

# Kompetitive Allele Specific PCR KASP markers

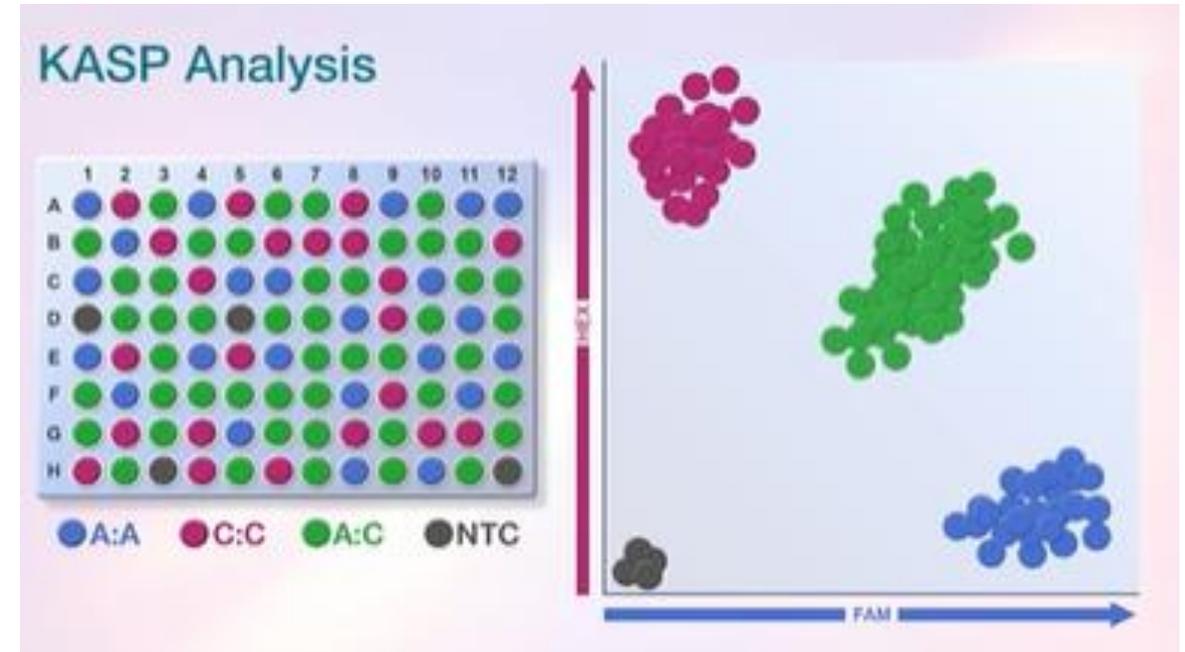
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# Kompetetive Allele Specific PCR: KASP

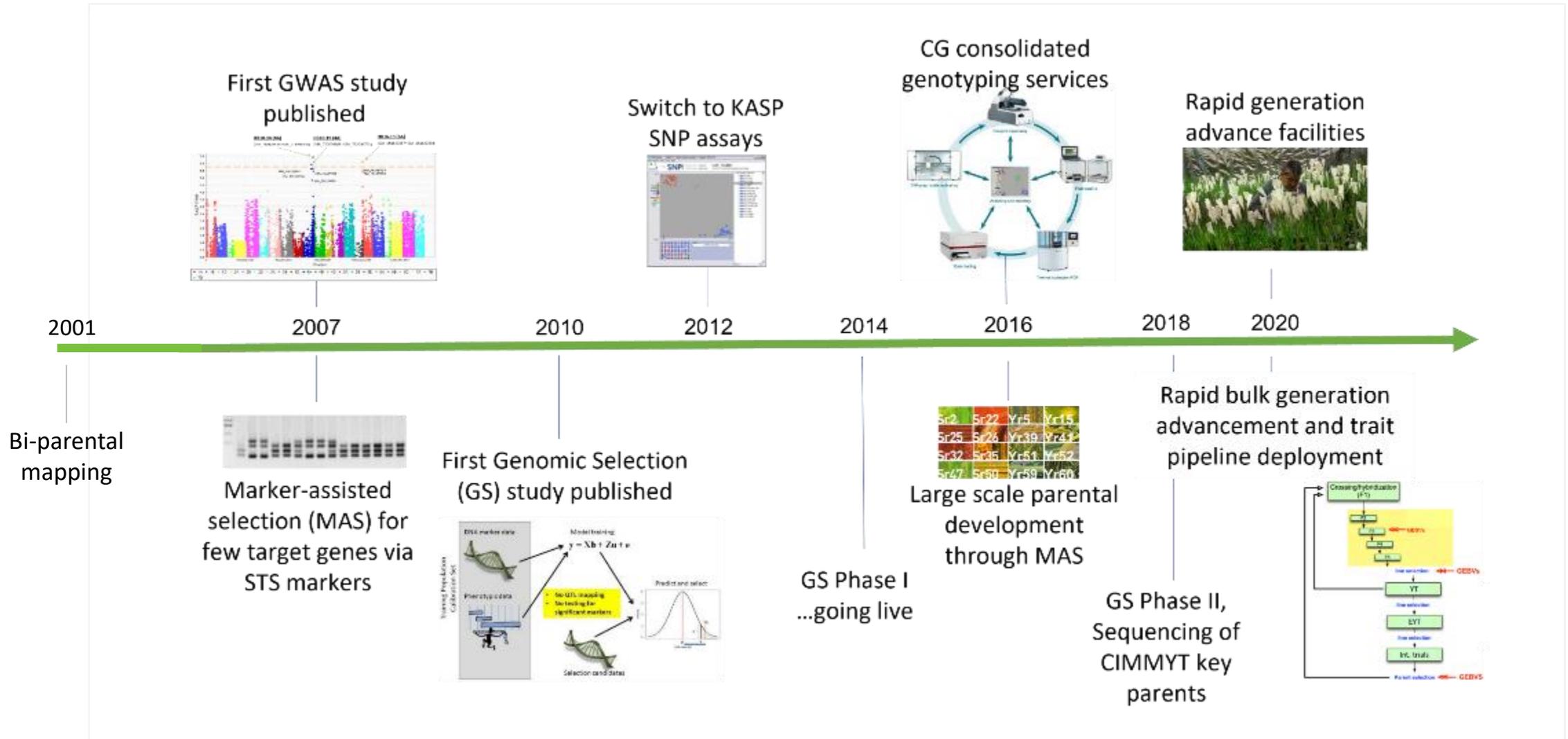
Monoplex SNP platform, supports mainly low- to mid-density SNP applications, e.g., MAS, MABC, parental characterization, QC

