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Common verification methods for sequencing results of full-length transcriptomes

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1. Gene structure verification

1) RT-PCR verification: Alternative splicing (AS), fusion genes, new genes and new transcripts can be performed on the full-length transcriptome

Structural analysis, the most common verification methods for structural analysis are reverse transcription PCR (RT-PCR) and agarose gel electrophoresis[1]. Design based on gene sequence

Specific primers at both ends were used for PCR amplification, and the amplified products were run on agarose gel electrophoresis, and the size of the electrophoresis band was compared with the size of the target fragment sequence.

Correspondence was performed to initially verify whether the sequencing results were consistent with the actual transcribed mRNA sequence.

① Verification of alternative splicing

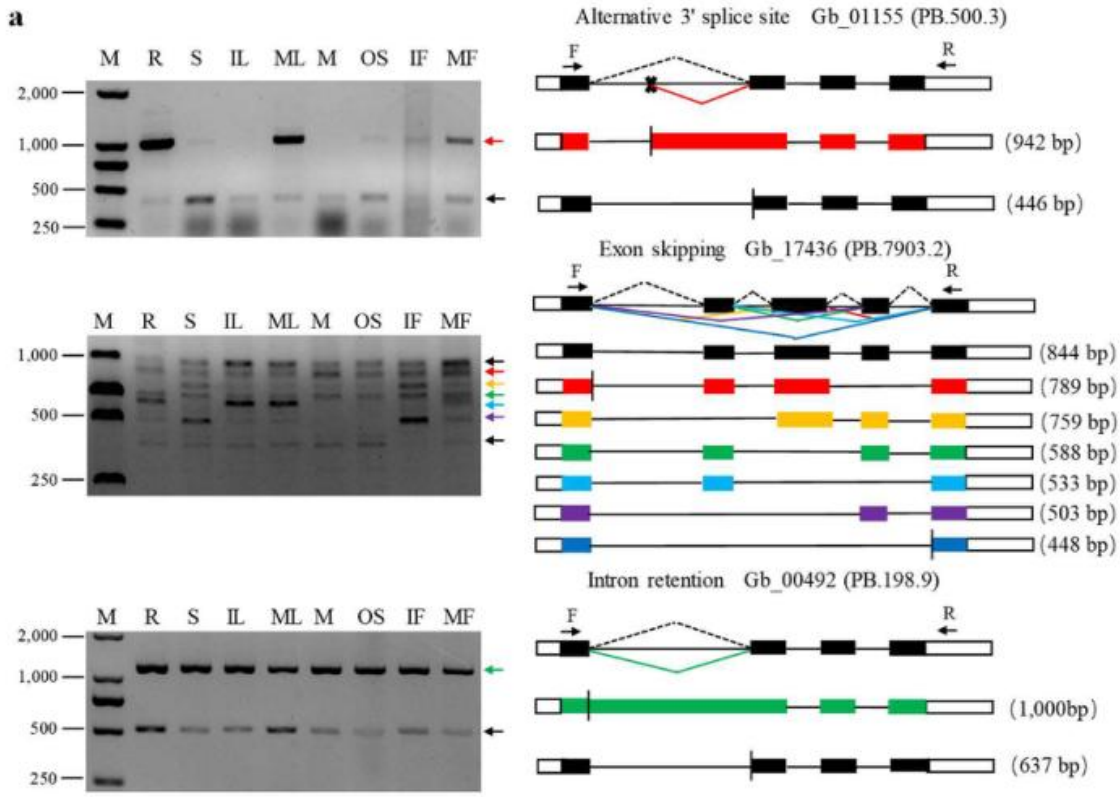

Figure 1 Verification of gene alternatively spliced transcripts in different tissues of Ginkgo biloba

(Left: Electrophoretic bands amplified in different tissues; Right: Schematic diagram of alternative splicing structures of different genes)

