Transcriptomic analysis of metabolic resistance in two F2-blackgrass populations segregating for resistance to ALS- and ACCase-inhibitors

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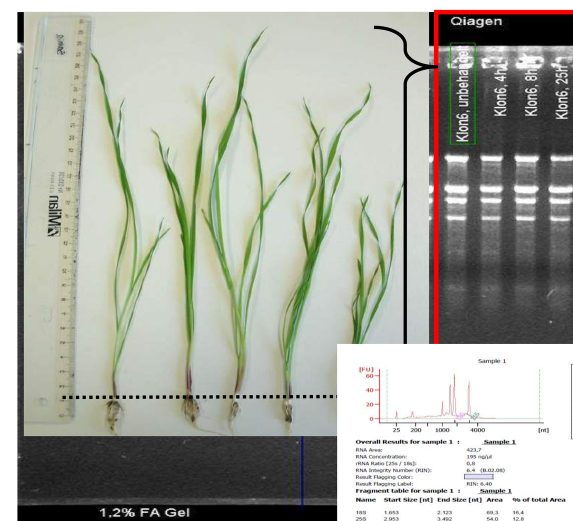
1. Introduction:

In order to identify candidate genes for metabolic herbicide resistance in blackgrass (*Alopecurus myosuroides*) a transcriptomics approach was chosen to determine genome-wide gene expression in green leaves of F2 plants which derived from single plant crosses of a sensitive wild type with two highly resistant blackgrass biotypes from Germany showing different resistance patterns to herbicides with different modes of action. The resistant parents were check for the absence of known target site mutations for ALS and ACCase. The two segregating F2-populations obtained were phenotypically assessed for resistance against ACCase- (fenoxaprop-P-ethyl) and ALS-inhibitors (mesosulfuron & iodosulfuron). Sensitive as well as the most resistant F2-individuals were pooled respectively and used for a bulked segregant analysis of gene expression by means of a 3´-specific next generation sequencing technique called MACE (= **m**assive **a**nalysis of 3´-**c**DNA **e**nds) on an Illumina Hiseq2000 with 1 x 100 bp reads.

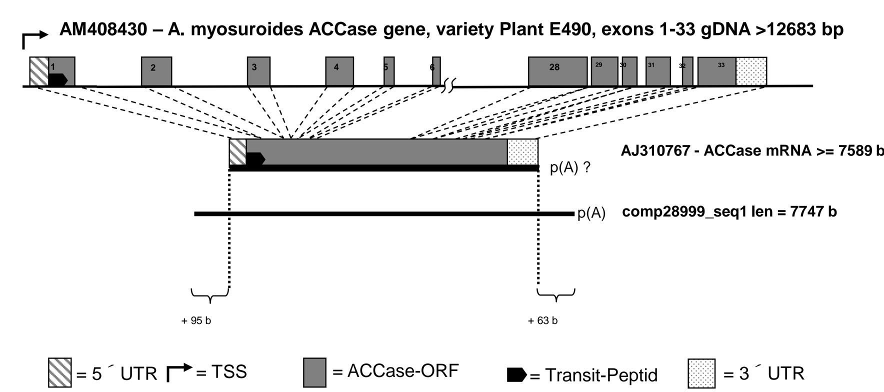
2. Reference transcriptome:

A reference transcriptome for blackgrass was established by means of a paired-end (2 x 100 bp) RNA-Seq protocol and four normalized RNA templates prepared from leaves of a metabolic resistant biotype. From a selected single resistant plant four two months old vegetative clones were chosen (one control, three after 4 h, 8 h & 25 h after treatment with Iodosulfuron + Mesosulfuron). Quality-checked and processed data comprised approx. 105 Mbp finally resulting in 51609 contigs which could be annotated to the SWISSPORT database.



- RNASeq (4x): control, 4h, 8h, 25 h normalized cDNAs
- Assembly: (control, 4h, 8h & 25 h) combined
- Longest Contig: 22033 bps
- Shortes Contig: 151 bps
- Mean Contig lenght: 803 bps
- All Contigs found: 130373
- Contigs identified: 51609 (Swissprot DB)

Context	Gene(super)-family	Contigs
TSR	Acetyl-CoA-Carboxylase	21
	Acetolactate-Synthase	7
MHR	ABC-Transporter	303
	Cytochrom P450 Monoxygenase	347
	Glutathion-S-Transferase	148
	Glutathion Peroxidase	9
	Peroxidase	207
	Glykolsyl-Transferase	216
Divers	Actin (inkl. „actin-related“)	22 (50)
	Elongation Factor 1-alpha	22
	Chlorophyll a-b binding protein (CAB)	27
	Ribulose bisphosphate carboxylase small chain, rbcS	2
	Receptor-like Protein Kinase	2855
	Transcription factor	1066