LGC Biosearch Technologies  
<https://www.biosearchtech.com>

Biallelic characterization of SNPs and Indels



# Integration of genomics tools in the CIMMYT GWP



# How does KASP work ?

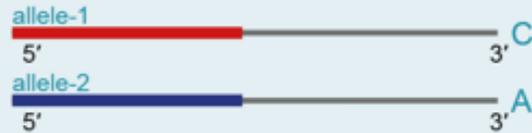
## 1. Assay components:

**A) KASP Assay Mix:** consists of 2 allele specific primers and 1 reverse primer.

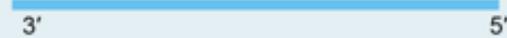
**B) KASP Master Mix:** contains universal fluorescent probes, Taq polymerase and dNTP's in an optimised buffer solution.

### A) KASP Assay Mix

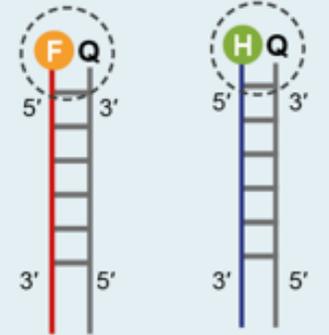
Allele-specific forward primers:



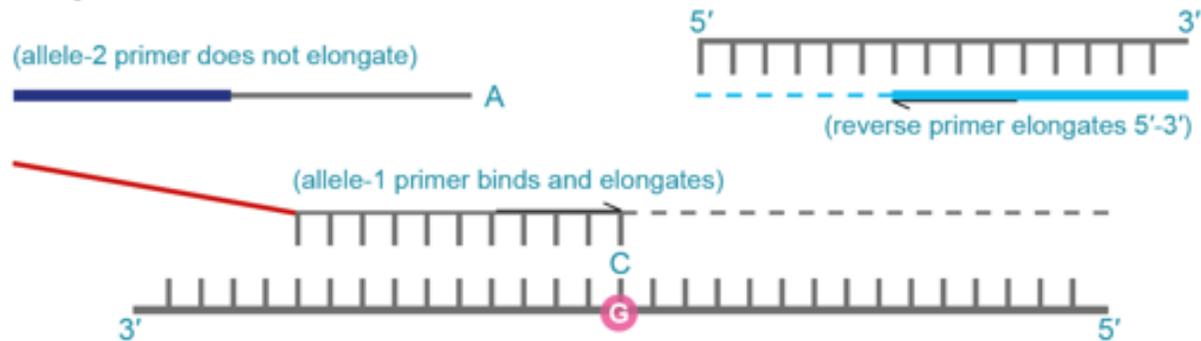
Reverse primer:



### B) KASP Master Mix

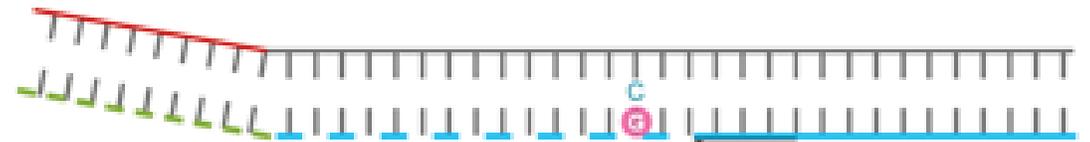


## 2. Denatured template and annealing components – PCR round 1:

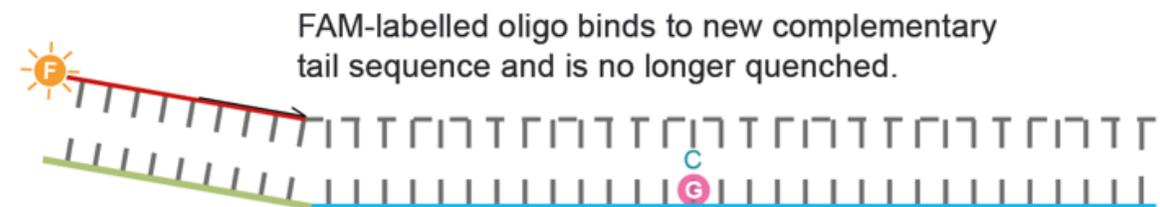


In the first round of PCR, one of the allele-specific primers matches the target SNP and with the common reverse primer, amplifies the target region.

## 3. Complement of allele-specific tail sequence generated – PCR round 2:



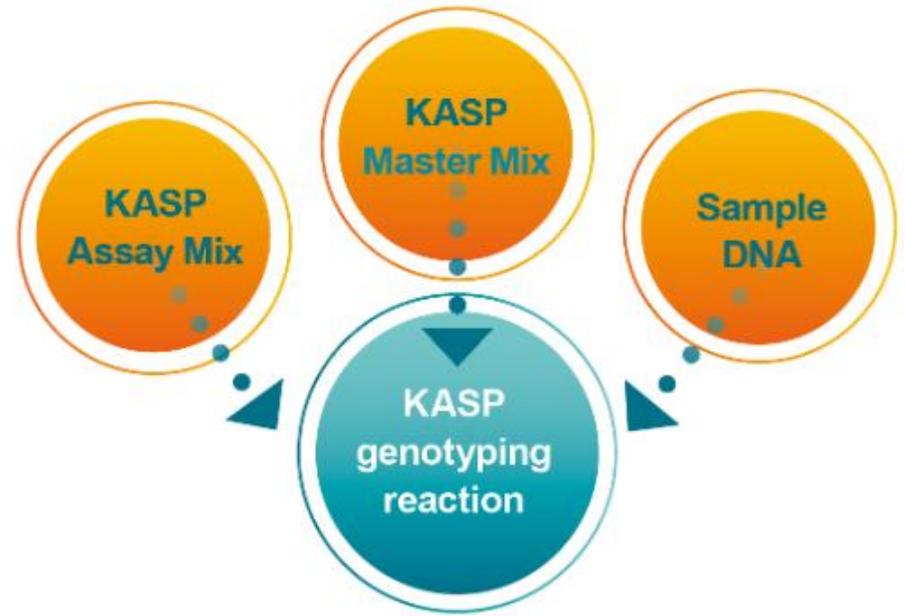
## 4. Signal generation – PCR round 3:



# KASP assay - advantages

## Easy to use

- ❖ Requires only two components: KASP assay and master mix
- ❖ Compatible with relative crude DNA extraction methods
- ❖ Flexible primer design
- ❖ Signal can be read on qPCR and FRET-capable plate readers
- ❖ Scaling up is possible



## Low cost

- ❖ Low reaction volumes – down to 1 uL - keep reagent costs to a minimum
- ❖ Relative robust assay means less time spent on repeats
- ❖ Cost benefits enable you to perform more assays overall, improving the quality of

# KASP assay - disadvantages

- ❖ Less competitive for medium- and high-density SNP applications
- ❖ Too expensive compared to upcoming medium-density platforms when  $> 100$  SNPs required.
- ❖ Cost- volume ratio of most service providers

# KASP genotyping in the CIMMYT GWP



Wheat Molecular Breeding Lab (WMBL)

- KASP design, initial verification and validation
- Small projects
- Complex production markers (more flexible protocols)
- (Gel-based markers)



Excellence in  
Breeding  
Platform

**intertek**

EIB Low density genotyping services (LDSG)

- Fully optimized production markers
- Fast turn-around (some recent tissues with customs release with longer turn-around)
- Min. or multiples of 384 samples



# KASP marker design – PolyMarker (<http://www.polymarker.info/>)

PolyMarker: bioinformatics pipeline for SNP assay development, via alignment to reference genomes

**PolyMarker** Create Primers Primers for wheat SNP-Chips About Cite

**Request primers**

PolyMarker is an automated bioinformatics pipeline for SNP assay development which increases the probability of generating homoeologue-specific assays for polyploid species. PolyMarker generates a multiple alignment between the target SNP sequence and the selected reference genome (from the drop off menu in green below). It then generates a mask with informative polymorphic positions between homoeologs which are highlighted with respect to the target genome.

