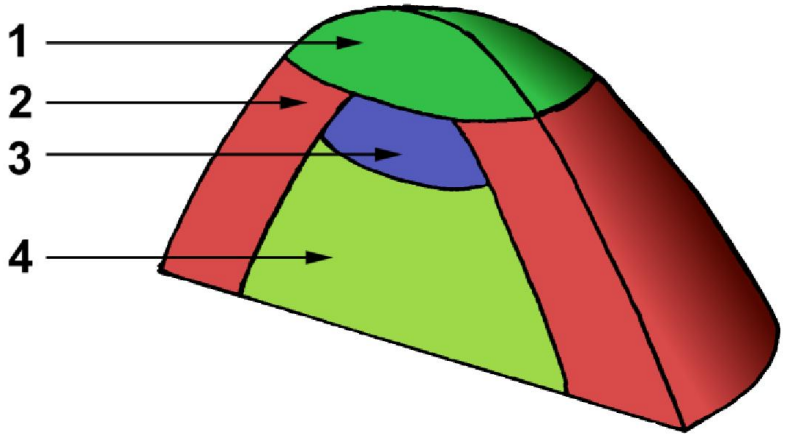
背景介绍

shoot apical meristem (SAM)

The SAM produces leaves and flowers from its peripheral zone（1） and replenishes itself in the central zone（1）.

Cells between the meristem and the organ primordium undergo growth arrest, forming a discrete boundary domain that separates the forming organ from the SAM



Axillary meristems (AMs) form in the boundary region in seed plants

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An organ boundary-enriched gene regulatory network uncovers regulatory hierarchies underlying axillary meristem initiation

器官边界基因富集的调控网络揭露腋生分生组织起始的调控

报告：韩勖
翻译：王栋

overview

特异细胞类型的基因表达信息

protein–DNA interaction (PDI) 数据

GRN

验证

从系统发育生物学理解边界器官和AM形成

对于边界器官和系统发育的新的观点

基因组调控核心区和网络构成

创新点

巧妙结合由TRAP-seq得到的翻译组数据和由Y1H得到的PDI数据建造GRN模型，并对植物发育学研究的相关问题提出新的看法。

TRAP-seq

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TARGETED PURIFICATION OF POLYSOMAL MRNA (TRAP-SEQ)

Targeted purification of polysomal mRNA (TRAP-Seq) maps translating mRNAs under various conditions[17]. In this method, tagged ribosomal proteins are expressed in cells. The tagged ribosomal proteins are then purified and the RNA isolated. RNAs are reverse-transcribed to cDNA. Deep sequencing of the cDNA provides single-base resolution of translating RNA.

Pros	Cons
• *Allows detection of translating RNAs* • *RNAs translated by specific targeted ribosomes can be assessed* • *No prior knowledge of the RNA is required* • *Genome-wide RNA screen*	• *Not as specific as more recently developed methods, such as Ribo-Seq*

TRAP-seq方法

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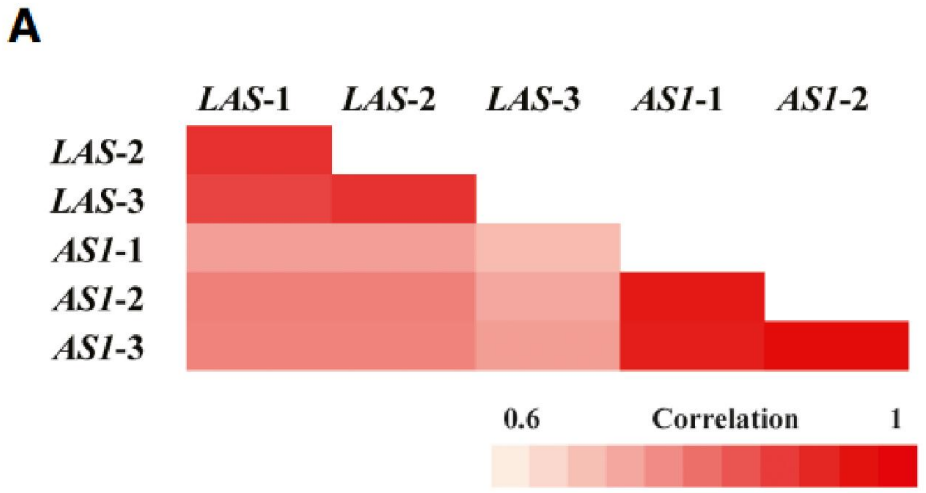
Report line：核糖体HF-RPL18　基于pOp promoter的控制

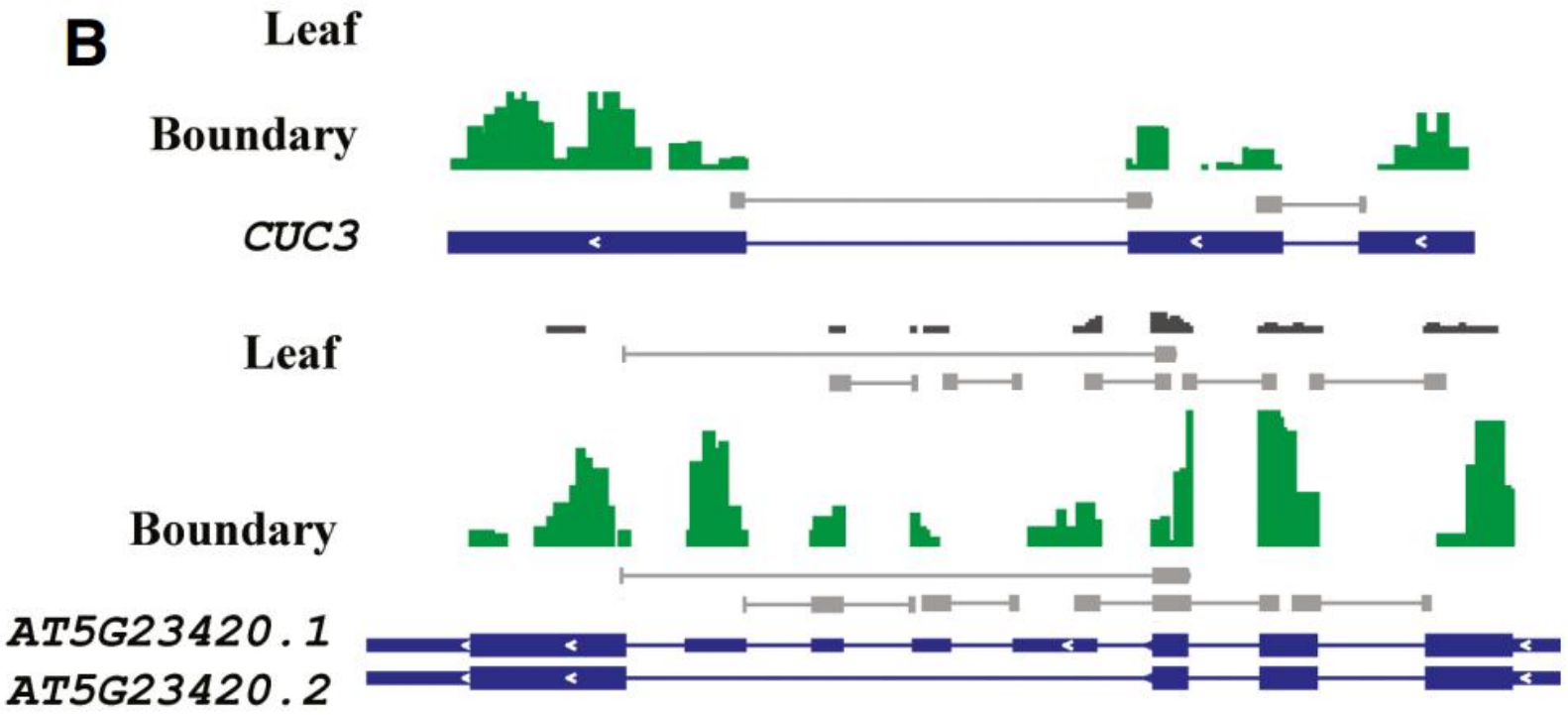
Drive line：TF LhG4　基于LAS promoter的控制
TF LhG4　基于AS1 promoter的控制

pLAS::LhG4 有边界区域特殊的活动
pAS1::LhG4 在叶原基出现的时候驱使pOp表达

isolate translating mRNA in the
LAS-expressing organ boundary cells
and AS1-expressing leaf primordia and cotyledon cells



