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BASC: an integrated bioinformatics system for *Brassica* research

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ABSTRACT

The BASC system provides tools for the integrated mining and browsing of genetic, genomic and phenotypic data. This public resource hosts information on *Brassica* species supporting the Multinational *Brassica* Genome Sequencing Project, and is based upon five distinct modules, ESTDB, Microarray, MarkerQTL, CMap and EnsEMBL. ESTDB hosts expressed gene sequences and related annotation derived from comparison with GenBank, UniRef and the genome sequence of *Arabidopsis*. The Microarray module hosts gene expression information related to genes annotated within ESTDB. MarkerQTL is the most complex module and integrates information on genetic markers, maps, individuals, genotypes and traits. Two further modules include an *Arabidopsis* EnsEMBL genome viewer and the CMap comparative genetic map viewer for the visualization and integration of genetic and genomic data. The database is accessible at http://bioinformatics.pbcbasc.latrobe.edu.au.

INTRODUCTION

Modern high throughput technologies deliver a wealth of biological data. The increased complexity and abundance of biological data has made it difficult for researchers to gain a comprehensive view of this information. In order to improve accessibility, numerous computational tools and databases have been established (1). However, their independent development has resulted in diverse data structures and formats, limiting the ability to query across data types. To overcome some of these limitations, we have developed a bioinformatics system that integrates gene and genome DNA sequence, gene expression, molecular genetic marker, phenotypic trait and population data (Figure 1). Where possible, established open source database systems have been incorporated, permitting the integration and exchange of public data. The use of bioperl and MySQL, together with EnsEMBL coding standards further promote the interpretability of this system with external databases.

DATA AND PROCESSING

The ESTDB module functionality is based on previous *Brassica* sequence databases (2,3) and consists of proprietary and public expressed sequences. New sequences are collated quarterly and processed through an automated annotation pipeline (Figure 2). Total sequence numbers within the database are *Brassica napus* (91 658), *Brassica oleracea* (6485), *Brassica juncea* (8576), *Brassica rapa* (21 256) and *Brassica nigra* (3267). Where the original trace files are available, the annotation pipeline uses Phred to call the bases (4). Crossmatch (http://www.phrap.org/) and RepeatMasker (http://www.repeatmasker.org/) are used to remove vector sequences and to identify and mask repeat sequences. Processed sequences are clustered using D2 cluster (5) and assembled using Phrap (http://www.phrap.org/). Each assembly is stored in the database along with individual EST and consensus sequences. Parameters relating to sequence processing, assembly and annotation are maintained and linked from the associated results pages. The current database (Version 4, August 2006) contains 47 555 unigenes made up of 17 939 consensus and 29 616 singleton sequences. Functional annotation is assigned to the unigenes by sequence similarity to entries within UniProt and GenBank, using BLAST (6). A cut-off value

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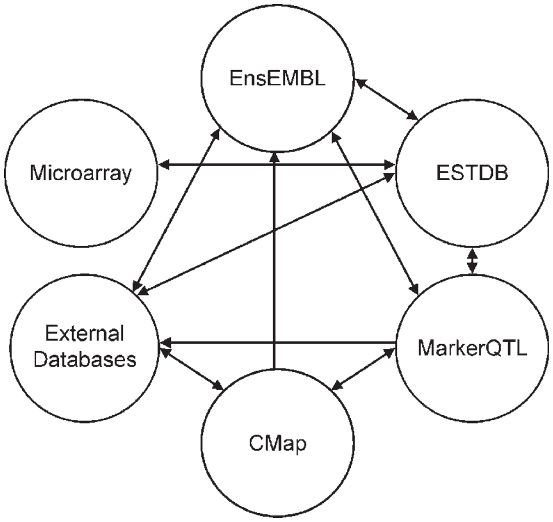


Figure 1. Overview of *Brassica* BASC module integration.

of $E < 10^{-5}$ is applied, with results parsed, stored and indexed in the database. Of the unigene sequences, 70% have a significant match with either UniProt or GenBank. Intermediate Gene Ontology (GO) annotation is derived via mapping to UniProt, with 40% of the unigenes annotated with at least one GO term. Sequences are compared with the *Arabidopsis* genome sequence using WU-BLAST (http://blast.wustl.edu/) to identify the best matching ortholog. These similarities are displayed using the *Arabidopsis* EnsEMBL genome viewer (7). Additional links to related molecular genetic marker and gene expression data are maintained. The Microarray module provides a platform for storing and visualizing gene expression data. The database stores both raw and normalized data and includes results from hydridizations of *Brassica* unigene cDNA microarrays representing 7000 unigenes.

