ

Title:

Filename:

Select file from Data Store Go to Data Store to Upload File

Plot Options

Plot Width:

Plot Height:

Font Size:

- 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

Dashboard

Job Status

Job Type

Job ID

Submission Date

Execution Time

Input Data and Parameters

COMPLETED

9

Jan 23, 2024, 8:38:31 AM

00 hours, 11 minutes, 28 seconds

Summary Figure for interactions

Scatterplot signalling role overall

Incoming pattern plots

Outgoing pattern plots

Aggregated cell-cell communication network interactions and probabilities

Aggregated cell-cell communication network for cluster for each cluster

Chord plots pathway

Circular plots pathway

Heatmap plots pathway

Grouped 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

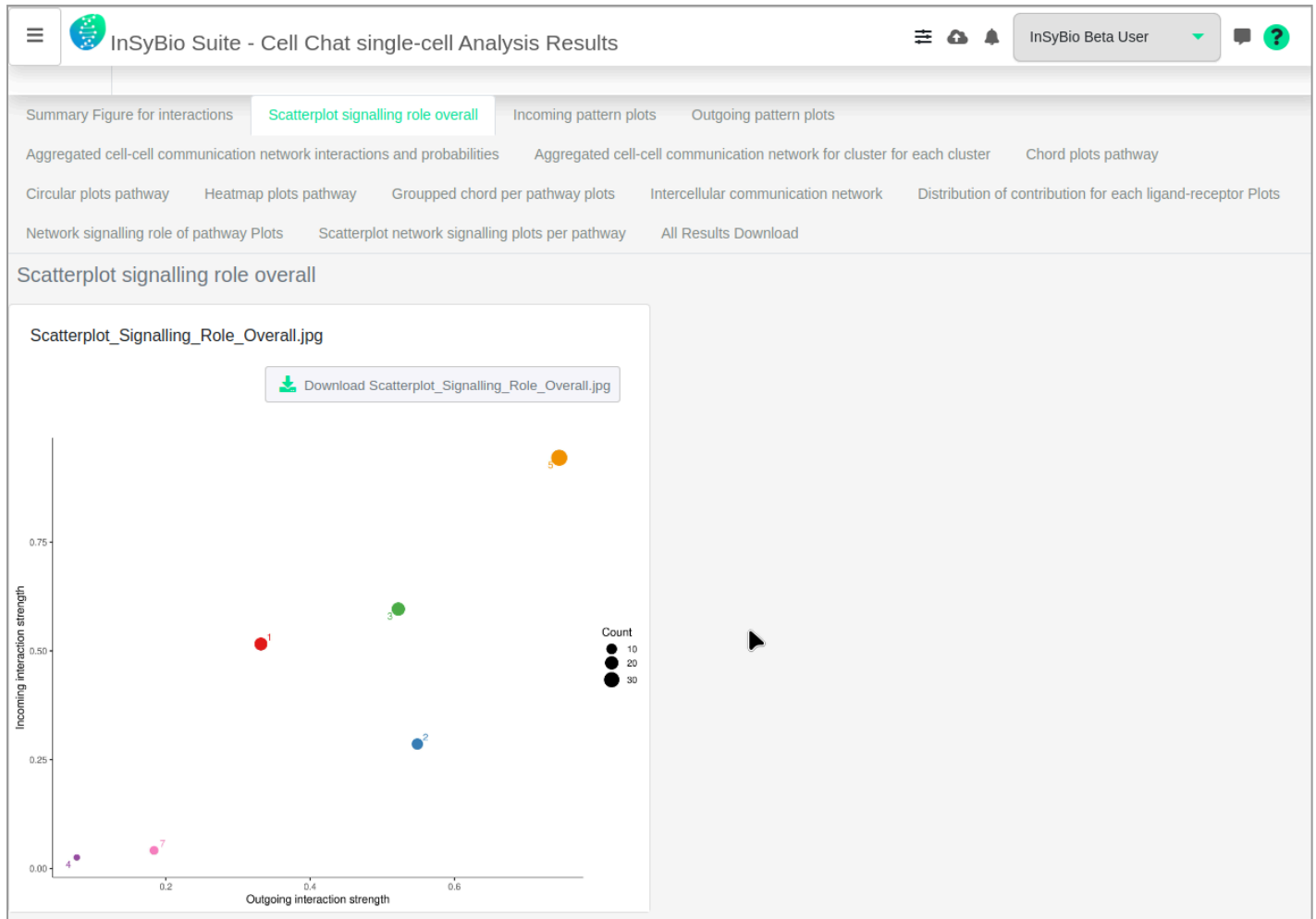
All Results Download

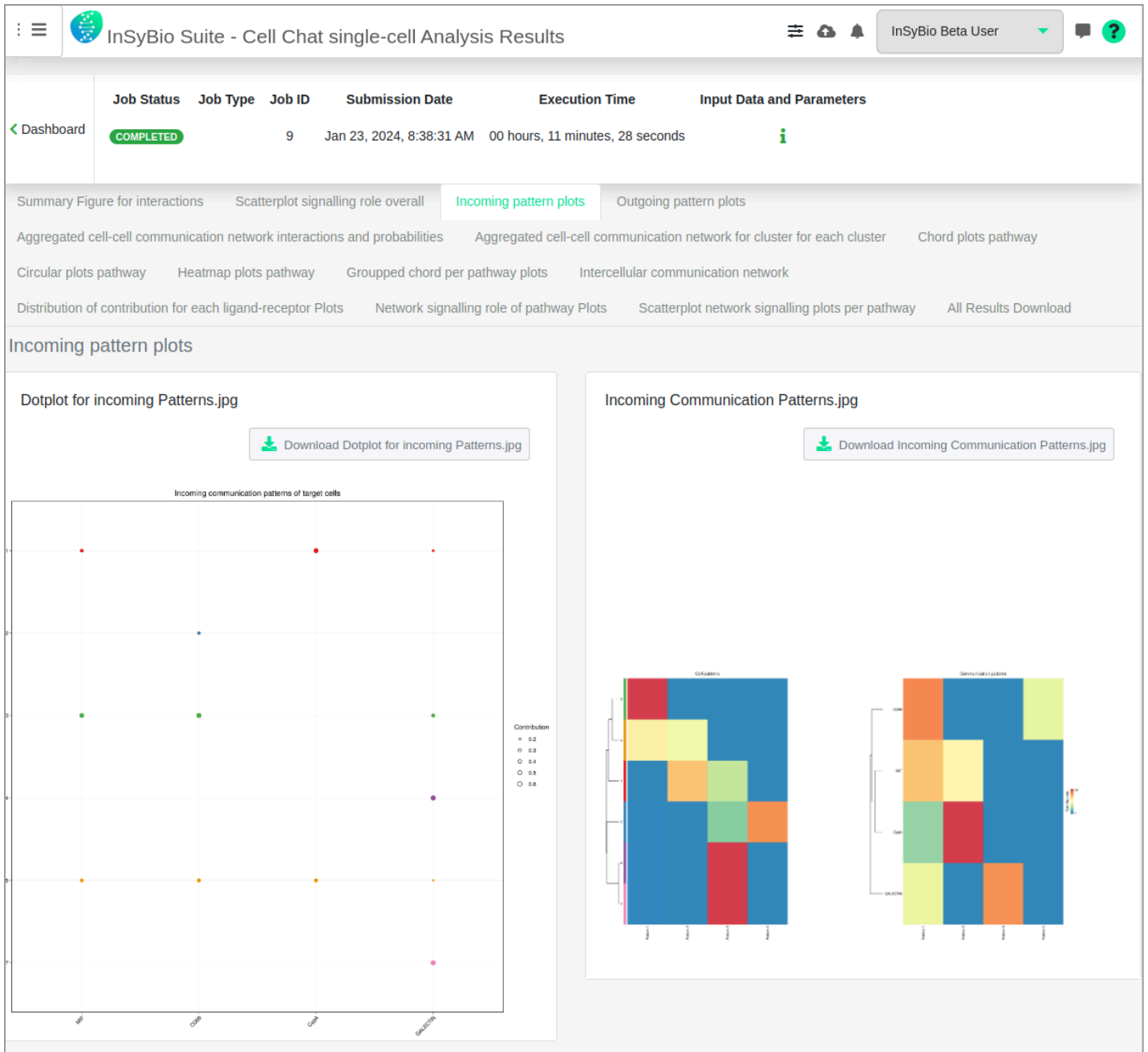
Summary Figure for interactions

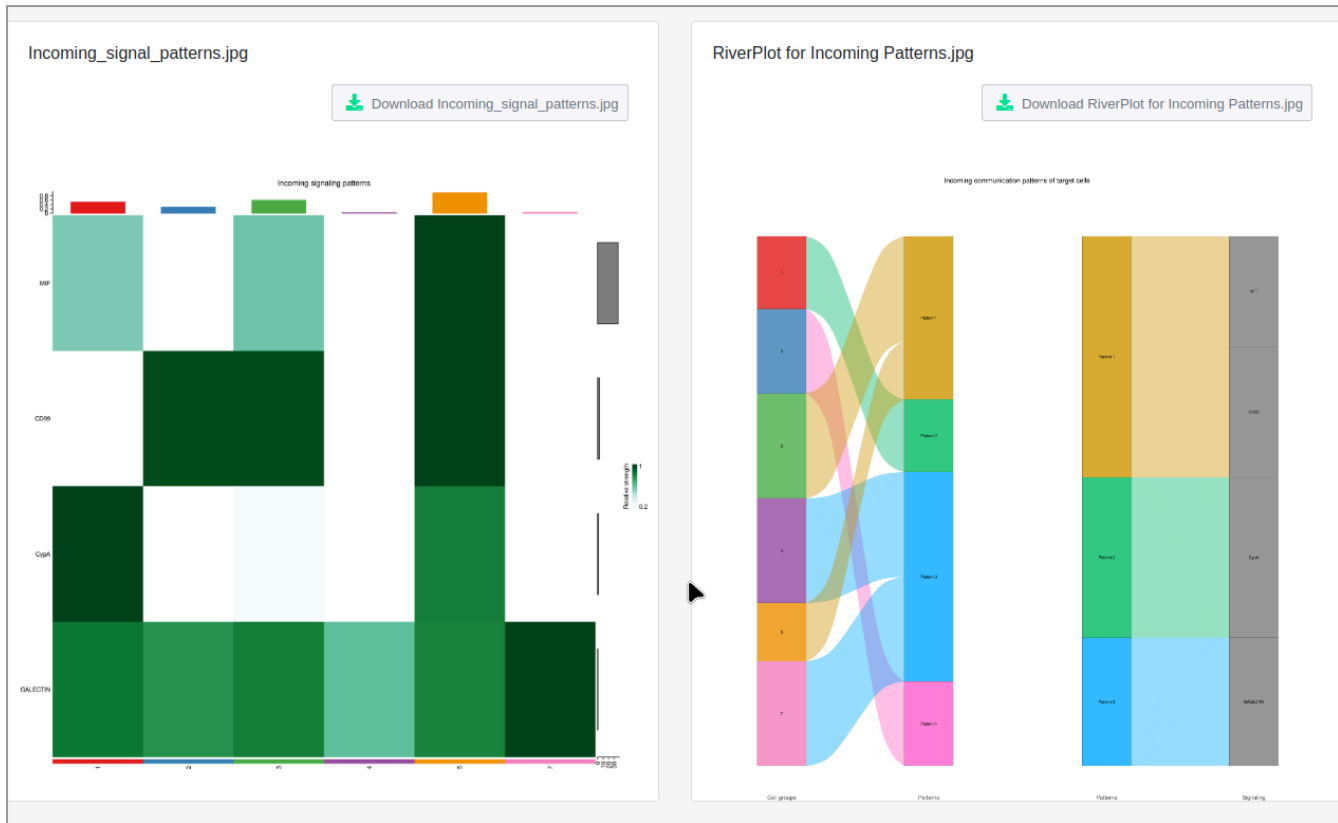
Download Summary Figure for interactions