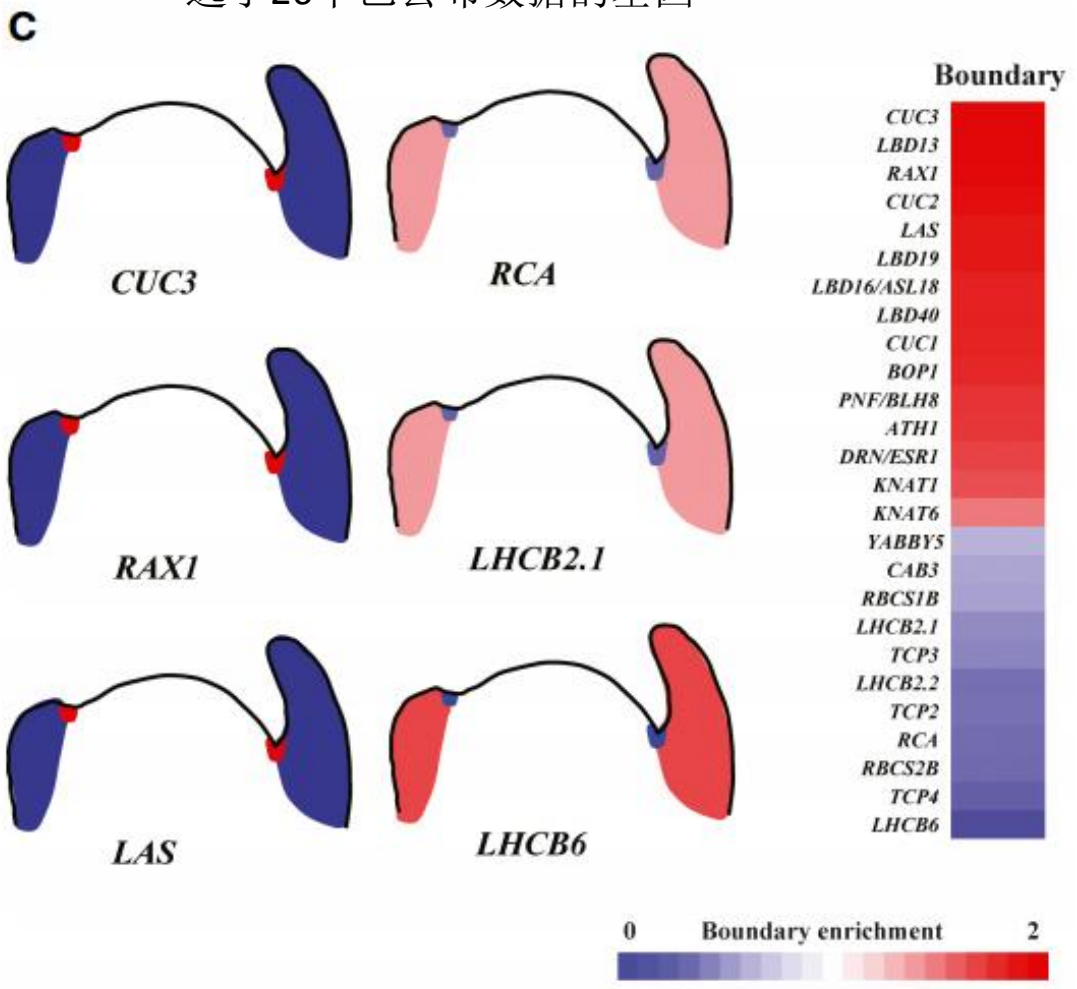
确保数据质量和可靠性

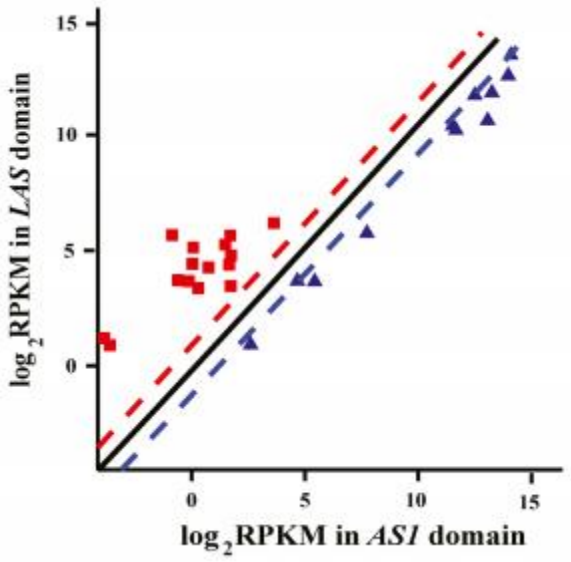


The CUP-SHAPED COTYLEDON3 (CUC3) TF gene is specifically expressed in the boundary domain

确保数据质量和可靠性

选了26个已公布数据的基因





boundary enrichment or depletion

RPKM：估计基因的表现量

边界区域富集的基因

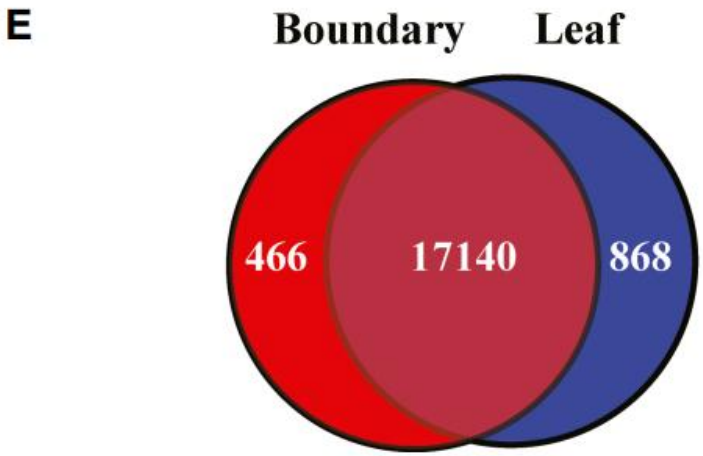


Table S4. Functional categories of boundary-enriched genes.

