

# Sequencing activities on Japanese malting barley Haruna Nijo



K. Sato<sup>1</sup>, Y. Kohara<sup>2</sup> and K. Takeda<sup>1</sup>

1. Research Institute for Bioresources, Okayama Univ.,  
Kurashiki, 710-0046, Japan
2. National Institute of Genetics, Mishima, 411-8540, Japan

## Morex

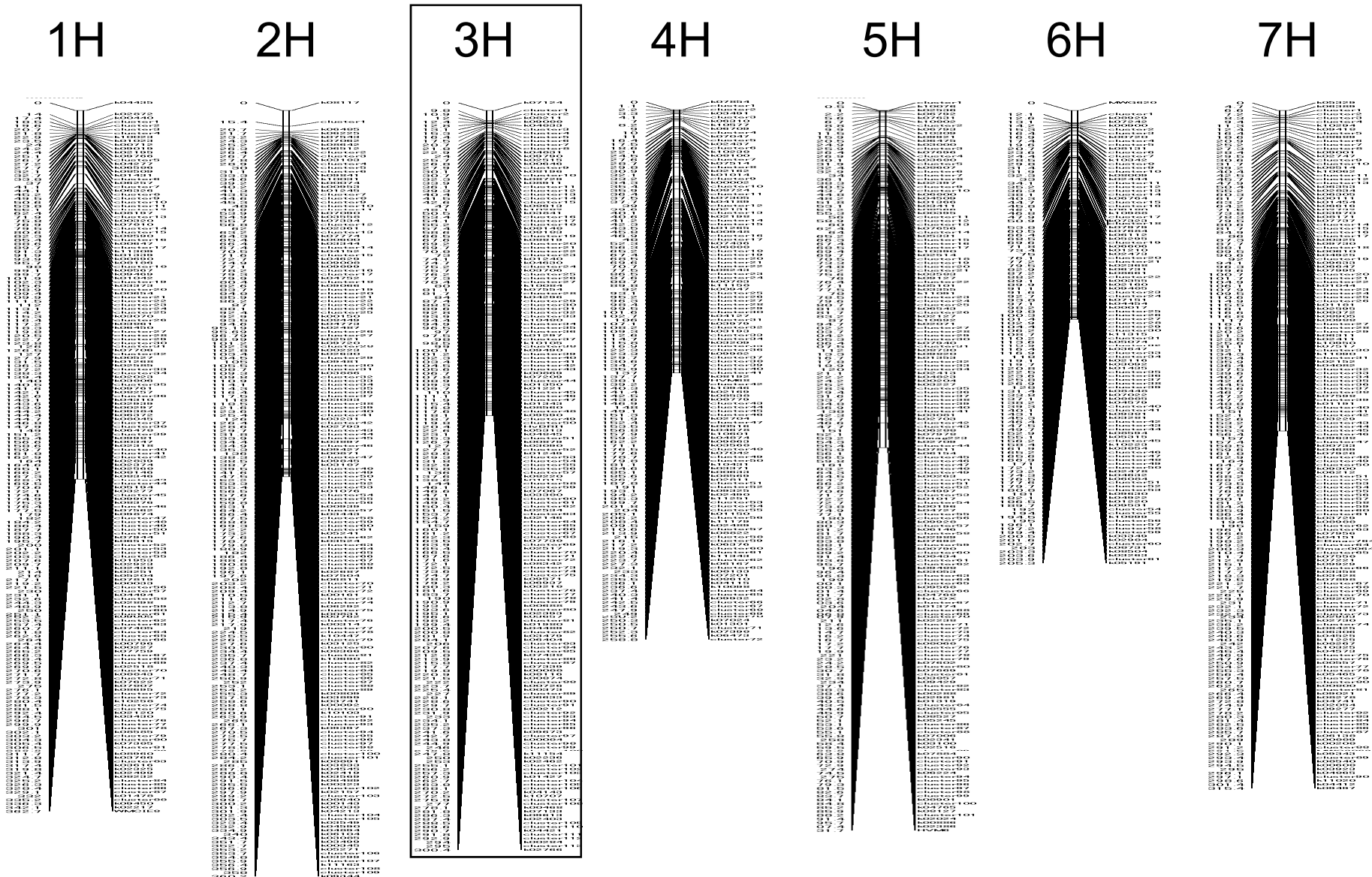
- American six-row malting barley with high malting quality profiles
- Standard for genetic map, EST, BAC lib. development
- International standard for genome sequencing

## Haruna Nijo

- Japanese two-row malting barley with high malting quality profiles of European origin
- EST & BAC lib. development at Okayama U.
- Full length cDNA development at Okayama U. (5k) & NIAS (20k, poster Kanamori et al.)
- High density EST map at Okayama U.
- 500 BAC sequencing on 3H by 454 at Okayama U.

