ContigScape User Manual 1.0

Introduction	2
Highlights	
Installation	
Overview	
Open files	
Open CRS file	
Open 454Contig.ace file	5
Open AGP file	7
Open mate-paired reads	7
Alignment Options:	
Quality threshold:	
Minimum read length:	
Minimum quality score to keep:	
Minimum percent of bases:	
Number of CPU for BWA:	
Visualization Options	9
Show contig width by ratio	9
Show contig sequences:	9
Hide contig link < {value}	9
Hide contig < {value} bp	9
Repeat contig threshold	9
Single contig threshold	9
Visualization appearance	11
Repeat contig color for >2x:	11
Probable contig color for >1.5x:	
Single contig color for <1.5x:	
Contig shape for <2kb:	
Link label size:	
Link color:	
Link label color:	
Link color for gap:	
Link label for gap:	
Set shape for plasmid:	
Maximum link width:	
Minimum link width:	
Show/Hide result	
Show intermediate results	
Estimate Plasmid/TIRs	14
Contig sequences	
Algorithm principle	
Finding connections between 454 reads	
Finding connections between mate-paired reads	
Genomic features prediction	
Copyright and Contact	
Frequently Asked Questions (FAQs)	19

Introduction

Thank you for choosing ContigScape, which is a Cytoscape plugin that offers construction and visualization of de novo genome assemblies. Its intuitive manner makes it very suitable for discovering contig relationships, and therefore helpful in gap-closing strategy. The program is freely available from <u>http://sourceforge.net/projects/contigscape/files/</u>.

Highlights

Automatically construct possible connections between scaffolds or contigs using assembly of reads (<u>ACE format</u>), mate-paired reads, scaffolding information (<u>AGP format</u>) or user defined CRS files. ContigScape lets you manipulate the appearance of the graph, as well as filter the contigs by their length and coverage. Meanwhile, the sequence contigs of plasmids and special repeats (IS elements, ribosomal RNAs, terminal repeats etc) can be displayed as well. The following figure illustrates network appearance upon loading ACE file, mate-paired reads and AGP file, respectively.



Installation

Before using ContigScape, you need to make sure you have **Java and Cytoscape2.8.3** installed. After that, you just need to put ContigScape.jar under installation directory cytoscape/plugins folder or browse to plugin using "**Install plugin from file**" which is under **Plugins** menu.

Overview

ContigScape is loaded automatically along with Cytoscape, which can be found in the left panel. The graphic interface is divided into four categories: "Open files", "Visualization Options", "Visualization appearance", "Show/Hide result", "Contig sequences". There are several options under each category. You can click the categories to make options hide or show up.



I Open files allows user to choose different file types to import into ContigScape. Currently ACE format, mate-paired reads, AGP format, and user defined tab-delimited files are supported.

II Visualization Options allows user to check which type of graphic object you wish to show on the graph, as well as see statistical information of the graph.In able to obtain the best visual effects, please use "Layout"—"Force-Directed

Layout" button and "Visual style" — "sample1"

III Visualization appearance allows user to control the appearance of the graph, such as color and size for different types of nodes/edges.

IV Show/Hide result allows user to export intermediate result and estimate plasmid/TIRs.

V Contig sequences allows user to check selected contig sequences. It is also possible for users to send the sequences to NCBI Blast upon selection.

I. Open files:

1 Open CRS file:

CRS format includes two files, and each contains three columns. 'tabbed.txt' represents the number of connections among contigs, and 'tabbedCov.txt' describes all the contigs' length and coverage. User can input 'tabbed.txt' guiding how contigs are connected to each other. This is usefully in creating connections using diverse data from other excellent software, or adds additional connections to the existed network. The following example shows how to prepare 'tabbed.txt' files using the beginning and end of the contig to create connected (the contig maybe a plasmid, etc), as contig140 in the following example. The format of 'tabbed.txt' is as follows:

Begin	End	Connections-ReadsNum (optional)
3S	119E	113
3E	58E	322
140S	140E	29

NOTICE: "S" represents "start position", "E" represents "end position"

'tabbedCov.txt' file can be imported as additional information by checking "**input tab-delimited contig coverage file**" and specify the location to the file. The file should also be tab-delimited and follow the format in the table below.

