

# Decreasing the risk of Coeliac disease in humans by modifying gliadin proteins in bread wheat, using the CRISPR/Cas9 technique

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# Outline

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- Wheat, Gluten & Coeliac disease
- Gene editing towards low gliadin immunogenicity
- Development of plant material & screening methods
- Results
- Conclusions & Future prospects



**Wheat,**

**Gluten**

**&**

**Coeliac disease**



# Wheat, gluten and Coeliac disease

- Polyploid complexity of wheat:

- Hexaploid bread wheat (*Triticum aestivum*, AABBDD)



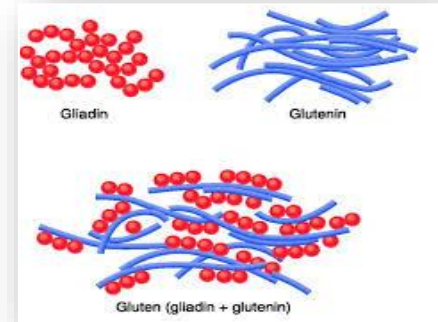
- Wheat grain gluten = Protein polymer

- Glutenins (HMW, LMW) → Elasticity

**Most important for bread**

- Gliadins ( $\alpha$ ,  $\gamma$ ,  $\omega$ ) → Viscosity

**Most immunogenic for CD**



- Gluten is present in most edible products

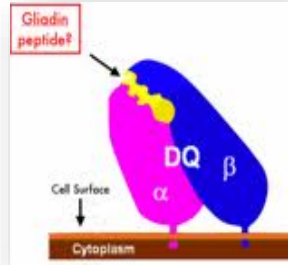


# Gliadin epitopes immunogenicity

- Gliadin epitope recognised = immunogenic epitope



Wheat  $\alpha$ -gliadin epitope



Human immune cell receptor

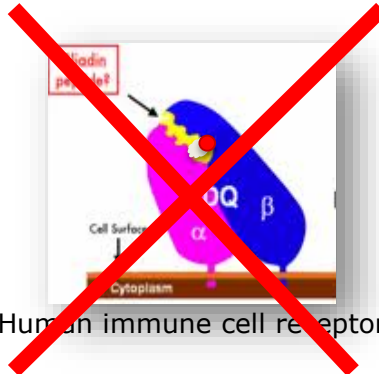


Coeliac disease

- Gliadin epitope **not** recognised = **non**-immunogenic epitope



Wheat  $\alpha$ -gliadin epitope  
with amino-acid deletion



Human immune cell receptor

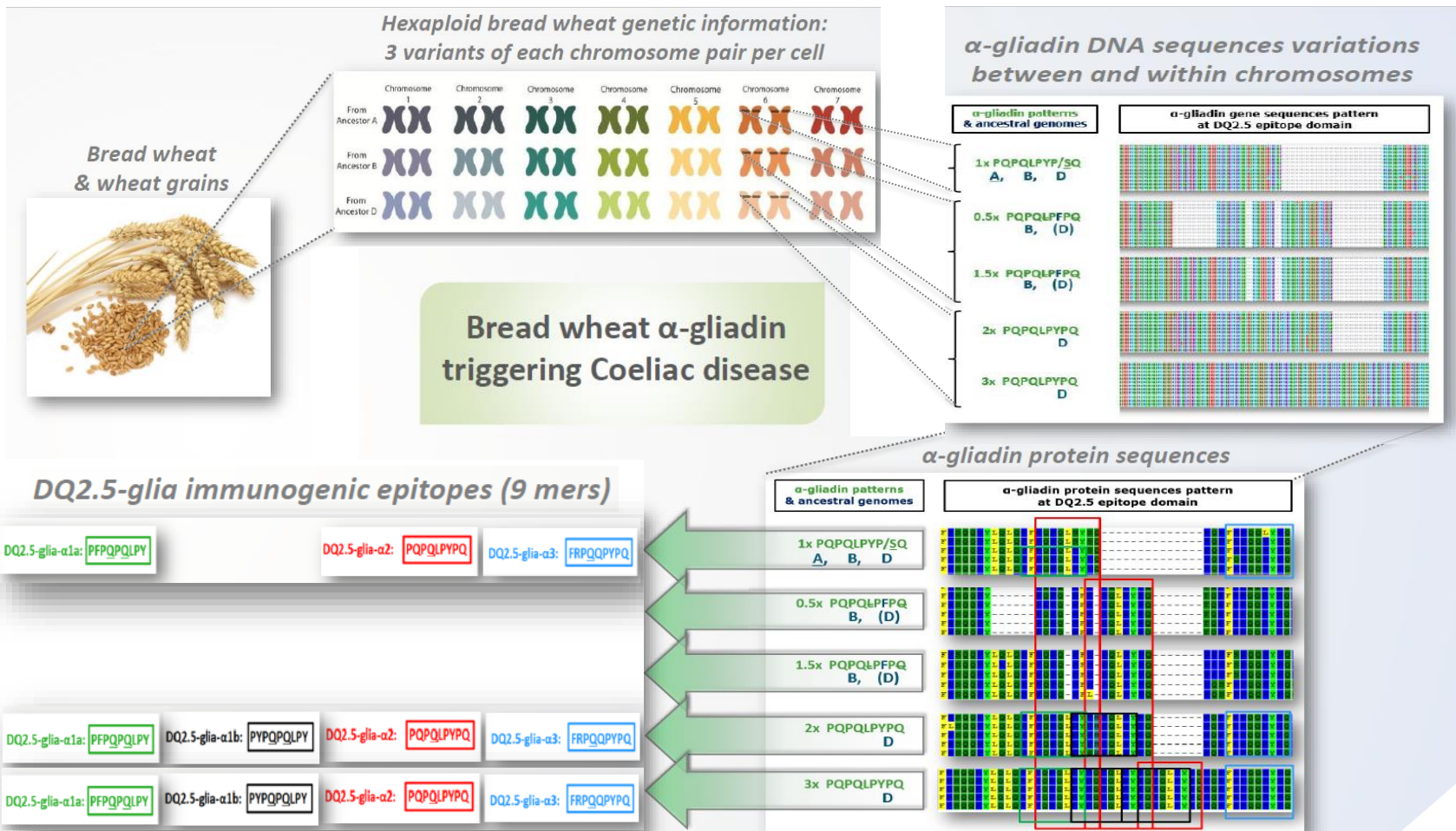