Figure ligand_receptor_interaction_database_summary_data.jpg

Category	Percentage
Secreted Signaling	29.4%
ECM-Receptor	13.1%
Cell-Cell Contact	10.8%
Non-protein Signaling	30.7%
Heterodimers	81%
Others	9%
KEGG	44%
Literature	96%







InSyBio Suite - Cell Chat single-cell Analysis Results

InSyBio Beta User

	Job Status	Job Type	Job ID	Submission Date	Execution Time	Input Data and Parameters
< Dashboard	COMPLETED		9	Jan 23, 2024, 8:38:31 AM	00 hours, 11 minutes, 28 seconds	i

Summary Figure for interactions

Scatterplot signalling role overall

Incoming pattern plots

Outgoing pattern plots

Aggregated cell-cell communication network interactions and probabilities

Aggregated cell-cell communication network for cluster for each cluster

Chord plots pathway

Circular plots pathway

Heatmap plots pathway

Grouped 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

Aggregated cell-cell communication network interactions and probabilities

Figure Aggregated cell-cell communication network_for cluster_0.jpg

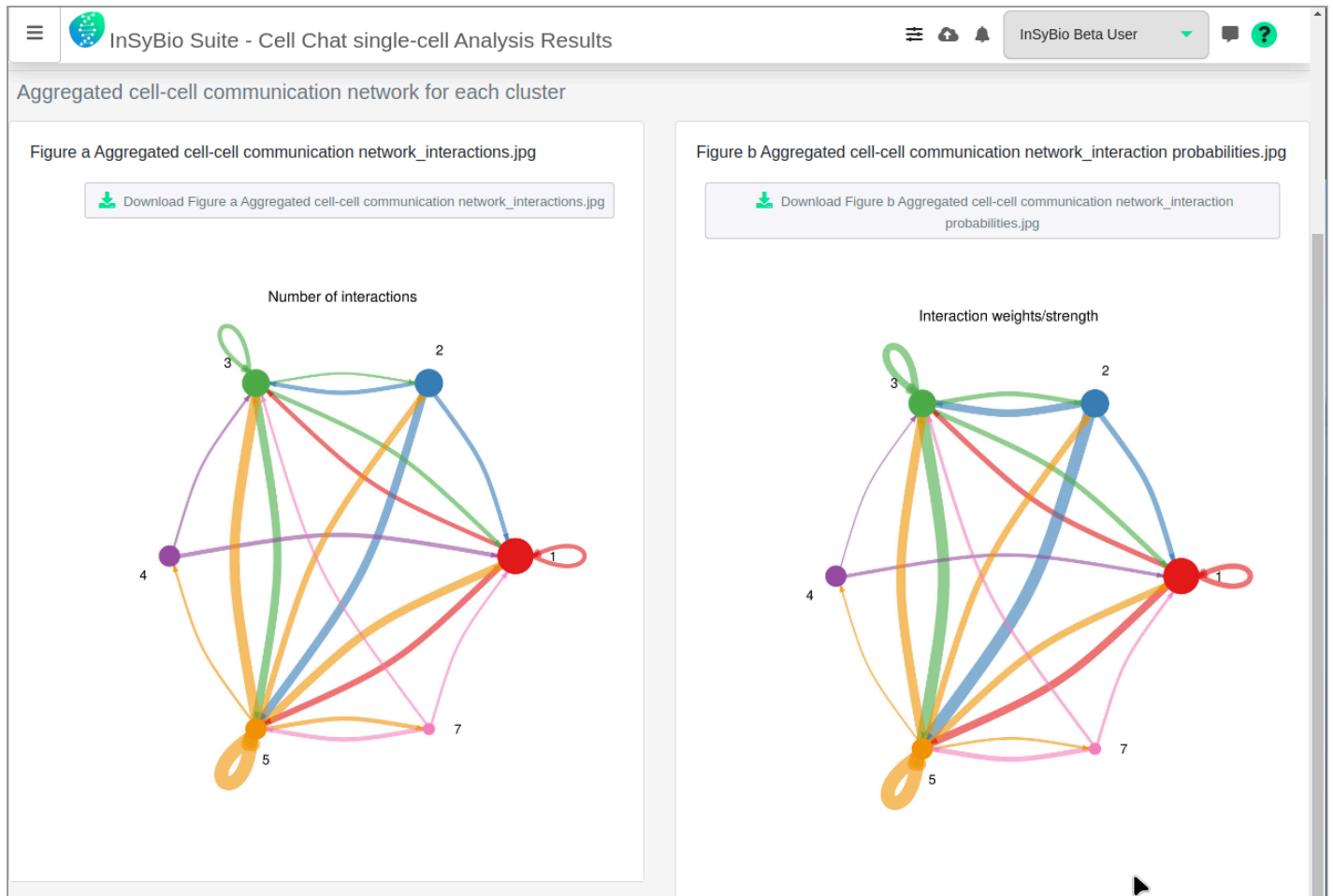
Download Figure Aggregated cell-cell communication network_for cluster_0.jpg