The MarkerQTL module stores a variety of data relating to molecular genetic markers, individuals, populations, genotypes, genetic and trait maps and phenotypic information. Data has been incorporated from the *B.napus* IMSORB project (http://Brassica.bbsrc.ac.uk/IMSORB/), the Biotechnology and Biological Sciences Research Council (BBSRC) (8) and the Osborn lab at the University of Wisconsin (http://osbornlab.agronomy.wisc.edu/).

The EnsEMBL and CMap modules are both open source tools developed by the European Bioinformatics Institute (EBI) (9) and The Generic Model Organism Database Project (GMOD) (10), respectively. The *Arabidopsis* EnsEMBL genome browser was developed by the Nottingham *Arabidopsis* Stock Centre (NASC) in the UK and allows the anchoring of biological features to the genome sequence of *Arabidopsis*. In collaboration with NASC, we have anchored the *Brassica* unigenes from the BASC ESTDB module and the *B.oleracea* whole genome shotgun sequences produced by The Institute for Genome Research (TIGR) and Cold Spring Harbor (http://www.tigr.org/tdb/e2k1/bog1/) to the *Arabidopsis* genome. *B.rapa* Bacterial Artificial Chromosomes (BAC) end sequences, produced by the Multinational *Brassica* Genome Sequencing Project, have also been mapped by their candidate syntenic locus (7). The *Brassica* BASC CMap displays genetic maps for *Brassica* and *Arabidopsis*. The current version hosts data for 6 *Arabidopsis*, 6 *B.juncea*, 9 *B.napus*, 3 *B.rapa* and 8 *B.oleracea* maps, with consensus maps generated using a rule-based approach. One QTL map for *B.napus* has been included, with measurements for 13 traits and 181 QTL. The genetic viewer and genome browser permit the identification of syntenic regions between *Brassica* and *Arabidopsis* and the translation of trait information in the form of QTL on genetic maps in CMap and MarkerQTL, to gene and genome information through the EnsEMBL browser.

