GEMtf - Database of Time-course Expression Profiles of Maize Transcription Factors by Using Biclustering Algorithm

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Abstract—Maize (Zea mays) is known to have the highest yield of grain crops and provides resources for food, fodder, chemicals, and biofuel. As a major crop, its genetics is well studied and its genome has been sequenced. Moreover, maize has been used as a model C4 plant. However, few studies of its regulatory network of leaf development have been conducted, although the regulation of Kranz anatomy development by transcription factors (TFs) is crucial for understanding maize leaf development.

A previous study has obtained 22 time-course transcriptomes of leaf development, starting from dry seeds to hour 192 post imbibition, including gene expression profiles for 1574 maize transcription factors (TFs). This is an excellent dataset for investigating the transcriptome dynamics of leaf development.

In gene regulation, different time frames correspond to sets of genes that participate in different biological functions. Biclustering is a technique which allows simultaneous clustering of gene expression profiles and developmental times; hence, it provides a better way for identifying genes with multiple functions.

We made use of the biclustering analysis tool, BiGGEsTS, to cluster the gene expression profiles of the 1574 maize TFs, revealing the process of maize leaf development features. A web-based service was set up to provide the results of our analysis.

Index Terms - Maize; Transcription factor; NGS; Time course microarray, Biclustering algorthm

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I. Introduction

Maize (*Zea mays*) is one of the most important grain crops in the world and, together with rice and wheat, provides food to more than half of the world population. It is also a key ingredient in animal feedstock. The demand for maize has increased in recent years because of its increasing use in the bio-energy industry. Moreover, maize is an outstanding model for research on seed development, and as a model system to study diverse biological phenomena, including transposons, paramutation, heterosis, imprinting and genetic diversity and allelic richness [1].

The expression dynamics of maize genes during leaf development have been studied. For instance, dramatic differences were found in the transcriptomes of the base of developing leaves with genes encoding enzymes involved in cell-wall biosynthesis, cell division, cellulose synthesis and auxin signaling and the tip was enriched in genes involved in photosynthesis and sugar metabolism/transport of developing leaves [2]. Pick's study [3] suggested that the *Ala* and *Asp*amino acids play an important role for metabolites shuttle between mesophyll and bundle sheath in maize leaves. Wang et al. [4] investigated the transcriptomes from maize foliar (with Kranz anatomy) and husk leaves (without Kranz anatomy) to identify cohorts of genes associated with procambium initiation and vascular patterning. Also, their work provides clues for finding possible key regulators of Kranz anatomy [4]. A more recent study examined maize earlier leaves developmental stages, embryonic leaf primordia of different times and displaying different levels of Kranz structure (KS) differentiation; the data provides an opportunity for hypothesizing the regulatory actions of KS development [5].

Liu et al. [5] showed that the expressed profiles of 1,238 maize TF genes can be classified into 16 co-expression clusters, which show the functional transition and gene expression pattern over a period of 72 hours.

Traditional clustering algorithms (e.g., K-means, hierarchical clustering) perform clustering based on one feature; for instance, those methods identify gene clusters under a given condition. Many clustering approaches have been proposed for the analysis of gene expression data, but the results from the application of traditional clustering methods are limited. In reality, genes bear different functions under different conditions or times. In order to measure the similarity between gene expression levels under different conditions, biclustering algorithms were employed in the present analysis.

Biclustering algorithms that specifically perform simultaneous clustering on the row and column dimensions of the data matrix have been proposed for biological data analysis [6]. Traditional clustering algorithms apply respectively to either the rows or the columns of the gene expression profiles, but biclustering performs clustering in these two dimensions simultaneously. This means that the traditional clustering approach derives a global model, while biclustering produces a local model to identify subgroups of genes and subgroups of conditions, by performing simultaneous clustering of both rows and columns of the gene expression profiles (Figure1).

Biclustering on biological applications have been applied in many studies. Gu and Liu [7] developed a Bayesian biclustering model (BBC) that can correctly evaluate diverse clusters of gene expression data and achieved a Gibbs sampling procedure for its statistical inference, such as enrichments of gene functions and transcription factor binding sites (TFBSs) in the relevant promoter sequences. Eren et al. [8] provided an excellent review on a collection of biclustering methods on analyzing gene expression data matrices. Yu et al. [9] combined the TFs' up and down expression signatures with the Gene Ontology (GO) annotations and the TF promoter motif contents to predict novel TFBSs and new TF–TFBS pairs in the maize genome.