Category	Gene No.
Structural proteins	190
Enzymes	124
TFs	46
Others	28
Unknown	78
Total	466

基因富集分析

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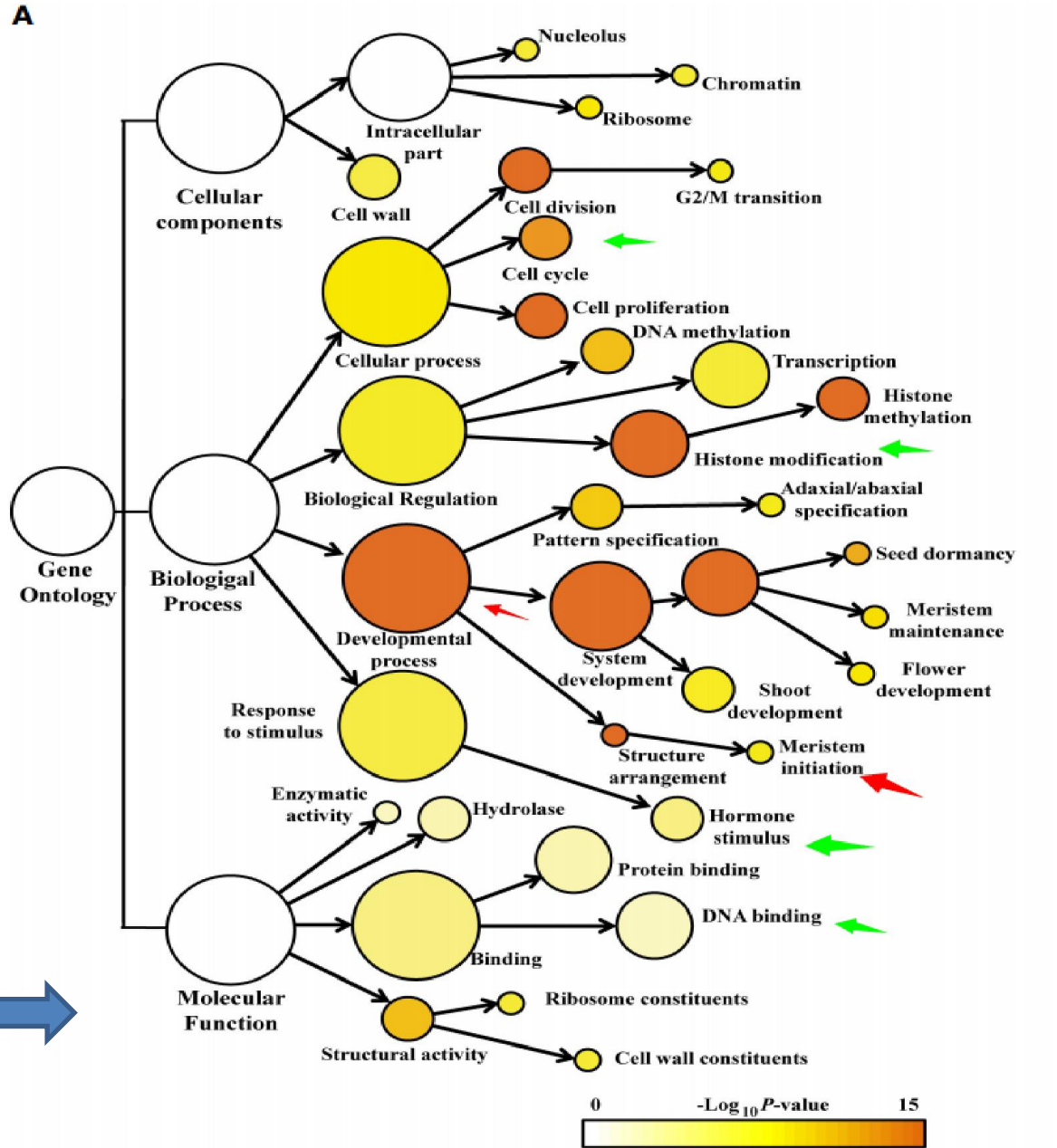
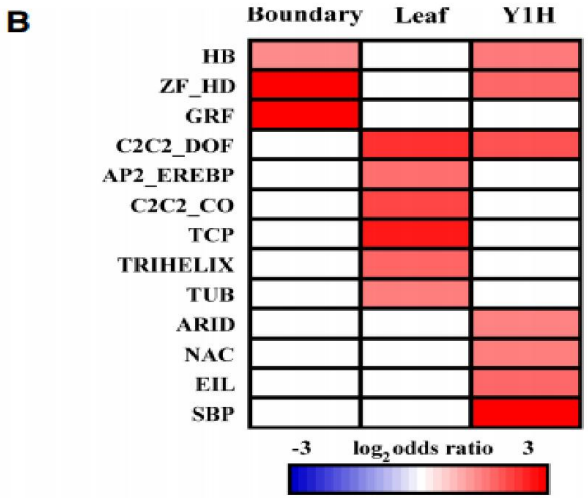
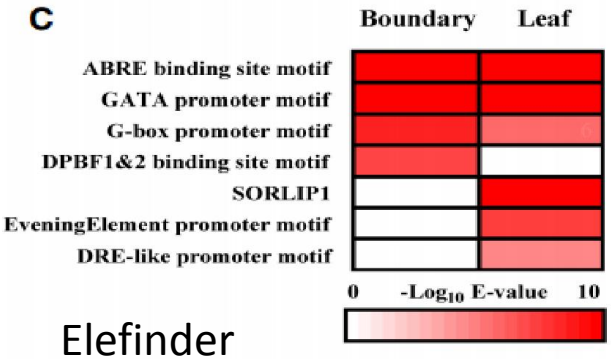
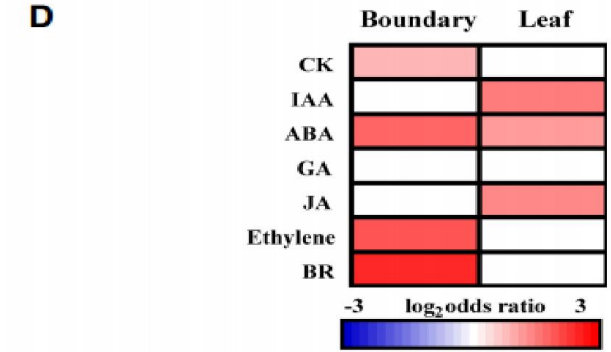