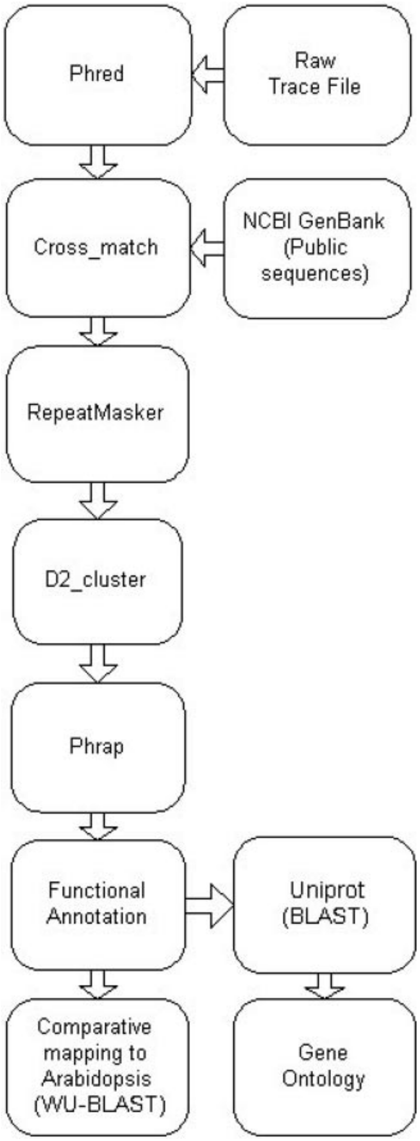
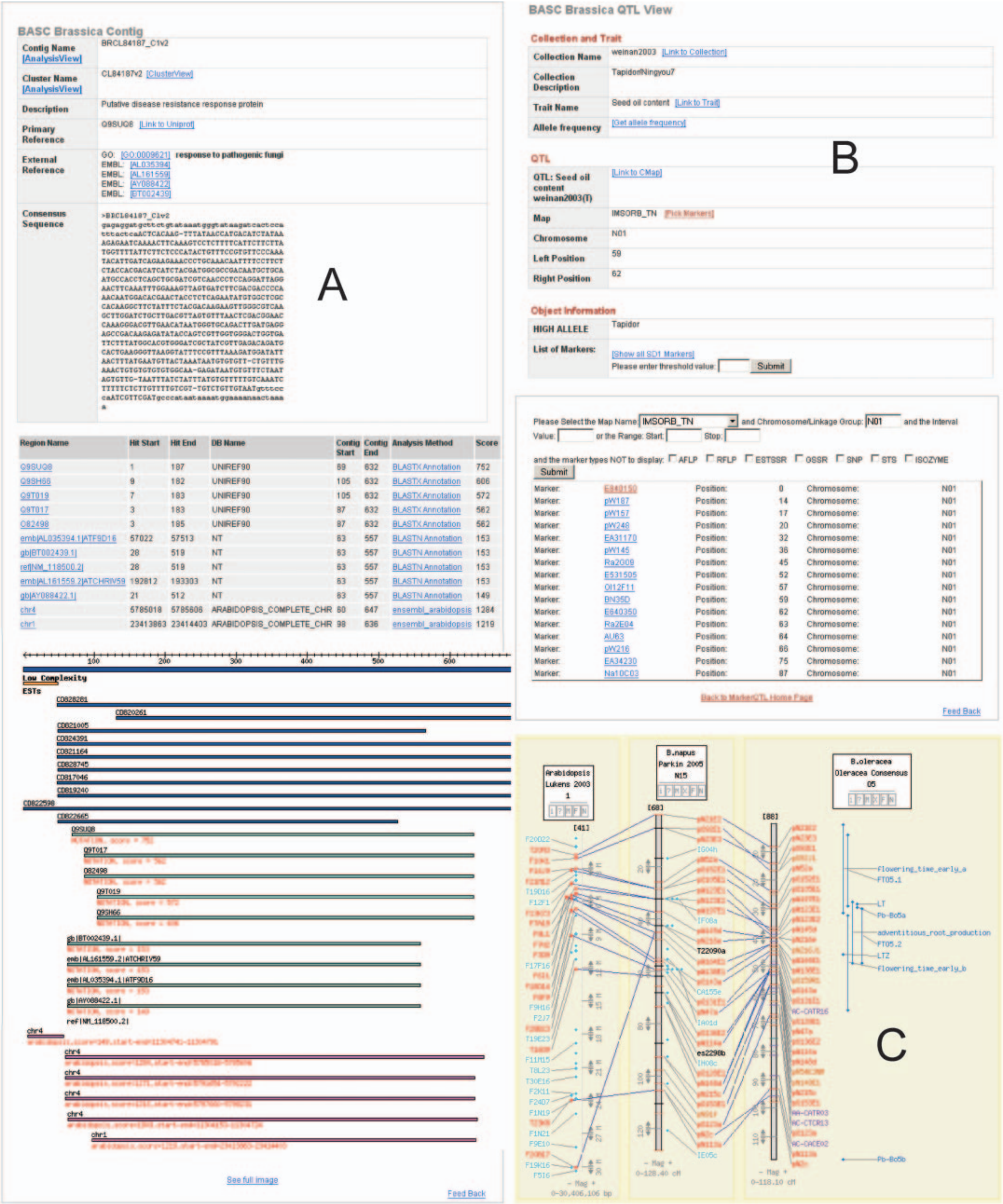


Figure 2. *Brassica* BASC EST sequence processing and annotation pipeline.