See [About](#) to know how to prepare your input. For wheat, we have [designed primers](#) for the iSelect 90K chip and the Axiom 820k chip.

If your request is terminated due a timeout, try with less markers and make sure the SNPs are not in low complexity regions.

File  No file chosen

OR Manual Input

**Reference**

Wheat cv Chinese Spring RefSeq v1.0

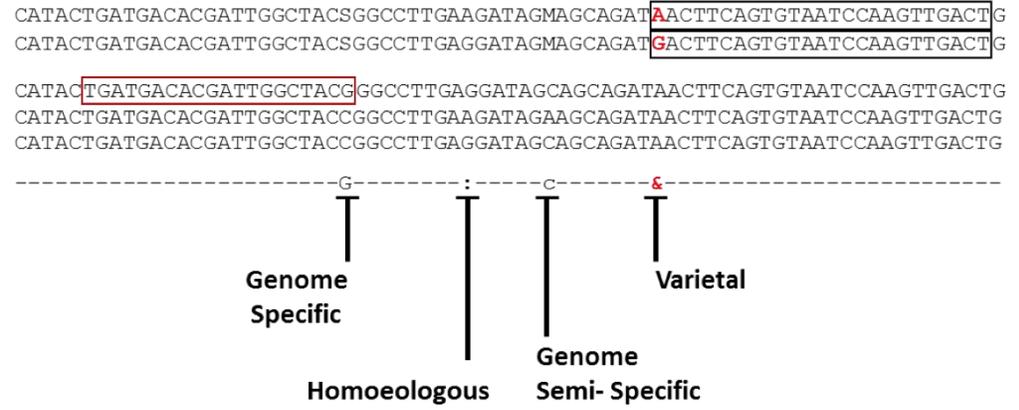
A whole genome assembly has been carried out by the IWGSC in collaboration with the company NRGene. Using a proprietary algorithm DeNovoMAGIC with Illumina sequencing data a 14.5 Gb assembly was produced. Sequences have been ordered using POPSEQ data and Hi-C (chromosome conformation capture) to generate 21 pseudomolecules representing the majority of the wheat genome. Reference sequence available in URGI The assembly is described in [IWGSC \(2018\)](#). Reference sequence available in [ensembl plants \(reference file\)](#) The repeats are masked to avoid multiple hits in repetitive regions.

[John Innes Centre](#) [BBSRC](#) [IWYP](#) [DFW](#)

```
>Ta#S58861868:A214G-Parent1
>Ta#S58861868:A214G-Parent2

>iwpsc_css_1AS_scaff_3271989
>iwpsc_css_1BS_scaff_3474683
>iwpsc_css_1DS_scaff_1913057

>MASK
```



- Use available genomics resources to support your SNP design e.g., GrainGenes, EnsemblPlants, etc,.
- Main problem in wheat is its polyploidy
- KASP Assay Cost: 40-45 USD (T4OLIGO, 100 μM, local provider)
- Other primer design software can be used, e.g., Primer3, WASP



# KASP genotyping



## PCR

- 384-well plate set-up for PCR
- KASP Master Mix: PACE™ Genotyping Master Mix, 3PC Bioscience: 4,920 USD (250ml)
- 4.25  $\mu$ l – PCR reactions