Coeliac disease



Wheat  $\alpha$ -gliadin epitope  
with amino-acid substitution

# $\alpha$ -gliadin: gene family & protein structures



# Solutions against CD

&

# Project presentation



# Which solutions against Coeliac disease???

- Gluten free diet

- Wheat, barley, rye free

➔ **Very challenging**



- Wheat varieties without immunogenic gliadin epitopes

- Conventional breeding



**Long, may not be possible**

- Mutagenesis



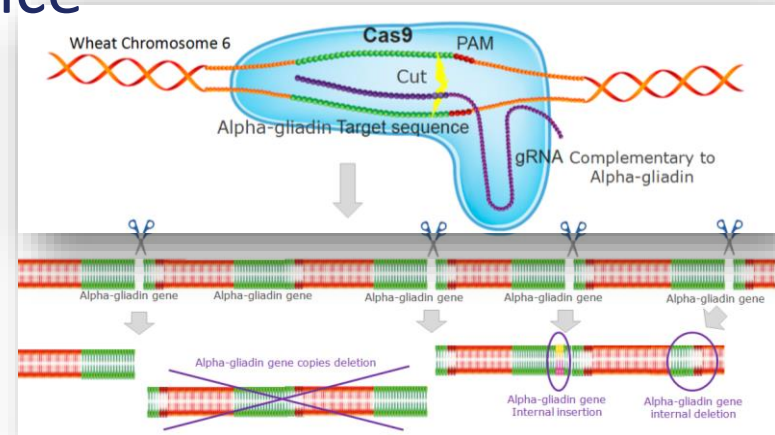
**Attractive alternative**

# Targeted mutagenesis via CRISPR/Cas9: mechanism

Clustered Regularly Interspaced Short Palindromic Repeats  
Associated with Cas9 endonuclease

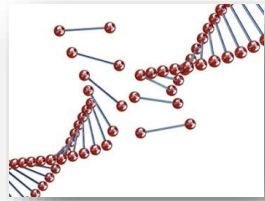
## Customised DNA scissors:

- **sgRNA** recognises chosen DNA sequence *e.g.  $\alpha$ -gliadins*
- **Cas9** cuts this DNA sequence
- Triggers mutations
  - Point mutations in gene
  - Large deletions in gene
  - Gene copy deletions



# Ultimate goal: using CRISPR/Cas9 on gliadins in wheat to decrease risks of Coeliac Disease

- Point mutations in  $\alpha$ - and  $\gamma$ -gliadin genes
  - Prevent epitopes to bind to immune cell receptors
  - Knock out genes preventing epitopes expression
- DNA fragment deletions at gliadin loci
  - Removing the epitope region from  $\alpha$ - and  $\gamma$ -gliadin
  - Decrease gliadin genes copy number



