Wheat HMW glutenins 2 genes are confirmed on chromosome 1A, 1B and 1D each of Wheat Chinese spring reference genome: TraesCS1A02G317311, TraesCS1A02G466500LC, TraesCS1B02G329711, TraesCS1B02G329992 TraesCS1D02G317211, TraesCS1D02G317301b corresponding to published HMW transcript sequences derived from different wheat varieties (Accessions: Chr.1A -JQ689003, M22208, X61009; Chr.1B -HQ731653, DQ119142,AY367771, EF540765; Chr.1D - X03346, KX375406, X03041, DQ000161)

```
TaHMW-Chr1A-scaffold002470  GCACCATGCGGCCAGCCTAAAGGTGGCAAAGGCACAGCAGCTCGCGGCACAGCTGCCGGCAATGT
TaHMW-Chr1D-scaffold063795  GCACCAGGCGGCCAGCCTAAAGGTGGCAAAGGCGCAGCAGCTCGCGGCACAGCTGCCGGCAATGT
TaHMW-Chr1B-scaffold031273  GTACCAGGCGGCCCGCCTAAAGGTGGCAAAGGCGCAGCAGCTCGCGGCACAGCTGCCGGCAATGT
TaHMW-Chr1B-scaffold030818  GCAGCAAACGGCCAGCCCAAAGGTGGCAAAGGTGCAGCAACCCGCGACACAGCTGCCGATAATGT
TaHMW-Chr1A-scaffold000320  GCAGCAAGCGGCCAGCCCAAAAGTGGCAAAGGCGCACCATCCGGTGGCACAGCTGCCGACAATGT
TaHMW-Chr1D-scaffold062418  GCAGCAAGCGGCCAGCCCAATGGTGGCAAAGGCGCAGCAGCCCGCGACACAGCTGCCGACAGTGT
                            * * **  ***** *** **  **********  ** ** *  * * ***********  * ***
TaHMW-Chr1A-scaffold002470  GCCGGCTAGAGGGCGGCGACGCATTGTTGGCCAGCCAGTGAtagaactctctgcagctcgcttggt
TaHMW-Chr1D-scaffold063795  GCCGGCTGGAGGGCGGCGACGCATTGTCGGCCAGCCAGTGAtagaactctctgcagctcgcatggt
TaHMW-Chr1B-scaffold031273  GCCGGCTGGAGGGCAGCGACGCATTGTCGACCAGGCAGTGAtagaactctctgcagcttgcatggt
TaHMW-Chr1B-scaffold030818  GTCGGATGGAGGGGGGCGACGCATTGTCGGCTAGCCAGTGAtagaactctctgcaacttgcatggt
TaHMW-Chr1A-scaffold000320  GCCAGATGGAGGGGGGCGACGCATTGTCGGCTAGCCAGTGAtagaactctctgcagcttgcatggt
TaHMW-Chr1D-scaffold062418  GTCGGATGGAGGGGGGCGACGCATTGTCGGCTAGCCAGTGAtagaactctctgcagcttgcatggt
                            * * * * *****  ************ * * ********************** ** ** ****
```

Stop codon TGA - sequence alignments to corresponding real time PCR primer are grey shaded.

TaHMW-ABrtFor2 5'-CACAGCTGCCGRCAATGTGYC-3' 21 bases Tm 63.2-67.0
TaHMW-ABrtRev2 5'-CCATGCRAGYTGCAGAGAGTTC-3' 22 bases Tm 62.1-65.8

Fragment size 81 bp

Wheat LMW glutenins

3, 4 and 7 LMW glutenins are present on chromosome 1 of the A, B and D genome, respectively, of the Chinese spring reference genome. The forward rel; time PCR primer covers all wheat LMW and the reverse primer all beside of TaLMW-1A-2.

