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Re-annotation of the maize oligonucleotide array

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Abstract

The microarray technology has become an established approach for large-scale gene expression analysis with mature protocols for sample, microarray, and data processing. The maize oligonucleotide array (maizearray) is one of the few microarray platforms designed for genome-wide gene expression analysis in *Zea mays* L. Many datasets addressing various genetic, physiological and developmental topics generated with this platform are available. The original 57,452 microarray probes were compiled based on expressed sequence tags (ESTs). Meanwhile the maize genome sequence became available providing the possibility for an improved annotation of the microarray probe set. In this study we determined the genome positions of all maizearray probes to obtain current gene annotations and generated current Gene Ontology (GO) annotations. These new data allow tracing redundancy of the probe set and interfering cross-hybridizations, and doubled the number of genes with functional GO data. Our re-annotation will largely improve the functional analysis of available and future datasets generated on this microarray platform.

Keywords: maize oligonucleotide array, microarray, annotation

Introduction

DNA microarrays have become a mature technology for gene expression analysis since their introduction in 1995 (Shena et al, 1995). DNA microarrays are generated by synthesizing or printing and cross-linking DNA elements (oligonucleotides) to a solid surface. These DNA elements serve as probes for hybridization with fluorescent-labeled cDNAs or RNAs generated from complex transcript samples (Phimister, 1999). There are few microarray platforms specifically for genome-wide gene expression analysis in maize including amongst others the Affymetrix Maize Genome Array (www.affymetrix.com), various custom Agilent arrays (Ma et al, 2006; Ma et al, 2008; Hayes et al, 2010), the Affymetrix Maize CornChip0 (Kirst et al, 2006), a custom NimbleGen array (Sekhon et al, 2011), and the 57K and 46K arrays from the Maize Oligonucleotide Array Project (Gardiner et al, 2005).

The maize oligonucleotide array is a long-oligonucleotide (~70 nt) microarray platform. The microarray was designed by the "maize oligonucleotide array project", which was carried out by a collaboration of the University of Arizona, The Institute for Genomic Research (TIGR), and the University of Wisconsin. The initial maizearray called 57K comprising 57,452 probes was printed on a two slides array set. The probes of the 57K maizearray were revised from oligonucleotides without expression or with hybridization problems. The reduced set comprising 43,536 oligonucleotides was distributed as single-slide microarray named 46K maizearray. The collaboration offered a large number of low cost microarray slides to the academic maize science community along with established protocols for sample processing and hybridization. The microarray platform was designed in absence of the maize' whole genome sequence based on expressed sequence tags (ESTs), the TIGR Assembled Zea mays (AZMs), and additional sequences from repeated elements, chloroplast and mitochondria (Gardiner et al, 2005). Currently there are 32 experiments generated on the maizearray accessible in NCBI GEO (Barrett et al, 2009), listed in Table 1.

The available annotation version v4 of the maizearray (www.maizearray.org) was also prepared before the release of the maize B73 genome. Although the microarray probes were designed to represent expression information of single genes this could not be ensured solely based on EST data and thus might not be the case for all of the probes. Multiple gene copies, alternative splicing forms of one gene and other cross-hybridization events may interfere with the gene expression information measured from probes designed without all relevant information.

We performed a new annotation of all the 57K maizearray probes by mapping the oligonucleotide sequences to the current maize genome assembly. With these data we can conclude if probes measure expression of single or multiple genes and/or loci respectively. In addition to the mapping we obtained gene information for the loci that are represented by the oligonucleotide. Based on those genes we gathered functional information by retrieving gene ontology (GO) terms using Blast2GO (Conesa and Gotz, 2008). Although the maizearray production was

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Table 1 - Published experiments generated on the 46K or 57K maizearray