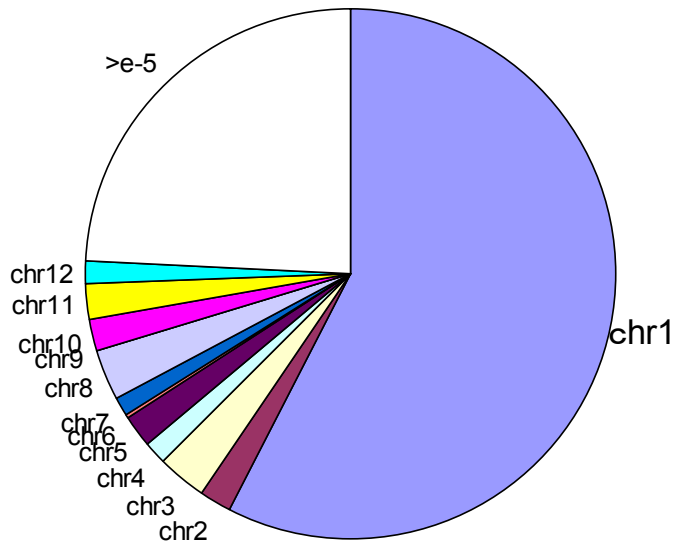
# Haruna Nijo/H602 EST map

2,890 non-redundant 3' ESTs mainly from Haruna Nijo



# High collinearity between ESTs on barley 3H and rice chrom 1

444 barley 3H EST vs. rice chromosomes



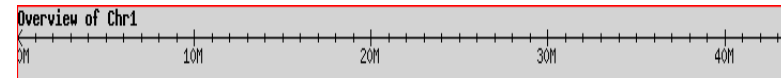
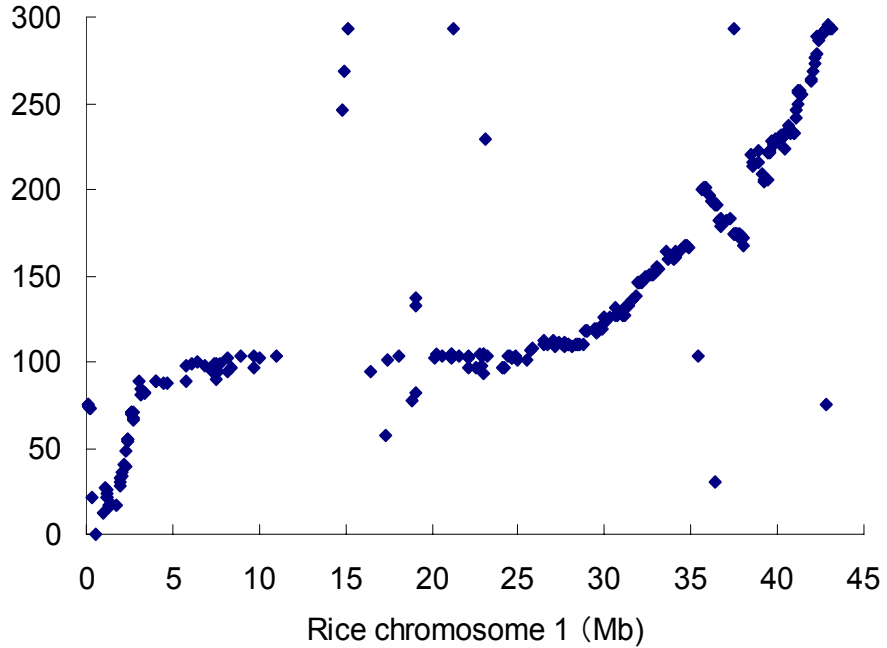
Barley 3H and rice chrom 1



3H



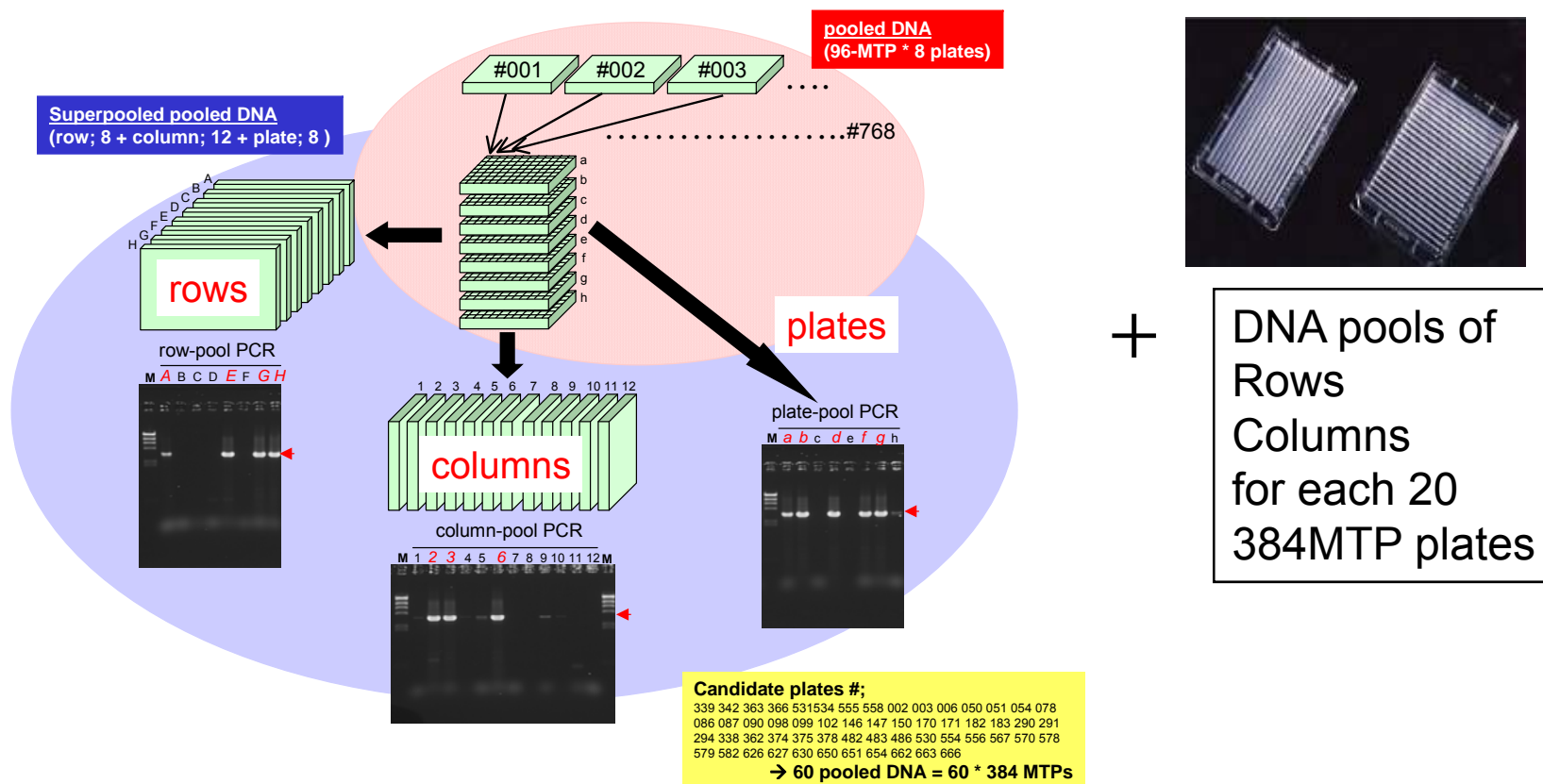
mapped ESTs on barley 3H (cM)



## Method of barley BAC sequencing analysis by 454

- 1) BAC clones were selected by mapped ESTs on barley chrom 3H.
- 2) 10 or 20 BAC clone DNAs were pooled in an order of short to long arm and used for shot-gun library development.
- 3) Library was sequenced by 454 GS20 (100bp) on either small (10Gbp) or large (20Gbp) plate, also sample sequenced by FLX (250bp).
- 4) Analyses were repeated to achieve more than 20x coverage.
- 5) Sequences were assembled by the standard assembler by 454.
- 6) Contig sequences were selected with the sequences more than 500bp.