The present work constructed a web site that utilizes a visualization interface to help researchers to view sets of time-course profiles for TF genes involved in the maize leaf development. To the best of our knowledge, this database is the first comprehensive database on the time-course expression profiles for maize TFs.

II. Materials & Methods

A. Materials

This study made use of the expression profiles of the 1574 TFs involved in maize leaf development, where the data were obtained from the Supplementary Dataset S1 file in Ref. [9]. To understand the regulation of the TFs in maize Kranz anatomy, they obtained 22 time-course transcriptomes of maize leaves developing from dry seeds to 192 hours post imbibition. Pair-end sequenced data were conducted by using the Illumina HiSeq 2000 machine. The RPKM(reads per kilobase of transcript per million mapped reads) values of gene expression were normalized, and kept RPKM value is ≥1 in at least 2 of the 22 time points in our analysis [9]. TFs in this category may play a key role in the differentiation of vascular cells and the development of Kranz anatomy.

The workflow of the present is depicted in Figure 2.

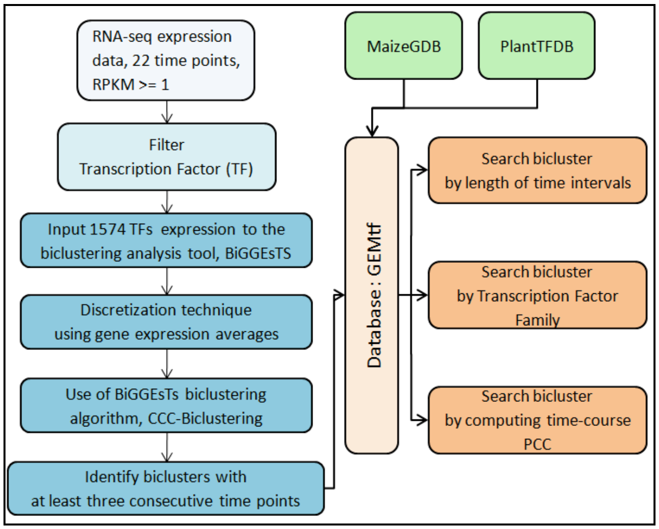

RNA-seq expression data, 22 time points, RPKM >= 1
Filter Transcription Factor (TF)
Input 1574 TFs expression to the biclustering analysis tool, BiGGEsTS
Discretization technique using gene expression averages
Use of BiGGEsTs biclustering algorithm, CCC-Biclustering
Identify biclusters with at least three consecutive time points
MaizeGDB
PlantTFDB
Database : GEMtf
Search bicluster by length of time intervals
Search bicluster by Transcription Factor Family
Search bicluster by computing time-course PCC

Figure 2. Workflow of the present study

B. Methods

In order to determine whether certain genes showed a similar trend between column and column values for the time-course profile matrix, we applied biclustering algorithms.

The traditional clustering algorithms assign the same weight to genes and into one single function grouping when processing expression data. In reality, however, many gene products are known to have more than one function, which means that a group of genes show similar expression profiles across a time interval.

Biclustering was developed originally by Hartigan in 1972 [10], which proposed a method for clustering cases and variables in the same step. The term biclustering was also used by Mirkin in 1996 [11] as a kind of output clustering structure. Furthermore, the biclustering algorithm was applied to study the gene expression profiles of *Saccharomyces cerevisiae* cell cycle and human B-cellin 2000 [12]. The work of Ref. 12 modeled the expression matrix with a block and a score called the mean-squared residue to measure the coherence of genes and conditions in the block [12].

