The Waksman Student Scholars Program (WSSP)

Student & Teacher Meeting 12/7/20



We hope that you are all staying safe for yourself and everyone else.

We are here to support you in how you want to run the WSSP this year.



The WSSP was initiated 28 years ago by the late George Pallrand (of the Graduate School of Education) and Bill Sofer (Waksman Institute)



Bring genuine <u>research</u> directly into the high school.

Students will learn science best by actually <u>doing</u> science



In previous years, in the students and teachers participated in a summer Institute



Students and teachers learned the background information and techniques necessary to carry out a <u>real</u> research project in molecular genetics









During the academic year, they go back to their schools and carry out the project there, recruiting additional students - You!!!

Different then most other outreach programs



Students come back to the Waksman several times during the academic year to review their progress, pick up supplies, learn new techniques, and to share data and ideas.



At the end of the academic year, you will present a poster on your research findings





Publish the DNA sequences in the public databases



S NCBI Resources 🗹	How To 🕑	
Nucleotide	Nucleotide C Advanced	
GenBank -		Send to: -
Landoltia punc chloroplastic-li	tata clone 06SET1.19 beta-carotene isomerase ke, mRNA sequence	D27,

GenBank: JZ981283.1

FASTA Graphics

<u>Go to:</u> 🖂

LOCUS JZ981283 737 bp mRNA linear EST 01-SEP-2019 DEFINITION Landoltia punctata clone 06SET1.19 beta-carotene isomerase D27, chloroplastic-like, mRNA sequence. ACCESSION JZ981283 VERSION JZ981283.1 DBLINK BioProject: PRJNA521257 KEYWORDS EST. SOURCE Landoltia punctata ORGANISM Landoltia punctata Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliopsida; Liliopsida; Araceae; Lemnoideae; Landoltia. REFERENCE 1 (bases 1 to 737) AUTHORS Tobia, S., Dang, M. and Mead, J. TITLE Transcript profiling of the Duckweed Landoltia punctuata JOURNAL Unpublished REFERENCE 2 (bases 1 to 737) AUTHORS Tobia, S., Dang, M. and Mead, J. TITLE Direct Submission JOURNAL Submitted (11-AUG-2019) Waksman Student Scholars Program, Rutgers University, 190 Frelinghuysen Rd, Piscataway, NJ 08854-8020, USA FEATURES Location/Qualifiers source 1...737 /organism="Landoltia punctata" /mol type="mRNA" /strain="RDSC-9264" /db xref="taxon:50518" /clone="06SET1.19" /clone lib="WSSP Duckweed cDNA library" <1..>737 misc feature /note="similar to beta-carotene isomerase D27, chloroplastic; involved in strigolactones biosynthesis" ORIGIN 1 agatacgttc tgcaaattcc gcttcttttc cccagtcgtg gtttcctttc tcccgggttc 61 cgcccagatg ggttcttctc tctatctgcg ccatcctctg caaatcccgt taccatcttc 121 cgcccggcgc cttatcctcc gcccacgccc aattcgcgtc ggattccgcc ccgtctccgc 181 cgcacttgat cgatcccggc cgggagatgt ttcggagagt aactaccagc ctggacctat 241 cgatgatete etceteaatt tetteeggeg ceagatggtg gaggaggttg gatgggaete 301 gcagaagcat gggtatgatg ggctaatgga agttgtcaat catctcatga tgaaagggag 361 caagcaagaa gttgagcaat cagcggtccg agtacttaga tctctcttcc ccccactgct 421 gctggaactc tattagatgc tcatcacacc catcaataag gggaaaccag ccgctctaat 481 gcttggcaag ctagtttctc tctctctc tagaaaactg aaacttgtag tgggtgatga 541 tatttatctt ccatcgcccc catcaacaag ggaaaaccag ctgctgtgat ggatggctcg 601 ccaqtttctc tttctctctc cqgaaaactc aaacctctta gaggccaggg cgacagctct 661 gttgtgccag tggttgatgg ggaaatgcac ggtcaactcc attgaccttc cagatgggtc 721 ctcttccaca accgggg

UTHORS Tobia,S., Dang,M. and Mead,J. ITLE Transcript profiling of the Duckweed Landoltia punctuata

WSSP Goal: Encourage students to continue their education and careers in the sciences

So how are we conducting the program this year?



Virtual summer Institute Virtual and In person (at schools) Academic Programs



20/21 Research Project:

DNA Sequence Analysis of the Duckweed Landoltia punctata



Duckweed:

- Smallest flowering plants,
- Grow in slow moving fresh water -world wide,
- Fast growing double in a few days







Duckweed:

- Potential biofuel source
 - Can accumulate 40%-70% starch
 - Sink to bottom of ponds
 - Starch can easily be converted to sugar for fermentation
 - Will not compete with food crop production
- Bioremediation
 - Grows in contaminated water
 - Sequesters or degrades contaminates such as lead, arsenate, halogenated compounds
 - Extract nitrogen and phosphate from waste water

WSSP site

Same link: <u>https://wssp.rutgers.edu</u>



The Waksman Student Scholars Program (WSSP) provides opportunities for high school students and their teachers to conduct an authentic research project in molecular biology and bioinformatics and publish their findings. This is a year-long program that begins each year with summer Institutes for teachers and one or two of their students. It continues back at each high school during the academic year, when more students can become involved in the research project. In the course of these studies, students, teachers, and research scientists work together on a genuine research problem. By actually doing science, students gain an understanding of how science operates and are encouraged to continue their education and careers in a science discipline. A video of an overview of the WSSP can be found here.