## Data visualization

- Initially qPCR, excel
- PHERAstar FSX plate reader, KlusterCaller

<https://repository.cimmyt.org/handle/10883/18822>

# KASP genotyping in the CIMMYT GWP

Type	CIMMYT SNP ID	Gene	SNP name	Species	Gene source	Category	Trait	Chr	SNP/indel (Ref/Alt)	FAM primer	VIC primer	Common primer	Direccion	Dominaa					
MAS	CIMwMAS0001	Lr34	Lr34_TCCIND	Blv'	Blv'	Rust	Rust	7DS	Ins/Del	GGTATGCCA777A4G47A47G47G4 A	GGTATGCCA777A4G47A47G47G4 T	TACTATATGGGGAGCA77ATTTTTTCC	R	Co-domin					
MAS	CIMwMAS0002	Pch1	CUB	Blv'	Blv'	Diseases and pests	Eyespot			AGCCTGGTTGGGTGCAAT	TCTCAGACATGCCCATCATT	GTTAGCTTGGCCCTTTGATCC	F						
MAS	CIMwMAS0003	Sr2	Sr2_ger9_3p	Blv'	Blv'	Rust	Rust	3B5	G	GTGGGAGACATCCG44C4CTG4 C	GTGGGAGACATCCG44C4CTG4 T	CTG44ATGGTGGAGCA44AGCTCT4	R	Co-domin					
MAS	CIMwMAS0004	Lr37/Yr17/Sr38	VPM_SNP	Blv'/Dlv'	T. ventricosum	Rust	Rust	2A5	C	CGCCGTTCCG44Y4CG4G A	CGCCGTTCCG44Y4CG4G G	CCCTGGCTTGGACCTTGG44C44	R	Co-domin					
MAS	CIMwMAS000	CIMMYT SNP ID	Status	Gene	SNP name	Trait	Chr	PCR Program	DMSO/MgCl2	Report Allele FAM	Report Allele VIC	Report NA/Null	Check FAM	Check VIC					
MAS	CIMwMAS000	CIMwMAS0001	In use	Lr34	Lr34_TCCIND	Rust	7DS	snp(td)	Not necessary	Lr34-	Lr34+	NA	Pastor, Pavon F76	Tonichi, Opata M85					
MAS	CIMwMAS000	CIMwMAS0002	Not diagnostic	Pch1	CUB	Eyespot				VPM1	Hsb1								
MAS	CIMwMAS000	CIMwMAS0003	In use	Sr2	Sr2_ger9_3p	Rust	3B5	snp(td)	2.5 MgCl2	Sr2+	Sr2+	Na or Null	Berkut1, Weebill	Pavon F76, Parula					
MAS	CIMwMAS000	CIMwMAS0004	In use	Lr37/Yr17/Sr38	VPM_SNP	Rust	2A5	snp(td)	Not necessary	VPM+	VPM-	NA	BW: Milan, DW: TRIDENT//CADO/B	DW: UC1113, Aconchi 89, BW:					
MAS	CIMwMAS000	CIMwMAS0005	Not applicable	Lr19/Sr25/Psy-E1	Psy1Da-g	Rust	7A	snp(td)	Not necessary	Sr25+	Sr25-	NA	DW: AG 1-22/2*ACONCHI//2*UC1	Pastor, Sokoll					
MAS	CIMwMAS000	CIMwMAS0006	Not diagnostic	Sr36/Pm6	Sr36/Pm6_8085	Rust	3B			Sr36-	Sr36+	NA	Berkut, Weebill	Sunco, Sr36#40					
MAS	CIMwMAS000	CIMwMAS0007	In use	Fhb1	UMN10_SNP	Fusarium	3B5	td) or td(snp61-	Not necessary	Fhb1-	Fhb1+	NA	Berkut1, Weebill	Sumai3					
MAS	CIMwMAS000	CIMwMAS0008	In use																
MAS	CIMwMAS000	CIMwMAS0009	Not ap																
MAS	CIMwMAS000	CIMwMAS0010	In						snpTA00446	snpTA00447	snpTA00029	snpTA00030	snpTA00031	snpTA00658	snpTA00065	snpTA00032	snpTA00621	snpTA00024	snpTA00037
MAS	CIMwMAS000	CIMwMAS0011	In						CIMwMAS0303	CIMwMAS0304	CIMwMAS0082	CIMwMAS0083	CIMwMAS0086	CIMwMAS0669	CIMwMAS0255	CIMwMAS0138	CIMwMAS0218	CIMwMAS0003	CIMwMAS0008
MAS	CIMwMAS000	CIMwMAS0012	In						Yr5	Yr5	Yr15	Yr15	Yr5T	Sr13 R/S	Sr22	Lr19/Sr25	Sr26	Sr2	Fhb1
MAS	CIMwMAS000	CIMwMAS0013	In						Yr5SPKASP3	Yr5New	Yr15-R3	Yr15-R8	BS00062676	Sr13R/S_SNP	Sr22_A_ML-Srs-MK5002524 (Sr2	Sr26_R-dom_A	Sr2_ger9_3p	snpSBS-8	
MAS	CIMwMAS000	CIMwMAS0014	In						Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant	Co-dominant
MAS	CIMwMAS000	CIMwMAS0015	In						INS:INS - Yr5+	-- Yr5-	A:A - Yr15-	C:C - Yr15+	C:C - Yr5T+	G:G - Sr13+	T:T - Sr22-	C:C - Lr19/Sr25-	T:T - Sr26-	G:G - Sr2-	C:C - Fhb1-