# PCR screening system for Haruna Nijo BAC library



67 PCR reactions can identify each clone

# Procedures for sequencing BAC pools by 454 sequencer (GS20)

1. BAC DNA isolation



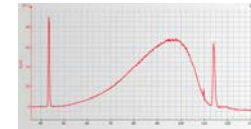
2. Insert size check by CHEF



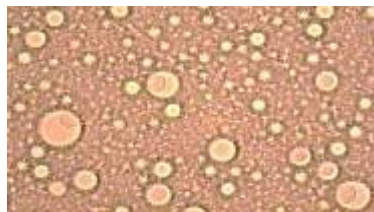
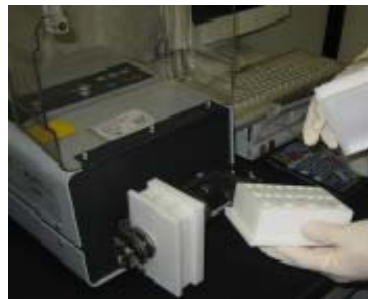
3. Fragmentation by nebulizer (N2 pressure)



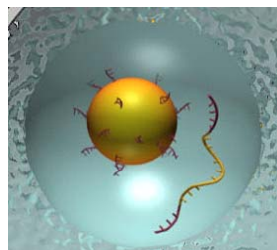
4. Fragment size estimation (peak at 500bp)



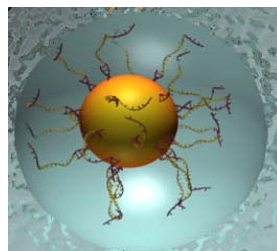
5. Emulsion development



6. Bead-fragment emulsion PCR



Before PCR



After PCR

7. Beads washing and amplification

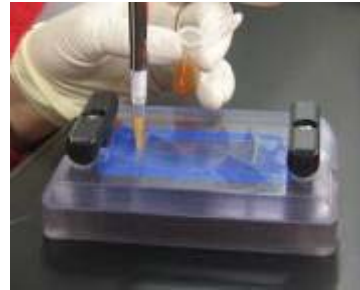


(Continued)

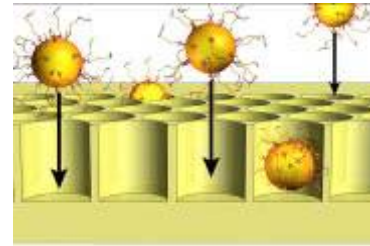
8. Bead number count



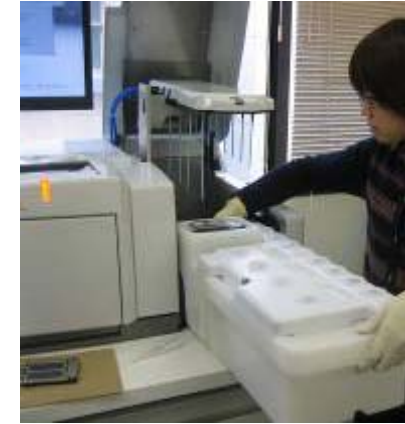
9. Beads application to the pico titer plate