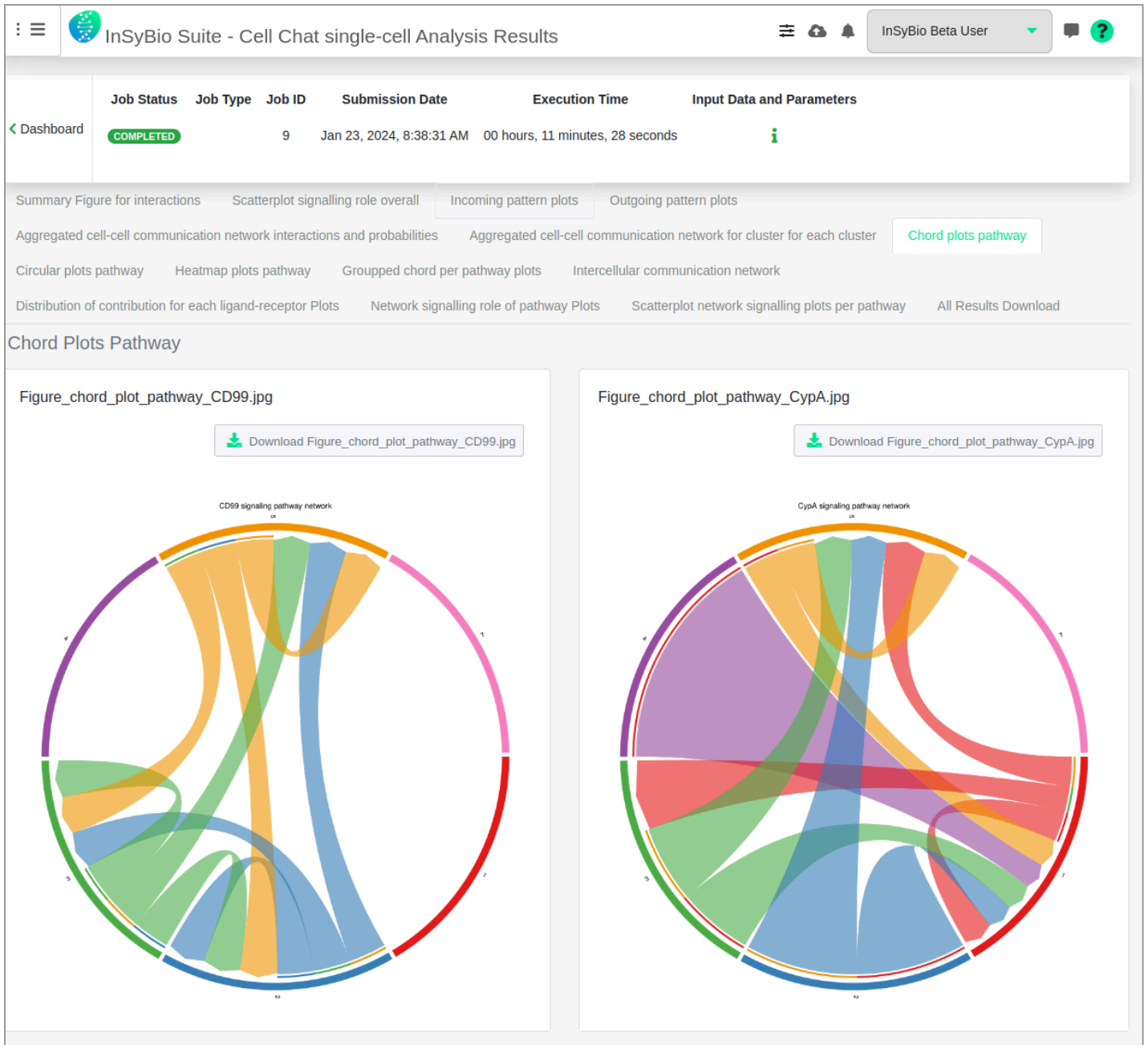
A network diagram for cluster_0. It features six nodes: node 1 (red), node 2 (blue), node 3 (green), node 4 (purple), node 5 (orange), and node 7 (pink). Node 1 is centrally located and has a self-loop. Red curved edges connect node 1 to nodes 2, 3, 4, and 5. There are also straight red edges between nodes 2 and 3, and between nodes 4 and 5.

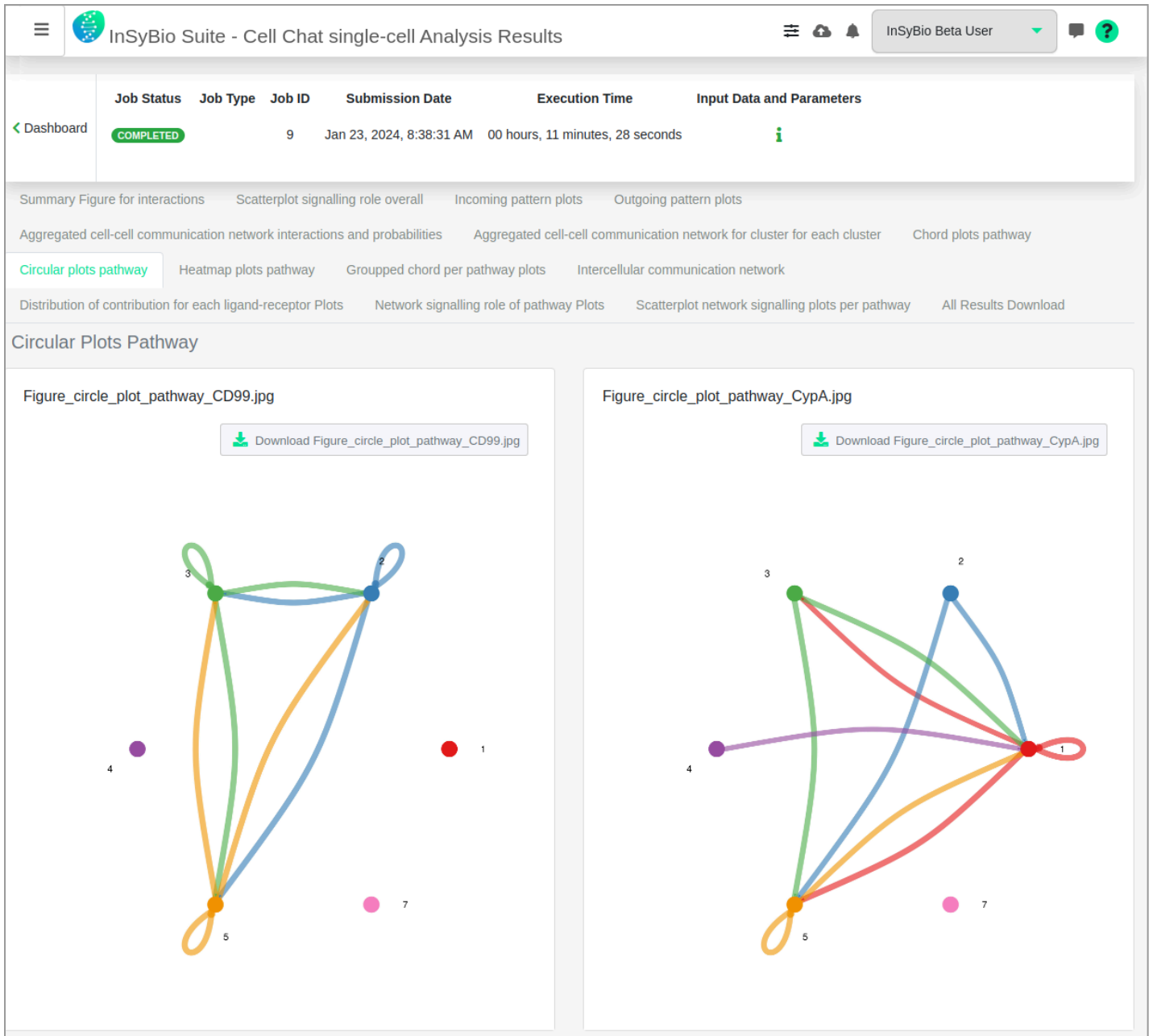
Figure Aggregated cell-cell communication network_for cluster_1.jpg