Let G' and S' denote the subsets of genes and samples. The pair (G',S') specifies a sub matrix with the mean-squared residue score, $\mathrm{H}(\mathrm{G}',\mathrm{S}')$ defined by [12],

$$\mathrm{H}(\mathrm{G}',\mathrm{S}') = \frac{1}{|G'||S'|}\sum_{i\in G',j\in S'}(w_{ij} - n_{iS'} - n_{jG'} + n_{G'S'})^2,$$

where

$$n_{iS'} = \frac{1}{|S'|}\sum_{j\in J} w_{ij}$$

$$n_{jG'} = \frac{1}{|G'|}\sum_{i\in I} w_{ij}$$

$$n_{S'G'} = \frac{1}{|G'||S'|}\sum_{i\in G',j\in S'} w_{ij}$$

where $n_{iS'}$ denotes the mean of the *i*th row in the bicluster w_{ij}; $n_{jG'}$ denotes the mean w_{ij} of the *j*th column in the bicluster w_{ij}; and$n_{S'G'}$represents the mean of all elements in the bicluster w_{ij} , and | G' | and | S' | denote the cardinality of G' and S'.

A sub matrix is called a δ-bicluster if H(G',S')$\leq \delta$ for some $\delta > 0$. A low mean-squared residue score together with a large variation from the constant suggests a good criterion for identifying a block [12].

Madeira et al. proposed the CCC-Biclustering algorithm [13], a discretization method based on transitions between time points, which obtain better results than those using absolute values for biclustering time-course gene expression data(Figure1).

C. Parameters

This study used a biclustering tool, named BiGGEsTS [14]. BiGGEsTS can annotate the dataset genes in terms of the Gene Ontology for certain organisms, such as *Saccharomyces cerevisiae, Oryza sativa, Arabidopsis thaliana*, but maize annotation is currently not available.

RNA-seques sequencing technologies to measure the level of gene expression. The quantity named reads per kilobase per million (RPKM) was proposed in 2008 by Mortazaviet et al. [15] to measure the level of gene expression. The definition of RPKM is given by:

$$\text{RPKM} = \frac{total\ exon\ reads}{mapped\ reads(millions) * excon\ length(KB)}$$

The TF genes with their corresponding RPKM values larger or equal to one were imported into BiGGEsTS, which utilized the biclustering algorithm, CCC-Biclustering [13, 16], to filter biclusters with at least three consecutive time points (window size of 3).

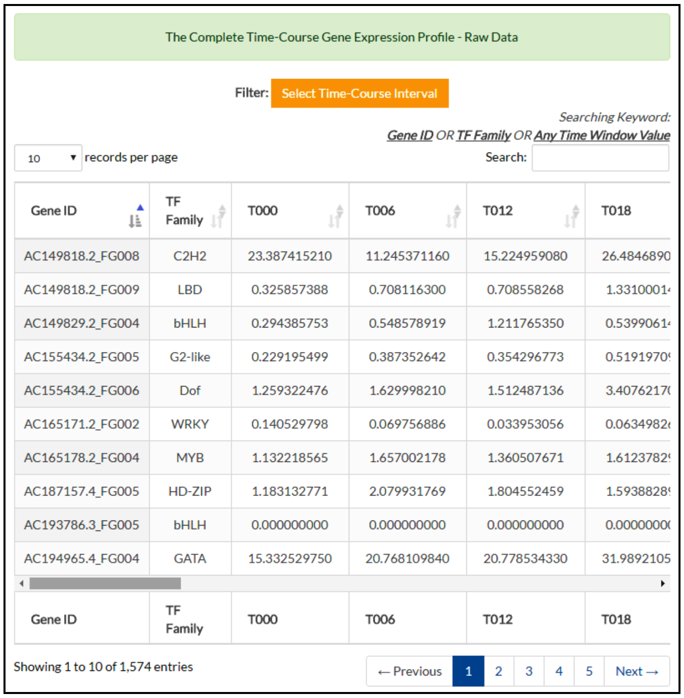
III. Results

A. Raw data search

The bicluster results were recorded in a database, named Genome-wide Expression Patterns of Maize Transcription Factor (GEMtf), which is accessible at http://ppi.bioinfo.asia.edu.tw/MaizeBase/. GEMtf includes the complete time-course gene expression profiles, and sort (A through Z) each column, search keyword by gene ID, TF family, or time-course intervals (Figure 3).

A total of 6543 biclusters were obtained, including the following five categories:
(i) time-course gene expression profiles of biclustering (BiGGEsTS label: Real Valued matrices),
(ii) discrete gene expression of biclustering (BiGGEsTS label: Discrete-Valued matrices),
(iii) graph contains curves of gene expression of biclustering (BiGGEsTS label: Graph by Real Valued matrices),
(iv) graph contains curves of full time-course gene expression of biclustering (BiGGEsTS label: Graph by Whole Real Valued matrices), and
(v) graph contains curves of discrete gene expression of biclustering (BiGGEsTS label: Graph by Discrete Valued matrices).