10. Bead fixation by centrifugation



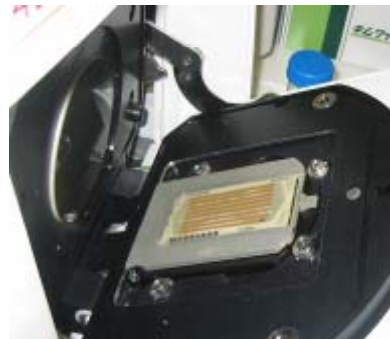
11. Washing the lines of sequencer



12. Reagent preparation



13. Sample plate application



14. Sequencing

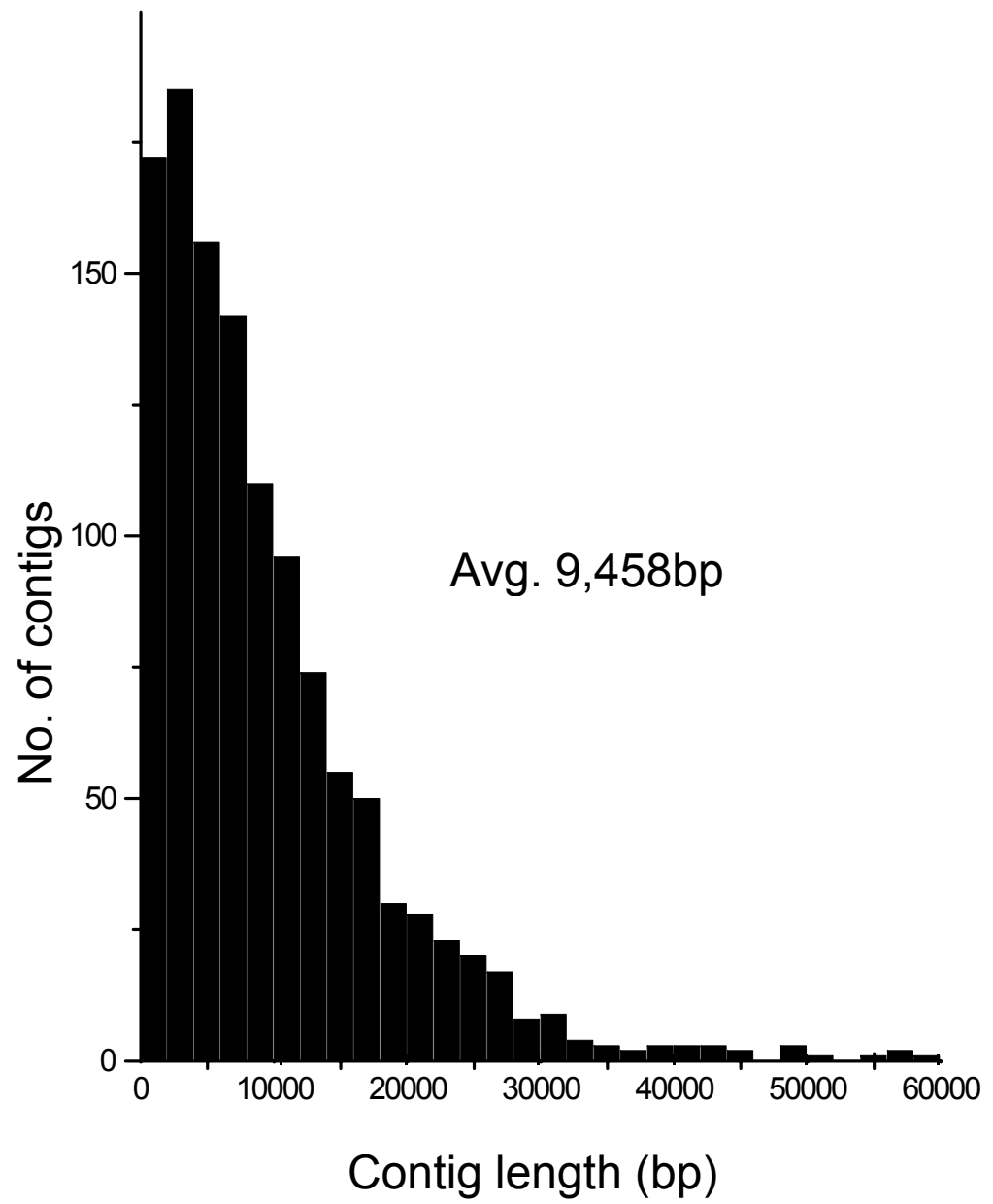


15. Sequence assembly

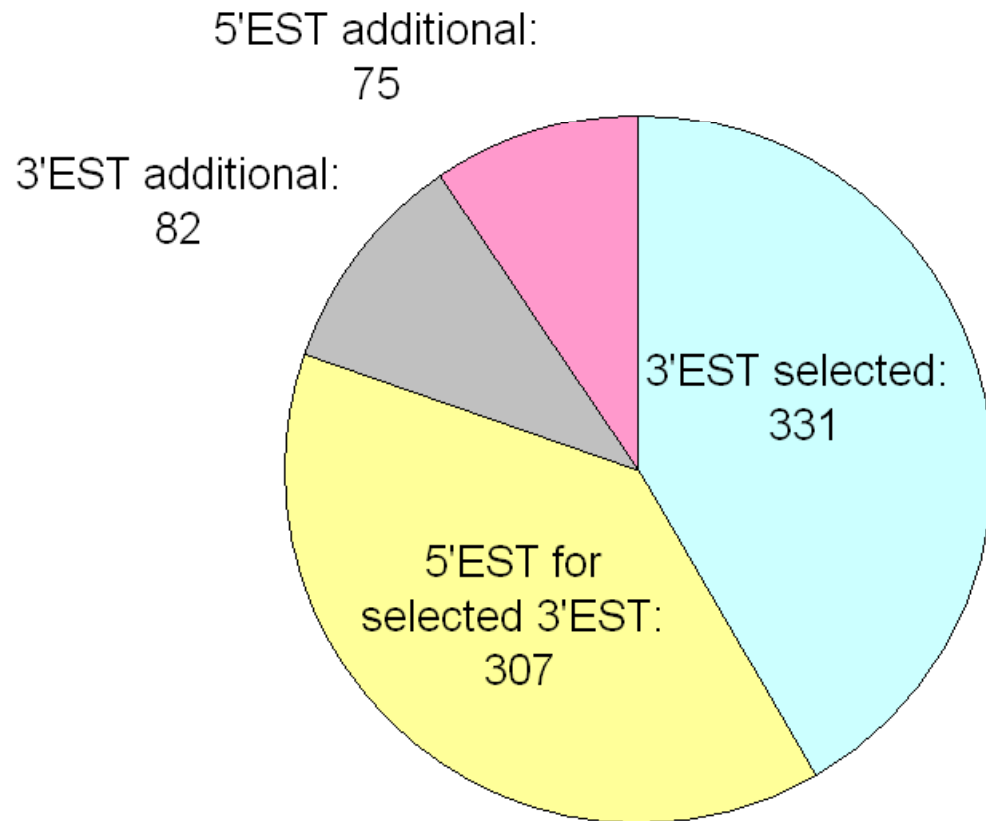




# Distribution of contig length (GS20)



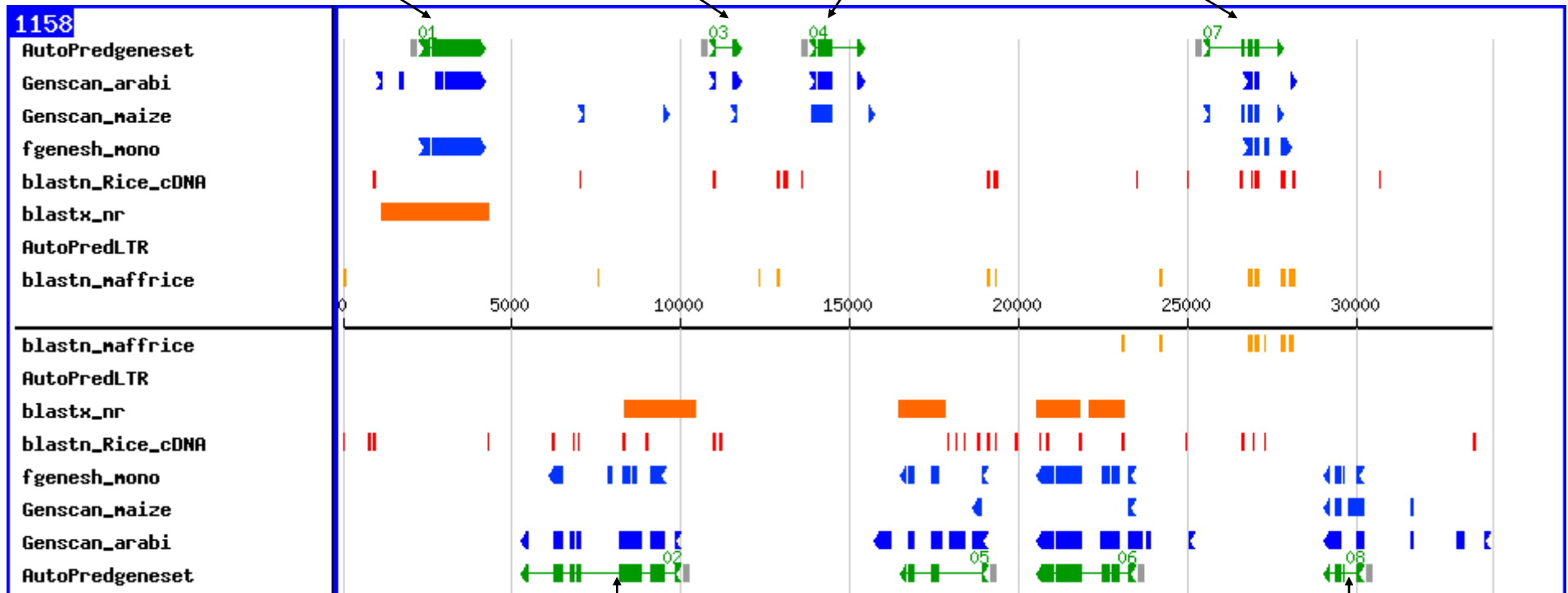
# Blast homology search between contig sequences and EST sequences used for 370 BAC clone selection



370 mapped EST sequences from 3' and 5' are blastn searched (e-30) against the contigs more than 500bp. 331 3'ESTs and 307 5' ESTs are identified in the contigs. Additional ESTs mapped on 3H (3':82, 5':75) are also found.