Access to the files and videos on this web site now require the username and password you used to

register on the 2016-2017 version of DSAP. Login information for the 2015-2016 will no longer work. If you are participating in the 2016-2017 WSSP please use the 2016-2017 WSSP Project version of DSAP to register. If you are still working on clones from 2015, please go to the 2015 version of DSAP. We will continue to review and submit sequences from 2015-2016 over the next four months.

Read more

2

Announcements

12/2/16 - 2016 Third Academic Year Follow Up Mtg 8/4/16 - 100 New 2016 Sequences Posted 7//1/6 - 197 New Sequences Posted!! 6/23/16 - 200 Sequences posted on NCBI 6/16/16 - 100 Sequences posted on NCBI

In the Media

2013 Waksman Forum Poster Session Old Bridge student finds unknown gene in duckweed

Programs

Waksman Institute Summer Experience (WISE) for High School Students: Two week summer research programs (June Session 6/19/17-6/30/17 or August Session 7/31/17-8/11/17) in molecular biology and bioinformatics for high school students.

Publish a cDNA Sequence (PACS): An online bioinformatics research project for students at schools who can not participate in the WSSP or WISE (Available in late fall 2017)

INFORMATION FOR	LOCATIONS	RESOURCES	274704
Alumni	Waksman Institute, Rutgers University	DSAP	SEARCH
Parents	John Hopkins University	Lecture & Lab Notes	
Students	Lawrence Livermore National Laboratory	PACS	
Teachers			If you have questions, please Contact Us

To Access Files from the WSSP site:



Home

Current Project

DSAP-16

DSAP-17

Toolbox 2

Modeling

Videos

at RU

SMART Protein

Challenges, Labs and

WSSP16 Published

2016 Summer Institute

Resources for Research

Lecture Notes

Teacher Notes

Lab Notes

Videos

Teachers

Equipment
Supplies

DNA Sequences

Overview

The 2017-2018 WSSP research project focuses on the DNA sequence analysis of genes from the duckweed pint. *Landolb gunctata*, and how these genes compare to those found in other species. Duckweed, a freshwater aquatic piant, is of interest to the scientific community because of its use in bioremediation, and its potential use a biofuel.

A mRNA population of *Landoltia punctata* will be used to explore the research question: Which genes are expressed in this organism, and how do they compare with expressed genes from other species?



Students in the project will lisolate and sequence genes from a cDNA library (a collection of mRNA's copied into DNA's). These sequences have never been determined before. If a student successfully analyzes a sequence, it will be published in GenBank, the international repository of all incown DNA sequences, for other scientists to study. Each published sequence will bear the student's name and higher teacher's name. In the occurse of their analyses, WSSP students will compare Landothis sequences with those from other eukaryotes to determine the evolutionary relationship of Landothis to other organisms. In addition, if their sequence codes for a protein from another organism and the three-dimensional stucture of that protein has been solved, student's may be able to use their sequence information to design a model of that protein and see how it differs from the same protein in Landottia.

A video overview of the WSSP and the research project here

The Summer Institutes

A teacher and one or two students from 44 high schools commute to the Waksman institute at Rutgers University in Piscataway, NJ daily for a three-week session in July. Two week summer institutes are laboratories in Livermore, CA, and Walpahu High School, HI. At each summer Institute students and teachers learn about a research project in melocular biology. Both the background material and the laboratory skills necessary to carry out the project are presented.

The Academic Year

Lawrence Livermore National

Laboratory

Teachers and students entum to their classrooms in September and begin working on the research project. Additional students will be recruited. Some schools offer a research course for high school oredit; others offer the research as an after-school activity. The students return to the sites of the summer institutes for meetings during the academic year. At the end of the year, in May or June, a forum is held where students present the results of their student at poster session.

Amino Acid Game (For student competition)

In the Media Programs 2013 Waksman Forum Poster Session Waksman Institute Summer Experience Old Bridge student finds unknown gene (WISE) for High School Students: Two week summer research programs (June Session 6/19/17-6/30/17 or August in duckweed Session 7/31/17-8/11/17) in molecular biology and bioinformatics for high school students. Publish a cDNA Sequence (PACS): An online bioinformatics research project for students at schools who can not participate in the WSSP or WISE (Available in late fall 2017) LOCATIONS RESOURCES Waksman Institute, Rutgers University DSAP Alumni Parents Lecture & Lab Notes John Hopkins University Students PACS

If you have questions, please

To access lab and lecture files

To Access Student Files from the WSSP site:

Home About Us Current Project Previous Projects Resources WISE

Home » Resources

Resources for Students

2017-2018 WSSP Lecture Notes, Presentations, and Videos

This web page provides links to materials for the Waksman Student Scholars Program for 2017. This information is intended for the students and faculty participating in the program during the 201. summer institute and through the 2017-2018 academic year. It is not intended for general public use and access is password protected. The material on these pages is provided for educational purposes only. Unauthorized use of this material is prohibited. If you are not a member of the WSSP and wish to download the files or have comments or questions about the material please contact <u>Sue Coleta</u>, Waksman Institute, Rutgers University.