Contig ID	Length	Coverage	e (or copy number)		
119	169	40.72	(1.94)		
3	3295	24.73	(1.18)		
58	31775	21.92	(1.04)		

Open CI	RS file	Open		×
Open 454C	ontigs.ace			
Open A	GP file	Input tab-delimited file	e_revised_picture\CRS\tabbed.txt	rowse
Open mate-p	aired reads	✓ Input tab-delimited contig coverage file:	evised_picture\CRS\tabbedCov.txt B	rowse
▲ Visualizat Show contigs width by	ion options ratio	O Add links to the following network	454Contigs_1.ace	
Show contigs sequences		Oreate new network using the links		
☐ Hide contig link < ☐ Hide contig(bp) <	10 2000 delete	Label of the links		
Repeat contig thredshold:	2.0	Width of the links	0	
Single contig thredshold:	1.5	Color of the links		
Visualizatio	on appearance			
Repeat contig color: Probable repeat contig	· · · · · · · · · · · · · · · · · · ·	是(1)] <u>香</u>	

The CRS test files of 10 454Contigs.ace can be downloaded from

http://sourceforge.net/projects/contigscape/files/datasets/454Contigs_CRS_test_files.zip

2 Open 454Contig.ace file:

ACE files are generated by various assembly programs, including Phrap, CAP3, Newbler, Arachne, AMOS, etc. ACE file not only includes contigs and reads of the assemblies, but also contains information of the connections between the contigs. Take 454Contig.ace for example, which was generated using 454 reads and Newbler program. Upon pressing "**Open 454Contig.ace**", a dialog promotes the user to input the locations to the sequence file. The graph below indicates the connections/copy number of the contigs after import 454Contig.ace.







Amycolatopsis mediterranei S699



Leptospirillum ferriphilum ML-04



Cotesia vestalis bracovirus



Ralstonia solanacearum



Bacillus thuringiensis BMB171



Mycoplasma bovis Hubei-1





Edwardsiella tarda

EIB202



Cordyceps militaris



Amycolatopsis orientalis Mycobacterium tuberculosis HCCB10007 CCDC5079



Acidianus hospitalis W1

3 Open AGP file:

<u>AGP format</u> describes the assembly of a larger sequence object from smaller objects, such as joining contigs into scaffolds. Here it is useful in forming larger assemblies using the reference sequences. You can apply BLAST alignment of the contigs onto close-related reference sequences using the following command.

blastall -p blastn -i contig.fas -d ref.fas -m 8 -e 1e-30 -F F -o output.txt

Then you need to remove both the duplicated and the ambiguous matches (score < 1000 in the following example):

```
perl -ne '@t=split(/\t/);next if $t[11]<1000;print unless defined $h{$t[0]};
$h{$t[0]}=1;' output.txt|sort -k9,9n > output2.txt
```

After that you can use <u>blast2agp.pl</u> to convert the BLAST output into AGP file, and then press button "open AGP file" and browse to its location:

Open A	GP file	Open 🛛
Open mate-p	aired reads	
Visualizat	ion options	Please specify the location of the AGP file
Show contigs width by	ratio	ised_picture\2999-scaffolding-tab\test.agp Browse
Show contigs sequences		- Add links to the following network
Hide contig link <	10	And THES to the following network
Hide contig(bp) <	2000 delete	454Contigs_1. ace
Repeat contig thredshold:	2.0	• Create new network using AGP file
Single contig thredshold:	1.5	
Visualizatio	on appearance	

4 Open mate-paired reads:

Mate-paired reads can be used to construct the relationship between the scaffolds. ContigScape needs several tools to do this (<u>SAMtools</u>, <u>bwa</u>, <u>FASTX-Toolkit</u>, <u>BEDTools</u>). In the **Open Dialog**, user needs to specify the sequences and the path to the executables. Press the **OK** button and ContigScape will precede the input sequences using the programs. After processing, the files generated will be saved in the same folder as the contig sequences you input.