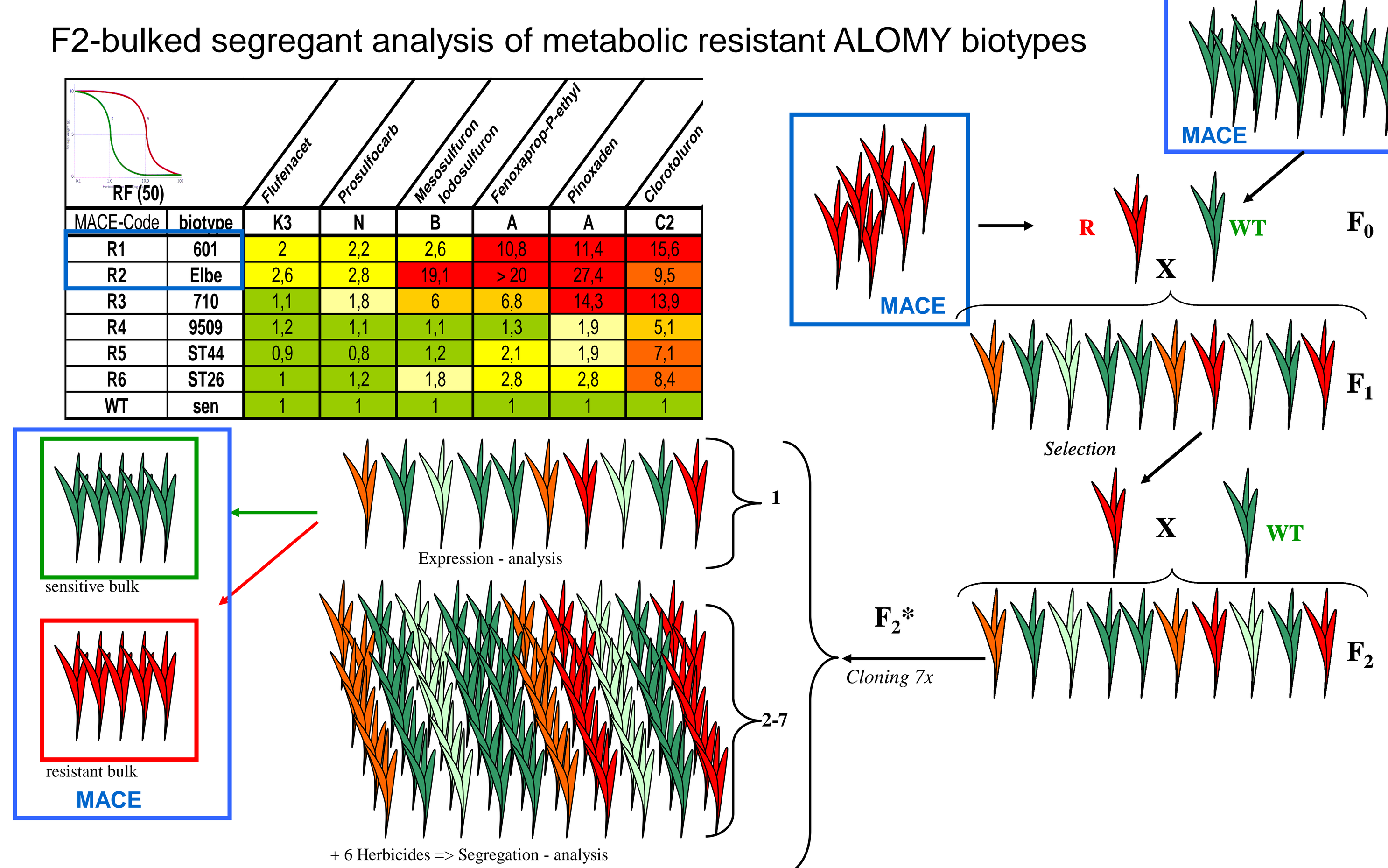


GSTs	ALOMY Contigs	Rice Gene	A.t. Gene
GSTF (Phi)	21	16	13
GSTU (Tau)	39	52	28
GSTT (Theta)	18	1	3
GSTZ (Zeta)	6	4	2
GSTL (Lambda)	1	3	1

3. Bulked segregant analysis of two F2 populations and MACE:

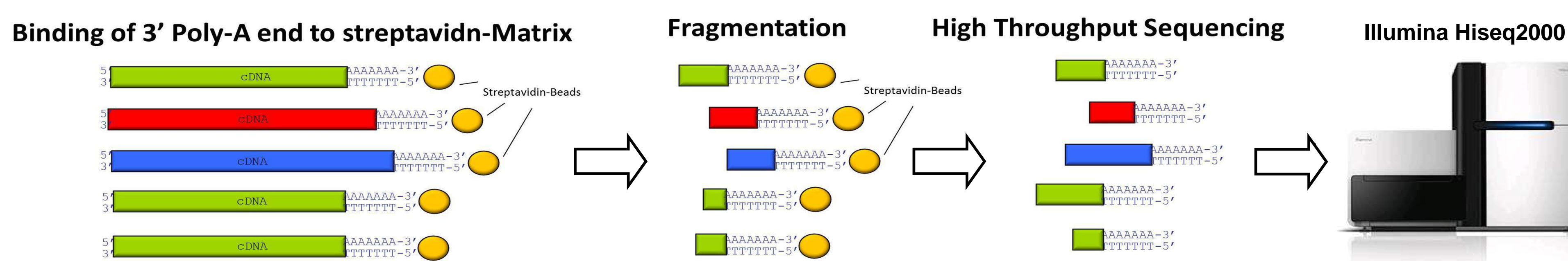
Out of 2 offsprings (n= 200) respectively leaf samples from the most resistant and sensitive F2-individuals were pooled and total RNAs were used for the construction of 3´-specific **M**assive **A**nalysis of **c**DNA **E**nds (MACE) libraries. Appr. 7 - 25 million MACE-reads corresponding to the same number of transcripts were obtained by means of Illumina HiSeq2000 sequencing. Quality checked MACE reads were mapped to the reference transcriptome for identification of the corresponding transcripts. Gene expression levels were determined by counting the frequencies of MACE reads found for each contig in the different metabolic resistant parental biotypes, the sensitive wild type and the derived bulks and normalized to “reads per million”.