		Ch.	2017 Lecture Topics Student Links	Notes (.pdf)	Videos (.mp4)	Figures (.pdf)	Figures (.ppt)	- Lecture Notes
		0	Introduction and overview of project		Ch0	Ch.0.pdf	Ch.0.ppt	Lecture notes
		0	Polymers and DNA Structure		-	Cpdf	Ch0.ppt	
		1	Vectors: Plasmids, Libraries, and DNA preps Background on Vectors Background on Libraries Plasmid DNA Minipreps	Ch. 1	Ch.1a Ch1.b Ch1.c	Ch1.pdf	Ch1.ppt	Powerpoints
		2	PCR, Mapping the size of an insert Background on PCR Analysis of an agarose gel of the PCR samples	Ch. 2	Ch.2a Ch.2b	Ch2.pdf	Ch2.ppt	(have hard copies)
Darwin		3	Restriction enzymes, Mapping the size of an insert Background on Restriction Enzymes Analysis of an agarose gel of the Restriction Digest samples	Ch. 3	Ch.3a Ch.3b	Ch3.pdf	Ch3.ppt	
		4	DNA Sequencing,	Ch. 4	Ch.4	Ch4.pdf	Ch4.ppt	
		5	Logging onto DSAP, Sequence quality, crop ends Determine waveform quality Analysis of a poor waveform Determining where the cDNA insert starts Determining where the cDNA insert ends	Ch. 5	Ch.5a Ch.5b Ch.5c Ch.5d	Ch5.pdf	Ch5.ppt	Videos
			Cropping the waveform		Ch.5e			
								Labs
	Lab	2017 L	aboratory Protocois				Protocols .pr/f	(have hand earlies)
	0	Introdu	ction to the WSSP laboratory - Overview of the laboratory experimer	nts (video)			Lab 0	(nave nara copies)
	1	Pipetin	g and weighing (video)				Lab 1	
		La	b 1D - DNA Spotting Assay (optional) Spot Template.ppt Google D	ocs Spot A	ssay Sheet			
	2	Sterile	technique and setting up ON cultures (video)				Lab 2	
	1	En	tering information into Google Docs Clone Report Sheet (video)				Lab	
	3	Setting NOTE: that yo	Up a PCR Reaction with Bacterial Cultures - (New!) Some schools will be using PCR beads while other schools will be u u are using the correct protocol!	sing a 2X F	PCR Mix. P	lease be sure	Lab 3-Bead Lab 3- Mix	
	4	Runnin	g an Agarose Gel on the PCR Samples				Lab 4	
		Po Los Mo PC Up	uring an agarose gel video. ading an agarose gel video. Examples of gel loading video. cking up a gel image in Powerpoint video. R Gel template (PCRG-Template.ppt) loading a gel image to Google Docs Report sheet video.					Videos

Resources Lab Notes Lecture & Lab Notes: Students Lecture & Lab Notes: Teachers Videos Toolbox 2 Download 4Peaks Download FinchTV DNAApp Equipment Supplies Publications Media Links Amino Acid Game Dot Plotter

My Name is Charles I

Use your <u>DSAP</u> Username and Password to download files



In person: Select clones from cDNA Library





Determine the size of the *Landoltia* cDNA by PCR and gel electrophoresis, purify the DNA and verify the insert size by restriction digests







Enter all the data about every clone into the Google Docs Clone Report Sheet

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2	Clone Name	Prepped By:	ON date	Box Location of Miniprep (School #:Box #-Position #)	PCR Gel Name	PCR Gel .jpeg Link	Insert size by PCR (bp)	Restriction Digest Gel	RDG .jpeg Link	Insert size by digest (bp)	Sequence Clone? (yes/no)	Date Sequenced (for WSSP staff only)	Sequence Reverse? (Yes/No)	Date REV Sequenced (For WSSP Staff only)	Actual Size of Insert (by Seq)
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12	20JB1.11	John Brick	6/26/2011	2 <mark>7</mark> -1-9	20PCRG2.11										
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17	20JM6.11	Janet Mead	6/27/2011	20-1-14	20PCRG3.11	https://picasa	400	20RDG2.11	https://picas	400	YES				
18	20JM7.11	Janet Mear	6/27/2011	20-1-15	20PCRG3.11	https://picasa	1000	20RDG2.11	https://picas	1000	YES				
19	20JM8.11	Janet Mead	6/27/2011	20-1-16	20PCRG3.11	https://picasa	300	20RDG2.11	https://picas	300	YES				
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20RDG1.10 (6/8/10) by Janet Mead, Andrew Vershon

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<u>All</u> gels and data have to be posted for a clone or else we will not be able to sequence the DNA.

Google Docs

Make sure the images are shared!!!

Evaluate DNA Sequence Data





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Conduct Bioinformatic Searches of the Databases

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gi 6265	7503	ref XP_221212.3 PREDICTED: similar to forkhead b	213	2e-54	G
<u>gi 5176</u> gi 3385	6585 4 emk	ref XP_126489.4 PREDICTED: forkhead box K2 [Mus mus CAA43200.1 transcription factor ILF [Homo sapiens]	<u>213</u> 213	2e-54 2e-54	G
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Query	255	TADKGWQNSIRHNLSLNRYFIKVPRSQEEPGKGSFWRIDPQSEAKLVEQAFRRR TADKGWQNSIRHNLSLNRYFIKVPRSQEEPGKGSFWRIDP SE+KL+EQAFR+R	RORGIA R RG+	434	
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Sbjct	352	CFRTPLGP-LSSRSAPASPNHAGVLSAHSSGAQTPESLSREG 392			



Perform searches of the scientific literature to determine the function of these genes



Figure 17-47. Two ways in which survival factors suppress apoptosis. (A) In mammalian cells, the binding of some survival factors to cell-surface receptors leads to the activation of various protein kinases, including protein kinase B (PKB), that phosphorylate and inactivate the Bcl-2 family member Bad. When not phosphorylated, Bad promotes apoptosis by binding and inhibiting Bcl-2. Once phosphorylated, Bad dissociates, freeing Bcl-2 to suppress apoptosis. As indicated, PKB also suppresses death by phosphorylating and thereby inhibiting gene regulatory proteins of the Forkhead family that stimulate the transcription of genes that encode proteins that promote apoptosis. (B) In *Drosophila*, some survival factors inhibit apoptosis by stimulating the phosphorylated, Hid promotes cell death by inhibiting IAPs. Once phosphorylated, Hid no longer inhibits IAPs, which become active and block cell death.