```
TaLMWG-1D-4_TraesCS1D02G007400  ATGAAGACCTTCCTCGTCTTTGCCCTTCTAGCCGTTGTGGCGACATCTGCCATTGCACAG
TaLMWG-1D-5_TraesCS1D02G007626  ATGAAGACCTTCCTCGTCTTTGCCCTTCTAGCCGTTGTGGCGACATGTGCCATTGCGCAG
TaLMWG-1D-6_TraesCS1D02G009400  ATGAAGACCTTCCTCATCTTTGCTCTCCTTGCCATTGCGGCGACAAGTGCCATTGCACAA
TaLMWG-1D-7_TraesCS1D02G009900  ATGAAGACCTTCCTCGTCTTTGCCCTCATCGCCGTTGTGGCGACAAGTGCCATTGCACAG
TaLMWG-1D-2_TraesCS1D02G000200  ATGAAGACCTTCCTCGTCTTTGCCCTCCTCGCCGTTGCGGCGACAAGTGCAATTGCGCAG
TaLMWG-1D-3_TraesCS1D02G000300  ATGAAGACCTTCCTCATCTTTGCCCTCCTCGCCGTTGCGGCAACAAGTGCCATTGCACAA
TaLMWG-1D-1 TraesCS1D02G015100  ATGAAGACCTTCCTCATCTTTGCCCTCCTTGCCGTTGCAGCGACAAGTGCCATTGCACAA
TaLMWG-1A-1_TraesCS1A02G010900  ATGAAGACCTTCCTCGTCTTTGCCCTCCTCGCCGTTGTGGCAACAAGTGCCATTGCGCAG
TaLMWG-1A-2_TraesCS1A02G008000  ATGAAGACCTTCCTCGTCTTTGCCCTCCTCGCTCTTGCGGCGGCAAGTGCCGTTGCGCAA
TaLMWG-1A-3_TraesCS1A02G010905  ATGAAGACCTTCCTCGTCTTTGCCCTCCTCACCGTTGTGGCGACAAGTACCATTGCGCAG
TaLMWG-1B-1_TraesCS1B02G013500  ATGAAGACCTTCCTCATCTTTGCCCTCCTCGCCGTTGCGGCGACAAGTGCCATTGCACAA
TaLMWG-1B-2_TraesCS1B02G011523  ATGAAGACCTTCCTCATCTTTGCCCTCCTCGCCGTTGCGGCGACAAGTGCCATTGCACAA
TaLMWG-1B-3_TraesCS1B02G011600  ATGAAGACCTTCCTCATCTTTGCTCTCCTCGCCATTGTGGCGACAAGTGCCATTGCACAA
TaLMWG-1B-4_TraesCS1B02G011700  ATGAAGACCTTCCTCATCTTTGCCCTCCTCGCCGTTGCGGCGACAAGTGCCATTGCACAA
                                *************** ******* **  *  *  ***  ** *** **** ***** **
TaLMWG-1D-4_TraesCS1D02G007400  ATGGAGACTAGCTGCATCCCTGGTTTGGAGAGACCATGGCAGCAACAACCATTAC-----
TaLMWG-1D-5_TraesCS1D02G007626  ATGGAGACTAGGTGCATCCCTGGTTTGGAGAGACCATGGCAGCAACAACCATTAC-----
TaLMWG-1D-6_TraesCS1D02G009400  ATGGAGACTAGCCGCGTCCCTGGTTTGGAGAAACCATGGCAGCAACAACCATTACCACCA
TaLMWG-1D-7_TraesCS1D02G009900  ATGGAGACTAGCTGCATCTCTGGTTTGGAGAGACCATGGCAGCAGCAACCATTAC-----
TaLMWG-1D-2_TraesCS1D02G000200  ATGGAGACTAGATGCATCCCTGGTTTGGAGAGACCATGGCAGCAGCAACCATTACCACCA
TaLMWG-1D-3_TraesCS1D02G000300  ATGGAGAATAGCCACATCCCTGGTTTGGAGAAACCATCGCAGCAACAACCATTACCACTG
TaLMWG-1D-1 TraesCS1D02G015100  ATGGAGACTAGCCACATCCCTGGCTTGGAGAAACCATCGCAACAACAACCATTACCACTA
TaLMWG-1A-1_TraesCS1A02G010900  ATGGATACTAGCTACATCCCTGGTTTGGAGAGACCATGGCAGCAGCAACCATTACCACCA
TaLMWG-1A-2_TraesCS1A02G008000  ATTTCACAGCAACAACAACAACCACCATTTTCACAGCAACAACAACCACCATTTTCGCAG
TaLMWG-1A-3_TraesCS1A02G010905  ATGGAGACTAGCTGCATCCCTGGTTTGGAGAGACCATGGCAGCAGCAACCATTACCACCA
TaLMWG-1B-1_TraesCS1B02G013500  ATGGAGACTAGCCACATCCCTAGCTTGGAGAAACCATTGCAACAACAACCATTACCACTA
TaLMWG-1B-2_TraesCS1B02G011523  ATGGAGAATAGCCACATCCCTGGTTTGGAGAGACCATCGCAGCAACAACCATTACCACCA
TaLMWG-1B-3_TraesCS1B02G011600  ATGGAGAATAGCCACATCCCTGGTTTGGAGAGACCATCGCAGCAACAACCATTACCACCA
TaLMWG-1B-4_TraesCS1B02G011700  ATGGAGAATAGCCACATCCCTGGTTTGGAGAGACCATCGCAGCAACAACCATTACCACCA
                                ******* ***   * ** **** ******* ***** *** ** **********
```

Start codon ATG - sequence alignments to corresponding real time PCR primer are grey shaded.

TaLMW-Glut-abFor3 GAA GAC CTT CCT CRT CTT TGC C 22 bases Tm 62.1-64.2
TaLMW-Glut-abRev3 TGG TCT CTC CAA ACC AGG GAT G 22 bases Tm 64.2

Frg size 96 bp

Wheat αβ-Gliadins specific real time PCR primer based on alignment of all wheat αβ-Gliadins in comparison to α-Gliadin. αβ-Gliadins contains an extended 5′ coding region which is absent in α-Gliadins leading to αβ-Gliadins specific forward real time PCR primer.