# Example of automatic contig sequence (33k) annotation by RiceGAAS

Barley FLcDNA e-130    Barley EST e-90    Barley EST e-159    Barley EST used for BAC clone selection e=0



Barley EST e-133

No barley candidates

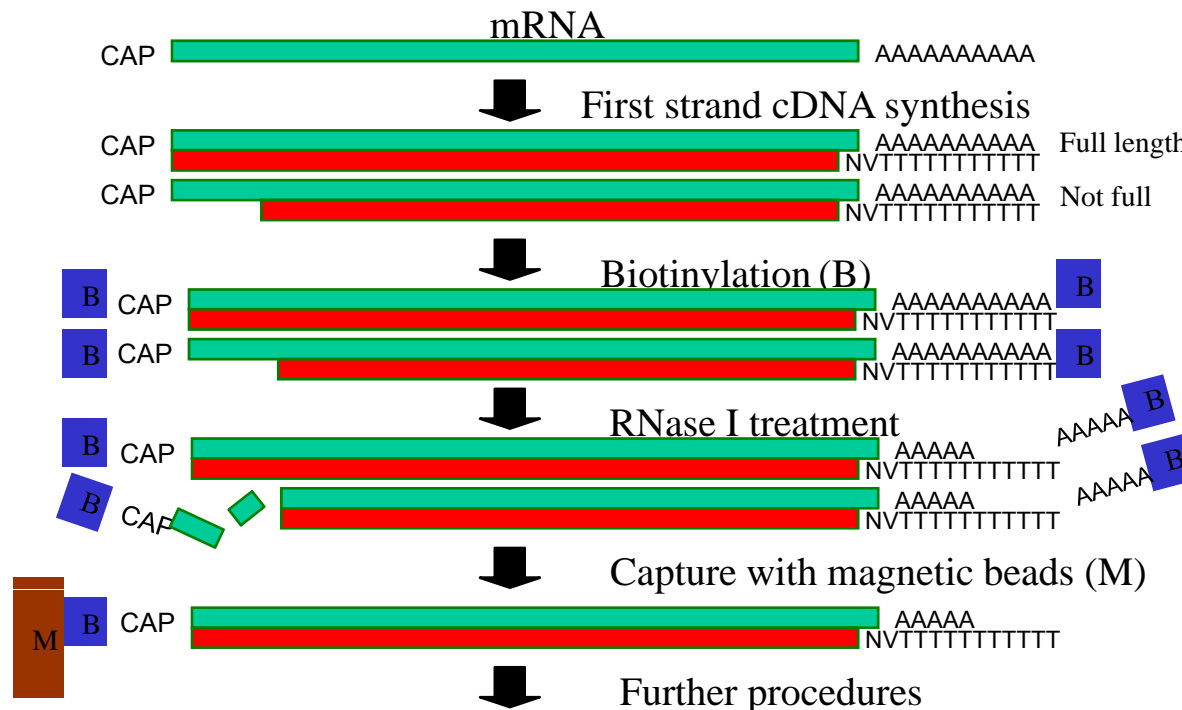
Barley EST e-117

# Full-length cDNA sequencing in Okayama Univ.

Biotynylated CAP trapper method (by RIKEN, Carninci et al. 1996)

Full sequencing of 5,006 clones selected from preliminary reads from both ends of 47,000 clones on one library developed by 16 different mRNA samples