What do you do if you are <u>not</u> permitted back at school to do the labs?

Analyze clone sequences on-line!!

The WSSP will provide each student with at least one clone that can be submitted to NCBI

Log onto the DSAP (no Chrome) (make sure that you are in the 2020-2021 DSAP)

DNA Sequence Analysis Program (DSAP)

Welcome to the Waksman Student Scholars Program Forgot your password | Register The research project for the 2020/20210 WSSP focuses on the genomic analysis of the duckweed Username: ٩v Landoltia punctata, and how the genes in this organism compare to other species. Password: Please visit Project overview and DSAP Demo. Remember me Sign In >> Please do not use Chrome to work on DSAP. It often does not save the data... PLEASE DO NOT RESET THE TAIR PASSWORD! Copyright© 2007-2020 Rutgers About DSAP Demo Announcements Downloads Privacy & Security | Terms of Service Contact Links

2020-2021 WSSP Project

WSSP F

Students should be doing PCs

Support

Do your own work!!!!

Changes for the 20-21 Program

NCBI has changed their interface

PDB has changed their interface

FLASH no longer works Toolbox -> ORFfinder

Interactome -> BioGRID

BLASTN Report

	BLAST [®] » blast	n suite » results for RID	CAVPGVTY014		Home Recer	t Results Saved Strategies Help
ΓΝ	< Edit Search	Save Search Search	Summary 💙	How to read this report?	BLAST Help Videos	DBack to Traditional Results Page
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RID W2C/PBJT016 (Expires on 09-20 23:05 pm) Query LD (clQuery, 16:297 Description None Molecule type nucleic acid Query Length 1059	Database Name nr Description Nucleot Program BLASTN	ide collection (nt) ↓2.7.0+ ▷ <u>Citation</u>	Sequence ID: <u>XM 0109442</u> Range 1: 296 to 951 Gen8 Score Ex 551 bits(610) 22 Query 137 cccAcccA3	df.2 Length: 1096 Number of Matches: 1 ank Graphics ▼ Next Matches: 1 pect Identities Gaps 152 528/678(78%) 25/678(3%) 0577CAACAGGCTTTTTGGCAAGCCAATGCTCTCOG PI	n Previous rand Is/Plus	
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			Sbjct 767	GUTTGARGAAUTGGAGUTGAUUTGAUUTTGAAGAAUTGGAGGGAGCCGAAT 	rgga 813	
			Query 677 GGAACAGCT		ATGC 736	
			Query 737 CCGACC	TGTCCGTCCTGCTGCTCAGAAGAACACCGCAGAAGAAGATGACTTG	CAGC 793	
			Sbjct 874 CAGGCAACA	GGTTCGGCCTGCTCCACAGAAAAATACAGCTGAAGAAGATGAACTTG	CAGC 933	
			Sbjct 934 ATTACAAG	AGAAATGGC 951		

BLAST[®] » blastn suite » results for RID-CAVPGVTY014

PDB Sequence Search is Currently Not Active



- · Chemical Formula or Molecular Weight
- Chemical Substructure or Similarity

Right image: MarvinJS by ChemAxon for Ligand Search of Lipitor

Start Ligand Search



Use the PDB Advanced Search to perform the search



To use the PDB Advanced Search

- Enter in your protein sequence into text box 1.
- 2. Select "Polymer Entities"
- 3. Select the con

	Q	ic
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Search	History	Browse Annota	tions MyF	DB He	elp							
Use the Adv	vanced Search	Query Builder too	I to create comp	oosite boolea	n queries. See th	e <mark>Help</mark> pa	age for more detailed	information.				
 Advance 	ed Search Q	uery Builder 🛛										
▼ Text 🕜												
▲ Sequence	0											
	MFNRLFGKPKQ KTMDEINEQTEN	etnalasldkmneale Imkqiqealsapigava	MLEKKEKVLLKK/ DLDDDELEAELEE	AAAEVERAKEF LEAELEELEGA	ARAKNKRAAKQCLI ELEEQLLQPATTAP/	KRKRLYE(APVHVP)	QQVEQLGNFQLRIHDQMI (ARPVRPAAQKNTAEEDE	ILLEGAKATTETVD ELAALQAVMAS	DALRSGAAAMQ	amhkatniddvd	Count	Clear
	PDB ID 1MBN	Target Protein	E-Value Cut	off 1000000	Identity Cutoff	0	% (Integer only) 🚱					
▼ Sequence	Motif 🕜											
✓ Structure S	imilarity 🕜											
Display Re	sults as G ✓ Stru Poly Ass	ictures /mer Entities emblies							-		Count Cle	ear Q
	Nor	n-polymer Entities						500				



First match on list (best match)



To perform the BLASTP Alignment:

Select the Sequence link in Tabular Report pull down menu
 Copy Sequence



To perform the BLASTP Alignment:

- 4. Connect to NCBI BLASTP page
- 5. Select "Align two or more sequences" button
- 6. Paste in copy of PDB FASTA sequence in bottom text box
- 7. Paste your protein sequence in top text box.

	blastn blastp blastx tblastn tblastx
	Enter Query Sequence
	Enter accession number(s), gi(s), or FASTA sequence(s) 🚱
#7	MFNRLFGKPKQETNALASLDKMNEALEMLEKKEKVLLKKAAAEVERAKEFARAKNKRAAKQCLK RKRLYEQQVEQLGNFQLRIHDQMILLEGAKATTETVDALRSGAAAMQAMHKATNIDDVDKTMDEI NEQTENMKQIQEALSAPIGAVADLDDDELEAELEELEAELEELEGAELEEQLLQPATTAPAAPVHVP YARPVRPAAQKNTAEEDELAALQAVMAS
	Or, upload file Choose File no file selected
	Job Title
#5 —	Enter a descriptive title for your BLAST search (G)
	Enter Subject Sequence
	Enter accession number(s), gi(s), or FASTA sequence(s) 🕢
#6	>5FD7_1 Chain A Vacuolar-sorting protein SNF7 Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (559292) GNAKNKESPTKAIVRLREHINLLSKKQSHLRTQITNQENEARIFLTKGNKVMAKNALKKKKTIEQ LLSKVEGTMESMEQQLFSIESANLNLETMRAMQEGAKAMKTIHSGLDIDKVDETMDEIREQVE LGDEISDAISRP
	Or, upload file Choose File no file selected Image: Selected

Output of Directed BLASTP

Range 1: 1 to 140 Graphics

Vext Match 🔺 Previou

Score		Expect Method	Identities	Positives	Gaps	-
71.6 bit	ts(174)	8e-21 Compositional matri	x adjust. 45/140(32%) 75/140(53%)	0/140(0%)	_
Query	7	GKPKQETNALASLDKMNEALEM	LEKKEKVLLKKAAAEVEI	RAKEFARAKNKRA A+ F NK	AKQCLKRK AK IK+K	66
Sbjct	1	GNAKNKESPTKAIVRLREHINL	LSKKQSHLRTQITNQEN	EARIFLTKGNKVM	AKNALKKK	60
Query	67	RLYEQQVEQLGNFQLRIHDQMI	LLEGAKATTETVDALRS	GAAAMQAMHKATN Ga am+ +H +	IDDVDKTM	126
Sbjct	61	KTIEQLLSKVEGTMESMEQQLF	SIESANLNLETMRAMQE	GAKAMKTIHSGLD	IDKVDETM	120
Query	127	DEINEQTENMKQIQEALSAP	146			
Sbjct	121	DEIREQVELGDEISDAISRP	140			

Toolbox: DNA Sequence Translation Program -NOT Functional without FLASH

WSSP Translator Toolbox

This program searches a DNA sequence for open reading frames (ORF's). Paste a DNA sequence into the textbox below and click one of the buttons.



			GE Healthcare
Frame 1	Frame 2	Frame 3	🔅 🍪 RUTGERS

NCBI ORFfinder: DNA Sequence Translation Program

NCBI Resource	s 🖸 How To 🗹			Sign in to NC
RFfinder	PubMed	0		Search
٥		Find NCB	COVID-19 is an emerging, rapidly evolv Get the latest public health information from CDC: <u>the</u> Get the latest research from NIH: <u>https://www.</u> SARS-CoV-2 literature, sequence, and clinical content:	ing situation. ps://www.coronavirus.gov nih.gov/coronavirus. https://www.ncbi.nlm.nih.gov/sars-cov-2/.
pen Reading F RF finder searches f ch ORF, along with coding segments, ve	rame Finder or open reading fra a its protein transla erify predicted prote	imes (ORFs) in the ation. Use ORF fir in using newly deve	DNA sequence you enter. The program returns the der to search newly sequenced DNA for potentia loped SMART BLAST or regular BLASTP.	range of protein
s web version of th sion, which doesn't	he ORF finder is I have query sequer	imited to the subra	nge of the query sequence up to 50 kb long. Sta is available for Linux x64.	nd-alone U R F I I II d e r
amples (click to set	values, then click \$	Submit button) :		ATGACGGATCAGCCGCAAGCGGAATTO CGACATAA
 NC_011604 Sal minimal ORF le NM_000059; get 	monella enterica pl ngth: 300 nt enetic code: 1; start	asmid pWES-1; ger codon: 'ATG only';	netic code: 11; 'ATG' and alternative initiation codons minimal ORF length: 150 nt	
Enter Query	Sequence -			
From:		🗘 То:	\$	
Choose Sea	arch Parameter	'S		
Minimal OR	F length (nt): 75	8		
Genetic cod	e: 1. Standard		e	
ORF start co	odon to use:			
 "ATG" of "ATG" a Any ser 	only and alternative initianse codon	tion codons		
⊌ Ignore nest	ed ORFs:			
- Start Searcl	h / Clear			
Submit C	lear			

Paste in sequence and use the default options if you have a the start of your protein