```
Ta-α-GliadinCl2-AS8        ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl7-AS11       ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl3-AS7        ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl5-AS7        ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl10-AS7       ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl9-AS7        ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl8-AS7        ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl11-AS7       ------------------------------------------------------------ATGGTTAGAG
Ta-α-GliadinCl1pA735-AS8   ATGAAGACCTTTCTCATCCTTGCCCTCCGTGCTATTGTAGCAACCACCGCCACAATTGCAGTTAGAG
Ta-αβ-GliadinCL6-AS8       ATGAAGACCTTTCTCATCCTTGCCCTCCGTGCTATTGTAGCAACCACCGCCACAATTGCAGTTAGAG
Ta-αβ-GliadinCL43-AS8      ATGAAGACCTTTCTCATCCTTGCCCTCCTTGCTATTGTAGCAACCACCGCCACAATTGCAGTTAGAG
Ta-αβ-Gliadin-24-Chr6AS    ATGAAGACATTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-26-Chr6AS    ATGAAGACCTTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACCGCAGTTAGAG
Ta-αβ-Gliadin-20-6AS       ATGAAGACCTTTCTCATCCTTGCCCTCCCTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-25-Chr6AS    ATGAAGACCTTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-21-6AS       ATGAAGACCTTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-ClassAI      ATGAAAACCTTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-27-Chr6AS    ATGAAGACATTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-GliadinCL19-AS7      ATGAAGACCTTTCTCATCCTTGCCCTCCTTGCTATTGTAGCAACCACCGCCACAATTGCAGTTAGAG
Ta-αβ-GliadinCL2-AS2       ATGAAGACATTTCTCATCATTTCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-28-Chr6AS    ATGAAGACATTTCTCATCATTTCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-ClassAIII    ATGAAGACCTTTCTCATCCTAGCCCTCCTTGCTATCGTGGCGACCACCGCCACAAGTGCAGTTAGAG
Ta-αβ-GliadinCL34-AS4      ATGAAGACCTTTCTCATCCTTTCCCTCCTTGCTATCGTGGCGACCACTGCCACAACTGCAGTTAGAT
Ta-αβ-Gliadin-30-Chr6BS    ATGAAGACCTTTCTCATCCTTTCCCTCCTTGCTATCGTGGCGACCACTGCCACAACTGCAGTTAGAT
Ta-αβ-Gliadin-29-Chr6BS    ATGAAGTCATTTCTCATCCTTGCCCTCCTTGCTATCGTGGCGACCACCGCCACAACTGCAGTTAGAG
Ta-αβ-Gliadin-23-Chr6BS    ATGAAGACCTTTATCATCCTTGCCCTCCTTGCTATTGTGGCGACCACCGCCACAACTGCAGTTACAG
                           *****  * *** *******  ****** ****** ** ** ***** ******* *   **** **
Ta-α-GliadinCl2-AS8       TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-α-GliadinCl7-AS11      TTCCAGTGCCACAATTGCAGCTACAAAATCCAT
Ta-α-GliadinCl3-AS7       TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-α-GliadinCl5-AS7       TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-α-GliadinCl10-AS7      TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-α-GliadinCl9-AS7       TTACAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-α-GliadinCl8-AS7       TTCCAATGCCACAATTGCAGCCACAAGATCCAT
Ta-α-GliadinCl11-AS7      TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-α-GliadinCl1pA735-AS8  TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-GliadinCL6-AS8      TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-GliadinCL43-AS8     TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-24-Chr6AS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-26-Chr6AS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-20-6AS      TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-25-Chr6AS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-21-6AS      TTCCAGTGCCACAATTGCAGCCACAACATCCAT
Ta-αβ-Gliadin-ClassAI     TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-27-Chr6AS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-GliadinCL19-AS7     TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-GliadinCL2-AS2      TTCCAGTGCCACAATTGCAGCCGCAAAATCCAT
Ta-αβ-Gliadin-28-Chr6AS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-ClassAIII   TTCCAGTGCCACAATTGCAGCCGCAAAATCCAT
Ta-αβ-GliadinCL34-AS4     TTCCAGTGCCACAATTGCAGCCGCAAAATCCAT
Ta-αβ-Gliadin-30-Chr6BS   TTCCAGTGCCACAATTGCAGCCGCAAAATCCAT
Ta-αβ-Gliadin-29-Chr6BS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
Ta-αβ-Gliadin-23-Chr6BS   TTCCAGTGCCACAATTGCAGCCACAAAATCCAT
                          ** ** ***************  *** ******
```

Start codon ATG - sequence alignments to corresponding real time PCR primer are grey shaded.

Ta-abGliaFor3 ATG AAG ACC TTT CTC ATC CTT GCC 24 bases Tm 63.6
Ta-abGliaRev3 TGT GGC TGC AAT TGT GGC ACT G 22 bases Tm 64.2
Frg size 92 bp

Wheat γGliadin – based on coding region insertion/deletion structure wheat γGliadins can be classified into to type 1 and type 2 allowing two type specific forward and a common reverse real time oligo nucleotide primer.