Open		×
Input contig sequence		Browse
Input scaffold. bed		Browse
Input mate-paired reads 1		Browse
Input mate-paired reads 2		Browse
Directory to SAMtools		Browse
Directory to bwa		Browse
Directory to FASIX-Toolkit		Browse
Directory to BEDIools		Browse
Alig Alig	n options	
Quality threshold	-[]	
Minimum quality score to keep		
Minimum percent of bases		
Minimum read length	40	
Num. of CPU for BWA	3	
是(Y)	否(N)	



Alignment Options

The options below are for setting the parameters used while mapping mate-paired reads onto the contigs. For the program used, please also refer to <u>http://hannonlab.cshl.edu/fastx_toolkit/commandline.html#fastx_trimmer_usage</u> and <u>http://bio-bwa.sourceforge.net/bwa.shtml</u>.

Quality threshold:

Set quality to be trimmed (from the end of the sequence) by fastq_quality_trimmer

Minimum read length:

Sequences shorter than this will be discarded by fastq_quality_trimmer.

Minimum quality score to keep:

Minimum quality score to keep by fastq_quality_filter.

Minimum percent of bases:

Minimum percent of bases filtered by fastq_quality_filter.

Number of CPU for BWA:

Number of threads used by bwa while doing alignment, see also "bwa aln".

II. Visualization Options:

Show contig width by ratio:

Check this option to apply modification to contigs width according to their length. Otherwise contigs are shown in the same width.



Show contig sequences:

Store contig sequences in memory while loading 454Contigs.ace files. The contig sequences are shown in the data panel below on click the nodes/edges representing each contig.

Hide contig link < {value}</pre>

Hide the connection for those contig connections having a reads count lower the value specified by user. Check this option if user want to leave high confident connections only.

Repeat contig threshold

Define repeat threshold, default value is 2

Single contig threshold

Define specific contig, default value is 1.5



A scaffolding with all contigs

B hide contigs (length<2kb)

III. Visualization appearances:

🧿 Cytoscape Desktop (Ne	🖷 Session)
File Edit View Select Layo	ut Plugins Help
	R 💼 🔯 🔜 🎠 🖪
Control Panel	Apply for
Retwork VizMapper™ ContigS	Scape Editor Filters
🔺 Open	files
Open C	RS file
Open 454C	ontigs.ace
Open A	GP file
Open mate-p	aired reads
Visualizat	ion options
Show contigs width by ratio	
Show contigs sequences	
Hide contig link <	10
Hide contig(bp) <	2000 delete
Repeat contig thredshold:	2.0
Single contig thredshold:	1.5
🔺 Visualizati	on appearance
Repeat contig color:	~
Probable repeat contig color:	· · ·
Single contig color:	· · · · · · · · · · · · · · · · · · ·
Contig shape for <2kb:	🔷 Diamond 🍟
Link label size:	13
Link color:	· · · · · · · · · · · · · · · · · · ·
Link label color:	~
Link color for gap:	¥
Link label color for gap:	~
Set shape for plasmid:	🗆 Rectangle 🛛 🍟
Maximum link width:	25.00
Minimum link width:	0.12
Hide 1	results
Show interme	diate results
Estimate p	lasmid/IIRs
Abou	it Vs
Welcome to Cytoscape 2.8.3 R	light-click + drag to ZOOM Middle-

In able to obtain the best visual effects, please use "Layout"—"Force-Directed Layout" button and "Visual style"—"sample1" Sumple1

The options below control the appearance of the graph, such as color, shape, size of the graph objects and labels according to constrains set by user.

Repeat contig color:

Set color for contigs which have coverage greater than two-folds of the overall coverage.

Probable repeat contig color:

Set color for the contigs whose coverage is greater than 1.5 and less than 2.

Single contig color for:

Set color for contigs which have coverage lower than 1.5-folds of the overall coverage.

Contig shape for <2kb:

Set shape for contigs which have length greater than 2k base pairs.

Link label size:

Set size for the labels of the contig connections.

Link color:

Set color for contig connections.

Link label color:

Set color for the labels of contig connections.

Link color for gap:

Set color for connections of gaps.

Link label for gap:

Set color for labels of connections of gaps.

Set shape for plasmid:

Set shape for the contigs predicted as plasmid.

Maximum link width:

Set maximum width for the connections between contigs. Set to 25 by default.

Minimum link width:

Set minimum width for the connections between contigs. Set to 0.12 by default.