GEO accession	Number of hybridizations	Array version	Year of publication	Authors	Field of research
GSE3890	24	57K	2007	Sawers et al	leaf development photosynthesis
GSE9341	16	57K	2008	Spollen et al	root development / water stress
GSE9352	16	57K	2008	Spollen et al	root development / water stress
GSE9369	16	57K	2008	Spollen et al	root development / water stress
GSE9379	48	57K	2008	Spollen et al	root development / water stress
GSE9386	24	57K	2008a	Liu et al	kernel development
GSE9453	64	57K	2008	Fernandes et al	stress response
GSE9698	24	57K	2008	Covshoff et al	photosynthesis
GSE10308	64	57K	2008	Maron et al	aluminium stress
GSE10449	4	57K	2008	Holding et al	opaque2 modifier
GSE10542	27	46K	2008	Stupar et al	heterosis
GSE10543	24	46K	2008	Stupar et al	heterosis
GSE10544	108	57K	2010	Morrison et al	nitrate reductase regulation
GSE10596	8	57K	2008	Yue et al	water stress
GSE11145	4	57K	2008b	Liu et al	root development / nitrate
GSE14728	96	57K	2009	Hayano-Kanshiro et al	water stress
GSE15853	36	57K	2010	Barros et al	comparison transgenic / non-transgenic plants
GSE17484	68	46K	2010	Soos et al	germination / stress
GSE17754	63	46K	2010	Thiemann et al	heterosis
GSE17932	16	57K	2010a[$]	Moose and Boddu	developing earshoot
GSE17953	32	57K	2010b[$]	Moose and Boddu	developing earshoot
GSE17971	22	57K	2009[$]	Moose and Zhao	leaf development
GSE18006	26	57K	2010[$]	Moose and Zhao	seed nitrogen metabolism
GSE18008	24	57K	2009[$]	Moose and Ayodeji	developing earshoot
GSE18011	37	57K	2009[$]	Moose and Church	nitrogen response
GSE19883	16	46K	2011	Luo et al	pathogen resistance
GSE24014	6	46K	2011	Bosch et al	cell wall biogenesis
GSE25526	45	57K	2010	Riddle et al	ploidy / hybridity
GSE27709	9	46K	2011	Johnson et al	pest resistance
GSE29132	36	46K	2012	Moriles et al	stress response
GSE33494	18	46K	2011[$]	Hansen et al	water stress
GSE36368	9	46K	2012[$]	Zhaoxia et al	root development / phosphate

[$]publication of the dataset only

closed recently, we expect our annotation to be of high value for currently ongoing experiments and re-analysis of existing datasets.

All data of the new annotation are assembled in one Supplemental Table and are accessible online.

Methods

Localization of the oligonucleotides and identification of their respective genes

The oligonucleotide sequences of the initial 57K maizearray, with all probes of the 46K maizearray included, were mapped to the maize genome to obtain genome positions for an association to specific gene loci. The whole procedure is outlined in Figure 1. The mapping of the oligonucleotides was performed on the maize genome sequence B73

RefGen v2 from ftp.maizesequence.org using BLASTn from standalone BLAST 2.2.26+ (Camacho et al, 2009) with a maximum e-value of 0.0001 and word-size set to 20. All matches with more than 3 mismatches, insertions or deletions (indels) were rejected from further analysis. These stringent parameters were chosen to comply to stringent hybridization conditions. All genes associated with full-length oligonucleotide matches were annotated by searching the maize „working gene set" (WGS) version 5a.59 GFF3 gene annotation (exon, intron) and the repeat/transposon annotation of the TE Consortium (ZmB73_5a_MTEC+LTR_repeats.gff) both datasets from ftp.maizesequence.org. The annotation comprises a search for exon, intron and repeat bodies with identical positional information in respect of chromosome and overlapping genome position for both strands with the mapping results. Alignments of oligonucleotides on the genome sequence that were shorter than the oligonucleotide length were analyzed for a second fragment within 20,000 bp on the same strand. This threshold was chosen to equal the maximum length of maize introns (Schnable et al, 2009). The summed up length of both fragments needed to reach at least the length of the oligonucleotide minus 10 bp to be taken into account for a following analysis for oligonucleotides overlapping splicing sites. In addition all matches covering at least the length of the oligonucleotide minus the BLASTn word-size of 20 and the maximum 3 indels/mismatches were taken into the analysis for oligonucleotides potentially overlapping a splicing site as well. For this subset BLASTn would miss the second fragment by the given minimum word-length. This splice-candidate oligonucleotide set was mapped to the maize cDNA sequences from the WGS dataset version 5a.59 using BLASTn to obtain gene annotation for oligonucleotides overlapping splice junctions. All settings and match criteria were identical to the initial mapping procedure. As microarray probes are intended to represent the expression of a single gene, we analyzed the re-annotation for information about number of genes per oligonucleotide, number of transcripts for genes or number of copies for repeats respectively as well as the number of oligonucleotides that represent a gene or repeat.