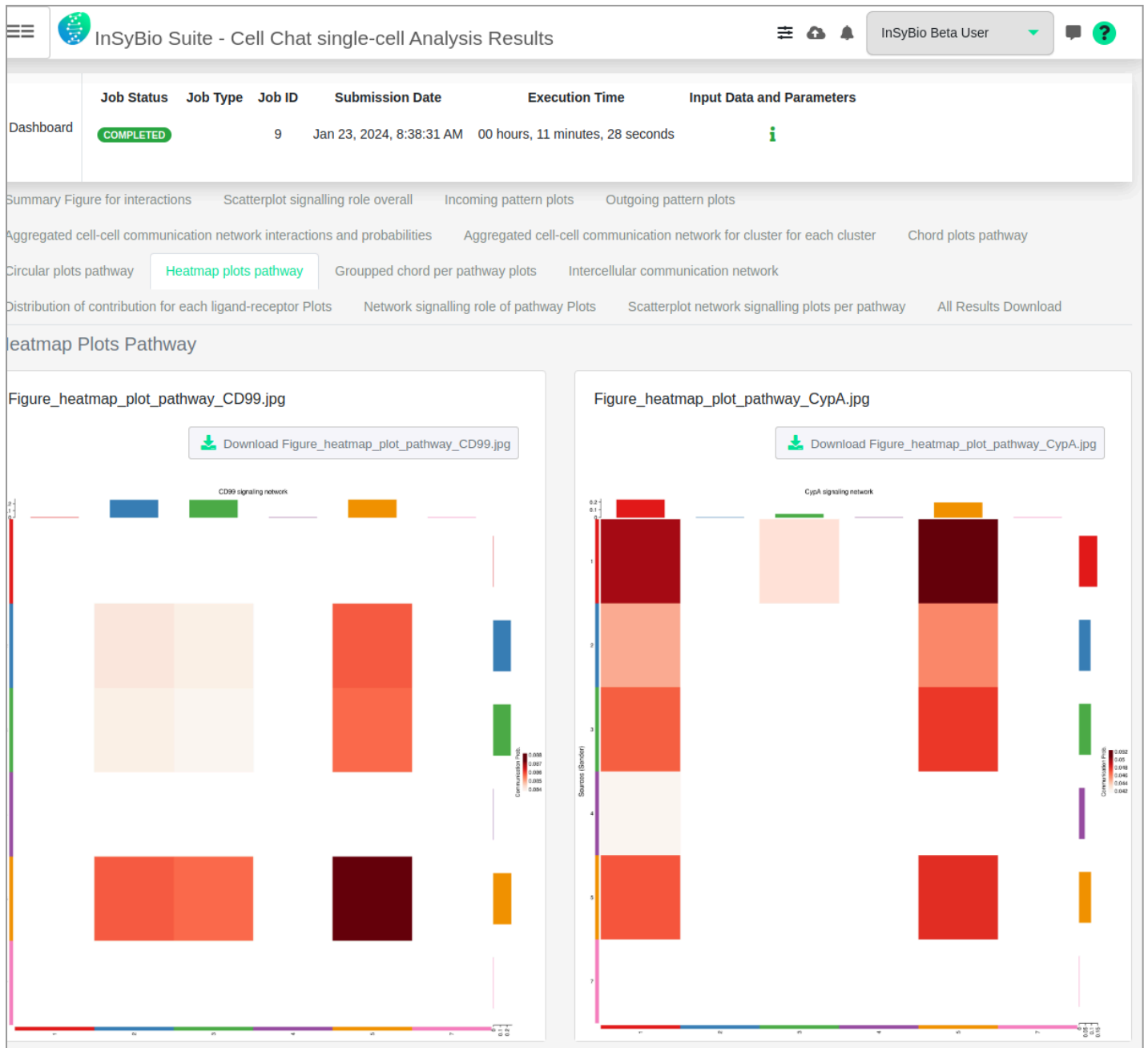
Download Figure Aggregated cell-cell communication network_for cluster_1.jpg