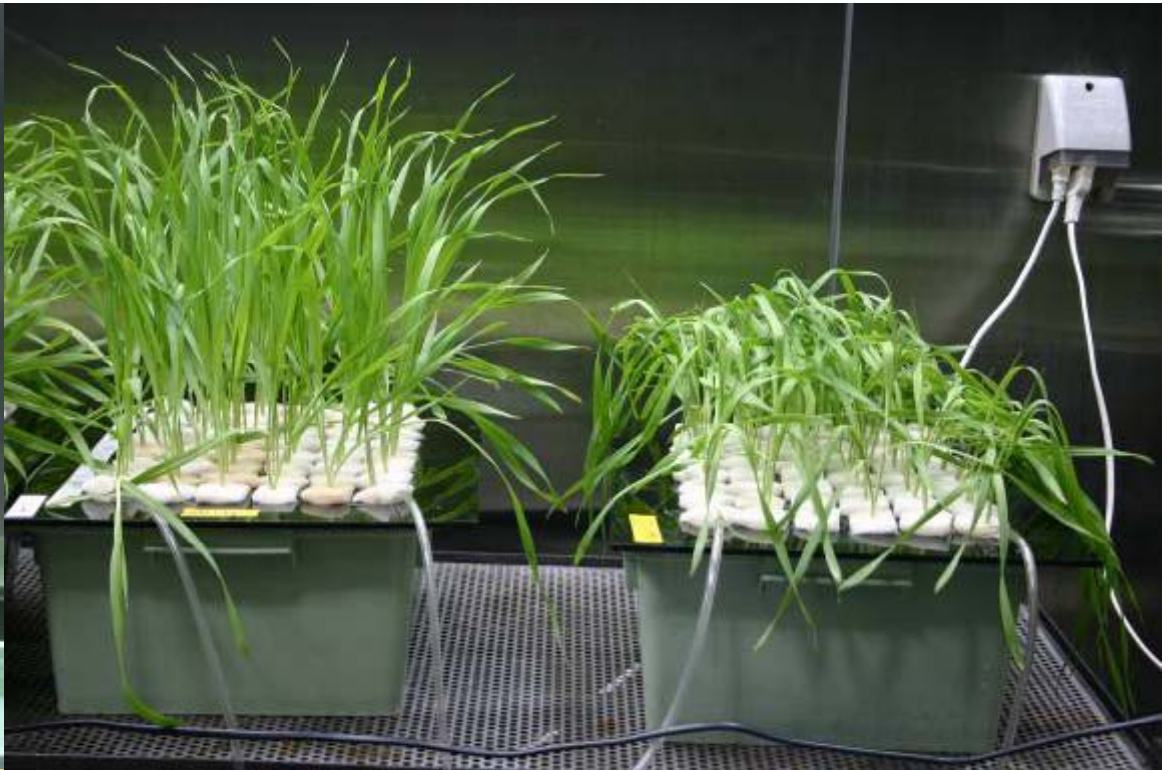
## Biotynylated CAP trapping



# mRNA samples for FL library development

Stress treated	Organ	Treatment duration	Treatment
1 AlK(SO4)·12H2O(30μM) pH4.3	Seedling root	6 hrs	Hydroponic + light
2 NaCl(0.1M) pH6.0	Seedling leaf	6 hrs	Hydroponic + light
3 NaCl(0.1M)	Seedling root	6 hrs	Hydroponic + light
4 Low temperature (-1°C)	Seedling leaf	24 hrs	Agar + light
6 High temperature (40°C)	Seedling leaf	24 hrs	Agar + light
7 Wounding (5cm) by cutting	Seedling leaf	12 hrs	Hydroponic + light
8 Drought on filter paper	Seedling leaf+root	2 hrs	Hydroponic + light

Stages	Organ	Condition
9 Germinating seed	Whole	2nd day
10 Germinating seed	Shoot	5th day
11 Heading	Leaf blade	Upper 3 leaves
12 Heading	Young spike	3~5cm
13 Vegetative	Culm	2 months after planting
14 Vegetative	Root	2 months after planting
15 Maturing	Spike	20 days after heading
16 Germinating seed	Embryo	2nd day



# Database release for 5,006 Haruna Nijo FL cDNAs





---

Home

**DB Contents**

- About
- Germplasm
- Core Collection
- cDNA/ESTs [New](#)
- Sequence
- Tools
- Site Map

---

• Reference Feedback [New](#)