The information was recorded in GEMtf.



The Complete Time-Course Gene Expression Profile - Raw Data

Filter:
Select Time-Course Interval
Searching Keyword:
Gene ID OR TF Family OR Any Time Window Value
10
records per page
Search:

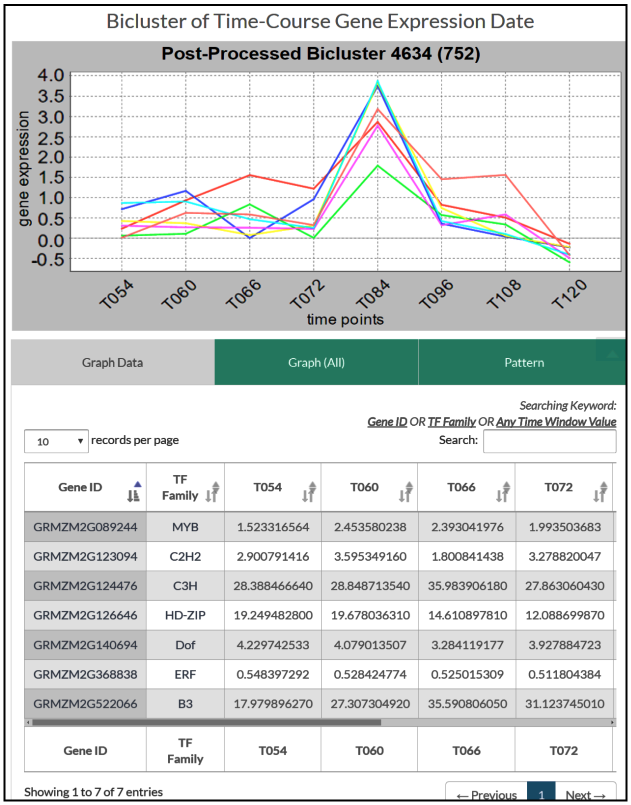
Gene ID	TF Family	T000	T006	T012	T018
AC149818.2_FG008	C2H2	23.387415210	11.245371160	15.224959080	26.4846890
AC149818.2_FG009	LBD	0.325857388	0.708116300	0.708558268	1.3310001
AC149829.2_FG004	bHLH	0.294385753	0.548578919	1.211765350	0.5399061
AC155434.2_FG005	G2-like	0.229195499	0.387352642	0.354296773	0.5191970
AC155434.2_FG006	Dof	1.259322476	1.629998210	1.512487136	3.4076217
AC165171.2_FG002	WRKY	0.140529798	0.069756886	0.033953056	0.0634982
AC165178.2_FG004	MYB	1.132218565	1.657002178	1.360507671	1.6123782
AC187157.4_FG005	HD-ZIP	1.183132771	2.079931769	1.804552459	1.5938828
AC193786.3_FG005	bHLH	0.000000000	0.000000000	0.000000000	0.0000000
AC194965.4_FG004	GATA	15.332529750	20.768109840	20.778534330	31.9892105
Gene ID	TF Family	T000	T006	T012	T018

Showing 1 to 10 of 1,574 entries

Figure 3. GEMtf web page for raw data search

B. Search bicluster by length of time points

The main function is the length of time points can be selected by user, and then choose time frame that the user was interest. To show the bicluster details on the web page, user can visualize the time expression profiles. User can click the "Graph (All)" box to view the expression profiles for all the 22 time points (Figure 4).