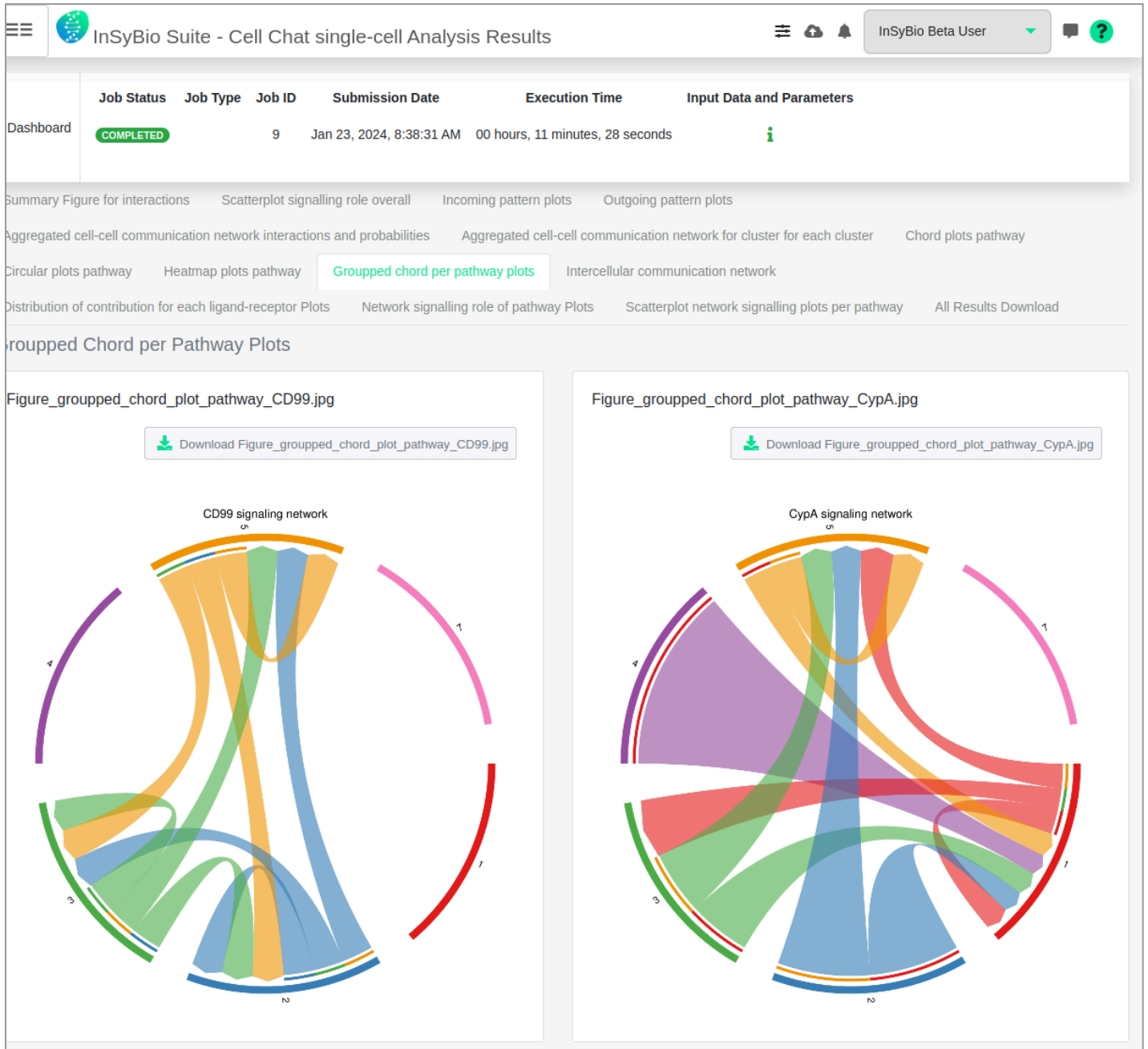
A network diagram for cluster_1. It features the same six nodes as cluster_0: node 1 (red), node 2 (blue), node 3 (green), node 4 (purple), node 5 (orange), and node 7 (pink). Blue curved edges connect node 1 to nodes 2, 3, 4, and 5. There are also straight blue edges between nodes 2 and 3, and between nodes 4 and 5.











InSyBio Suite - Cell Chat single-cell Analysis Results

InSyBio Beta User

Dashboard

COMPLETED

9

Jan 23, 2024, 8:38:31 AM

00 hours, 11 minutes, 28 seconds

Summary Figure for interactions

Scatterplot signalling role overall

Incoming pattern plots

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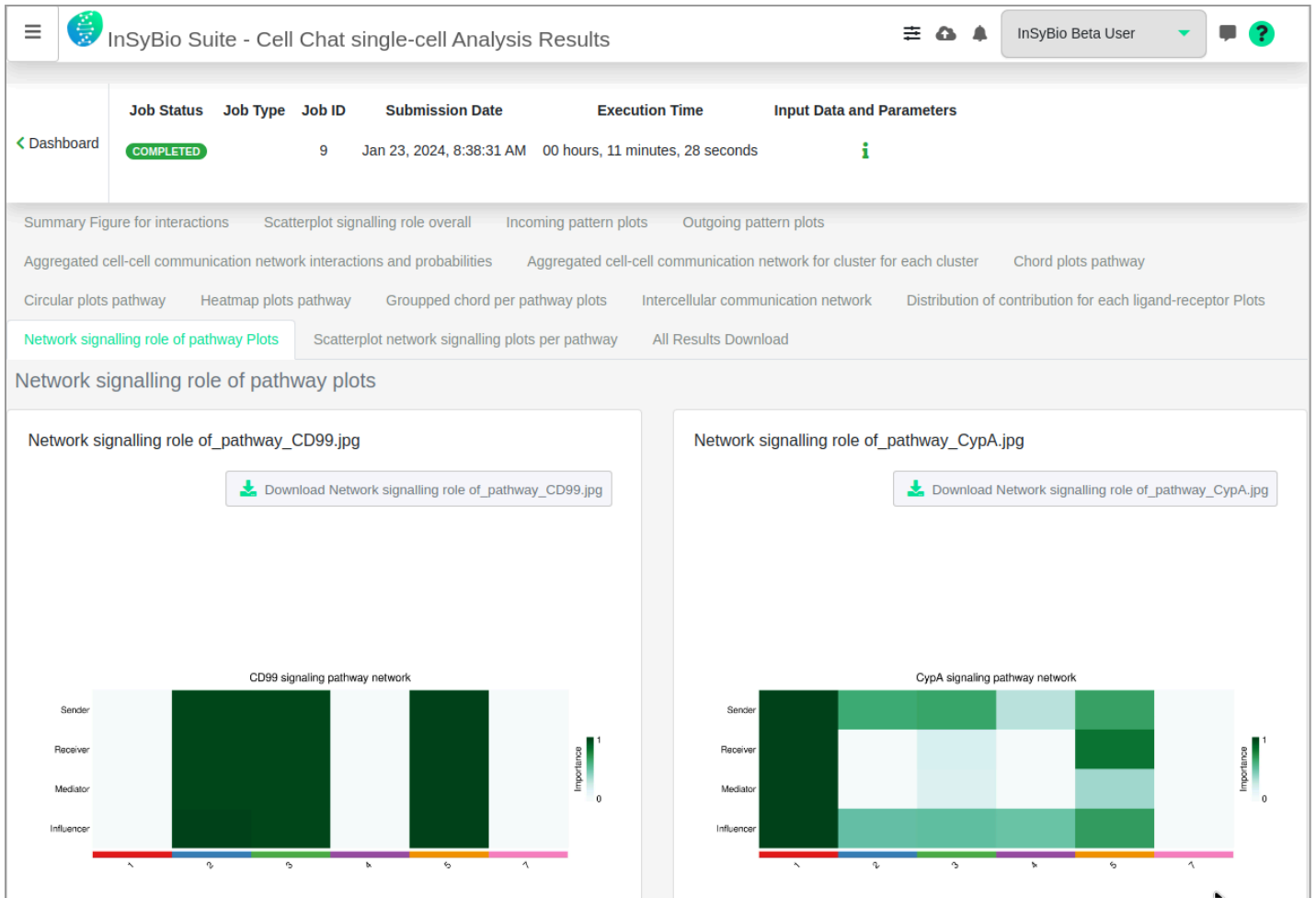
Supplementary CSV files