MAS	CIMwMAS000	CIMwMAS0016	In						Del:Del - Yr5-	INS:INS - Yr5+	G:G - Yr15+	T:T - Yr15-	T:T - Yr5T-	A:A - Sr13-	A:A - Sr22+	T:T - Lr19/Sr25+	G:G - Sr26+	A:A - Sr2+	G:G - Fhb1+
MAS	CIMwMAS000	CIMwMAS0017	In						-:INS - Het	INS: - - Het	G:A - Het	T:C - Het	T:C - Het	A:G - Het	A:T - Het	T:C - Het	G:T - Het	A:G - Het	G:C - Het
MAS	CIMwMAS000	CIMwMAS0018	In	SampleID	StudyID	GID	Plant Nr	Entry	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA (Null)	NA
MAS	CIMwMAS000	CIMwMAS0019	In	Bw21FV000294	682594	9360813	1	2014	snpTA00446	snpTA00447	snpTA00029	snpTA00030	snpTA00031	snpTA00658	snpTA00065	snpTA00032	snpTA00621	snpTA00024	snpTA00037
MAS	CIMwMAS000	CIMwMAS0020	In	Bw21FV000295	682595	9360813	2	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000	CIMwMAS0021	In	Bw21FV000296	682596	9360813	3	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	NA	G:G	C:C
MAS	CIMwMAS000	CIMwMAS0022	In	Bw21FV000297	682597	9360813	4	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	NA
MAS	CIMwMAS000	CIMwMAS0023	In	Bw21FV000298	682598	9360813	5	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000	CIMwMAS0024	In	Bw21FV000299	682599	9360813	6	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	NA
MAS	CIMwMAS000	CIMwMAS0025	In	Bw21FV000300	682600	9360813	7	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000	CIMwMAS0026	In	Bw21FV000301	682601	9360813	8	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	NA
MAS	CIMwMAS000		In	Bw21FV000302	682602	9360813	9	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000303	682603	9360813	10	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000304	682604	9360813	11	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000305	682605	9360813	12	2014	-:INS	INS:INS	G:A	T:C	NA	G:G	T:T	NA	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000306	682606	9360813	13	2014	NA	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000307	682607	9360813	14	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000308	682608	9360813	15	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000309	682609	9360813	16	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000310	682610	9360813	17	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000311	682611	9360813	18	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000312	682612	9360813	19	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000313	682613	9360813	20	2014	NA	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000314	682614	9360813	21	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000315	682615	9360813	22	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000316	682616	9360813	23	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000317	682617	9360813	24	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C
MAS	CIMwMAS000		In	Bw21FV000318	682618	9360813	25	2014	-:INS	INS:INS	G:A	T:C	G:G	G:G	T:T	T:C	T:T	G:G	C:C