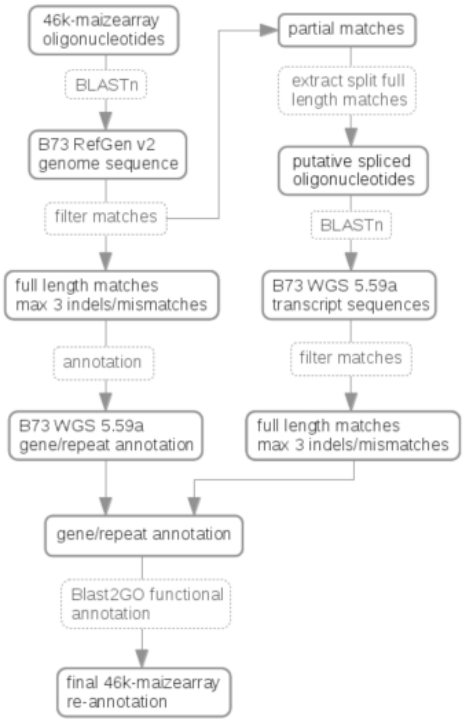

Figure 1 - The maizearray oligonucleotide sequences were mapped to the B73 Refgen v2 genome using BLASTn. Full-length matches were annotated using the B73 WGS 5.59a gene and repeat annotations for both sense and anti-sense strand. Partial matches that reassemble the oligonucleotide sequence were mapped to the B73 WGS 5.59a transcript sequences using BLASTn. All full-length matches are annotated by the transcript. The functional re-annotation of these datasets was performed using Blast2GO.

Functional annotation using Blast2GO

The functional annotation of the maizearray was conducted with Blast2GO version 2.5.1 that obtains sequence annotation data by a three-step approach. The first step is searching for homologous gene products in the NCBI non-redundant protein sequences (nr) database using BLASTx. All sequences with successful identification of homologous protein sequences are forwarded to a mapping step that collects GO-terms that are assigned to the blast hits and a final annotation by scoring the obtained GO-terms (Conesa and Gotz, 2008). The Blast2GO analysis was performed for all genes identified in the localization of the oligonucleotides except the repeated sequences. The analysis was performed with Blast2GO default settings using the most recent Blast2GO PRO database b2g_apr12.

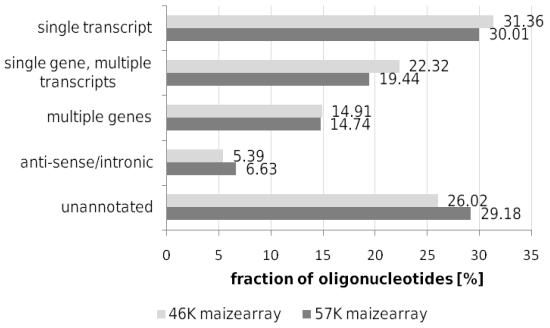

Figure 2 - Oligonucleotide gene annotation results. Distribution of annotations to both maizearray platforms. Half of annotations of oligonucleotides of both arrays allot to single genes with a larger fraction representing single transcripts and a smaller set of multiple transcripts of one gene. Nearly 15% of all oligonucleotides of both arrays correspond to multiple genes. The smallest number of oligonucleotides maps anti-sense to genes or intronic regions. One-third of all oligonucleotides remains unannotated.