```
* Conserved nucleotide for type 1 or type 2
| conserved nucleotides for both type 1/2

                                        ******** ******* * * *****  ****************
Type 1 γGlia FJ006592 ------------AGAACAACAACAAGGCGTGCCGATCCTGCGGCCACTATTTCAGC-------------
Type 1 γGlia FJ006612 ------------AGAACAACAACAAGGCGTGCCGATCCTGCGGCCACTATTTCAGC-------------
Type 1 γGlia FJ006589 ------------AGAACAACAACAAGGCATACAGATCCTCCGGCCACTGTTTCAGC-------------
Type 1 γGlia FJ006601 ------------AGAACAACAACAAGGCATACAGATCCTCCGGCCACTGTTTCAGC-------------
Type 1 γGlia FJ006606 ------------AGAACAACGACAAGGCGTGCAGATCCGGCGGCCACTGTTTCAGC-------------
Type 1 γGlia FJ006611 ------------AGAACAACAACAAGGCATACAGATCCTCCGGCCACTGTTTCAGC-------------
                                   |          |  ||  | |  |||||    ||||| | |||||
Type 2 γGlia FJ006595 GCAGCAACAACAACAACAACAACAAGGCATGCATATCTTTCTGCCACTATCTCAGCAGCAACAGGTGGG
Type 2 γGlia FJ006603 GCAGCAACAACAACAACAACAACAAGGCATGCATATCTTTCTGCCACTATCTCAGCAGCAACAGGTGGG
Type 2 γGlia FJ006591 AG---AACAACAA---------CAAGGCATGCATATCCTGCTGCCACTATATCAGCAGCAACAGGTGGG
Type 2 γGlia FJ006604 AG---AACAACAA---------CAAGGCATGCATATCCTGCTGCCACTATATCAGCAGCAACAGGTGGG
Type 2 γGlia FJ006599 AG---AACAACAAGAACAACTACAGGGTGTGCAAATCCTGGTGCCACTGTCTCAACAGCAACAGGTGGG
Type 2 γGlia FJ006607 AG---AACAACAAGAACAACTACAGGGTGTGCAAATCCTGGTGCCACTGTCTCAACAGCAACAGGTGGG
                           ********         *****  **** *** *  ******* * *** **************
                                      ***  ** **** *** ****************************  **** ******