IV. Show/Hide result:

Show intermediate results:

Intermediate results contain information generated while loading ACE files, AGP files and mate-paired reads. The following example shows contig and contig connections information for ACE file loaded. User can choose either "**Export**" to save current table, or "**Export All**" to save all the tables.

Intermediate	results		X	Intermediate	results		X
Contigs-F:\2999	2999-454Contigs.ace	Linkers-F:\2999\2999-454Contigs.ace		Contigs-F:\2999	2999-454Contigs.ace Lind	kers-F:\2999\2999-454Contigs.ace	
Contig	Length	Coverage		Start	End	Count	
1	474	51.70	~	93E	137E	6	~
2	6205	20.33		20E	143S	29	
3	6170	17.78		64E	142S	15	
4	8878	58.73		65E	94S	17	
5	22867	34.21		595	815	6	
6	1281	56.89		149S	153S	115	
7	322735	21.46		195	1 43E	23	
8	95957	25.33		60S	159S	20	
9	273677	23.58		65E	66E	16	
12	932	25.91		70E	117S	10	
13	9033	51.95		315	142S	22	
14	35543	21.42		255	78E	31	
15	230734	22.98		79E	80E	7	
16	776	61.78		157E	158E	26	
17	241957	25.34		1E	2S	24	
18	159510	25.26		117E	1 43E	29	
19	93497	20.78		59S	62S	11	
20	869	33.98		8E	325	5	
21	185781	24.90		95	1 49E	29	
22	168853	26.56		16S	18S	18	
23	4846	32.79		6S	53S	52	
24	1063	4.14		55S	57S	10	
25	368	22.70		75S	76S	1	
27	51615	17.52		5S	6S	24	
28	187024	25.12	~	385	78E	33	~
	Export	Export All			Export	Export All	
		角定			确定		

Estimate Plasmid/TIRs:

Plasmid results include predicted plasmids with coverage and type information.



V. Contig sequences

Show sequence in the data panel field below upon clicking corresponding contigs and edges.