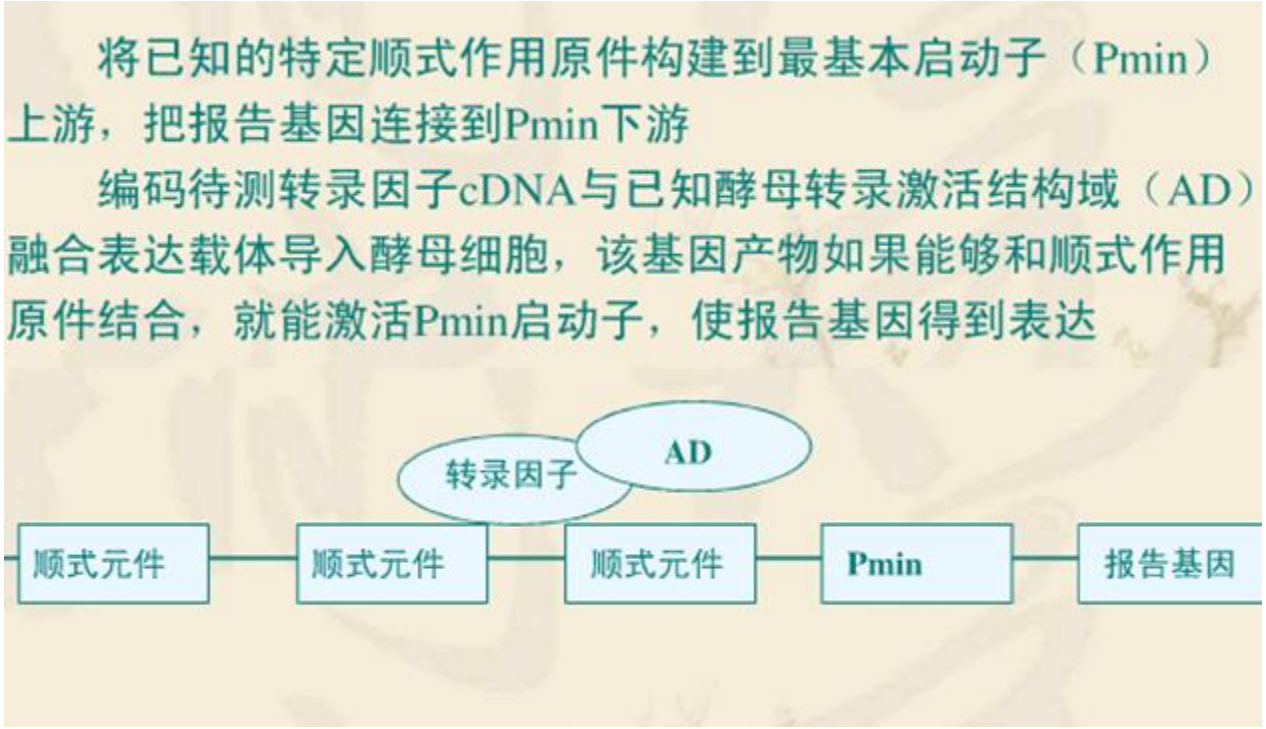




Elefinder



Y1H 酵母单杂交



Recent developed TF library + boundary domain expressing TF = 1184 protein preys
34 regulatory genomic regions

检测了40256（fragment*TF）PDIs

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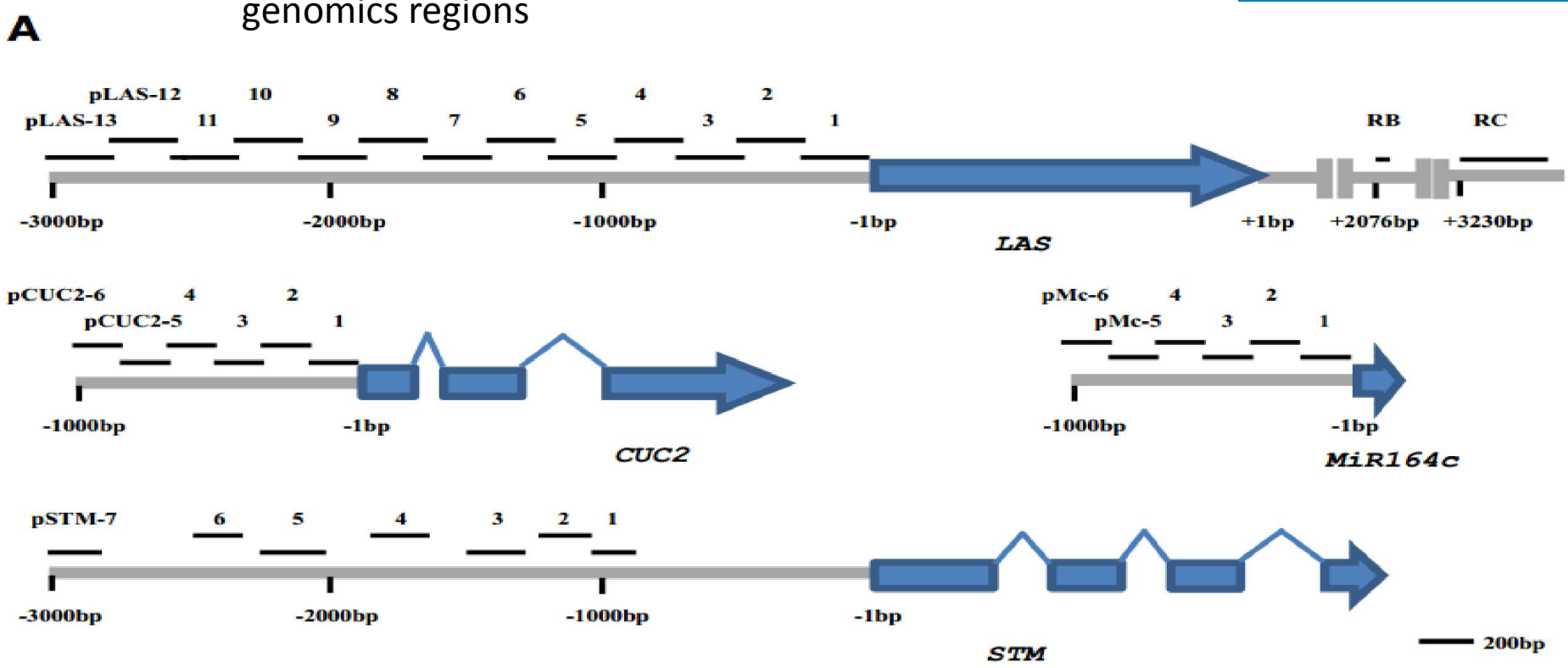
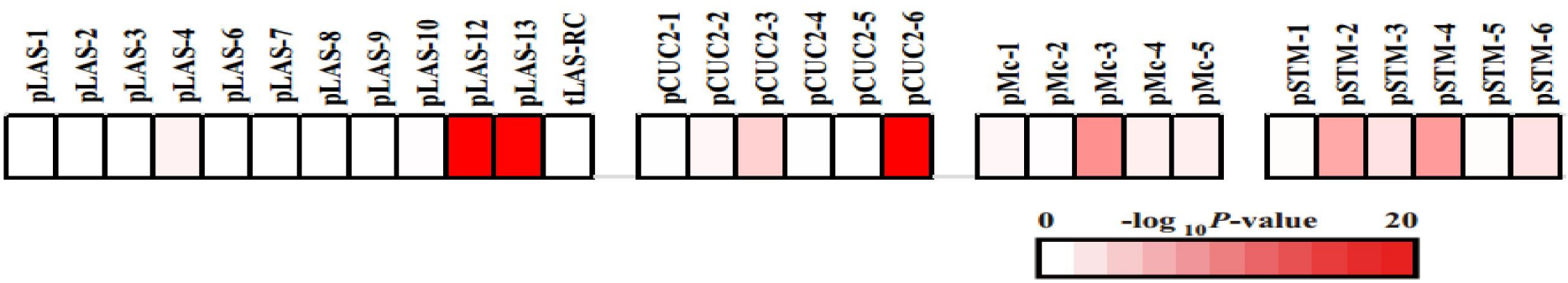
genomics regions



PDI enrichment among tested genomics regions



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在23个基因组调控区（ regulatory genomic regions）的103个TFs中，得到了180PDIs
其中67.7%的基因组调控区至少有一个TF
8.7%的TF和一个以上的基因组调控区相对应
63.1%的TF唯一地结合在一个基因组调控区

PDI network. Circle, transcription factor (TF), diamond; promoter fragment; edge, PDI. Boundary-enriched TFs are shown in red, and boundary-depleted TFs are shown in blue. Circles of the same color represent promoter fragments of the same gene.