Type 1 γGlia FJ006592 -----------TCGCCCAGGGTCTGGGTATCATCCAACCTCAACAACCAGCTCAATTGGAGGGGATCAG
Type 1 γGlia FJ006612 -----------TCGCCCAGGGTCTGGGTATCATCCAACCTCAACAACCAGCTCAATTGGAGGGGATCAG
Type 1 γGlia FJ006589 -----------TCGTCCAAGGTCAGGGCATCATCCAACCTCAACAACCAGCTCAATATGAGGTGATCAG
Type 1 γGlia FJ006601 -----------TCGTCCAAGGTCAGGGCATCATCCAACCTCAACAACCAGCTCAATATGAGGTGATCAG
Type 1 γGlia FJ006606 -----------TCGTTCAGGGTCAGGGCATCATCCAACCTCAACAACCAGCTCAATTGGAGGTGATCAG
Type 1 γGlia FJ006611 -----------TCGTCCAAGGTCAGGGCATCATCCAACCTCAACAACCAGCTCAATATGAGGTGATCAG
                                  |||  || ||||  || || |||||||| |||||||||||||||  |||| ||||||
Type 2 γGlia FJ006595 TCAAGGTTCTCTAGTCCAAGGCCAGGGCATCATCCAACCACAACAACCAGCTCAATTGGAGGCGATCAG
Type 2 γGlia FJ006603 TCAAGGTTCTCTAGTCCAAGGCCAGGGCATCATCCAACCACAACAACCAGCTCAATTGGAGGCGATCAG
Type 2 γGlia FJ006591 TCAAGGTACTCTCGTCCAGGGCCAGGGCATTATCCAACCCCAACAACCAGCTCAATTGGAGGCGATCAG
Type 2 γGlia FJ006604 TCAAGGTACTCTCGTCCAGGGCCAGGGCATCATCCAACCCCAACAACCAGCTCAATTGGAGGCGATCAG
Type 2 γGlia FJ006599 TCAAGGTATTCTCGTCCAGGGTCAAGGCATCATCCAACCTCAACAACCAGCTCAATTGGAGGTGATCAG
Type 2 γGlia FJ006607 TCAAGGTATTCTCGTCCAGGGTCAAGGCATCATCCAACCTCAACAACCAGCTCAATTGGAGGTGATCAG
                       *******  *** ***** ** ** ***** ******** ****************  **** ******
                       ********* ***  *** ******* ******************  *
Type 1 γGlia FJ006592 GTCATTGGTGTTGCAAACTCTTCCAACCATGTGCAACGTGTATGTCCCACCTTACTGCTCCACCATCAG
Type 1 γGlia FJ006612 GTCATTGGTATTGAAAACTCTTCCAACCATGTGCAACGTGTATGTGCCACCTGACTGCTCCACCATCAA