Repeat contig color:	
Probable repeat contig	
Single contig color:	
Contig shape for <2kb: Oi amond	
Link label size: 13	
Link color:	
Link label color:	
Link color for gap:	Data Panel
Link label color for gap:	Contig linker 156 to 29 TGATGAACCGCTCCTTCGACTCGCTGCACCTGGTCAACACCTACGGTGCCTTCGGCTCCGTCAGCCGGATCCGCCACGAGGTGGTGGTCGAGGGCACGG
Set shape for plasmid: Rectangle	CCGACGAGGCGCCCCGCGAGGACGGGGACTGGCGGGGGGAGTACGAGTTCAAGGGGAAACCCGGTGATCCCCGGCGCTGGCCGCGCGCG
Maximum link width: 25.00	CATCTGCGGCTCGACTGGATGATGTGGTTCGCCGCCGCGTGGCCGCGGACCGTGGTTCGCCACCTTCGCGGAACGGCTCCTGGAGGACGA CCGCGCCACCCTGCGCGCTGCGCCGCCGCCCGCCCGCCCG
Minimum link width: 0.12	GCGGGAGCTGCGGGAGACGGGGGGGGGGGGGGGGGGGGG
Hide results	AGTTCGCAGGCGAGCGCGTGGGCGATCAGCCCGCCGGCGGGCCACGACCAGCAGCGGCCGGC
Show intermediate results	AGTCGGCGGCCATCTCCTCGAAGGACGCCGGCAGGGGTTCGGGGG Design Priners GTGCCTGGATGGCGTAGACCGGGGGCGGGGGGCGATGTG
Estimate plasmid/IIRs	GTTCAGGAGCCCGCTGTACGACCAGCTGATGCCGCCGCGCGGGGGGGG
About Us	GTCTCGCGGACGCGGGGGGGCGGCGGCGGCGAGCAGCGAGGAGCGCGCCGC
	CCTCCCCGAAGAGGCTCCCACACGCACTTCCCCCGCGCCTCCGCGCCCCCCCC
Welcome to Cytoscape 2.8.3 Right-click + drag to ZOOM	Middle-click + drag to PAN
Repeat contig color:	
Probable repeat contig	
Single contig color:	
Contig shape for <2kb: Oliamond	
Link label size: 13	
Link color:	primer pair start direct. len. distContigEnd Im score sequence
Link label color:	
Link color for gap:	Data Panel 19 285 60.95 530.00 CGGCICGACIGGAIGAIGI right: 155 19 136 61.47 181.00 CCGACCAGAICCGCAAGAI
Link label color for gap	Contig linker 156 to 29
Set shape for plasmid: Restands	TGATGAACCCGTCCTTCGACTCGCTGCACCTGGT right: 154 < 19 135 59.76 161.00 CGACCAGATCCGCAGATC
Maximum link width: 25.00	
Minimum link width: 0.12	CGCGCGCCACCCTGCGGCTGCGCCGCCGCCGCCCC right: 155 < 19 136 61.47 181.00 CCGCCGCGCAGTCCGCAGTC
Hida raenlte	GCCGGGAGCTGCCGGGAGACCGGGGCGTGCTGGGA
Show intermediate results	AGTTCGCAGGCGAGCGCGCGCGCGCGACCAGCGCGACGACGACG
Estimate plasmid/IIRs	
About Us	GTTCAGGAGCCCGCTGTACGACCAGCTGATGCCG CCAGCGCGTCGGCGGGGTCGTCCATGACCAGGCC
Welcome to Cytoscape 2.8.3 Right-click + drag to ZOOM	Middle-click + drag to PAN
Repeat contig color:	
Probable repeat contig	
Single contig color:	
Contig shape for 2kb: 🔷 Diamond 👻	
Link label size: 13	
Link color:	
Link label color:	
Link color for gap:	Data Panel Options for blastn
Link label color for gap:	
Set shape for plasmid: Rectangle	TGATGAACCGCTCCTTCGACTCGCTGCACCGCTGCCACCGCTGCC
Maximum link width: 25.00	CCGACGAGGCGCCCCGCGAGGACGGGGACTGGCGGGGAGTACGAGGTC/ CATCTCGCGCTCGACGAGGACGAGGACGGCGCGCGCGCGC
Minimum link width: 0.12	CGCGCCACCCTGCGGCTGCCGCCGCCCGCCCGCCCGGCCCC CCGCGCCACCCTGCGGCTGCCGCCGCCCGCCCCGC
Ni da saculta	GCGGGAGCTGCGGGAGACGGGGGGGGGGGGGGGGGGGGG
Show intermediate results	CCCCCCCGCGCGCCCCGCGCCCCGCGCGCCCGCGCGCG
Estimate plasmid/IIRs	AGTCGGCGGCCATCTCCTCGAAGGACGCCGGCAGGGGTTCGGGGCGG(
About Us	GTICAGGAGCCCGCIGIACGACCAGCIGAIGCCGCCGCGGGGGGGG
Welcome to Cytoscape 2.8.3 Right-click + drag to ZOOM	Middle-click + drag to PAN

When viewing the graph, the 1,000 base pairs of both 5'-end and 3'-end can be loaded, with 20 "N" linking them representing the middle sequences. Clicking the edge of two contigs, the sequence containing corresponding contigs' ends can also be displayed. The displayed sequence can be used to design primers in ContigScape and perform blast against NCBI database.

Algorithm principle

Finding connections between 454 reads:

To our knowledge, Roche 454 shotgun sequencing is the most suitable strategy for de novo genome sequencing. The reads length of Roche 454 reached 700bp now and could resolve small repeats caused gaps. But reads of longer repeats would be assembled into only one contig and thus produced gaps in other repeat regions. The 'Newbler Assembler' could produce a '454Contigs.ace' file, which contained all the assembly information and could be opened by 'Consed' (Gordon, D. *et al.* 1998). As we can see from the following, when a read was separated into two contigs, the coordinate of the read in each contig will be shown after the read name, followed by the contig number this read linked, using 'fmX' representing 5' end of the read located in contigX and 'toY' representing 3' end of the read located in contigY. This label showed a unique advantage of 'Newbler Assembler' endowed by 454 long reads. We can extract all the 'fm' and 'to' information from '454Contigs.ace' file, and arrange them into a relationship table, such as '5'-end-Contig1' linked '3'-end-Contig2'.

Then the table could be displayed by ContigScape.