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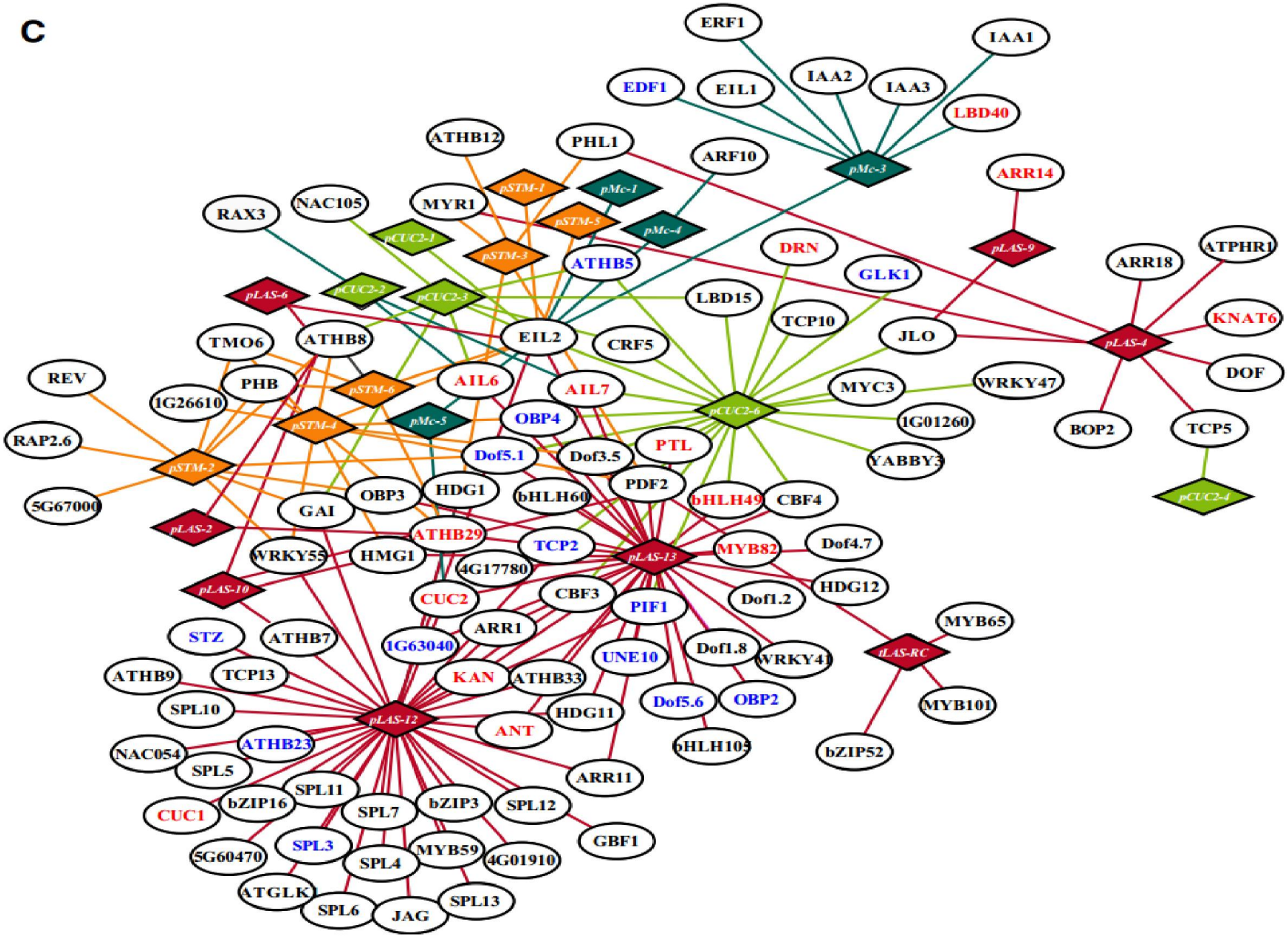

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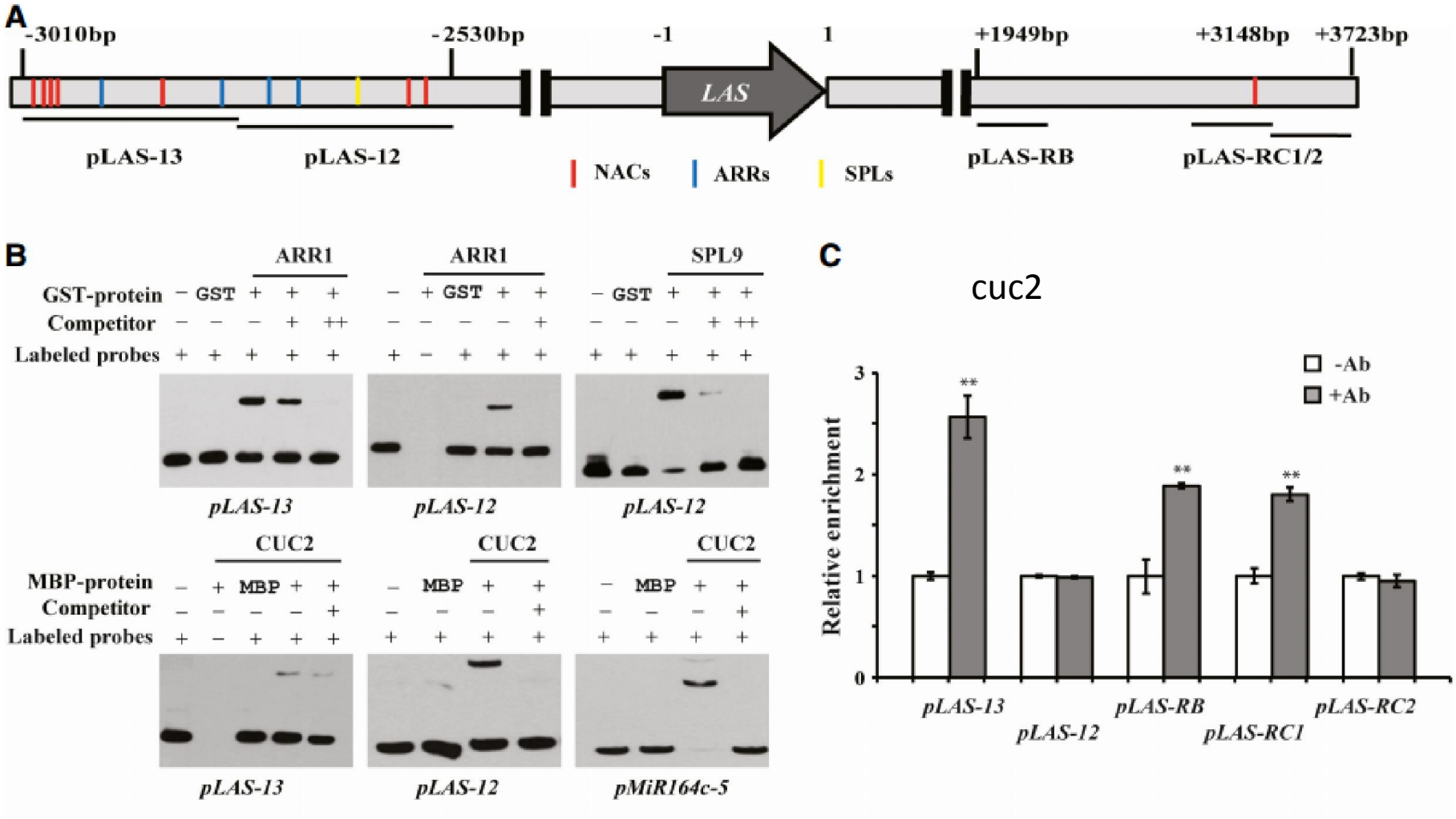
Validation of protein–DNA interactions (PDIs)
凝胶迁移或电泳迁移率检测(Electrophoretic Mobility Shift Assay, EMSA)
CHIP-PCR

Both CUC2 and ARR1, which activate LAS expression, and SPL9 and SPL15, which suppress LAS expression, interact with the overlapping pLAS-12 and pLAS-13 genomic fragments in Y1H assays.