Bicluster of Time-Course Gene Expression Date

Gene ID	TF Family	T054	T060	T066	T072
GRMZM2G089244	MYB	1.523316564	2.453580238	2.393041976	1.993503683
GRMZM2G123094	C2H2	2.900791416	3.595349160	1.800841438	3.278820047
GRMZM2G124476	C3H	28.388466640	28.848713540	35.983906180	27.863060430
GRMZM2G126646	HD-ZIP	19.249482800	19.678036310	14.610897810	12.088699870
GRMZM2G140694	Dof	4.229742533	4.079013507	3.284119177	3.927884723
GRMZM2G368838	ERF	0.548397292	0.528424774	0.525015309	0.511804384
GRMZM2G522066	B3	17.979896270	27.307304920	35.590806050	31.123745010
Gene ID	TF Family	T054	T060	T066	T072

Showing 1 to 7 of 7 entries

Figure 4. GEMtf web page for results that meet the search criteria

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The length of time points is 3 to 22. After selecting the length, it will automatically display the corresponding expression profiles corresponding to the input time frame. Figure 5 illustrates the results of a bicluster (id=752) using eight time points, i.e. a time frame from T054 to T120.

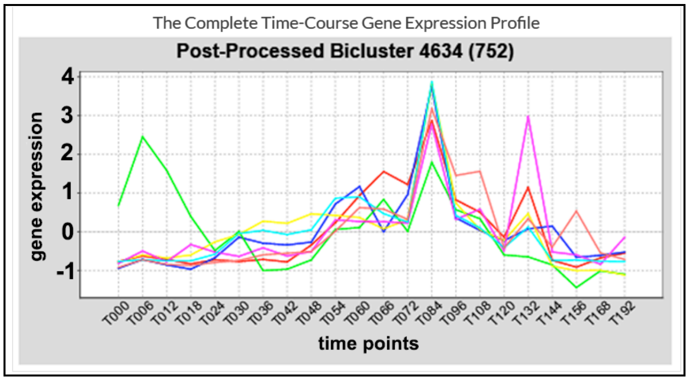
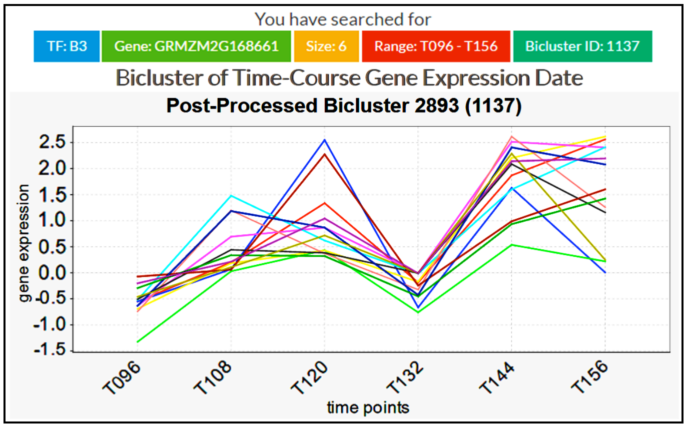


Figure 5. GEMtf– gene expression profiles of all the 22 time points of a bicluster (id = 4634)

C. *Search bicluster by Transcription Factor Family*

When biologists interested in some of the TFs, this feature helps them to identify those TFs [17] belong to a specific group of biclusters. Users can also filter out specific information by selecting the TF family, Gene ID of TF family and length of time interval. The genes found in the biclusters may help to dissect the roles of the TFs in maize leaf development.

Figure 6 depicts the results from ‘Graph of bicluster’ that show the oscillatory gene expression behavior for a TF family (*Arabidopsis* best hit: transcription factor B3 family protein) i.e. down-regulated at time T132, and then up-regulated at time T144. A more detailed understanding of this pattern remains to be examined by the biologists.



You have searched for

TF: B3 | Gene: GRMZM2G168661 | Size: 6 | Range: T096 - T156 | Bicluster ID: 1137

Bicluster of Time-Course Gene Expression Date

Figure 6. GEMtf web page for the oscillatory gene expression behavior for a TF family, i.e. transcription factor B3 family protein.

D. *Search for similarTFexpression patterns*

Biologists can upload their own experimental data to the server and compare the time-course biclusters in the server. GEMtf had already computed and saved the average time-course gene expression profile for each bicluster, and each bicluster that has an average time-course profile is used to compare with the up loaded time-course data by computing the Pearson Correlation Coefficient (PCC).

GEMtf will report the PCC scores for the upload data and biclusters with the same number of time points, ranging between T000 and T192. This service could possibly help biologists to quickly identify *novel* TFs with similar time-course profiles for their own experimental measurements.