**Gliadin sequence alignments,**



**sgRNAs design & cloning,**

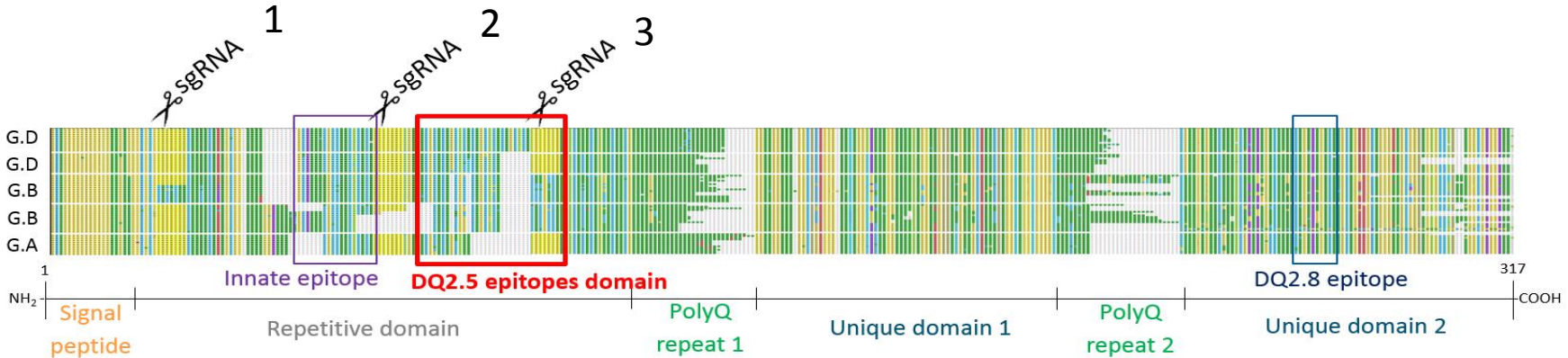


**Wheat transformation**

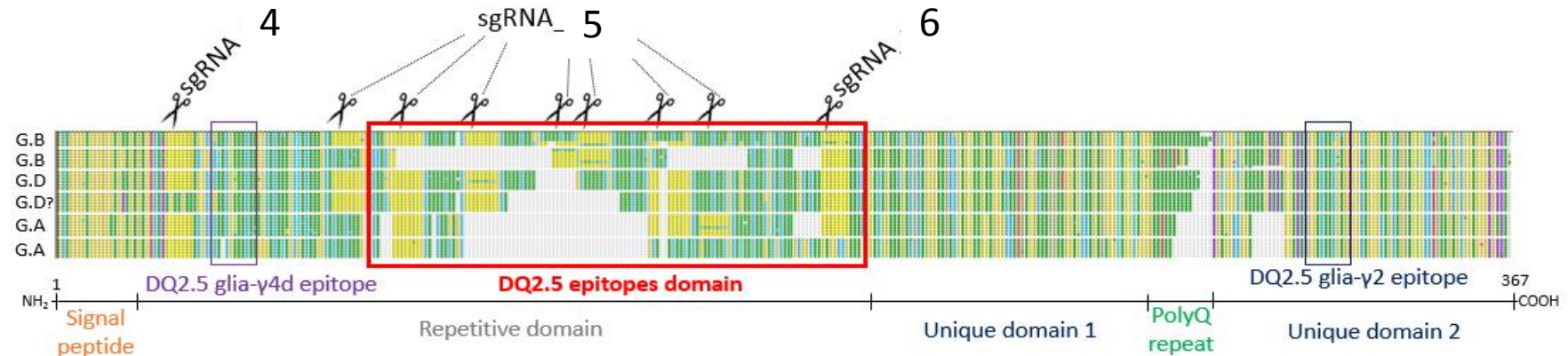


# $\alpha$ - and $\gamma$ -gliadin gene conserved regions & sgRNAs

## ■ $\alpha$ -gliadin protein sequences & sgRNAs

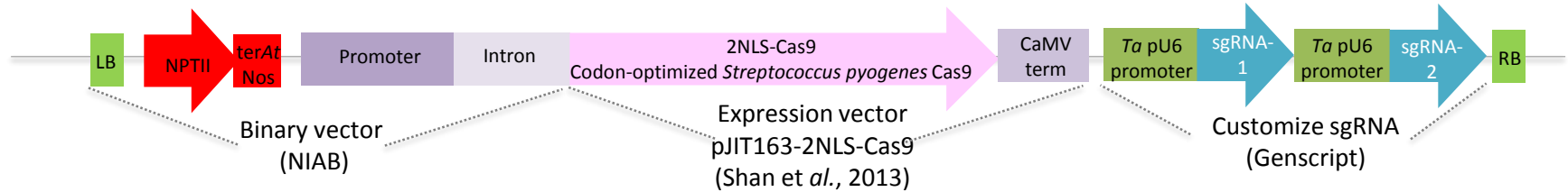


## ■ $\gamma$ -gliadin protein sequences & sgRNAs



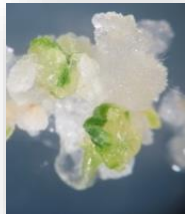
# CRISPR/Cas9 constructs & Fielder transformation