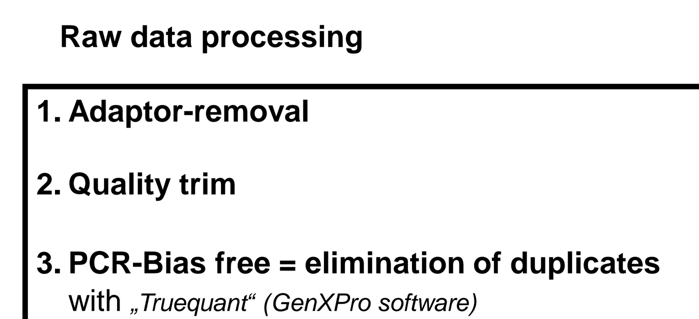
F2-bulked segregant analysis of metabolic resistant ALOMY biotypes

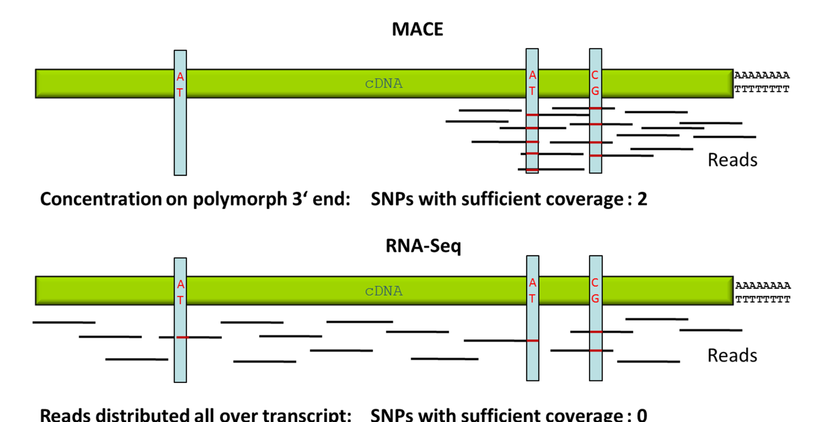


RNASeq	ALOMY	Phenotype	# reads
Parents	R1	MHR	7241528
	R2	MHR	12976726
	WT	sensitive	10731780
F2-Bulks	R1-r	ACCase res. (1)	9260415
	R1-s	sensitive	6495252
	R2a-r	ACCase res. (2)	25405504
	R2b-r	ALS res. (3)	12901536
	R2c-r	ACCase-ALS-PSII (4)	17307530
	R2-s	sensitive	9909368

(1) FenoxapropP-ethyl
(2) FenoxapropP-ethyl, Pinoxaden
(3) Ido-/Mesosulfuron
(4) Chlortoluron



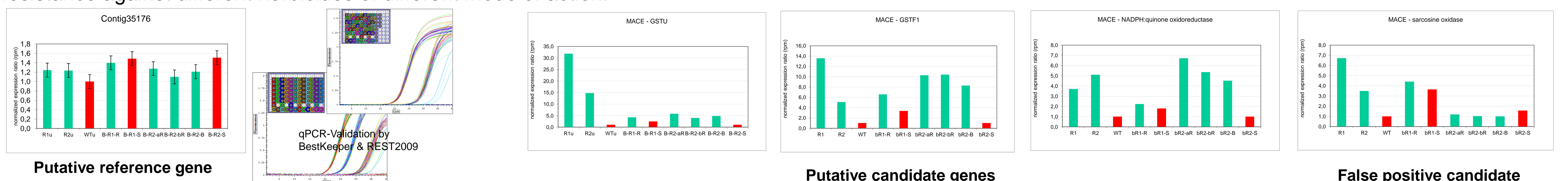




Schematic representation of MACE (massive analysis of cDNA ends) and comparison with standard RNASeq

4. Preliminary expression results and outlook:

By means of comparison of gene expression levels in sensitive and resistant bulks as well as in their corresponding resistant and sensitive parents putative candidate genes involved in detoxification pathways can be detected and may correlate with the metabolic resistance phenotype. Candidate genes involved in herbicide resistance are currently tested by qPCR using the original set of individual RNA samples as well as on a broader set of F2-individuals exhibiting varying degrees of metabolic resistance against different herbicides of different mode of action.



Putative reference gene **Putative candidate genes** **False positive candidate**

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