Enter Query Sequence

AGTGTTTGAAGCGGAA	AAGGCTGTACGAACAGCAAGT	TGAGCAGCTGGGAAATTTCC/	ACTGCGGATCCATGATCAGATG
TACTCCTGGAAGGTGC/	AAAAGCCACTACAGAGACTGT	TGACGCACTGAGAAGTGGAG	CAGCAGCGATGCAGGCCATGCAG
AAGCCACTAACATTGA	TGATGTCGACAAAACCATGGA	CGAAATCAATGAGCAGACAGA	GAATATGAAGCAAATTCAGGAG
CACTGTCTGCCCCAAT	TGGCGCTGTAGCCGACTTAGA	CGATGATGAACTGGAAGCTGA	AGCTTGAAGAACTGGAAGCTGAG
TTGAAGAACTGGAGGG/	AGCCGAATTGGAGGAACAGCT	CTTGCAGCCTGCCACCACCG	GCCCGCCGCCCTGTGCATGT
CCTATGCCCGACCTGT	CCGTCCTGCTGCTCAGAAGAA	CACCGCAGAAGAAGATGAACT	TGCAGCATTGCAAGCTGTAATO
СТТСТТАААТСААТСА	GATGGACTGAGAGGAAGTCAT	TGCCGCAGCGATTTGTCGTT	GCTTCATAATGGACAGGTAAAAG
TTGCTTTCTAGTTCAT	CCTTCCACTCACTCCTTGTGT	CGAAGCACGCTTTGTATCTAT	AGCTTATTCATAGGGTCATCAT
CGGGTGGCACATTTGT	GGATCTTGGtGtgATTTCAAT	CTGTTGCTTCCTGTCTTGTT1	СААААААААААААААААааааА
ΑΑΑΑΑΑ	<u> </u>		
From:	🙃 То:		
	· · · · ·	~	

	Choose Search Parameters	
0	Minimal ORF length (nt): 75 😌	
0	Genetic code: 1. Standard	
0	ORF start codon to use:	
	 "ATG" only "ATG" and alternative initiation codons Any sense codon 	
0	Ignore nested ORFs:	

ORFfinder Output

Open Reading Frame Viewer

Sequence



ORF2	(224 aa)	Display ORF as	Mark
>lcl](MFNRLF ARAKNH DALRSC ADLDDU VRPAAC	DRF2 GKPKQETNALASI GRAAKQCLKRKRL GAAMQAMHKATN DELEAELEELEAEI DKNTAEEDELAAL	LDKMNEALEMLEKKEKVLLK YEQQVEQLGNFQLRIHDQMI IDDVDKTMDEINEQTENMKQ IEELEGAELEEQLLQPATTA QAVMAS	KAAAEVERAKEF LLEGAKATTETV IQEALSAPIGAV PAAPVHVPYARP

Mark subs	et Mark	(ed: 0	Download marked set			as Protein FASTA		
Label	Strand	Frame	Start		Stop		Length (nt aa)	
ORF2	+	3		144	818		675 224	
ORF4	-	2		795	529)	267 88	
ORF1	+	1		607	807		201 66	
ORF6	-	3		395	228		168 55	
ORF5	-	3		725	600)	126 41	
ORF3	-	1		520	398		123 40	

Six-frame translation...

Longest ORF first

ORF2

Marked set (0)

SmartBLAST

SmartBLAST best hit titles... 😡

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BLAST

BLAST

BLAST Database:

UniProtKB/Swiss-Prot (swissprot)

Does this ORF agree with the results from the BLASTx matches?



Enter in data from ORFfinder into DSAP Define ORF page

URF2 (224 a	a) Display ORF as Mark	Mark subs	iet	viarked: 0	Download ma	rked set a	IS Protein FASTA
>lcl 0RF2 MFNRLFGKPKQE	TNALASLDKMNEALEMLEKKEKVLLKKAAAEVERAKEF	Label	Strand	Frame	Start	Stop	Length (nt aa)
ARAKNKRAAKQC DALRSGAAAMQA	QCLKRKRLYEQQVEQLGNFQLRIHDQMILLEGAKATTETV QAMMKATNIDDVDKTMDEINEQTENMKQIQEALSAPIGAV		+	3	144	818	675 224
VRPAAQKNTAEE		ORF3	-		795	529	267 88
		ORF1	+		607	807	201 66
		ORF5	-	2	395	228	168 55
		ORF4	-	2	725	600	126 41
		ORF6	-	3	520	398	123 40
Define OI	RF						
In this step sequence	p you will determine if there is an open rea	iding frame	e (ORF	F) in your e	edied DNA		P
Paste the edged DNA sequence into the Toolbox, where it can be translated in each of the reading frames. Ret ieve sequence							e
2	2 Which reading frame is most likely to code for a +1 +2 • +3 None protein? (+ stop coden)						
3	Paste the most likely potein sequence or	a portion	of a pr	otein sequ	ence here		
	MFNRLFGKPKQETNALASLDKMNEALEML KQCLKRKRLYEQQVEQLGNFQLRIHDQMIL DDVDKTMDEINEQTENMKQIQEALSAPIGA LLQPATTAPAAPVHVPYARPVRPAAQKNTA	EKKEKVLL LEGAKATI VADLDDD EEDELAAL	KKAA/ TETVD/ ELEAEI QAVM	AEVERAKE ALRSGAAA LEELEAELI AS	FARAKNKI MQAMHK EELEGAEL	RAA ATNI EEQ	
	Number of characters: 224 Cursor is	on residue	: 225	Range n	narked:		

. .



