1, Two default parameters explained
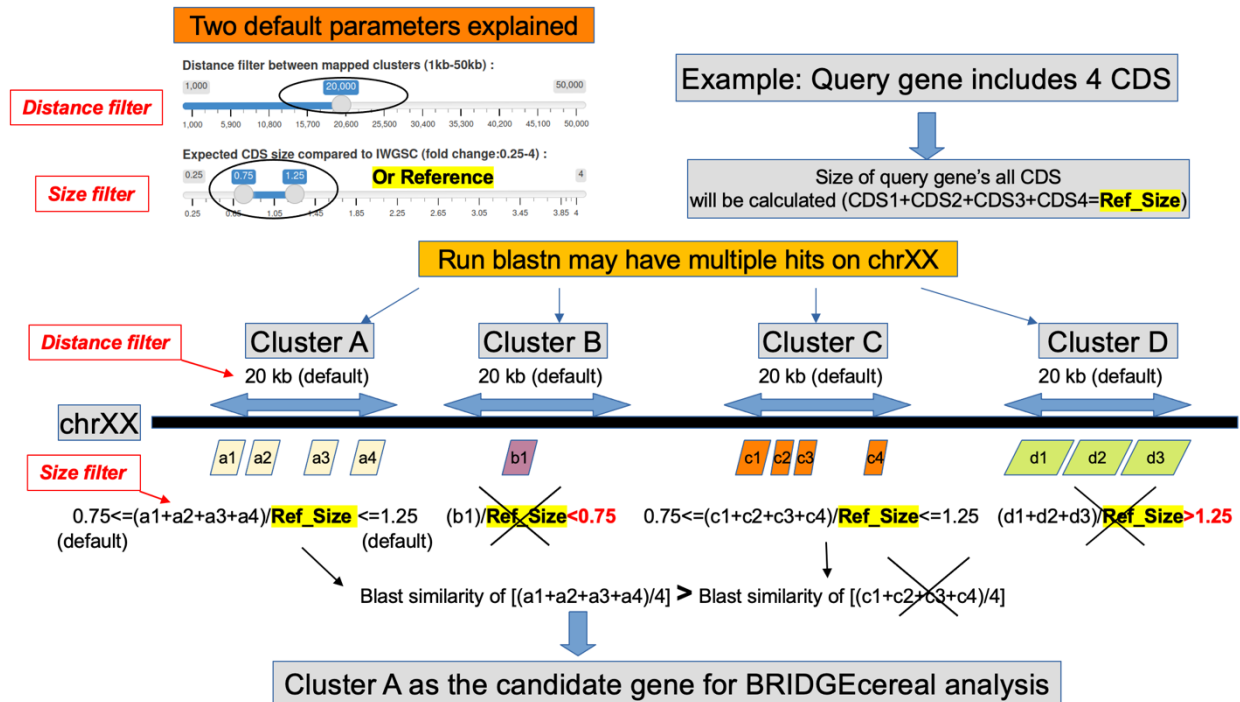
1, If you expect large insertions in orthologs, please increase the distance filter.

2, If you expect that the ratio of (ortholog's coding region) / (query's coding region) is too large or too small, please adjust the size filter accordingly.

2, Paste sequence

Users want to blast a transcript sequence against pan-genomes

1). Input your sequence ID name, such as YourID, in the Gene Name box.

DO NOT click Button "(1) Check Gene ID"!

2). Manually select the Reference and Chromosome, select "fasta_seq" in the "CDS" box, then paste the sequence in the "Your fasta sequence" box. For your fasta sequence, the first line should be >YourID, the same name as the input shown in the Gene Name box.

3). Click "(2) Submit" button to start the process. User's "YourID" query sequence together with other pan-genomes will be plotted in Panel 1.

Gene name (such as TraesCS4A02G058900 for IWGSC) or YourID for fasta sequence

YourID ◄━━━━ Job's name

(1) Check Gene ID ☒ **DO NOT click this button**

Pick Genome (Please select one!) :

IWGSC ◄━━━ Reference genome (Wheat)

Chromosome (Please select one!)

chr4A ◄━━━ Target chromosome (Wheat)

CDS (Coding sequence); OR your fasta sequence :

fasta_seq ◄━━━ Select this option

Your fasta sequence (Please add first line: >YourID Before pasting your DNA sequence!)

>YourID ◄━━━ Paste your sequence here with the name "YourID"
ATGGGTCGGGGGAAGGTGGAGATGAGGCGGATCGAGAACAAGATAAGCCGGCAGGTGACGT
TCGCCAAGCGCCGGAATGG
GCTGCTCAAGAAGGCCTACGAGCTCTCGCTGCTCTGCGACGCCGAGGTCGCCCTCATCATCTT

3, Upload a contig or a chromosome

Users want to compare their sequenced chromosome (or large DNA fragment/contig) with pan-genomes on specific gene.

Prepare your ***.fa and ***.fa.gz files following this instruction: Rename query chromosome.fa file (Wheat example):

> >chr4A
>
> ATGCATGC...

Here, chr should be in lowercase!