Type 1 γGlia FJ006589 GTCATTGGTATTGAGAACCCTTCCAAACATGTGCAACGTGTATGTCCGACCTGACTGCTCCACCATCAA
Type 1 γGlia FJ006601 GTCATTGGTATTGAGAACCCTTCCAAACATGTGCAACGTGTATGTCCGACCTGACTGCTCCACCATCAA
Type 1 γGlia FJ006606 GTCATTGGTATTGAGAACTCTTCCAACCATGTGCAACGTGTATGTCTCACCTGACTGCTCCACCATCAA
Type 1 γGlia FJ006611 GTCATTGGTATTGAGAACCCTTCCAAACATGTGCAACGTGTATGTCCCACCTTACTGCTCCACCATCAG
                       |||||||| |||  ||| ||||||  |||||||||||||||||| ||||  | ||||||| |||||
Type 2 γGlia FJ006595 ATCATTGGTATTGCAAACTCTTCCATCCATGTGCAACGTGTATGTCCCACCAGAGTGCTCCATCATGAG
Type 2 γGlia FJ006603 ATCATTGGTATTGCAAACTCTTCCATCCATGTGCAACGTGTATGTCCCACCAGAGTGCTCCATCATGAG
Type 2 γGlia FJ006591 GTCATTGGTGTTGCAAACTCTTCCAACCATGTGCAACGTGTATGTCCCACCTGAGTGCTCCATCATCAA
Type 2 γGlia FJ006604 GTCATTGGTGTTGCAAACTCTTCCAACCATGTGCAACGTGTATGTCCCACCTGAGTGCTCCATCATCAA
Type 2 γGlia FJ006599 GTCATTGGTGTTGCAAACTCTTCCAACCATGTGCAACGTGTATGTCCCACCTTACTGCTCCACCATCAG
Type 2 γGlia FJ006607 GTCATTGGTGTTGCAAACTCTTCCAACCATGTGCAACGTGTATGTCCCACCTTACTGCTCCACCATCAG
                       ******** *************** *************************  * ******* *****
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Real-time PCR primer Type I and Type II wheat γ-Gliadins
(based on sequences from Wang et al. 2012, Theoretical and Applied Genetics 125, pp 1371-1384)
Type 1
TaGlia1For 5’- CCT GCG GCC ACT ATT TCA GCT C-3’ 22 bases Tm 65.8

Type 2
TaGlia2For 5’- GCA GCA ACA GGT GGG TCA AGG T -3’ 22bases Tm 65.8

Type 1/2
TaGlia1/2Rev 5’- GGG ACA TAC ACG TTG CAC ATG G -3’ 22 bases Tm 64.2