Finding connections between mate-paired reads:

material: matePairedReads1.fq, matePairedReads2.fq, matePairedContigs.fas, scaffold.pl

The most common way to assemble contigs into scaffolds is through mate-pair information. Depending on the fragment size

of mate-pair reads, scaffolding program can identify how far two contigs should be apart from each other. For example, if two contigs were separately mapped by a pair of 3kb apart mate-pair reads, the two contigs could be joined into a scaffold and the gap size should be 3kb minus distance from mapping loci to the end of contigs. The repeat region less than 3kb could be spanned using this method. But if the repeat region was longer than the fragment size of mate-pair library, ambiguous linkage information would occur. Same as 'Newbler' result of 454 reads, the ContigScape could display a network relationship within scaffolds by counting mate-pair reads number linking LargeContigs (>500bp). Detailed method was described in scaffold.pl (http://sourceforge.net/projects/contigscape/files/datasets/).

Genomic features prediction:

Genomic features can be categorized into several groups: plasmids, phage elements, and terminal repeats, which can be accessed through "show Plasmid/TIRs" in the control panel. See the following figure for details. In Figure **A**. The interface of ContigScape. The left part was the control panel, the window on the right shows a sample genome. Contigs were indicated by red (repeated contig), dark blue (unique contig) and orange(probable repeats). B. Zoomed image of some contigs (light blue frame in panel A). B1. A linear plasmid formed by three contigs. B2. Repeats (Contig28) in the end of chromosome. B3. A circular plasmid with high copy number formed by three contigs (Contig141,142,143). B4. Two high-copy number circular plasmids each formed by a single contig. B5. A linear plasmid with high copy number formed by one contig. B6. A circular plasmid with single copy number composed of one contig.



Judging whether a repeat contig was from chromosome or plasmid mainly depended on the linkage information of two ends of this contig. Four different types were shown in **Figure**, 1). Repeat contigs connected in a circular fashion (Panel 3), 2). Individual contig connected itself without anyone else (Panel 4 and 6), 3). One end of repeat contig having no linkage to any other contigs, usually representing linear chromosome telomere or linear plasmid end (Panel 1 and 2), 4). A linear plasmid composed of only one repeat contig without connections to any contigs (Panel 5). While if a plasmid is linear and single copy, ContigScape cannot distinguish it. We can estimate whether or not a contig was a plasmid effectively based on above described situation in our experience. Of course researcher must confirm whether it is a plasmid or not by PCR, sequencing and annotation.

In **Figure B**, 143E has connections with 142E and 144E (Panel 3). But the number of connections (800) between 143E and 142E is more than that (10) between 143E and 144E. In this case, the latter might be a nonspecific connection caused by little overlap among the reads. Additionally, Figure 6B shows that contig78 in the linear plasmid 80E-80S-78E-78S-54E-54S also has another copy in the chromosome (Panel 1).

Copyright

ContigScape is a free software; you can redistribute it and/or modify it under the terms of the Lesser GNU General Public License as published by the Free Software Foundation; either version 2 of the License, or (at your option) any later version. This program is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the Lesser GNU General Public License for more details.

You should have received a copy of the Lesser GNU General Public License along with this program; if not, write to the Free Software Foundation, Inc., 59 Temple Place, Suite 330, Boston, MA 02111-1307 USA

Contact

You may email any questions/suggestions to: qwang.big@gmail.com, tb_411@163.com You're welcome to report bugs at <u>ContigScape Discussion Forums</u>.

Frequently Asked Questions(FAQs)

1. Why the network looks messed up after I opened 454Contigs.ace?

A: You have to do layout manually after loading the network, one way is to use "Force directed layout" in Cytoscape menu. In able to obtain the best visual effects, please use "Layout"—"Force-Directed Layout" [35], button and

VizMapper-"Visual style" — "sample1"

2. How do I make an AGP file by comparing the contigs to the reference sequences?

A: You may do a local BLAST between the contig and the reference sequences with "-m 8" parameter, then use a script called "blast2agp.pl" included in this software package to convert the BLAST output to an AGP file.

3. Have GRASS, SSPACE and OPERA been integrated into the package of ContigScape?

A: Sorry, we have not integrated them. User can prepare scaffold result handled with these tools to CRS format file mentioned in the above, and then ContigScape will provide a Global visualization.