QUERY TOOLS AND USER INTERFACE

The web interface provides multiple routes to query the various databases. The inter-related structure of the system permits cross-linking and browsing between divergent data and cross searching of complex data types (Figure 2). Modules may be searched independently or together. All



BASC Brassica Contig

Contig Name [AnalysisView]	BRCL84187_C1v2
Cluster Name [AnalysisView]	CL84187v2 [ClusterView]
Description	Putative disease resistance response protein
Primary Reference	Q9SUQ8 [Link to Uniprot]
External Reference	GO: [GO:0009621] **response to pathogenic fungi** EMBL: [AL035394] EMBL: [AL161559] EMBL: [AY088422] EMBL: [BT002439]
Consensus Sequence	see below

```
>BRCL84187_C1v2
gagaggatgcttctgtataaatgggtataagatcactcca
tttactcaACTCACAAC-TTTATAACCATGACATCTATAA
AGAGAATCAAAACTTCAAAGTCCTCTTTTCATTCTTCTTA
TGGTTTTATTCTTCTCCCATACTGTTTCCGTGTTCCCAAA
TACATTGATCAGAAGAAACCCTGCAAACAATTTTCCTTCT
CTACCACGACATCATCTACGATGGCGCCGACAATGCTGCA
ATGCCACCTCAGCTGCGATCGTCAACCCTCCAGGATTAGG
AACTTCAAATTTGGAAAGTTAGTGATCTTCGACGACCCCA
AACAATGGACACGAACTACCTCTCAGAATATGTGGCTCGC
CACAAGGCTTCTATTTCTACGACAAGAAGTTGGGCGTCAA
GCTTGGATCTGCTTGACGTTAGTGTTTAACTCGACGGAAC
CAAAGGGACGTTGAACATAATGGGTGCAGACTTGATGAGG
AGCCGACAAGAGATATACCAGTCGTTGGTGGGACTGGTGA
TTCTTTATGGCACGTGGGATCGCTATCGTTGAGACAGATG
CACTGAAGGGTTAAGGTATTTCCGTTTAAAGATGGATATT
AACTTTATGAATGTTACTAAATAATGTGTGTT-CTGTTTG
AAACTGTGTGTGTGTGGCAA-GAGATAATGTGTTTCTAAT
AGTGTTG-TAATTTATCTATTTATGTGTTTTTGTCAAATC
TTTTTCTCTTGTTTTGTCGT-TGTCTGTTGTAATgtttcc
caATCGTTCGATgcccataataaaatggaaaanaactaaa
a
```

Region Name	Hit Start	Hit End	DB Name	Contig Start	Contig End	Analysis Method	Score
Q9SUQ8	1	187	UNIREF90	69	632	BLASTX Annotation	752
Q9SH66	9	182	UNIREF90	105	632	BLASTX Annotation	606
Q9T019	7	183	UNIREF90	105	632	BLASTX Annotation	572
Q9T017	3	183	UNIREF90	87	632	BLASTX Annotation	562
O82498	3	185	UNIREF90	87	632	BLASTX Annotation	562
emb|AL035394.1|ATF9D16	57022	57513	NT	63	557	BLASTN Annotation	153
gb|BT002439.1|	28	519	NT	63	557	BLASTN Annotation	153
ref|NM_118500.2|	28	519	NT	63	557	BLASTN Annotation	153
emb|AL161559.2|ATCHRIV59	192812	193303	NT	63	557	BLASTN Annotation	153
gb|AY088422.1|	21	512	NT	63	557	BLASTN Annotation	149
chr4	5785018	5785606	ARABIDOPSIS_COMPLETE_CHR	60	647	ensembl_arabidopsis	1284
chr1	23413863	23414403	ARABIDOPSIS_COMPLETE_CHR	98	636	ensembl_arabidopsis	1219

BASC Brassica QTL View

Collection and Trait

Collection Name	weinan2003 [Link to Collection]
Collection Description	Tapidor/Ningyou7
Trait Name	Seed oil content [Link to Trait]
Allele frequency	[Get allele frequency]