For other crops (chromosome 4): Maize (>chr4); Sorghum (>chr4); Rice (>chr4); Barley (>chr4H)

For a contig or a large fragment containing your interested gene, just use chromosome as fasta file's first line. For example, your sequenced wheat contig/fragment contains a gene, which is known located at chromosome4A in the default reference genome IWGSC, your first fasta line should be written as >chr4A

Rename this file as:

> Parent1_chr4A.fa (the second one as Parent2_chr4A.fa)

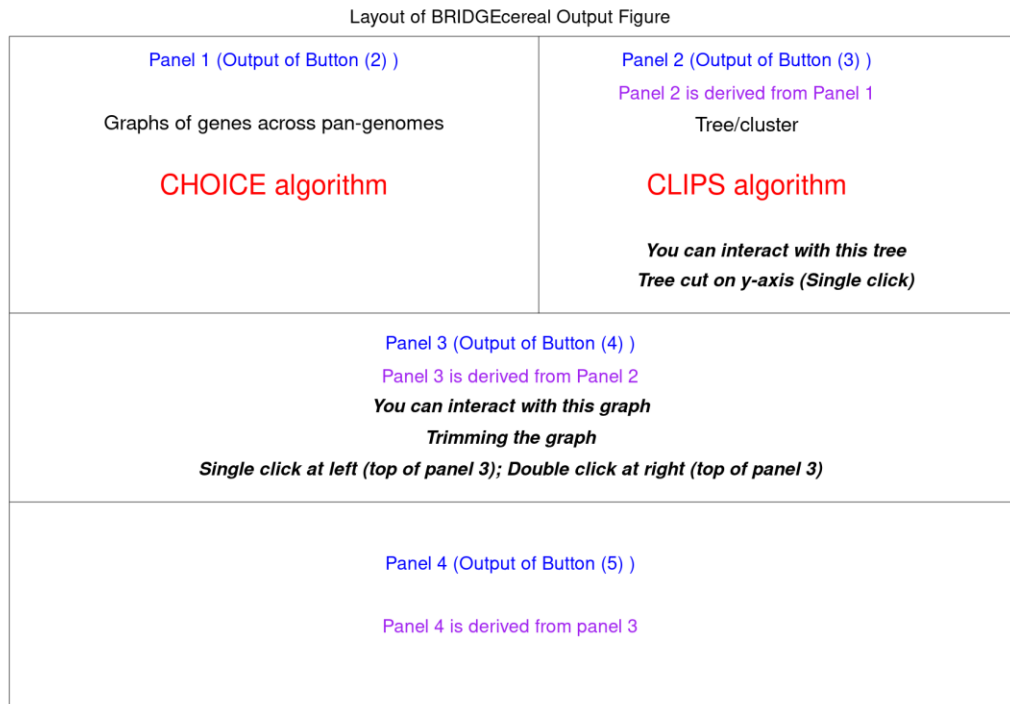Then Run (to get Parent1_chr4A.fa.gz):

> bgzip Parent1_chr4A.fa

1). Input the gene model ID in the Gene Name box, then click "(1) Check Gene ID" button to fill the Boxes for Reference and Chromosome. A hyperlink, connected to the corresponding crop's genomic database, will be available (in blue color) for the query gene ID.

2). To upload the compressed Parent1_chr4A.fa.gz file (max size=300 MB), click "Browse" button and upload your file, and BRIDGEcereal will let you know the file's uploading progress ... (Time depends on your bandwidth speed).

3). When you see the progress bar (blue) showing "Upload complete", then click "(2) Submit (large file)" Button (yellow color) to run BRIDGEcereal. Your "Parent1" genome together with other pan-genomes will be plotted in panel 1.

3

4, Figure layout

Layout of BRIDGEcereal Output Figure

Panel 1 (Output of Button (2)) Graphs of genes across pan-genomes **CHOICE algorithm**	**Panel 2 (Output of Button (3))** Panel 2 is derived from Panel 1 Tree/cluster **CLIPS algorithm** *You can interact with this tree* *Tree cut on y-axis (Single click)*

Panel 3 (Output of Button (4))
Panel 3 is derived from Panel 2
You can interact with this graph
Trimming the graph
Single click at left (top of panel 3); Double click at right (top of panel 3)

Panel 4 (Output of Button (5))

Panel 4 is derived from panel 3

5, Arrangement of tables

Arrangement of BRIDGEcereal Output Tables

Variety Table (From CLIPS algorithm and User's tree cut)

Varieties in each group based on User's tree cut

Table1 (From CHOICE algorithm)
Summary of all mapped positions (HSPs) on target chromosome
and candidate gene selected for downstream analysis

Table2 (From Table1's candidate cluster(gene))
Candidate gene selected for plotting all figures

Table3 (From Table1's Non-candidate clusters)
Not used for BRIDGEcereal downstream analysis
BUT, maybe helpful for troubleshooting
And, provide all information about mapped positions (HSPs) for query gene

6, Downloadable files

	Output files in the compressed .zip	Details
1	GeneID.png	Trimmed figure (Panel 4) in .png format
2	GeneID_Blast_Original	blast output (original), all HSPs on target chromosome
3	GeneID_Haplotype_syn	CHOICE processed blast output, filtered HSPs on target chromosome
4	GeneID_CDS.fa	Gene's CDS fasta file
5	GeneID_Haplotype.fa	genomic sequences (.fa) extracted from pan-genomes
6	GeneID_Haplotype_N_Gaps	Gaps found in pan-genomes
7	GeneID_Haplotype-Self_out_m8	blast output of genomic DNA in pan-genomes (pairwise)
8	GeneID_ref_CDS-Haplotype_out_m8	blast output of CDS and pan-genomes
9	GeneID_repMask2	blast output of repeats
10	GeneID_User_Selected.fa (optional)	output of User selected DNA (.fa) based on trimmed coordinates