Enter in the range of the ORF (includes the Stop codon)

Possible types of clones in the cDNA library



BLASTX results of a partial coding sequence



If you have partial sequence select the "Any sense codon" option

Enter Query Sequence

AGTGTTTGAAGCGGAAA	GGCTGTACGAACAGCAAGTT	GAGCAGCTGGGAAATTT	CAACTGCGGATCCAT	SATCAGATGA
TACTCCTGGAAGGTGCA	AAGCCACTACAGAGACTGTT	GACGCACTGAGAAGTGG/	AGCAGCAGCGATGCAG	SCCATGCACA
AAGCCACTAACATTGAT	GATGTCGACAAAACCATGGAC	GAAATCAATGAGCAGAC/	AGAGAATATGAAGCAA/	ATTCAGGAGG
CACTGTCTGCCCCAATT	GCGCTGTAGCCGACTTAGAC	GATGATGAACTGGAAGC	GAGCTTGAAGAACTG	GAAGCTGAGC
TTGAAGAACTGGAGGGA	CCGAATTGGAGGAACAGCTC	TTGCAGCCTGCCACCAC	GCGCCCGCCGCCCCT	STGCATGTCC
CTATGCCCGACCTGTC	GTCCTGCTGCTCAGAAGAAC	ACCGCAGAAGAAGATGA	ACTTGCAGCATTGCAAG	GCTGTAATGG
CTTCTTAAATCAATCAG	TGGACTGAGAGGAAGTCATT	GCCGCAGCGATTTGTCG	TGCTTCATAATGGAC	AGGTAAAACG
TTGCTTTCTAGTTCATC	CTTCCACTCACTCCTTGTGTC	GAAGCACGCTTTGTATC	TATAGCTTATTCATAG	GTCATCATA
CGGGTGGCACATTTGTG	GATCTTGGtGtgATTTCAATC	TGTTGCTTCCTGTCTTG	Ttcaaaaaaaaaaaaa	AAAaaaaAA
ΑΑΑΑΑΑ				
From:	🗘 То:			



ORFfinder output for a partial



ORF8 (132 aa)	Displa	y ORF as	Mark		Mark subs	set N	larked: 0	Download ma	rked set	as Protein FASTA 🔇			
>lcl 0RF8 HPDHVIVIKYVPYV0	GDSKRAMDEYT	SEIFMGGRSTIV	LHNTCEDSLLAAP		Label	Strand	Frame	Start	Stop	Length (nt aa)			
TILDLVLLAELSTRI VNALSKQRAMLENIN	IQLKAEGESKF MRACIGLAPEN	HTFHPVASILSY	LSKAPLVPPGTPV		ORF8	+	3	<3	401	399 132			
					ORF18	-	3	500	174	327 108			
					ORF6	+	2	212	448	237 78			
					ORF11	-	1	484	260	225 74			
					ORF5	+	2	<2	205	204 67			
					ORF19	-	3	173	>3	171 56	F	ran	1e
					ORF1	+	1	25	177	153 50		• ••••	
	\mathbf{N}				ORF9	+	3	444	590	147 48			
			,	8	ORF12	-	1	259	116	144 47			
Score		Expect	Method				Identitie	es.	Posi	tives	Gaps	Frame	Ð
262 bits(669)	1e-85	Composition	al mat	rix adju	st.	123/13	2(93%)	131/	132(99%)	0/132(0%)	+3	
		4											
Query	3	HPDHV	IVIKYVPYV	GDSK	RAMDE	YTSE	IFMGGF	STIVLE	INTCE	DSLLAAPI	ILDLVLLAR	5 18	2
C 1	100	HPDHV	IVIKYVPYV	GDSK	RAMDE	YTSE	IFMGG-	-STIV+H	HNTCE	DSLLAAPI	ILDLVLLA	3 17	~
Sbjct	120	HPDHV	IVIKIVPIV	GDSK	RAMDE	YTSE	IFMGGF	STIVM	INTCE	DSLLAAPI	ILDLVLLA	5 17	9

The first bases of a partial clone are part of the reading frame



Protein Interaction Searches



Interactome Requires FLASH



BioGRID

BioGRID Interaction Database



Enter in At Gene Name



Select Arabidopsis from the pull down menu

BioGRID Output

SNF7.2 F6F22.14, F6F22_14, VPS32, AT2G19830		Arabidopsis thaliana (Columbia)	Interact	or Statistics	
vacuolar protein sorting-associated pro	otein 32-1			Proteins/Genes 11	Publications 3
GO Process (1)	GO Function (1)	GO Component (3)			
TAIR C Entrez Gene C RefSeq C	UniprotKB 🖓				
🕹 Down	load Curated Data for t	this Protein			
			O Phy	sical Interactors (11)	
Switch View: Interactors 11 Interaction	ns 25 Network				
Showing 1 to 11 of 11 unique inte	ractors			Filter Interactions	ADV 🕢
Interactor	Organism / 🛛 🖨 Chemical Type	Aliases	\$	Description	¢ Evider
SNF7.1	A. thaliana (Columbia)	VPS32, AT4G29160, F19B15.190, F19B15_190	`	vacuolar protein sorting-associated protein	n 32-2 3 View
SNF7.2	A. thaliana (Columbia)	VPS32, F6F22.14, F6F22_14, AT2G19830	Ň	vacuolar protein sorting-associated protein	n 32-1 3 View
VPS20.1	A. thaliana (Columbia)	VPS20, MGI19_8, MGI19.8, AT5G63880	`	vacuolar protein sorting-associated protein	n 20-1 3 View
VPS20.2	A. thaliana (Columbia)	VPS20, T2K12.4, AT5G09260, vacuolar protein sorting associated protein 20.2	g- ,	vacuolar protein sorting-associated protein	n 20-2 3 View
AT1G15130	A. thaliana (Columbia)	F9L1_7, F9L1.7, AT1G15130	6	endosomal targeting BRO1-like domain-co	ontaining protein View
AT4G32760	A. thaliana (Columbia)	F4D11_40, F4D11.40, AT4G32760	1	ENTH/VHS/GAT family protein	1 View
CFIM-25	A. thaliana (Columbia)	F27B13_60, AT4G29820, F27B13.60, ATCFIM-25, hor CFIM-25, ARABIDOPSIS THALIANA HOMOLOG m	molog of nore 🖶	CFIM-25-like protein	1 View
VPS2.2	A. thaliana (Columbia)	VPS2, MFC16_25, MFC16.25, AT5G44560	`	vacuolar protein sorting-associated protein	n 2-2 1 View
VPS2.3	A. thaliana (Columbia)	VPS2, F21M11.12, AT1G03950, F21M11_12, vacuolar sorting-associated protein 2.3	r protein	vacuolar protein sorting-associated protein	n 2.3 <mark>1</mark> View
VPS24.1	A. thaliana (Columbia)	VPS24, MRN17.18, MRN17_18, AT5G22950	`	vacuolar protein sorting-associated protein	n 24-1 1 View
VPS60.1	A. thaliana (Columbia)	AT3G10640	v	vacuolar protein sorting protein 60.1	1 Viev