Properties of the boundary-enriched protein–DNA interaction (PDI) network .

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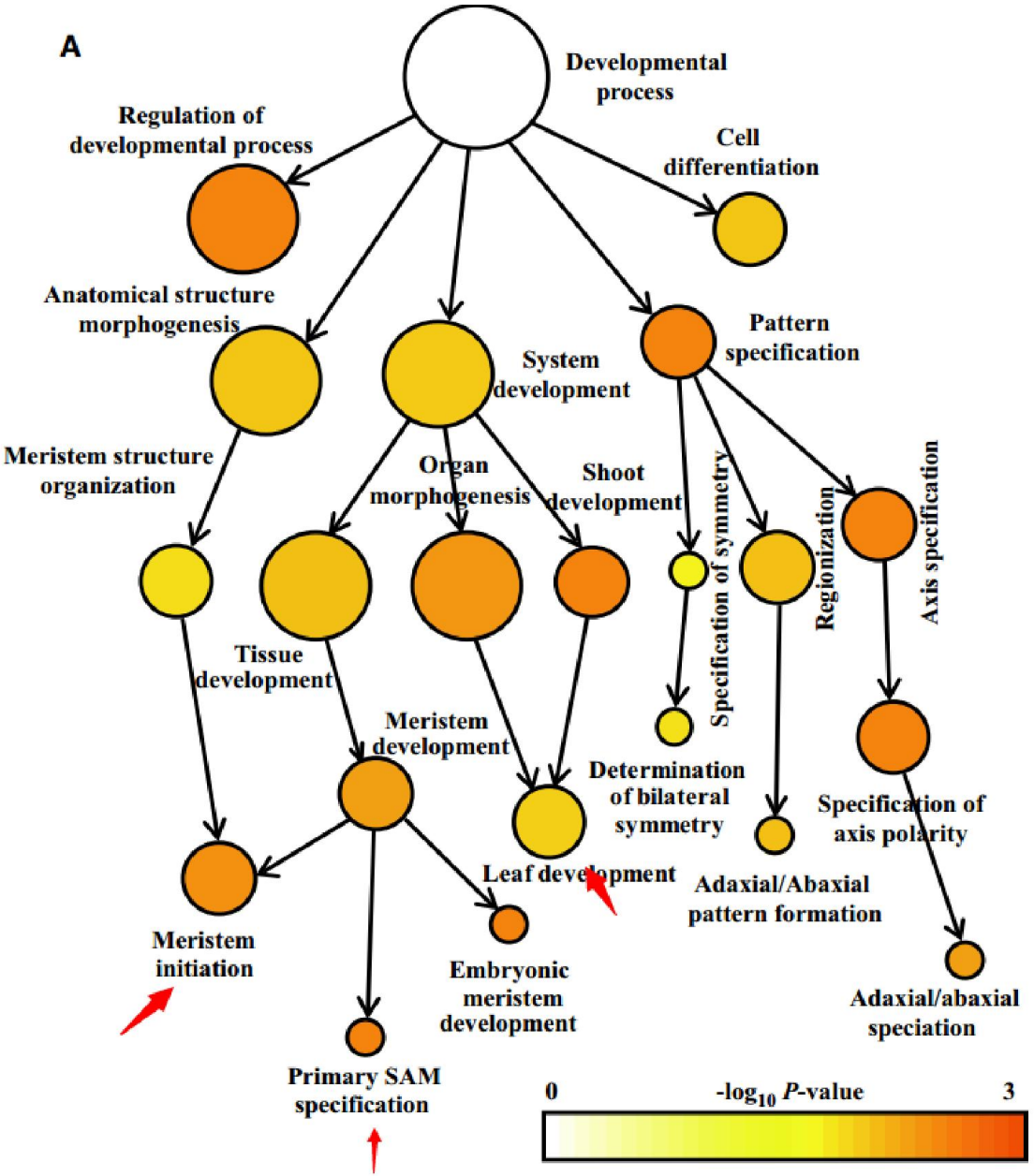
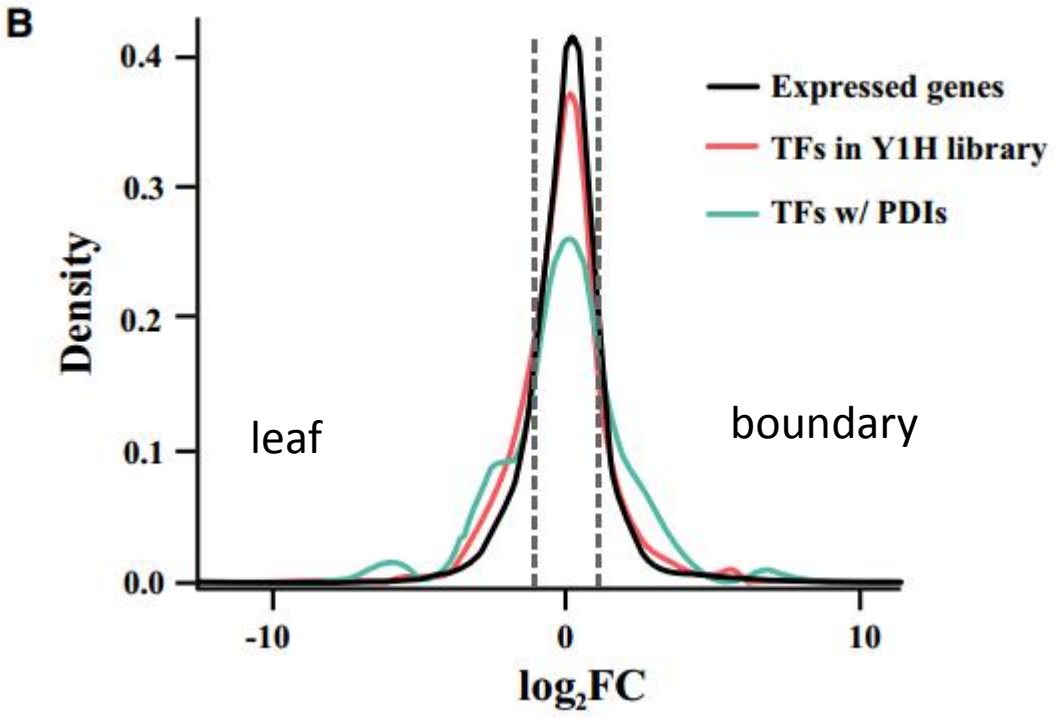


