T3 Wheat Tutorial – Primer Design

1. Predesigned primers for 2017_WheatCAP experiment

The PolyMarker program was used to design primers on all the markers in the 2017_WheatCAP experiment. This table below gives a good summary of the results. PolyMarker automates the design of genome specific primers. Primer3 is used to pick candidates then PolyMarker selects a primer pair with the highest specificity.

SNP_type	primer_type	total	percentage
		32466	2.92920590
homoeologous		3778	0.34086552
homoeologous	chromosome_nonspecific	616	0.05557786
homoeologous	chromosome_semispecific	10575	0.95411669
homoeologous	chromosome_specific	5202	0.46934421
non-homoeologous		189269	17.07656843
non-homoeologous	chromosome_nonspecific	170574	15.38983448
non-homoeologous	chromosome_semispecific	436538	39.38611726
non-homoeologous	chromosome_specific	259337	23.39836966

2. Three ways to access the designed primers in T3 Wheat

a. Go to Reports => JBrowse => RefSeq_v1. Make sure the "Primers 2017_WheatCAP" is selected in available tracks. Navigate to the region of interest then click on an item in the "Primers 2017_WheatCAP" track to view primer information. Within the popup box there is a "T3_link" to more information on the associated SNP.
b. Go to Select => Markers. Select "Wheat CAP 2017" Map, then chromosome, then enter a range, then "show markers". Highlight up to 10 markers and click on the "Select markers" button. Then go to Reports => Designed Primers.
c. Go to Analyze => BLAST. Enter a marker sequence, select "RefSeq_v1", then select "Basic Search". On the results page click on the best hit in the "T3 JBrowse" column. In JBrowse click on an item in the "Primers 2017_WheatCAP" track.

3. Selecting markers not in WheatCAP_2017 experiment for primer design

If you have two lines (germplasm) and you want to find which markers are polymorphic in those lines use the following procedure.

a. Select the first line (germplasm) using "Quick search". Click on the Marker Alleles Show link. The web page will now show the genotype experiments where that line was genotype. Record these experiments.

b. Select the second line using “Quick search”. Click on the Marker Alleles Show link. The web page will now show the genotype experiments where that line was genotype. Record these experiments.
c. Then find the common experiments that genotyped both lines.
d. Select one of those experiment either using “Quick search” or clicking on the link in “Show markers” page.
e. On the “Genotyping experiment” page click on the “Select experiment” button.
f. Go to Select => Map and select the RefSeq v1.0 map
g. Go to Select => Subset Marker by Polymorphisms
h. Select Chromosome, Start, Stop, then Query
i. The output is the markers that are polymorphic. Click on “Save marker selection”.
j. Go to Download => Genotype and Phenotype Data. Then click “Create file” VCF format.
k. These results can be manually formatted then submitted to PolyMarker for design