Interaction summary

SNF7.2 F6F22.14, F6F22_14, VPS32, AT2G19830	BAIT	SNF7.1 F19B15.190, F19B15_190, VF	PS32, AT4G29160		PREY
vacuolar protein sorting-associated protein 32-1		vacuolar protein sorting-as	sociated protein 32-2		
GO Process (1) GO Function (1) GO Component (3)		GO Process (2)	GO Function (1)	GO Component (2)	
TAIR C* Entrez Gene C* RefSeq C* UniprotKB C*	TAIR C Entrez Gene C	RefSeq 🖉 UniprotKB 🗷			
Arabidopsis thaliana (Columbia)		L L L L L L L L L L L L L L L L L L L	Arabidopsis thaliana (Colum	bia)	

PCA

A Protein-Fragment Complementation Assay (PCA) is a protein-protein interaction assay in which a bait protein is expressed as fusion to one of the either N- or C- terminal peptide fragments of a reporter protein and prey protein is expressed as fusion to the complementary N- or C- terminal fragment of the same reporter protein. Interaction of bait and prey proteins bring together complementary fragments, which can then fold into an active reporter, e.g. the split-ubiquitin assay.

Publication

The Arabidopsis ESCRT protein-protein interaction network.

Shahriari M, Richter K, Keshavaiah C, Sabovljevic A, Huelskamp M, Schellmann S

In yeast, endosomal sorting of monoubiquitylated transmembrane proteins is performed by a subset of the 19 "class E vacuolar protein sorting" proteins. The core machinery consists of 11 proteins that are organised in three complexes termed ESCRT I-III (endosomal sorting complex required for transport I-III) and is conserved in eukaryotic cells. While the pathway is well understood in yeast and ... [more]

Unknown Mar. 26, 2011; 0(0); [Pubmed: 21442383]

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Throughput Low Throughput

Related interactions

Interaction	Experimental Evidence Code	Dataset	Throughput	Score	Curated By	Notes
SNF7.1 → SNF7.2	PCA	Shahriari M (2011)	Low	-	BioGRID	-
SNF7.2 -> SNF7.1	Two-hybrid	Braun P (2011)	High	-	BioGRID	-
SNF7.2 -> SNF7.1	Two-hybrid	Richardson LG (2011)	Low	-	BioGRID	-
SNF7.1 -> SNF7.2	Two-hybrid	Richardson LG (2011)	Low	-	BioGRID	-

Curated By

• BioGRID

BioGRID Network



Unknown Clones will not be released for analysis until:

PC1 to PC3 are completed and reviewed.
 i.e. Clone Status = "Reviewed and correct"

2. The Post Survey is completed.

Please do not email until these are done. Send a message requesting the release of your unknowns to <u>wssp@waksman.rutgers.edu</u>

Students should include their name, school and clone names

E-mail links on DSAP or WSSP web site



All caught up!! Most of the 2020 summer sequences already published!!

(Using old sequences)

Clones
106TG3.15, 106TG4.15
107SMC2.15, 107SMC4.15
109LB1.15, 109MX1.15
108ALR8.15, 108LA11.15, 110RGC1.16
109CC7.15, 109JS2.16
108AL1.15
108LA5.15, 108LA7.15, 130SM5.19
109SP2.15, 109XZ2.16
106TTM6.14, 107MS1.15

Timeline of the 2020/2021 WSSP Project



WSSP Summer Institute: If in person: 7/6/21-7/16/21 One student per school Students will be leaders back at their schools

If virtual: 7/12/21-7/16/21 Two students per school Students will be leaders back at their schools

Waksman Institute Summer Experience (WISE), Open to any student, Fee

Spring Session (virtual): 1/25/21-3/25/21 (Mon/Wed 7:00-8:30 PM)

Virtual: June Session: 6/21/21-6/25/21 August Session: 7/26/21-7/30/21

In person: June Session: 6/21/21-7/2/21 August Session: 7/26/21-8/6/21

Have fun

Send us your data!!

Update your Google Docs Supply sheet on your plans

Stay safe!!