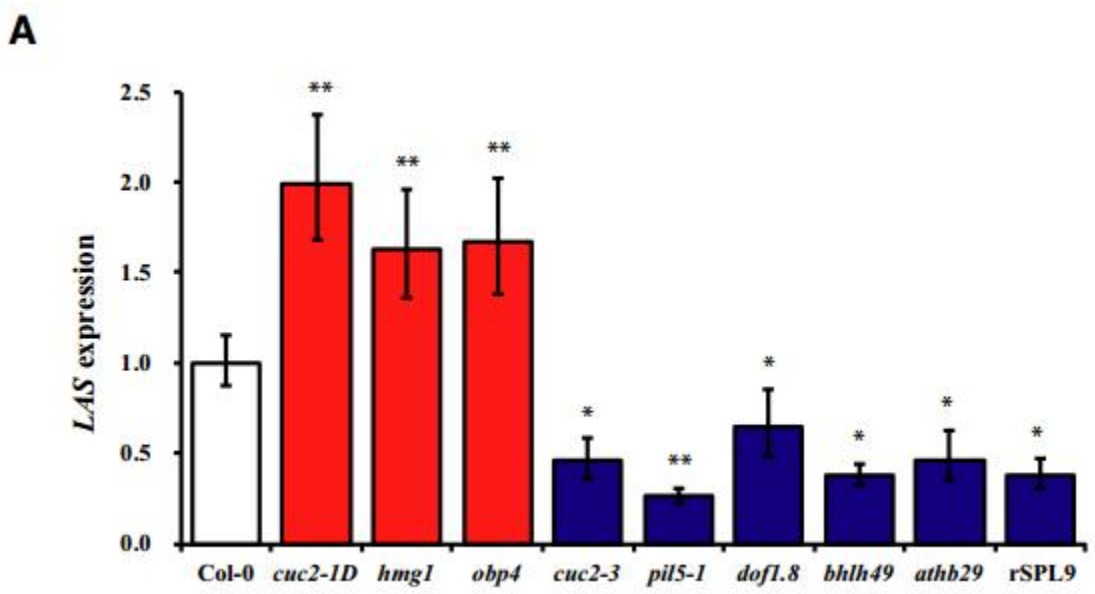
核密度估计(Kernel Density Estimates)



PDI-associated TFs have obvious differential expression patterns

Inferring GRN by data integration

使用qRT-PCR进行定量检测突变体和超表达的TFs在目标区域的表达情况，并在野生型和突变型植株中，对每个PDI使用最小二乘回归建立TF和其目标基因间表达的模型。



A Real-time RT–PCR analysis of target gene expression in wild-type and in transcription factor (TF) mutant or over-expression lines. Error bars indicate s.d., a double asterisk (**) represents *P*-value < 0.01, and an asterisk (*) represents *P*-value < 0.05 between wild-type and a mutant or over-expression line.

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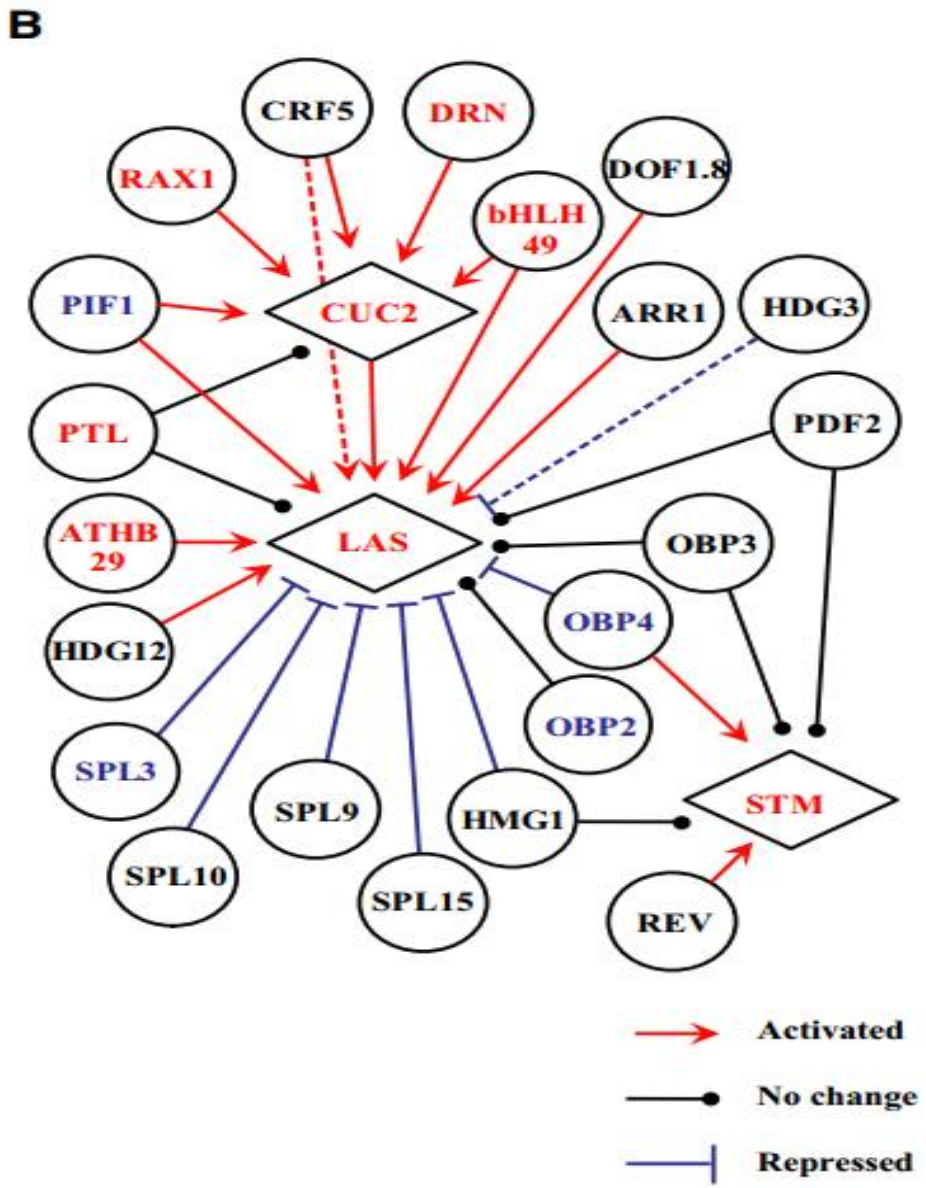
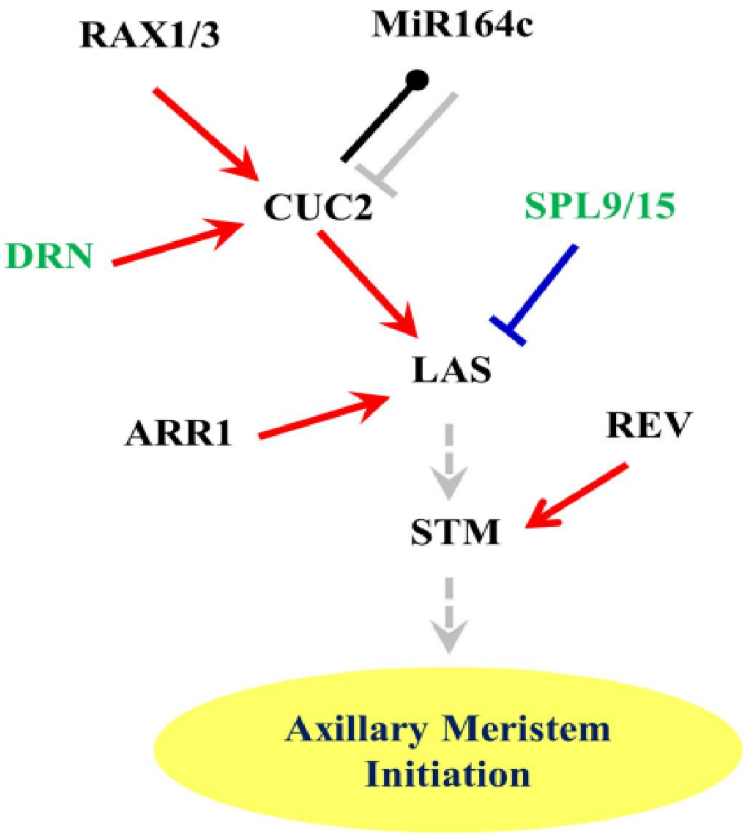