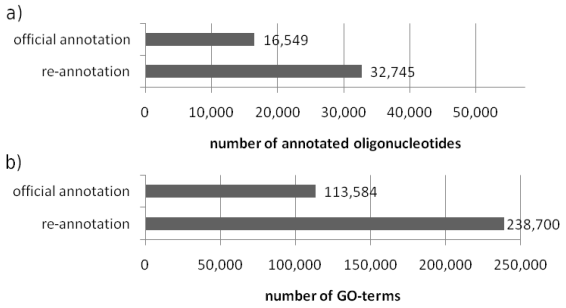

Figure 3 - GO-annotation results. a) The number of oligonucleotides of all maizearray probes with GO-annotation of our re-annotation could be two-fold increased compared to the official annotation. b) The total number of GO-terms obtained for the maizearray by the functional re-annotation was raised by factor 2.1.

Results

Localization of the oligonucleotides and identification of genes

The initial BLASTn-search of the oligonucleotide sequences to the maize genome sequence resulted in 3,376,312 alignments. 1,339,243 of these alignments covering 48,756 (84.86%) distinct oligonucleotides fulfilled the threshold of maximum 3 indels or single nucleotide mismatches. The filtering for full-length matches resulted in 584,589 alignments corresponding to 37,351 (65.01%) of all oligonucleotides. 9,962 of these oligonucleotides matched to multiple gene loci. 214,386 fragmented or partial alignments corresponding to 10,873 distinct oligonucleotide sequences were filtered as candidates potentially overlapping splice sites.

The annotation of the full-length alignments using the maize WGS 5a.59 GFF3 gene annotation results in 40,975 exons that are covered by 30,733 distinct oligonucleotides. A set of 13,014 intronic gene regions is represented by 5,405 oligonucleotides. The mapping of the fragmented oligonucleotides to the B73 WGS transcripts for the discovery of oligonucleotides overlapping splice sites resulted in 7,125 transcripts represented by 5,355 distinct oligonucleotides that were confirmed as oligonucleotides spanning an intronic region. The annotation of oligonucleotides to the anti-sense strand of genes resulted in 5,103 exons covered by 5,101 oligonucleotides and 8,049 introns covered by 2,099 oligonucleotide sequences. The annotation of the oligonucleotides to the repeat position data from the TE Consortium resulted in 3,306 repeated or transposable elements covered by 1,190 oligonucleotide probes. The corresponding anti-sense repeat annotation resulted in 1,796 repeats covered by 673 distinct oligonucleotides. The annotation results are illustrated in Figure 2.

In total 40,692 (70.82%) of all oligonucleotides on the 57K maizearray and 32,210 (73.98%) on the 46K maizearray respectively, were annotated to at least one known gene or repeat including annotations of introns as well as oligonucleotides oriented to the anti-sense strand of genes. 36,881 (64.19%) of the 57K array probes and 29,861 (68.59%) of the 46K maizearray oligonucleotides respectively reflect at least one gene (exon, spliced exon, repeat). 28,408 (49.45%) of the 57K and 23,369 (53.68%) of the 46K oligonucleotides account the expression of a single gene. 17,241 (30.01%) of the 57K array and 13,654 (31.36%) of the 46K array oligonucleotides respectively could be furthermore assigned to a single splice-form of the gene. The numbers of these results are summarized in Table 2.

Functional re-annotation

The Blast2GO annotation of all oligonucleotides that were associated to a gene resulted in 47,562 annotated genes covered by 32,745 distinct oligonucleotides corresponding to 57.00% of the microarray probes. 38,144 GO-term annotations collate to 30,546 distinct transcript-associated (exon/spliced

Table 2 - Total and relative annotation results for both microarrays

	57K maizearray	46K maizearray
unannotated	16.760 (29.18%)	11.326 (26.02%)
anti-sense/intronic	3.811 (6.63%)	2.349 (5.39%)
multiple genes	8.473 (14.74%)	6.492 (14.91%)
single gene, >1 transcript	11.167 (19.44%)	9.715 (22.32%)
single transcript	17.241 (30.01%)	13.654 (31.36%)

exon) oligonucleotides representing 53.17% of the whole set. The total number of annotated GO-terms is 238,700 resulting in 5.92 GO-terms per annotated oligonucleotide. The GO-annotation results are summarized in Figure 3.