• Related Sites

---

Last Update:  
Nov. 2007

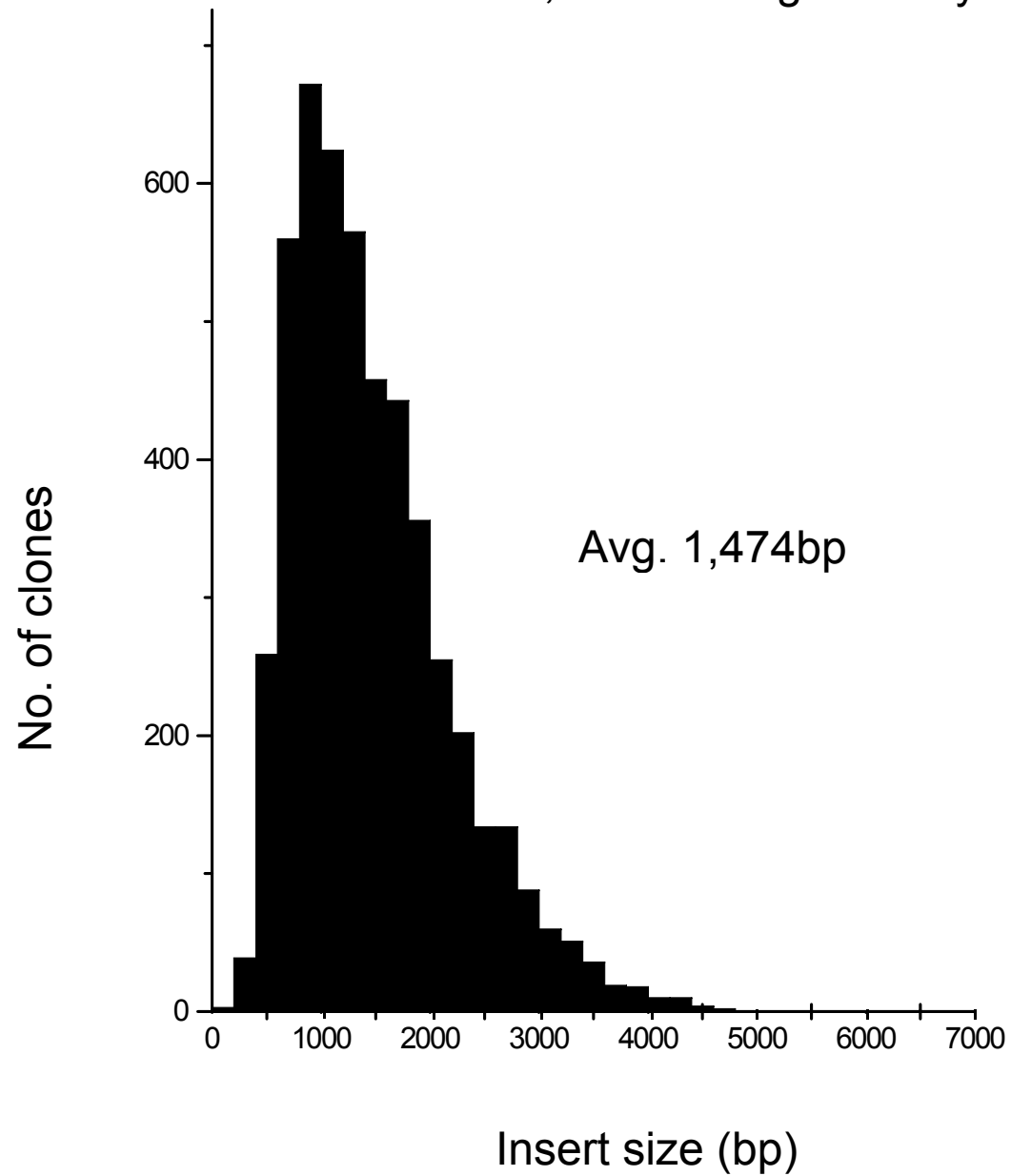


## Result of Library Retrieval From barley\_pub

Go To [MAIN](#) 1-50 / Hit:5006 [Next](#)

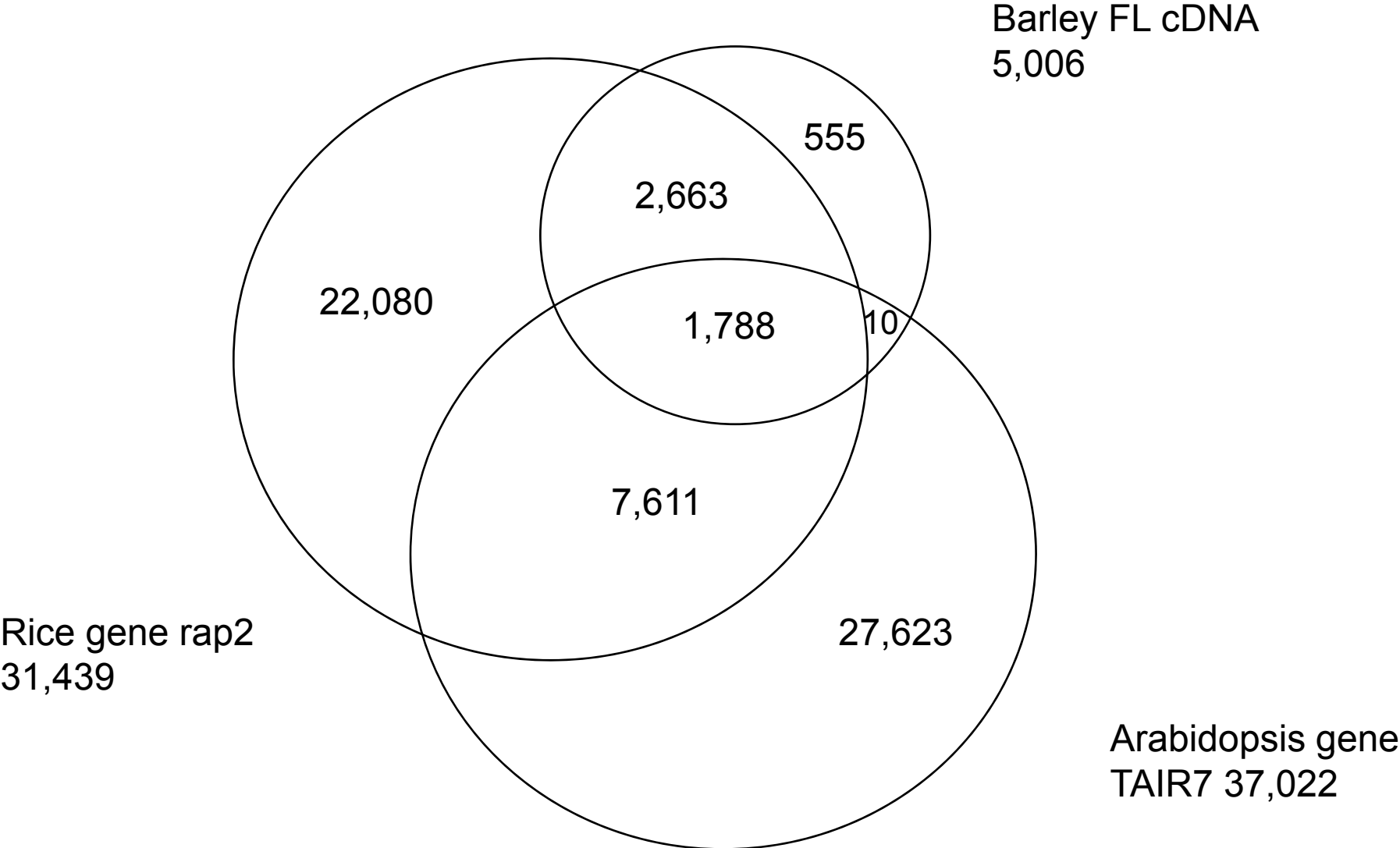
Clone name	Length	Accession No.	full/not_full	Definition	nt	uniprot	TIGR-ORF	RAP-ORF
<a href="#">FLbaf100a02</a>	1294	<a href="#">AK250858</a>	not_full	<a href="#">dbj AK060859.1  Oryza sativa (japonica cultivar-group) cDNA clone:001-034-F07, full insert sequence Length = 1212</a>	<a href="#">33</a>	<a href="#">44</a>	<a href="#">18</a>	<a href="#">9</a>
<a href="#">FLbaf100a04</a>	864	<a href="#">AK250860</a>	not_full	<a href="#">gb AG151567.1  Pan troglodytes chromosome X clone RP43-007H19 map human ortholog c23, complete sequence Length = 200796</a>	<a href="#">8</a>	<a href="#">15</a>	<a href="#">444</a>	<a href="#">50</a>
<a href="#">FLbaf100a05</a>	1370	<a href="#">AK250862</a>	not_full	<a href="#">ref NM_001072383.1  Oryza sativa (japonica cultivar-group) Os11g0169400 (Os11g0169400) mRNA, complete cds Length = 1396</a>	<a href="#">396</a>	<a href="#">24</a>	<a href="#">270</a>	<a href="#">32</a>
<a href="#">FLbaf100b05</a>	1047	<a href="#">AK250863</a>	full	<a href="#">cb DQ245093.1  Zea mays clone 12935 mRNA sequence Length = 803</a>	<a href="#">486</a>	<a href="#">156</a>	<a href="#">44</a>	<a href="#">21</a>
<a href="#">FLbaf100b21</a>	773	<a href="#">AK250866</a>	not_full	<a href="#">dbj AB180900.1  Brassica oleracea S-12 SP11 gene for S locus protein 11, complete cds Length = 17252</a>	<a href="#">7</a>	<a href="#">3</a>	<a href="#">79</a>	<a href="#">17</a>
<a href="#">FLbaf100c06</a>	1768	<a href="#">AK250867</a>	not_full	<a href="#">emb CT830183.1  Oryza sativa (indica cultivar-group) cDNA clone:OSIQOEAC13A05, full insert sequence Length = 1879</a>	<a href="#">185</a>	<a href="#">500</a>	<a href="#">453</a>	<a href="#">346</a>
<a href="#">FLbaf100c08</a>	984	<a href="#">AK250869</a>	not_full	<a href="#">ref NM_001055285.1  Oryza sativa (japonica cultivar-group) Os03g0115200 (Os03g0115200) mRNA, complete cds Length = 956</a>	<a href="#">61</a>	<a href="#">45</a>	<a href="#">88</a>	<a href="#">25</a>
<a href="#">FLbaf100d03</a>	849	<a href="#">AK248374</a>	not_full	<a href="#">ref NM_001055283.1  Oryza sativa (japonica cultivar-group) Os03g0115000 (Os03g0115000) mRNA, partial cds Length = 768</a>	<a href="#">212</a>	<a href="#">20</a>	<a href="#">76</a>	<a href="#">47</a>