# Rust resistance in bread wheat

## Resistance genes

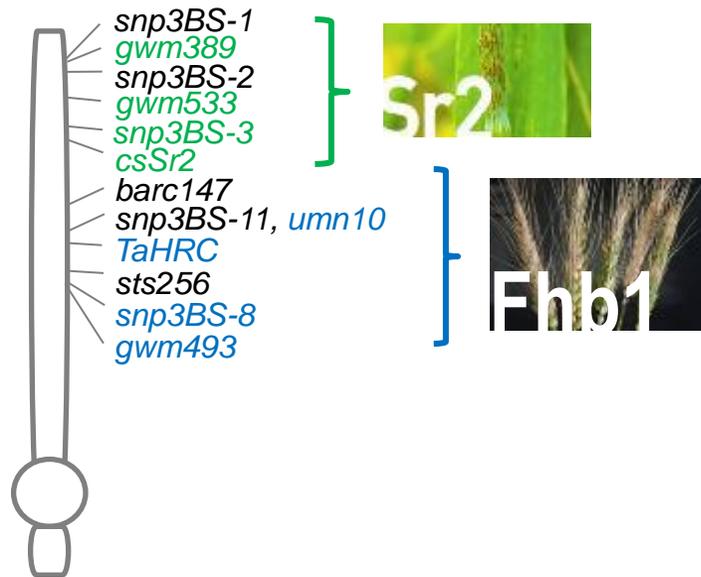


## Lines in current yield trials

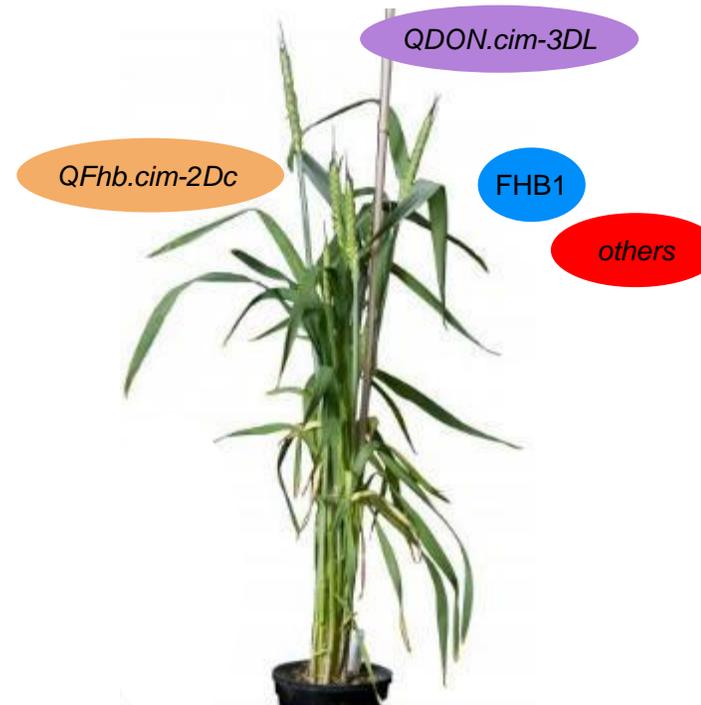
Cross	Gene	# lines YT18-19
BOKOTA*3//RL6077/AOC-YR	Lr67	1
BORL14*3/SUJATA	Lr67 + QTL.7BL	1
HARTOG_SUMAI3 (LINE B)/2*NAVJ07/5/KIRITATI//HUW234+LR34/PRINIA/3/FRANCOLIN #1/4/BAJ #1/6/2*TAM200/PASTOR//TOBA97/3/FRNCLN/4/WHEAR//2*PRL/2*PASTOR	Sr2 + Fhb1	2
HARTOG_SUMAI3 (LINE B)/2*NAVJ07/4/MUTUS//KIRITATI/2*TRCH/3/WHEAR/KRONSTAD F2004/5/2*TAM200/PASTOR//TOBA97/3/FRNCLN/4/WHEAR//2*PRL/2*PASTOR	Sr2 + Fhb1	1
SWSR22T.B.//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/MUNAL #1/5/2*MUNAL*2/WESTONIA	Sr22	1
SWSR22T.B.//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/SUP152/BAJ #1/5/2*KUTZ	Sr22	1
SWSR22T.B./FRANCOLIN #1//2*FRNCLN/3/3*WBLL1*2/BRAMBLING*2//BAVIS	Sr22	2
MUTUS*2/HARIL #1*2/3/SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU/4/MUCUY	Sr22	4
SWSR22T.B.//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/3*KACHU/3/WHEAR//2*PRL/2*PASTOR	Sr22 + Sr25	2
BORL14*3/4/KACHU/3/WHEAR//2*PRL/2*PASTOR	Sr25	3
SHORTENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65531/3/MISR 2/5/2*BAJ #1	Sr26	1
SHORTENED SR26 TRANSLOCATION//2*WBLL1*2/KKTS/3/BECARD/4/3*BORL14	Sr26	1
FRANCOLIN #1*2//ND643/2*WBLL1/3/SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU/5/SHORTENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65531/3/MISR 2/6/SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU	Sr26 + Sr22	4
BORL14*2/SR50	Sr50	7
BORL14*3/SR50	Sr50	7
BORL14*3/BLANCA GRANDE 515	Yr5 + Yr15	13
BORL14*2/BLANCA GRANDE 515	Yr5 + Yr15	6
BLANCA GRANDE 515/2*BAJ #1	Yr5 + Yr15	4
BORL14*3/KOELZ W 11192:AE	Yr52	3
YR57#5474-6/3*BAJ #1	Yr57	2
BORL14*3/IRAGI	Yr59	2
BORL14*2/IRAGI	Yr59	1
BORL14*3//LALBMONO1*4/PVN	Yr60	3
BORL14*2//LALBMONO1*4/PVN	Yr60	1
LALBMONO1*4/PVN//3*BAJ #1	Yr60	1

# Combining genes for fusarium head blight resistance

*Sr2-Fhb1* recombinants  
introgressed in CIMMYT elite  
backgrounds



Pyramiding of complementary  
genes



# Centralized marker-assisted trait development pipelines

<b>Parent development pipeline</b> (Trait deployment)	<b>Trait augmentation pipeline</b> (Trait augmentation)	<b>Discovery pipeline</b> (Trait Discovery)
Parent development by embedding or new genetic variants in diverse genetic backgrounds	Frequency augmentation of relevant genetic variants, final product development	Identification of new diversity, trait donors and genetic variants

Previous parent development pipelines based on the shuttle through DGGW, IWYP, SEED, CRP and other projects