## ■ Combining CRISPR/Cas9 & sgRNAs into binary vector



## ■ Stable transformation of Cas9-sgRNAs into Fielder bread wheat

From immature embryos using *Agrobacterium* under Japan Tobacco Inc. licence



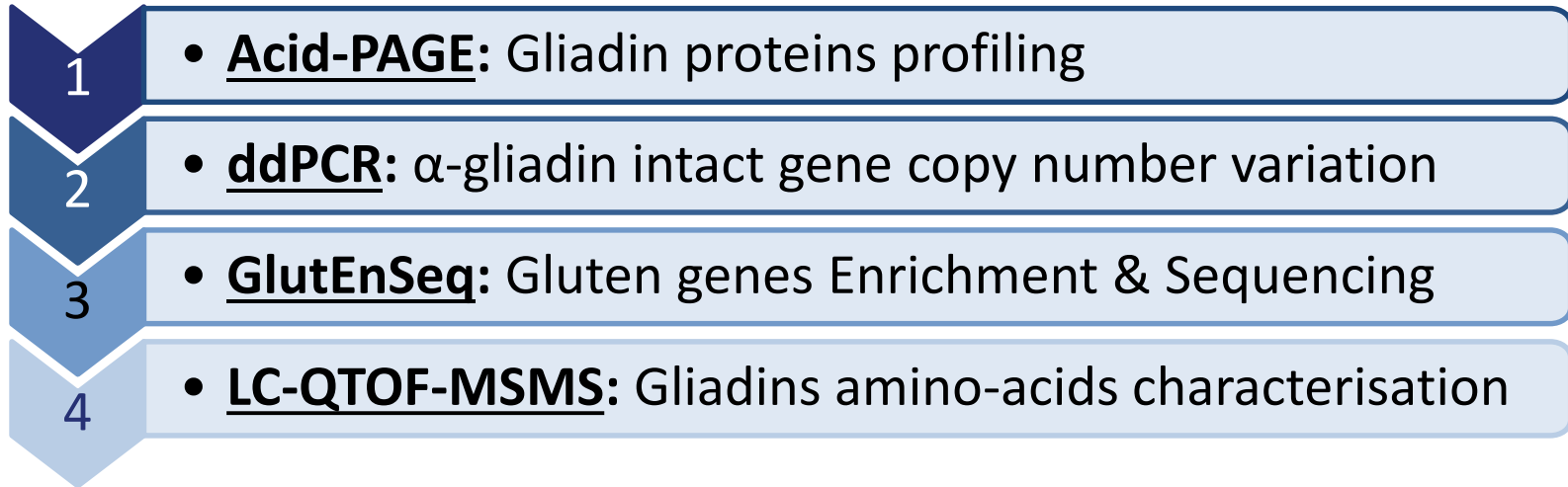
Pictures: Emma Wallington's transformation team, NIAB, UK