For Type I and Type 2

Alignment 5'coding region wheat omega-gliadins - conserved region (grey shaded) for identical forward primer and degenerative revers real time PCR primer sequence (data accessions: KF412579, KF412580, KF412581, KF412582, KF412583, KF412584, KF412585, KF412586, KF412587).

```
Omega-Gliadin-Cl5      ATGAAGACCTTCCTCATCTTTGTCCTCCTTGCCATGGCGATGAACATCGCCACTGCTGCT
Omega-Gliadin-Cl8      ATGAAGACCTTTCTCATCTTTGTCCTCCTTGCCATGGCGATGAAGATCGCCACTGCCGCT
Omega-Gliadin-Cl3      ATGAAGACCTTCCTCATCTTTGTCCTCCTTGCCATGGCGATGAAGATCGCCACTGCCGCT
Omega-Gliadin-Cl6      ATGAAGACCTTTCTCATCTTTGTCCTCCTTGCCATGGCGATGAAGATCGCCACTGCCGCT
Omega-Gliadin-Cl1      ATGAAGACCTTTCTCATCTTTGTCCTCCTTGCCATGGCGATGAACATCGCCACTGCTGCT
Omega-Gliadin-Cl2      ATGAAGACCTTCCTCATCTTTGTCCTCCTTGCCATGGCGATGAAGATCGCCGCTGCCGCT
Omega-Gliadin-Cl7      ATGAAGACCTTCCTCATCTTTGTCCTCCTTGCCATGGCGATGAAGATCGCCACTGCCGCT
Omega-Gliadin-Cl4      ATGAAGACCTTTCTCATCTTTGTCCTCCTTGCCATGGCGATGAACATCGCCACTGCTGCT
                       *********** ******************************** ****** **** ***

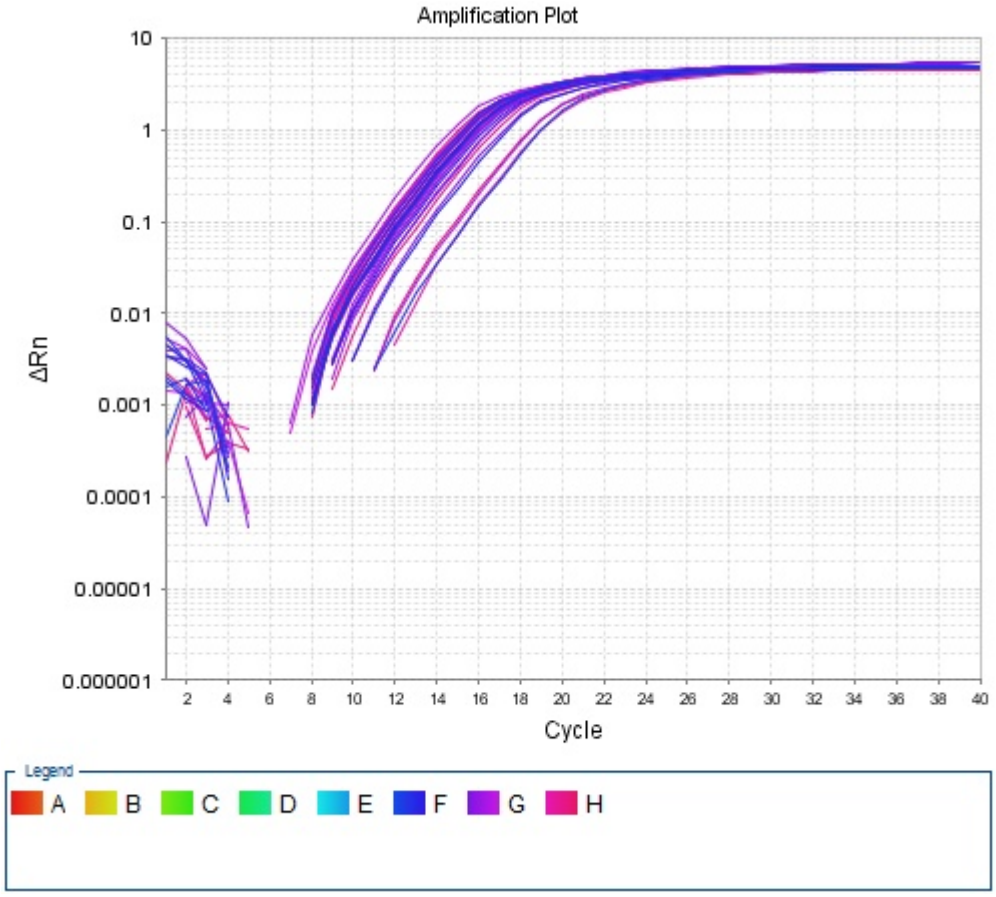
Omega-Gliadin-Cl5      AGGCAGCTAAACCCTAGCAACAAAGAGCTACAATCACCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl8      AGGGAGTTAAACCCTAGCAACAAAGAGTTACAATCACCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl3      AGGGAGTTAAACCCTAGCAACAAAGAGTTACAATCACCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl6      AGGGAGTTAAACCCTAGCAACAAAGAGTTACAATCACCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl1      