IV. DISCUSSION

The tool, GEMtf, is set up by using time-course NGS gene expression profiles for maize leaf development and 6543 biclusters were identified. By integrating those pieces of information, GEMtf provides a tool to show the results of biclustering analysis and provides data query. User can input a fixed length of time-course expression vector, and GEMtf provides an online service, which computes the PCC values between the input vector and the average expression profile of biclusters with the same length of time interval. A large PCC value suggests that the query gene exhibits similar behavior to certain TF families; hence hidden biological function is detected.

To examine the roles of TFs in maize, we compare the biclusters’ TFs with the maize pathways collected from the KEGG GENOME T01088database. Among the 134 pathways in the database, four pathways are associated with at least five TFs. These four pathways are: MAPK signaling pathway (zma04016), plant hormone signal transduction (zma04075), plant-pathogen interaction(zma04626) and circadian rhythm (zma04712). Table 1 summarized the number of biclusters compose of two to 16 TFs found in the KEGG T01088 database.

It is known that growth hormones are important biomolecules that regulate plant development. Shin et al., [18] has reported that once the MYB77 gene knockout, the expression level of auxin response factors (ARFs) gene is dramatically suppressed. Both of light and TFs influence every major developmental transition process like the plant hormone [19], this suggested that the ‘Plant Hormone Signal Transduction Pathway’(zma04075) consists of a larger number of TFs found in the biclusters.

In summary, our GEMtf results showed that the bicluster method is capable of identifying co-expressed TFs for time-course RNA-seq data. The database provides an easy means of investigating the regulatory role of TFs for maize leaf development.

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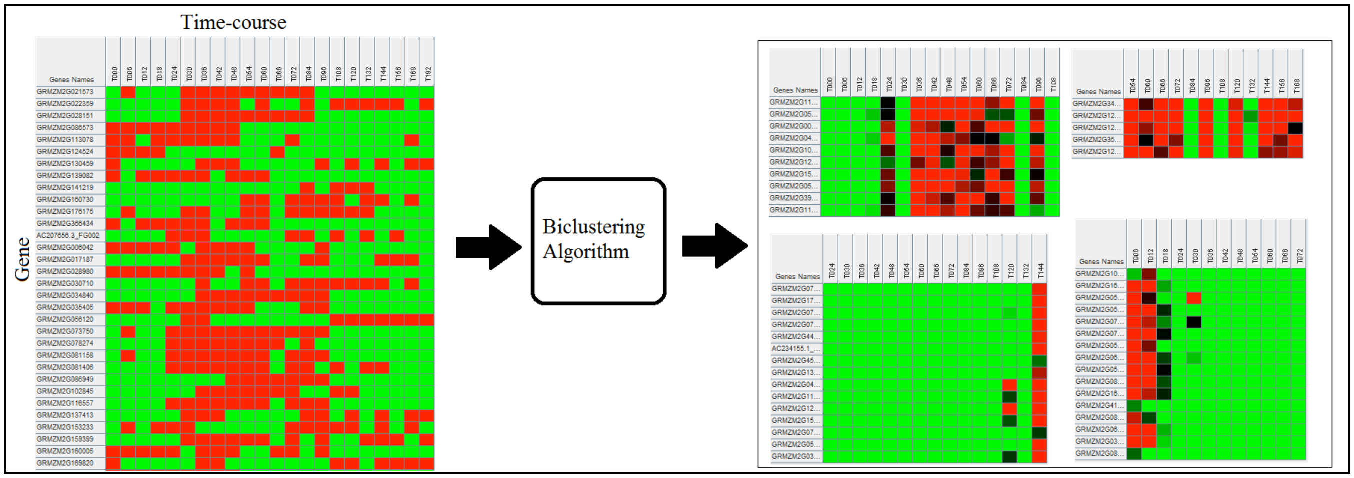

Figure 1. Gene setsidentified in different subsets of gene expression profiles

Table 1. Number of biclusters compose of two to 16 TFs in four pathways: zma04016, zma04075, zma04626 and zma04712.

Pathway\TFs	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
zma04016	155	67	66	27	11	30	27	3	1	0	0	0	0	0	0
zma04075	361	126	30	55	44	14	23	22	15	20	12	15	5	6	3
zma04626	73	39	19	10	0	0	0	0	0	0	0	0	0	0	0
zma04712	160	94	52	16	20	8	0	0	0	0	0	0	0	0	0