# Pipeline development for screening gliadin wheat mutants

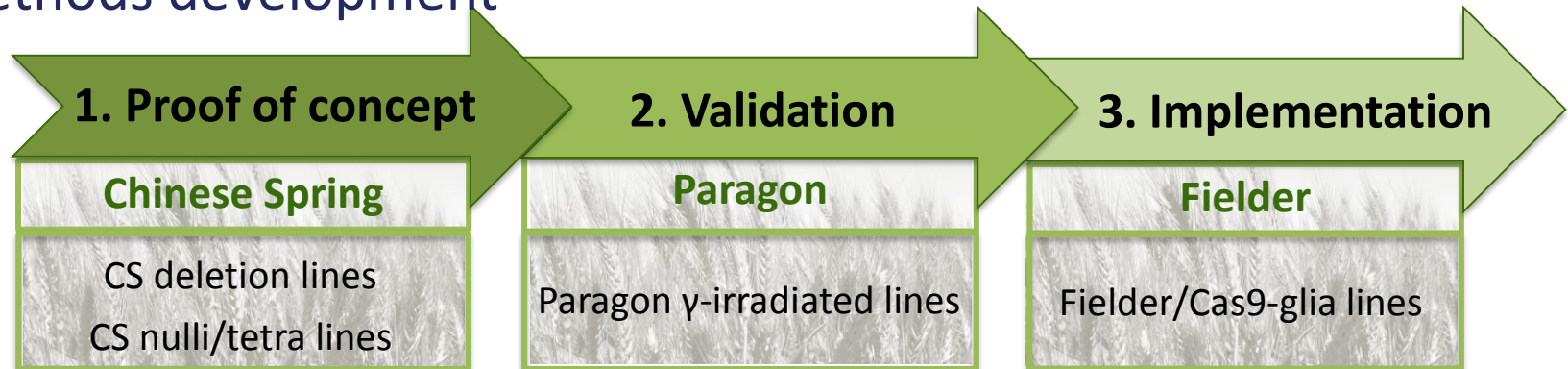


# Mutants screening: methods pipeline

## ■ Methods pipeline

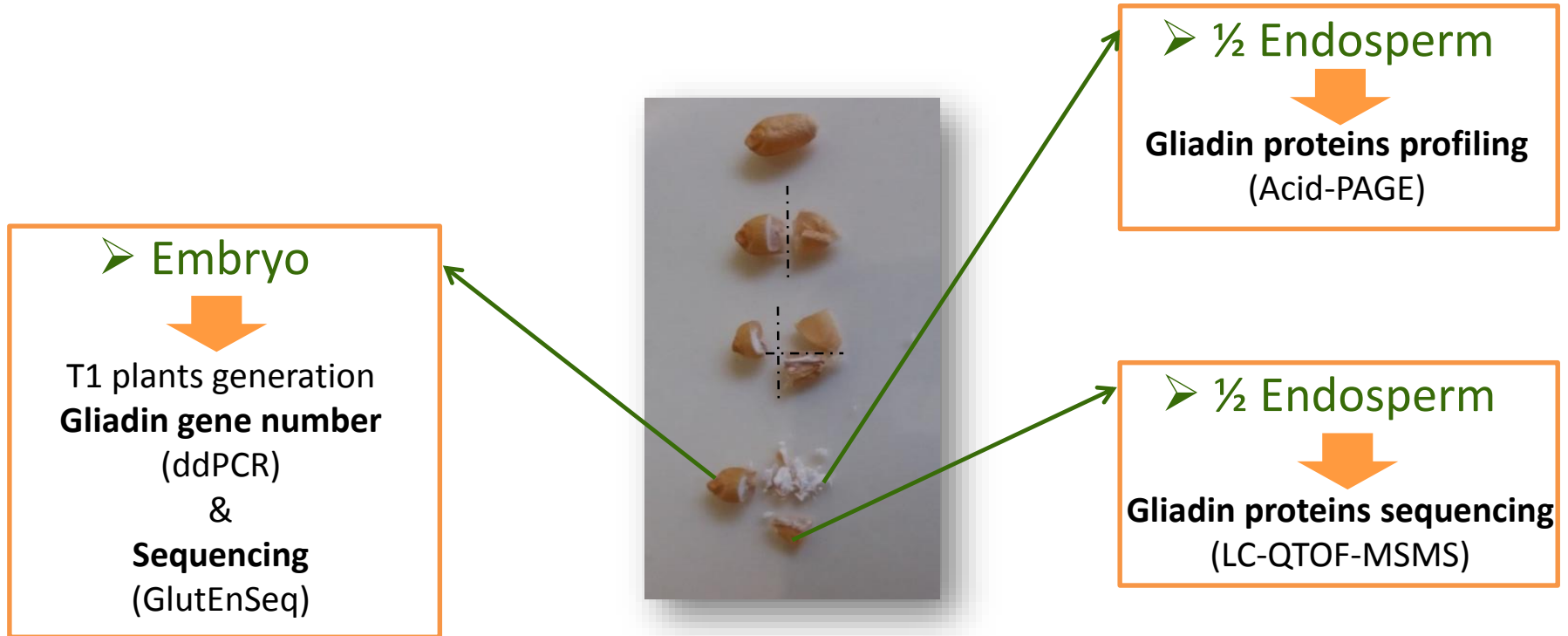


## ■ Methods development



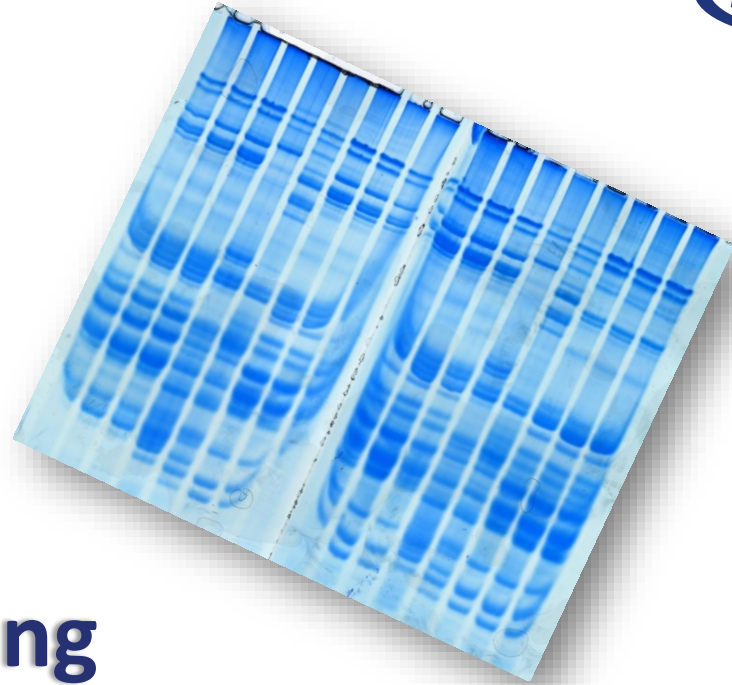
# Unique T0 wheat grains: 1 grain, 4 methods