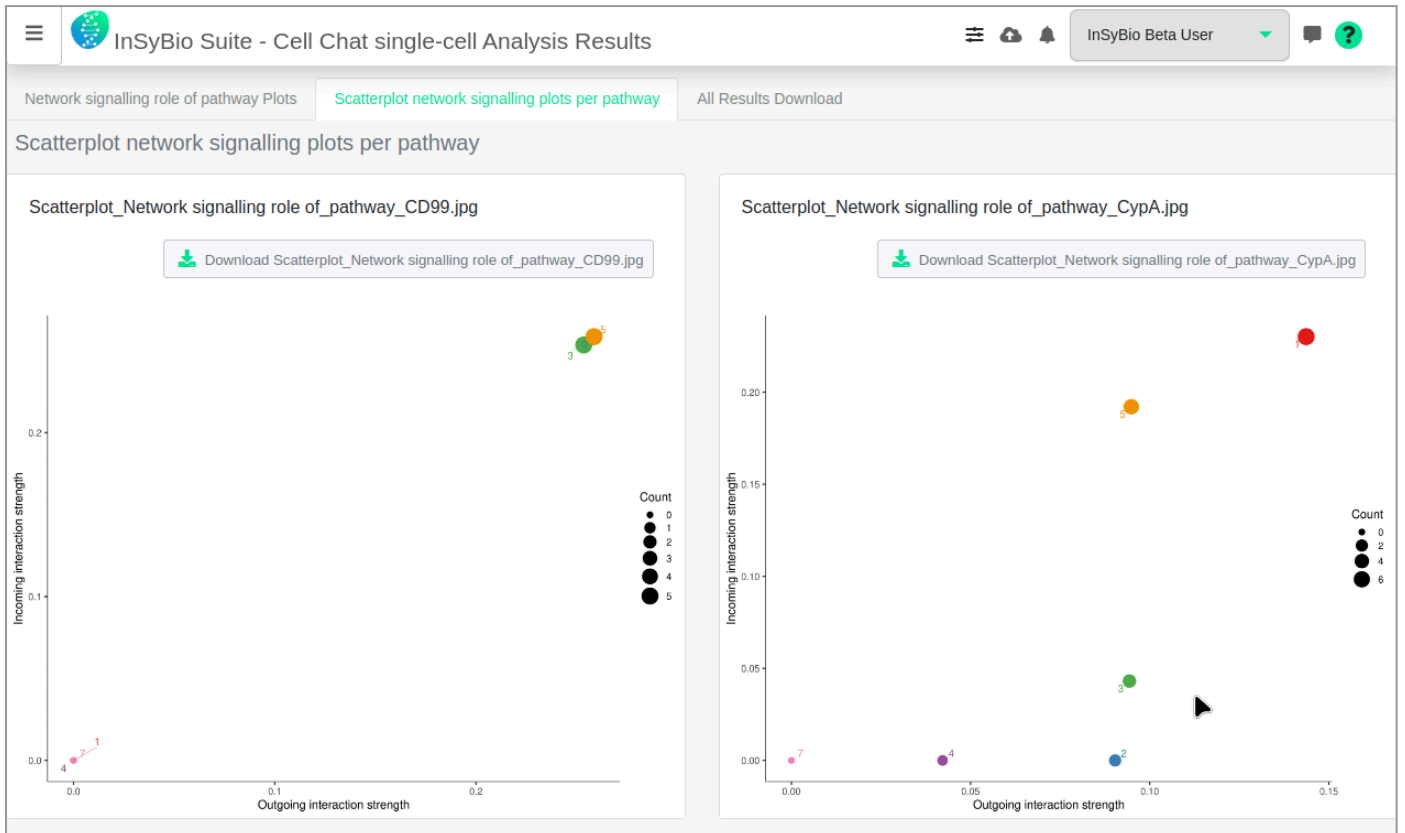
Supplementary Table 1 intercellular communication network

Download Supplementary Table 1 CSV

Supplementary Table 2 significant pathways

Download Supplementary Table 2 CSV





InSyBio Suite - Cell Chat single-cell Analysis Results

Dashboard **COMPLETED** 9 Jan 23, 2024, 8:38:31 AM 00 hours, 11 minutes, 28 seconds