Discussion

Localization of the oligonucleotides and identification of genes

The localization of the oligonucleotide sequences on the B73 genome resulted in a large number of alignments for nearly 86% of all microarray probes. The unmapped oligonucleotides were most probably designed from ESTs that have been sequenced from other maize lines than B73 and that are lacking in B73 or exhibit a large number of indels or SNPs that exceeded the mapping thresholds. A large number of oligonucleotides matched to multiple genome loci. These oligonucleotides are expected to account for repeats and genes that were multiplied due to transposition or genome duplication events that maize has undergone (Schnable et al, 2009).

The annotation of oligonucleotides mapping to introns as well as the anti-sense strand of genes revealed oligonucleotides that were not optimally positioned during the microarray design and thus are not able to reflect intended gene expression. The anti-sense to the protein-coding genes located oligonucleotides are assumed to either may have arisen from miss-orientation of the ESTs which the oligonucleotides were designed from or cover natural anti-sense transcripts (NATs) (Jin et al, 2008). The oligonucleotide probes located anti-sense to transposons and repeated elements are able to reflect intermediate products by the RNA interference pathway (Ito, 2012) that will be detected depending on the hybridization-sample preparation. Our re-annotation resulted in a slightly lower number of annotated genes compared to the current official annotation but the localization to the maize genome and knowledge about cross-hybridization allows a more precise analysis of gene expression data obtained from the maizearray. Here we show that 30,655 (53.36%) of the 57K probes and 23,262 (53.43%) of the 46K probes of all oligonucleotides correspond to a single gene, demonstrating the high information content in the expression data generated on the maizearray.

Functional re-annotation

The current official annotation of the maizearray version 4 provided by the collaboration covered 43,381 gene-associated oligonucleotides and comprised a GO annotation for 16,549 of these oligonucleotides with a total of 113,584 GO-terms. This implies an average annotation of 6.86 GO-terms per oligonucleotide. As a consequence of our re-annotation, the number of GO-annotated gene-associated oligonucleotides was increased nearly by factor two, the average number of GO-terms per annotated oligonucleotide was slightly decreased.

The analysis of high-throughput experiments as microarrays aim to discover key genes responsible for a certain process or condition. The Gene Ontology Consortium provides a uniform, dynamic, and controlled vocabulary represented in GO-terms for gene function, localization and biological processes for all eukaryote species (The Gene Ontology Consortium 2000). This standardized nomenclature is applied by a large number of tools e.g. for ontology visualization (Day-Richter et al, 2007; Carbon et al, 2009), or enrichment analysis (Gentleman et al, 2004; Alexa et al, 2006). In many microarray experiments a subset of genes with similar expression pattern is analyzed by GO-term enrichment that finally results in a set of overrepresented gene functions. Our functional annotation enables to perform GO enrichment analyses covering more genes of the maizearray than the current available annotation and thus allows more accurate and detailed GO enrichment analyses. By integrating the redundancy information obtained by the oligonucleotide localization these analyses will furthermore be less biased.

Conclusion

We performed a re-annotation of the maizearray by mapping the microarray probe sequences to the B73 maize genome and annotating them to the known genes and repeats, resulting in an annotation for 70.82 % of all oligonucleotides of the 57K maizearray and 73.98% of the 46K array respectively. A subsequent functional re-annotation using Blast2GO nearly doubled the number of oligonucleotides with functional information. Our newly generated annotation allows a more precise analysis of gene expression based on the microarray platform by knowing the genes that account for a certain expression. The additional information about cross-hybridization of multiple genes to oligonucleotides allows an assured analysis of gene expression and the elimination of ambiguous expression values. Our annotation with Blast2GO results in nearly twice as much genes with gene ontology function annotation. Together, these data will enable more precise downstream functional characterization of expression data generated on the maizearray platforms. We expect our re-annotation to be of high value for the maize community by supporting the re-analysis of previously published data and ongoing analyses based on either the 46K or the 57K maizearray.

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