- Unique wheat grain cut in 3



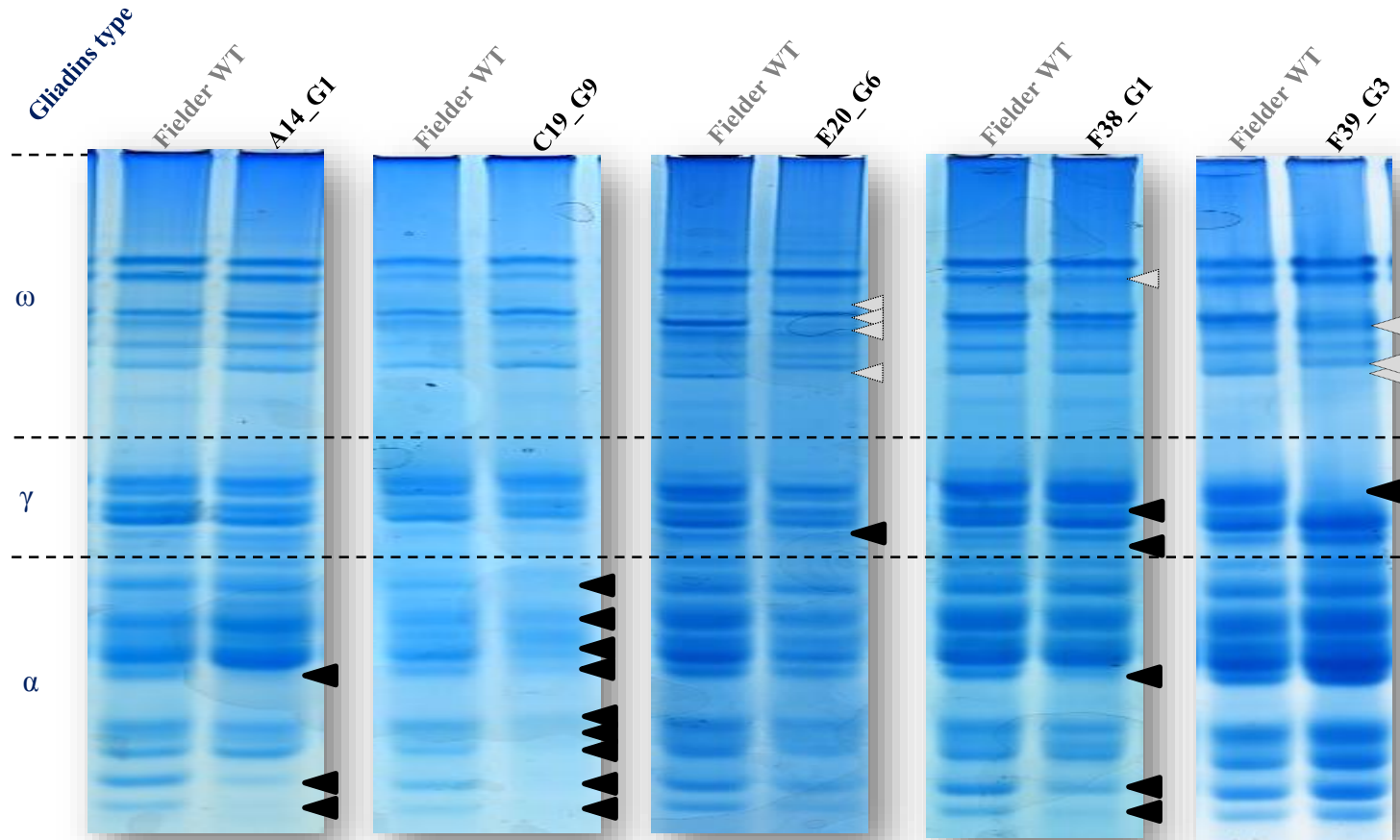
## **Acid-PAGE:**

# **Gliadin protein profiling**



# Acid-PAGE: gliadin protein profiling

- T1 grains screening: Fielder-Cas9 lines vs wild type



## Grains screened

Per constructs

30 plants

8 grains/plant

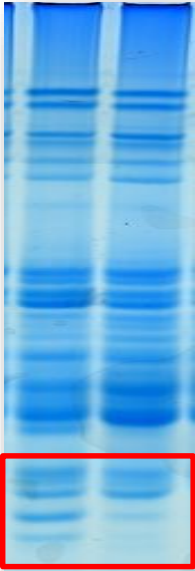
**= 960 grains**  
**(500 gels)**

# Gene editing vs mutation breeding

## ■ Fielder/Cas9-glia lines vs Paragon $\gamma$ -irradiated lines

### ➤ CRISPR/Cas9 lines

$\alpha$ -gliadins



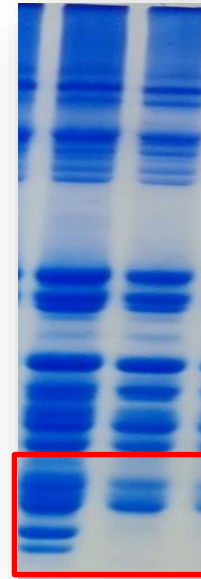
$\gamma$ -gliadins



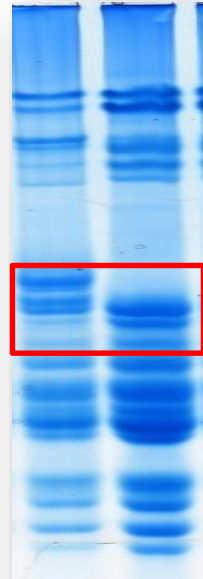
Gliadin genes editing only

### ➤ $\gamma$ -irradiated lines

$\alpha$ -gliadins



$\gamma$ -gliadins