Summary Figure for interactions Scatterplot signalling role overall Incoming pattern plots Outgoing pattern plots

Aggregated cell-cell communication network interactions and probabilities Aggregated cell-cell communication network for cluster for each cluster Chord plots pathway

Circular plots pathway Heatmap plots pathway Grouped 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**

All Results Download

Cell Chat single-cell Analysis Results [Download](#)

Compressed Folder [Folder](#)

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

The screenshot shows the InSyBio Suite web interface for RDS File Conversion. The header includes the InSyBio logo, the title 'InSyBio Suite - RDS File Conversion', and user information 'InSyBio Beta User'. A sidebar on the left has a 'Dashboard' link. The main content area is titled 'RDS vs Triple files Conversion analysis' and contains the instruction 'Convert RDS file to matrix, feature and barcode datasets (triple) and vice versa.' Below this, there are two radio button options: 'RDS to Triple files' (selected) and 'Triple files to RDS'. A green 'Submit Job' button is located at the bottom left of the main content area.

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.

 InSyBio Suite - RDS File Conversion

 InSyBio Beta User  

[< Dashboard](#)

RDS vs Triple files Conversion analysis

Convert RDS file to matrix, feature and barcode datasets (triple) and vice versa.

☒ RDS to Triple files ☐ Triple files to RDS

RDS File 

Title:

Filename:

 Select file from Data Store  Go to Data Store to Upload File

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

InSyBio Suite - RDS File Conversion

Dashboard

RDS vs Triple files Conversion analysis

Convert RDS file to matrix, feature and barcode datasets (triple) and vice versa.

☐ RDS to Triple files
 ☒ Triple files to RDS

Matrix	Features	Barcodes
Matrix Title: *	Features Title: *	Barcodes Title: *
Matrix Filename: *	Features Filename: *	Barcodes Filename: *
<button>Select from Data Store</button>	<button>Select from Data Store</button>	<button>Select from Data Store</button>
<button>Upload to Data Store</button>	<button>Upload to Data Store</button>	<button>Upload to Data Store</button>

Advanced Options +

Submit Job

- 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

Secondary filtering

nFeature_RNA ? : Yes

Lower limit: 200

Upper limit: 10000

nCount_RNA ? : No

Feature Extraction Method Umap

Shared Nearest Neighbor (SNN) Graph

k parameter (k-nearest-neighbor): 20

Clustering

Resolution parameter ? : 0,8

- 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.

InSyBio Suite - Single Cell RNA-Seq Differential Expression Pipeline Dashboard

InSyBio Beta User

+ Add new Job

Filter Jobs Show All 13 1 0 4
Completed Running Pending Error

Status	Job ID	Job Type	Input File(s)	Submission Date	Start Execution Date	Completion Date	Current Step	Actions
Completed	21	RNASeq Single Cell Velocity Analysis		11/26/73, 3:22 AM	1/16/24, 1:59 PM	-	Secondary Single Cell Analysis	View Results
Completed	20	Deconvolve Data against single-cell RNA-seq Analysis		8/12/11, 6:41 AM	1/15/24, 2:14 PM	1/15/24, 2:15 PM		View Results
Completed	19	RNASeq Single Cell Velocity Analysis		3/29/88, 9:11 PM	1/12/24, 8:51 AM	1/12/24, 9:06 AM	Secondary Single Cell Analysis	View Results
Completed	18	Cell Chat Analysis		12/19/68, 6:13 AM	1/12/24, 8:29 AM	1/12/24, 8:33 AM	Single Cell Alignment	View Results
Completed	17	RDS Conversion		2/9/59, 5:58 AM	1/11/24, 11:16 AM	1/11/24, 11:18 AM	Secondary Single Cell Analysis	View Results
Completed	15	RDS Conversion		9/21/76, 5:35 AM	1/17/24, 10:54 AM	1/17/24, 10:55 AM	Secondary Single Cell Analysis	View Results

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.

The screenshot displays the 'InSyBio Suite - RDS File Conversion Results' interface. At the top, the job status is 'COMPLETED' for Job ID 8, submitted on Jan 23, 2024, at 8:27:10 AM, with an execution time of 00 hours, 03 minutes, and 11 seconds. The 'Results Files' tab is active, showing three download links: 'Download Features File', 'Download Matrix File', and 'Download Barcodes File'.

Job Status	Job Type	Job ID	Submission Date	Execution Time	Input Data and Parameters
COMPLETED		8	Jan 23, 2024, 8:27:10 AM	00 hours, 03 minutes, 11 seconds	

Results Files

Fastq Dataset Download

Features File Download Features File

Matrix File Download Matrix File

Barcodes File Download Barcodes File

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

InSyBio Suite - RDS File Conversion Results

InSyBio Beta User

< Dashboard

Job Status

Job Type

Job ID

Submission Date

Execution Time

Input Data and Parameters

COMPLETED

7

Jan 22, 2024, 9:23:37 AM

00 hours, 01 minutes, 54 seconds

Results Files

Seurat object

Download

Seurat object

Download Seurat object

Cloud computing Infrastructurer and Security Certifications

InSyBio Suite and all its tools are running over the cloud computing as a service infrastructure of Vultr (<https://www.vultr.com>), at the Amsterdam (Netherlands) facilities, offering the following security attestations and certifications (SOC 2+ (HIPAA), PCI (Merchant), CSA Star Level 1, ISO/IEC 20000-1:2018, ISO/IEC 27001:2022, ISO/IEC 27017:2015, ISO/IEC 27018:2019).

How to get InSyBio Interact

To request a free one month license of InSyBio Suite please email us at info@insybio.com.

To purchase InSyBio Interact commercial version 4.0 please contact us at sales@insybio.com.

About Us

InSyBio Inc 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.

InSyBio Suite consists of tools for providing integrated biological information from various sources, while at the same time it is empowered with robust, user-friendly and installation-free bioinformatics tools based on intelligent algorithms and methods.

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