# Wheat breeding scheme optimization in Toluca

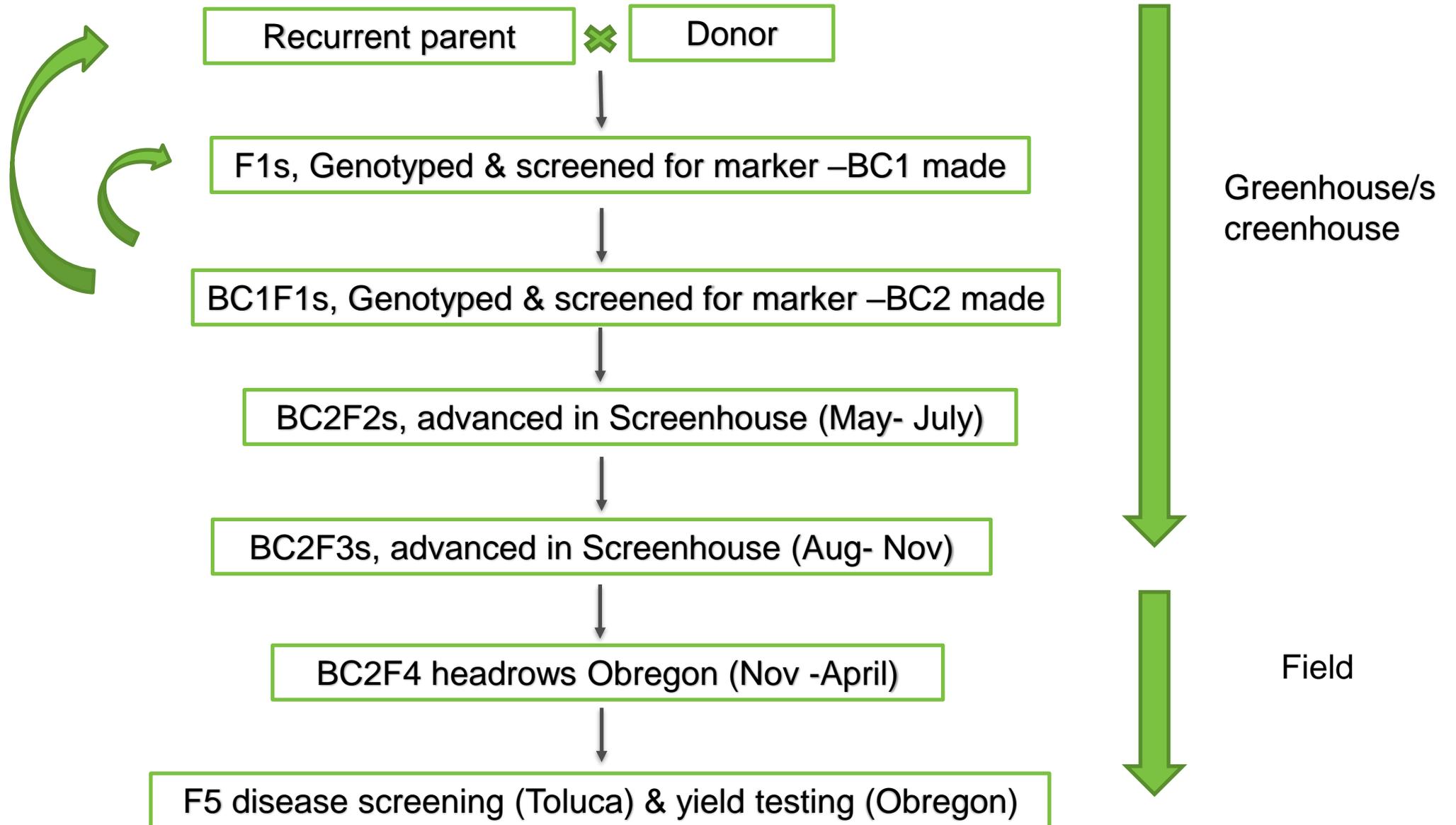


# Trait augmentation pipelines: priorities 2020 - 2022

Crop	Project Value	Product Profile	Trait	Genes
BW	Improved and diversified rust resistance	HW-OE-NM, HW-HT-EM, HW-DT-NM, HW-DT-EM	Stem and yellow rust	<i>Sr22, Sr50, Sr2, Yr57, Yr59, Sr35, Yr15, Yr5, Sr47, Sr25, Sr13, YrSP</i>
BW	Enhanced Fhb resistance	HW-HiR_NM	Fusarium head blight	<i>Fhb1, Qfhb.cim-2DLc</i>
BW	Improved STB resistance	HW-HiR_NM, HW-DT-NM	Septoria tritici Blotch	<i>Stb6, Stb16</i>
BW	Improved insect resistance	HW-OE-NM, HW-DT-NM	Green bug	<i>Gb7/Gba, Gb5, QRp.slu-5AL, QRp.slu-5BL-R</i>
BW	Improved TKW	HW-OE-NM, HW-HT-EM, HW-DT-NM, HW-DT-EM	TKW	<i>TaGW2, Qgw-jic-6A, Qgl-jic-5A</i>
BW	Novel diversity for stress tolerance (heat drought)	HW-DT-EM, HW-DT-NM, HW-HT-EM	Heat/drought tolerance	<i>LTP-HB3B, LTP-HB4B, LTP-HB5B, LTP-HB6D, LTP-HB7D-1, LTP-HB7D-2</i>



# Speed breeding for rapid introgressions of specific genes







**Thank you  
for your  
interest!**