Gliadin & other genes deleted

360 lines  
14 mutants

➡ **Might be regulated as GM**

Additional risk assessments and labelling

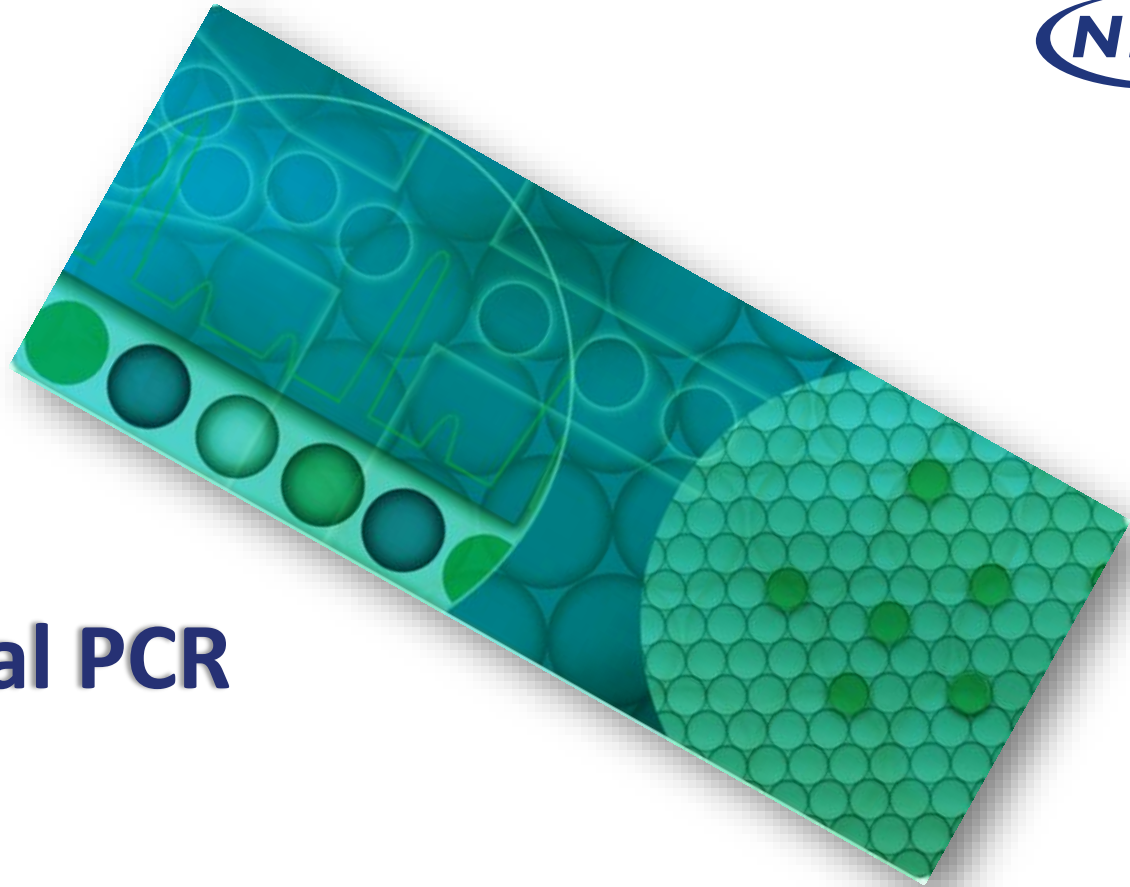
➡ **Exempted from GM regulation**

No additional risk assessments, no labelling



**ddPCR:**

**droplet digital PCR**



# ddPCR: $\alpha$ -gliadin gene copy number variation

## ■ droplet digital PCR = accurate type of quantitative PCR

- Low level of gene expression
- Gene Copy Number Variation (CNV)
  - BUT never tested for CNV in large gene families in hexaploid

➡ **Intact  $\alpha$ -gliadin Gene Copy Number Variation in mutants vs wild type**

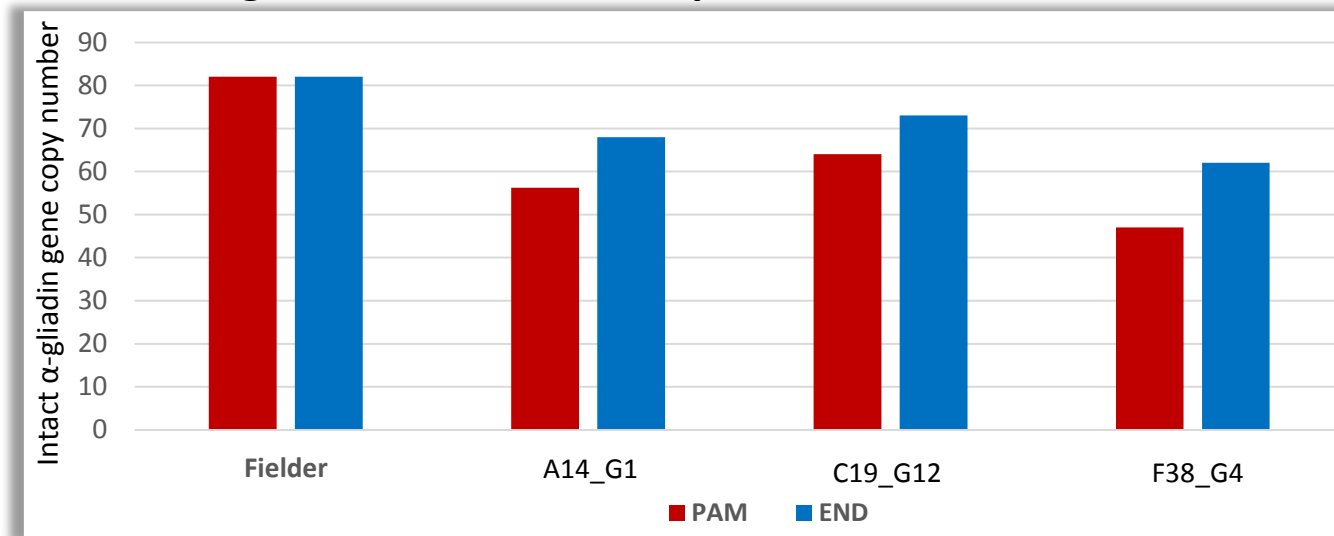
## ■ droplet digital PCR: experiment design