QTL

QTL: Seed oil content weinan2003(T)	[Link to CMap]
Map	IMSORB_TN [Pick Markers]
Chromosome	N01
Left Position	59
Right Position	62

Object Information

HIGH ALLELE	Tapidor
List of Markers:	[Show all SD1 Markers] Please enter threshold value: [ ] Submit

Please Select the Map Name: IMSORB_TN ▾ and Chromosome/Linkage Group: N01 and the Interval Value: [ ] or the Range: Start: [ ] Stop: [ ]

and the marker types NOT to display: □ AFLP □ RFLP □ ESTSSR □ GSSR □ SNP □ STS □ ISOZYME

Submit

Marker:	E840150	Position:	0	Chromosome:	N01
Marker:	pW187	Position:	14	Chromosome:	N01
Marker:	pW157	Position:	17	Chromosome:	N01
Marker:	pW248	Position:	20	Chromosome:	N01
Marker:	EA31170	Position:	32	Chromosome:	N01
Marker:	pW145	Position:	36	Chromosome:	N01
Marker:	Ra2G09	Position:	45	Chromosome:	N01
Marker:	E531505	Position:	52	Chromosome:	N01
Marker:	Ol12F11	Position:	57	Chromosome:	N01
Marker:	BN35D	Position:	59	Chromosome:	N01
Marker:	E640350	Position:	62	Chromosome:	N01
Marker:	Ra2E04	Position:	63	Chromosome:	N01
Marker:	AU63	Position:	64	Chromosome:	N01
Marker:	pW216	Position:	66	Chromosome:	N01
Marker:	EA34230	Position:	75	Chromosome:	N01
Marker:	Na10C03	Position:	87	Chromosome:	N01

Back to Marker/QTL Home Page

Feed Back

Figure 3. Examples of data visualization, Sequence annotation in tabular and graphical format (**A**), *Brassica* QTLview (**B**) and *Brassica* CMap (**C**).

relevant data is indexed, and searching by keyword using MySQL Boolean operators or accession number will retrieve associated sequence and annotation data, as well as molecular marker, gene expression or phenotypic trait information. A BLAST interface permits a similarity based search with an amino acid or DNA sequence. Analysis parameters for sequence assembly and annotation are linked from the relevant pages and help pages. Example searches are also provided.

Typical results from the ESTDB module would include EST or consensus gene sequence, cDNA library information, sequence annotation, including UniRef, GenBank and significant GO categories and links to syntenic regions within *Arabidopsis*. The results are displayed in both tabular and graphical format (Figure 3A). Microarray results include gene expression values across treatments for a gene, experiment and sample details. The MarkerQTL MarkerView displays information related to a marker assay such as SSR, SNP, RFLP or AFLP parameters, including sequence information, amplification primers and any associated maps or locus information where available (Figure 3B). Markers may be selected within a defined region of a map, by association with other mapped markers, through associated QTL loci, or with a defined distribution across a genome. CMap enables the comparison of genetic markers and traits between maps (Figure 3C) as well as the exploitation of information from the complete genome sequence of *Arabidopsis*. By using this comparative map viewer, users can quickly identify traits in similar positions on other genetic maps. The development of consensus maps further enables the identification of molecular markers around the trait of interest for further characterization in specific populations. The integrated nature of the BASC bioinformatics system allows users to traverse between annotated gene sequence data, gene expression information, molecular genetic marker, genetic map and trait data with a few clicks of the mouse.

FUTURE DEVELOPMENTS

The structure of the database and application programming interface (API) offers the flexibility to add additional datasets and expand the current capabilities, including additional species such as wheat, barley and legumes. As the *Brassica* genome sequencing progresses, EnsEMBL based *Brassica* genome views will be included to display and compare this information and extend comparisons between *Brassica* species and *Arabidopsis*. Additional gene expression, gene, trait and genetic map information will be incorporated as it becomes available.

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Conflict of interest statement. None declared.

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