B PDIs that result in activating (red line), repressive (blue line), and no effect (black line) in target expression were determined using qPCR of the TF and its target as shown in (A) and in Supplementary Fig S5. Dotted lines represent referred interaction from homologous TFs. Boundary-enriched TFs are shown in red, and boundary-depleted TFs are shown in blue.

discussion

molecular systems biology

Systems developmental biology for understanding organ boundary and AM formation



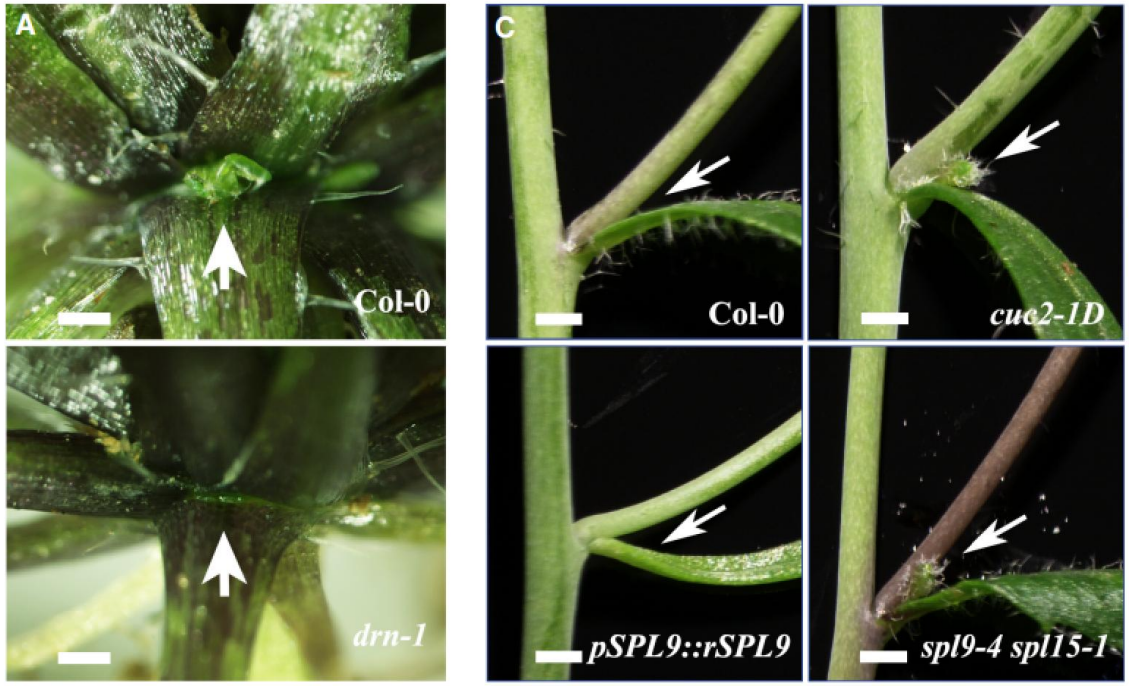
Network architecture and regulatory genomic region hubs

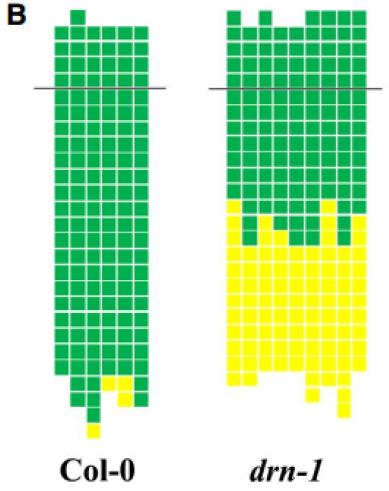
In fact, the majority (53.9%) of PDIs were associated with one promoter region of each of two key regulators, CUC2 and LAS.

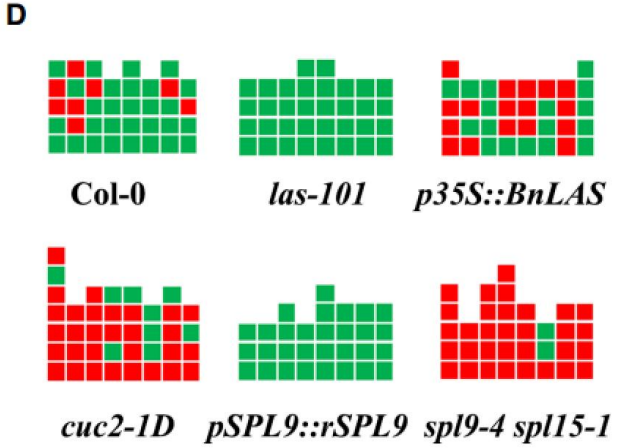
Figure 8. Summary of known and newly identified regulators and regulatory relationships controlling AM initiation.
Gray solid line, known direct interaction between miRNA and targeting mRNA; gray dotted line, known genetic interaction; red arrow, activating PDI identified in this study, blue bar, repressive PDI identified in this study; black line, PDI identified in this study, unknown regulatory relationship. New regulators of AM initiation are shown in green.

discussion

molecular systems biology







discussion

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New views of boundary and AM development

对已有的结论进行总结：

First, we confirmed boundary enrichment or depletion of a large number of genes (Fig 1C).

Furthermore, independent GO analysis of genes enriched in the boundary domain and GO analysis of TFs bound to promoters of key regulators of boundary specification and AM initiation separately identified meristem-related GO functions (Figs 2A and 5A).

Additionally, we provided genome-scale support for the recent finding that a low auxin niche is required for AM initiation , which is followed by a cytokinin signaling pulse

新的观点：

1、细胞循环调控，转录调控，表观调控以及细胞壁自动平衡都影响边界区和AM的形成；

2、除了生长素和细胞分裂素，我们还鉴定出了5种主要的植物激素对植物的边界区和AM发育有正调控或负调控作用。

3、我们得到了边界区富集的TFs和其在相关PDIs中的功能富集，其中有一些关键的TF直接与植物的发育和生理过程相关，这将有助于我们在反向遗传学的研究。

启发：
文章启示我们在进行数据分析时，加入大量的分子生物学实验进行验证，可以增加结果的可靠性，并弥补数据分析上的缺陷和不足。

改进:
仅仅在植物发育空间层次上进行了研究，可以加入一些时间上的，得到一个空间和时间上更完整的系统;
模型的建立相对简单，可以推导相关函数进行补充说明;
还可考虑环境因素对发育调控的影响。

Thanks for Watching!