- Reference = **PFT1** present in **1 copy** per haploid genome
- Targets =  $\alpha$ -gliadin **PAM** → decreases both if **small indels** or **large deletions**  
 $\alpha$ -gliadin **END** → decreases only if **large deletions**



# ddPCR: $\alpha$ -gliadin gene copy number variation

ddPCR:  $\alpha$ -gliadin PAM & END amplicons in Fielder & Fielder-Cas9

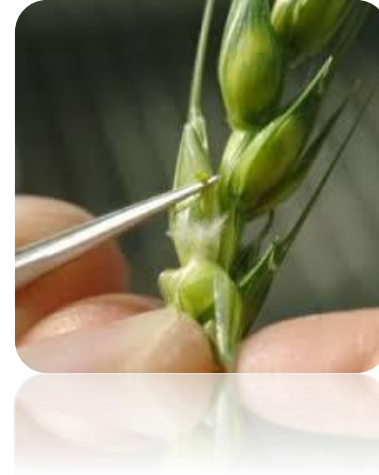


- Reduction of PAM and END amplicons in mutants
  - Both small indels and large deletions occurred
- Greater reduction of PAM than END amplicons in mutants
  - Small indels at targeted site more frequent than large indels

# Conclusions

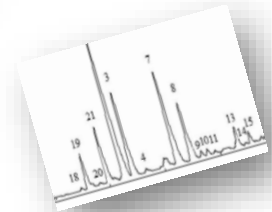
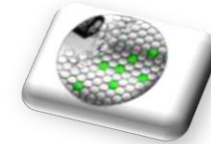
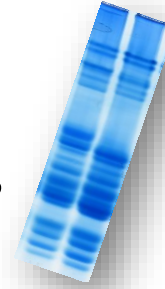
# &

# Further steps



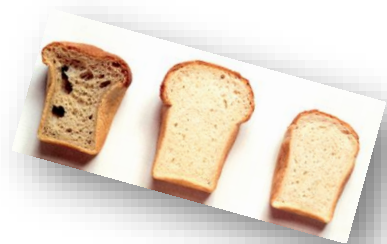
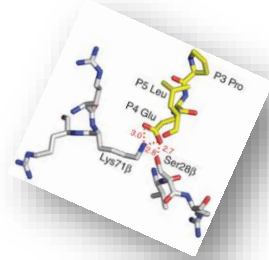
# Summary & further steps

- Acid-PAGE: Gliadin protein profile mutants  
→ CRISPR/Cas9 generate mutant phenotypes
- ddPCR: Reduction of intact  $\alpha$ -gliadin genes  
→ More targeted indels than large deletions
- GlutEnSeq: DNA sequences gliadins WT + mutants
- LC-QTOF-MSMS: Proteomics for epitopes characterisation



# Long term prospects

- Toxicity prediction of mutant epitopes
- Immunogenicity testing with anti-gliadin antibodies
- Immunogenicity testing with specific T-cells
- Rheology: bread dough quality assessment



# Acknowledgments



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& her transformation team
- Dr. James Cockram
- Dr. Lesley Boyd
- Dr. Bruno Santos
- Dr. Lawrence Percival-Alwyn

## Varieties & seeds

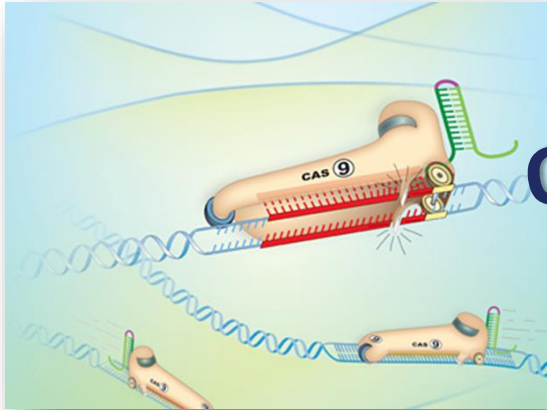
- Ms. Helen Appleyard
- Ms. Pravina Solanki
- Ms. Sofia Alves



# Thanks for your attention



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## Questions?



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