AGGCAGCTAAACCCTAGCAACAAAGGGCTACAATCACCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl2      AGGGAGTTAAACCCTAGCAACAAAGAGTTACAATCACCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl7      AGGGAGTTAAACCCTAGCAACAAAGAGTTACAATCGCCTCAACAATCATTTTCCCATCAA
Omega-Gliadin-Cl4      AGGCAGCTAAACCCTAGCAACAAAGAGCTACAATCACCTCAACAATCATTTTCCCATCAA
                       *** ** ***************** * ******* ************************
```

ATG start codon 5' coding

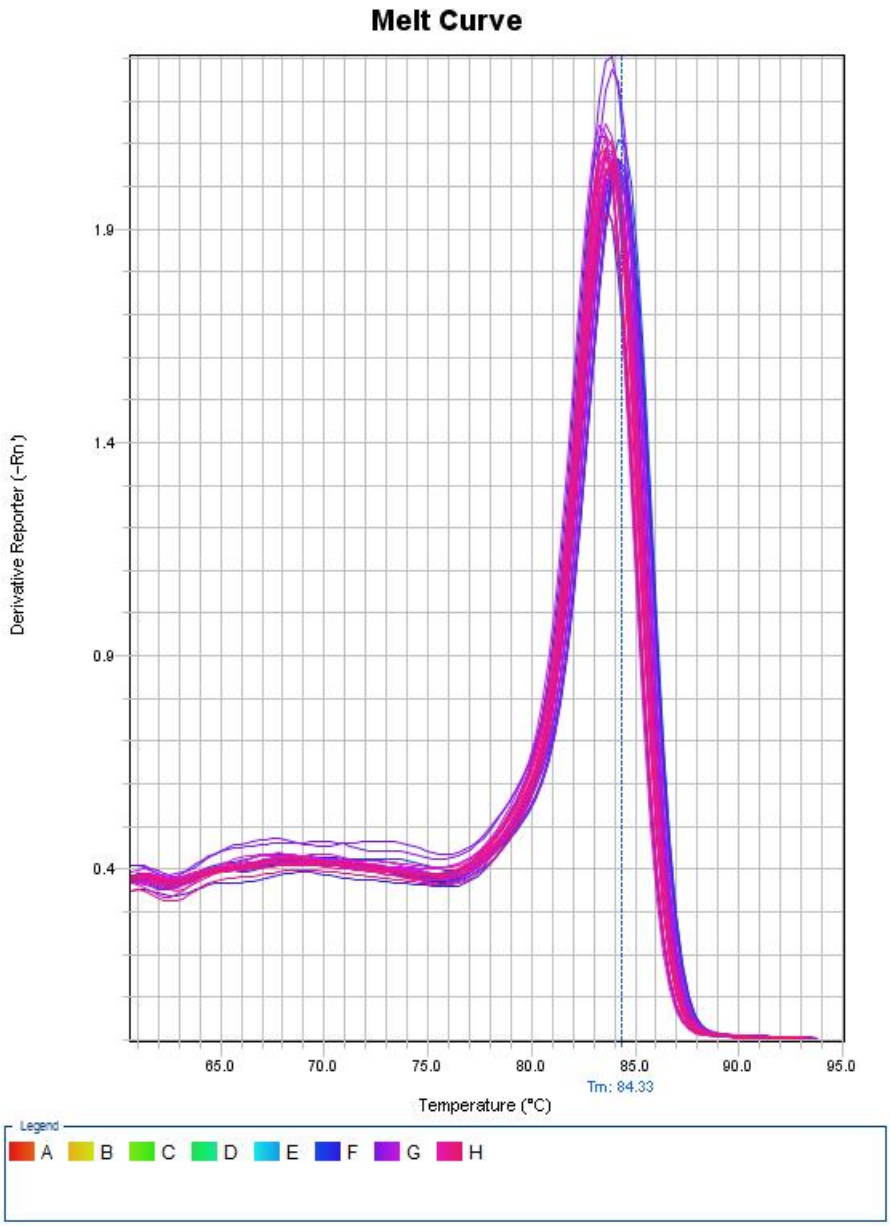
Ta-OmegaGliaFor1 TGT CCT CCT TGC CAT GGC GAT G 22 Bases Tm 65.8
Ta-OmegaGliaRev1 TGA GGT GAT TGT ARC TCT TTG TTG C 25 bases Tm 62.5-64.1

Frg size 80 bp

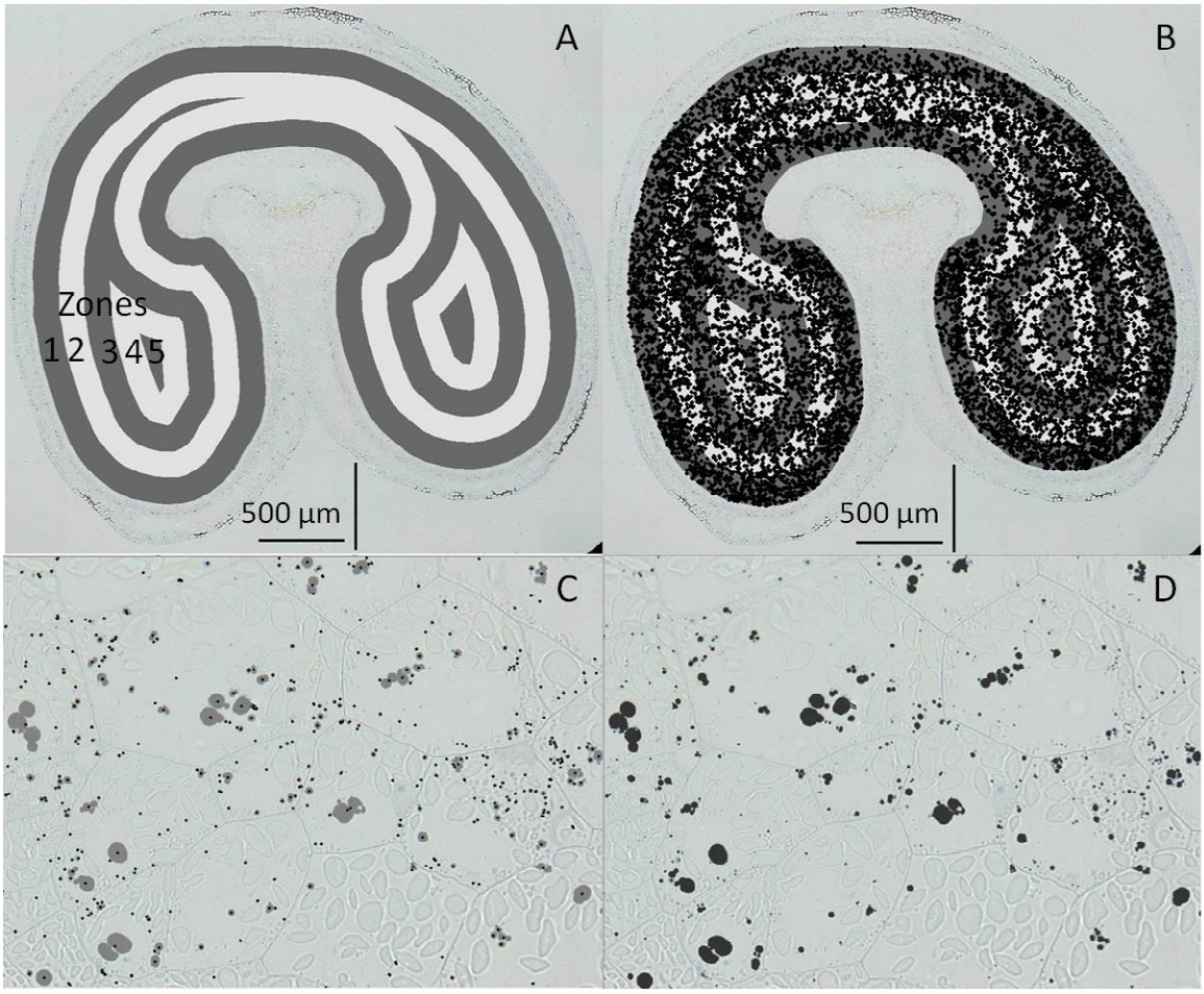
Example for amplification plot and melting curve:



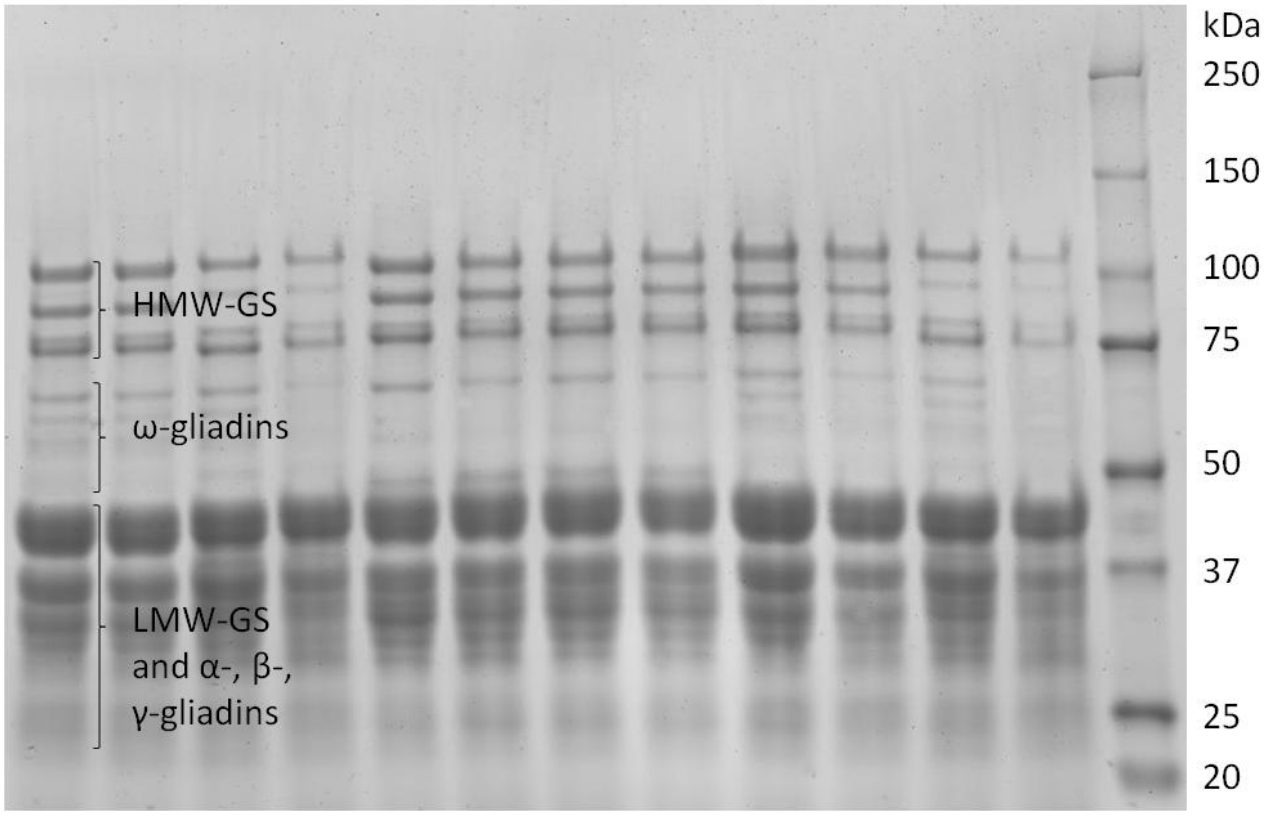
Amplification plot for LMW-GS in Skyfall at N100



Melting curve for LMW-GS in Skyfall at N00



Overview of the grain protein gradient analysis. (A) A composite light-microscopy image of a 1µm thick wheat grain cross section with five concentric zones from the outer to the inner endosperm (labelled 1-5). (B) The zones from image (A) are overlaid on the detected protein bodies. (C) and (D) show the same region of a grain. (C) Detected protein bodies were marked with a central dot. (D) For the analysis of protein body size distribution, the area and distance from the aleurone layer of protein bodies was measured.



A representative SDS-PAGE gel image with marked protein groups that have been identified according to Wan et al. (2014).