4. I had some problem to install the java class. It doesn't work on my Mac, and gives me an error. On a Linux system I got it to run. I loaded the 454 contigs as ace file into ContigScape. Further, it is extremely "slow". My java job is always on 99%..

A: It looks that you have not install java correctly. Please download Java 1.6 or higher version from http://java.sun.com and install it properly.

5. You mentioned contigs can be reordered, broken and the results can be saved. I haven't found those functionalities in ContigScape. Can the results be saved?

A: Those functions can be found in Editor panel of Cytoscape. Cytoscape is a software package with perfect visualization network and high operability. That is why we select it.

6. Would it be possible to import bam files? Those contain most of the relevant information, and it is a widely used format. A: You can use bamToBed in BEDTools package to convert a sorted bam file into a bed file, then specify both the contig sequence and the bed file from "open mate-paired reads" dialog to load.

7. I saw the scaffold.pl script, but why can't it be run within ContigScape?

A: Scaffold.pl is a perl script used in processing 'fastq' file and corresponding contig file with the purpose of gaining a scaffold.bed and tabbed.txt including contigs' relationships. Scaffold.pl needs Fastx_toolkit, BEDTools, BWA, Samtools which were developed only based on Linux platforms. In fact, user can run scaffold.pl in "open mate-paired reads" of ContigScape on Linux platforms.

8. In the abstract you said that you can find repeats like IS elements, Ribosomal RNA etc. This is detection purely done by coverage of reads? If due to long reads like PacBio, those are assembled into their single copies, will you still detect them? A: It's true. The contig number of bacterial genome will greatly reduced using PacBio technology. ContigScape won't be able to detect small repeat like IS elements, Ribosomal RNA. But, large repeat contig still exist in several bacterial genome, fungi genome and all plant and animal genome. Large repeats more than 10kb usually have important biological significance.

9. The scope is supposed to be microbial genomes gap closing, but would the tools be able to be applied to scope with plant and animal genome with ten thousands of contigs?

A: User may convert the result to CRS format file mentioned in the above, and then ContigScape provides a Global visualization. Though thousands of contigs may look complicated in the image, it is the real relationship among these contigs.

10. How do I get "454Contigs.ace" for my assembly?

A: You have to ensure you checked "Single ACE file" or "Single ACE file for small genomes" in GS De novo Assembler Output Sub-Tab, see http://454.com/my454/documentation/gs-flx-system/emanuals/Part_C/wwhelp/wwhimpl/common/html/wwhelp.htm# href=PartC.1.016.html&single=true

11. Why I cannot obtain the result after input scaffold.bed and corresponding contig sequence ? A: Please check the integrity of the scaffold.bed and make sure that the sequence title in scaffold.bed is same as corresponding contig sequence.

12. Can I install it in Cytoscape 3.0?

A: Sorry, ContigScape 1.0 don't support Cytoscape 3.0 now, we will update it in version 1.1.

13. I have not clearly understood whether the program was specific to Newbler assembler or not.

A: The "open 454Contigs.ace" option can be only used to input 454Contigs.ace, which had been modified in ContigScape v1.0. Ace format is a very large file with various contents. We cannot process so many formats sand we provided CRS format described in the manuscript to fit different program. CRS format contains two files, and users can refer to http://sourceforge.net/projects/contigscape/files/datasets/454Contigs_CRS_test_files.zip/download.

14. How it provides help in finishing after viewing a graph?

A: Contigs displayed in ContigScape were better operational, as the repeat contigs, gaps and even plasmids can be highlighted, filtered, and customized. So, graph itself will facilitate a faster and more precise determination of the linkages among contigs and greatly improve the efficiency of gap closing.

After view a graph, we can get the sequence of contigs' ends which may be used to blast and designing primer. Click the edge of two contigs, the sequence containing corresponding contigs' ends can be used to blast on NCBI to judge the edge is true or not. Other, if user needs to edit the connections of the network, the user can open "edit panel" to edit. All the gaps needs to be filled by PCR and sequencing using ABI3730 sequencer. We have added this point in part "Display functionality of ContigScape" of our revision.

In our finishing strategy, all contigs together with ABI3730 data must be assembled using phred, phrap, consed at last. Our plugin cannot replace "consed" program. It looks like a canvas which used to edit and judge the order among contigs and can evaluate the complexity of assembly visually.