Distribution of insert sizes of 5,006 full length barley clones





Blastn search among barley FL-cDNAs,  
rice genes and Arabidopsis genes (e-5)



## GO annotation for 555 FL clones with low homology with rice and Arabidopsis

no hit	325
metabolic_process	74
cellular_process	70
gene_expression	25
response_to_stimulus	18
biological_regulation	13
establishment_of_localization	13
localization	13
developmental_process	2
immune_system_process	1
multicellular_organismal_	
process	1
<b>BIOLOGICAL PROCESS</b>	

no hit	355
cell_part	45
antioxidant_activity	2
molecular_transducer_activity	2
transporter_activity	13
structural_molecule_activity	11
translation_regulator_activity	1
enzyme_regulator_activity	15
catalytic_activity	61
nutrient_reservoir_activity	1
binding	86
transcription_regulator_activity	8
<b>MOLECULAR FUNCTION</b>	

no hit	453
cell	45
organelle	23
extracellular_region_part	1
extracellular_region	8
envelope	2
macromolecular_complex	16
organelle_part	7
<b>CELLULAR COMPONENT</b>	

# GBrowse annotation of Haruna Nijo sequence contigs with barley cDNAs and other genome information

## Barley contig viewer

---

BLAST Search

Program :

Database :

Please specify the sequence data to reference.

**COPY & PASTE :**

```
GTTTACATGTCGTGCTGCATTTTCCCGGTTCCCTCCGTCAAAAAATTCGGT
GATTCCATCCTCCTTGTATAACAAATTTGCAGACCACCTCTCTACCTGGA
CATTCCCAGAAAAATAATGCAAAGGAAAAAAAATAAACTGTTACTCCTT
CAAGACATGTTGCAGTTTTCTCTCGAAACAAAGAAACAAGTTCAGCTAC
ATGACTATATCCTTTCTTCCACGACGGTGGTCGTTAGTTGCCGGCTGCTC
CACTCCAGCACGATGGTCTCCCTGTGCTGCACCGCCTTCGAGTTCGAGTT
```

# Contig identification by query sequence

## Barley contig viewer

---

BLAST Result

Position	Contig	Score	E-val
3H_131-150	contig01225	983	0.0
3H_231-250	contig02257	32	2.5
3H_31-50	contig01483	32	2.5
3H_191-210	contig00160	32	2.5
3H_151-170	contig02449	32	2.5
3H_291-310	contig01384	30	9.9
3H_291-310	contig00950	30	9.9
3H_251-270	contig01493	30	9.9
3H_71-90	contig01369	30	9.9
3H_51-70	contig02313	30	9.9
3H_51-70	contig00645	30	9.9
3H_211-230	contig02216	30	9.9
3H_211-230	contig02197	30	9.9
3H_211-230	contig01300	30	9.9
3H_21-30	contig01536	30	9.9
3H_191-210	contig01367	30	9.9
3H_151-170	contig01865	30	9.9
3H_151-170	contig01729	30	9.9
3H_131-150	contig01553	30	9.9
3H_111-130	contig00745	30	9.9

# Conting sequence annotation by GBrowse

## Current db contents

- 1) ESTs: 2,890 genetically mapped ESTs
- 2) FLengthNIG: 5,006 FL cDNAs
- 3) OkEST: ESTs by Okayama Univ.
- 4) HarvEST: HarvEST unigene #31
- 5) INE\_AA: rice FLcDNA-AA (tblastx)
- 6) RepeatMasker (repeat, tRNA, rRNA)
- 7) Genscan\_Arabidopsis
- 8) Genscan\_maize
- 9) DNA/GC Content
- 10) 6-frame AA translation

# GBrowse mapping of barley cDNAs on a sequenced contig