② Fusion gene verification

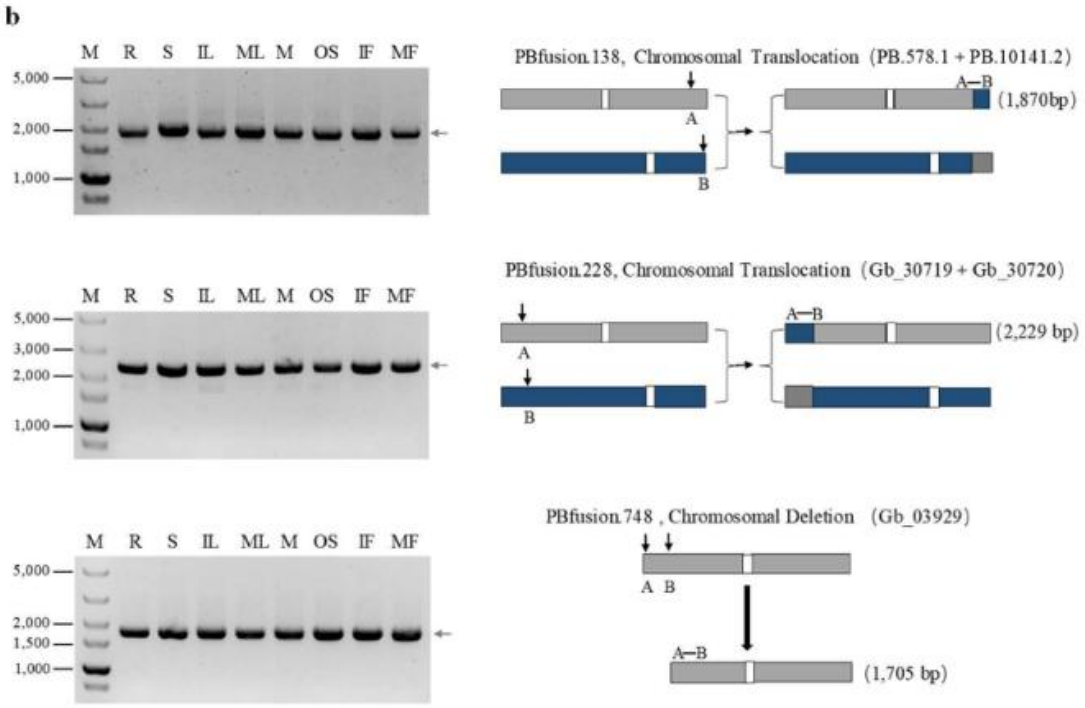

Figure 2 Verification of fusion genes in different tissues of Ginkgo biloba

(Left: Electrophoretic bands amplified in different tissues; Right: Schematic diagram of the structure of different fusion genes)

③ New gene verification

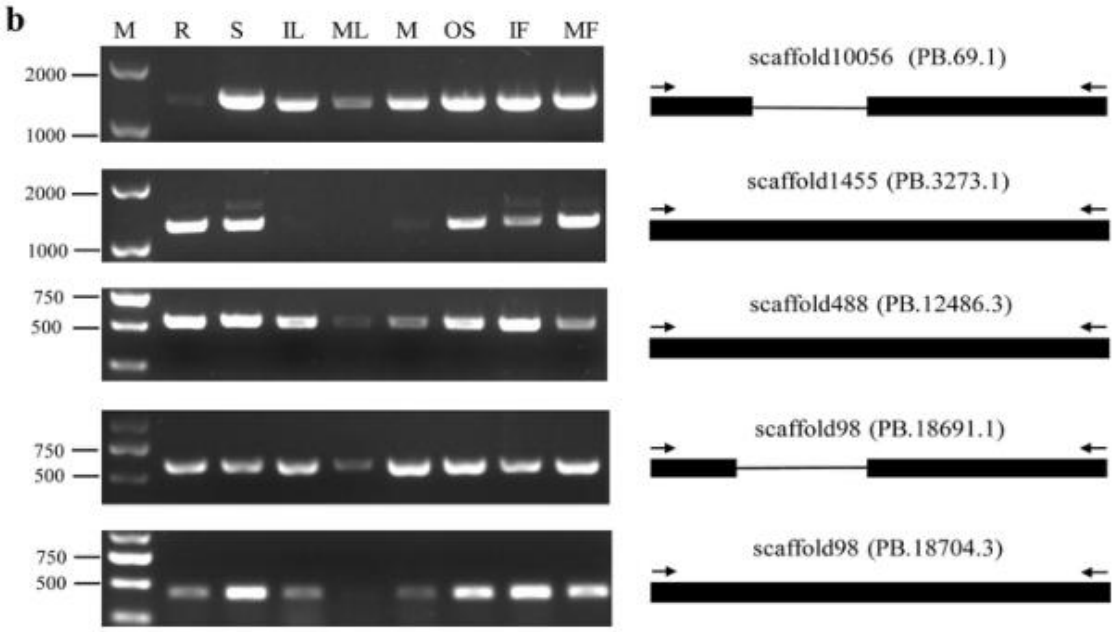

Figure 3 Verification of new genes in different tissues of Ginkgo biloba

(Left: Electrophoretic bands amplified in different tissues; Right: Schematic diagram of the structure of different new genes)

2) RACE-PCR verification: Rapid-amplification of cDNA ends (RACE) technology is the rapid cloning of cDNA ends by PCR

Technology. RACE is based on a known cDNA fragment based on PCR technology, using anchor PCR to rapidly amplify the cDNA end from

And obtain the cDNA sequence technology of a small sequence and 3' or 5' in the known mRNA. RACE-PCR can be used to quickly verify the APA

result.

① Verification of variable polyadenylation

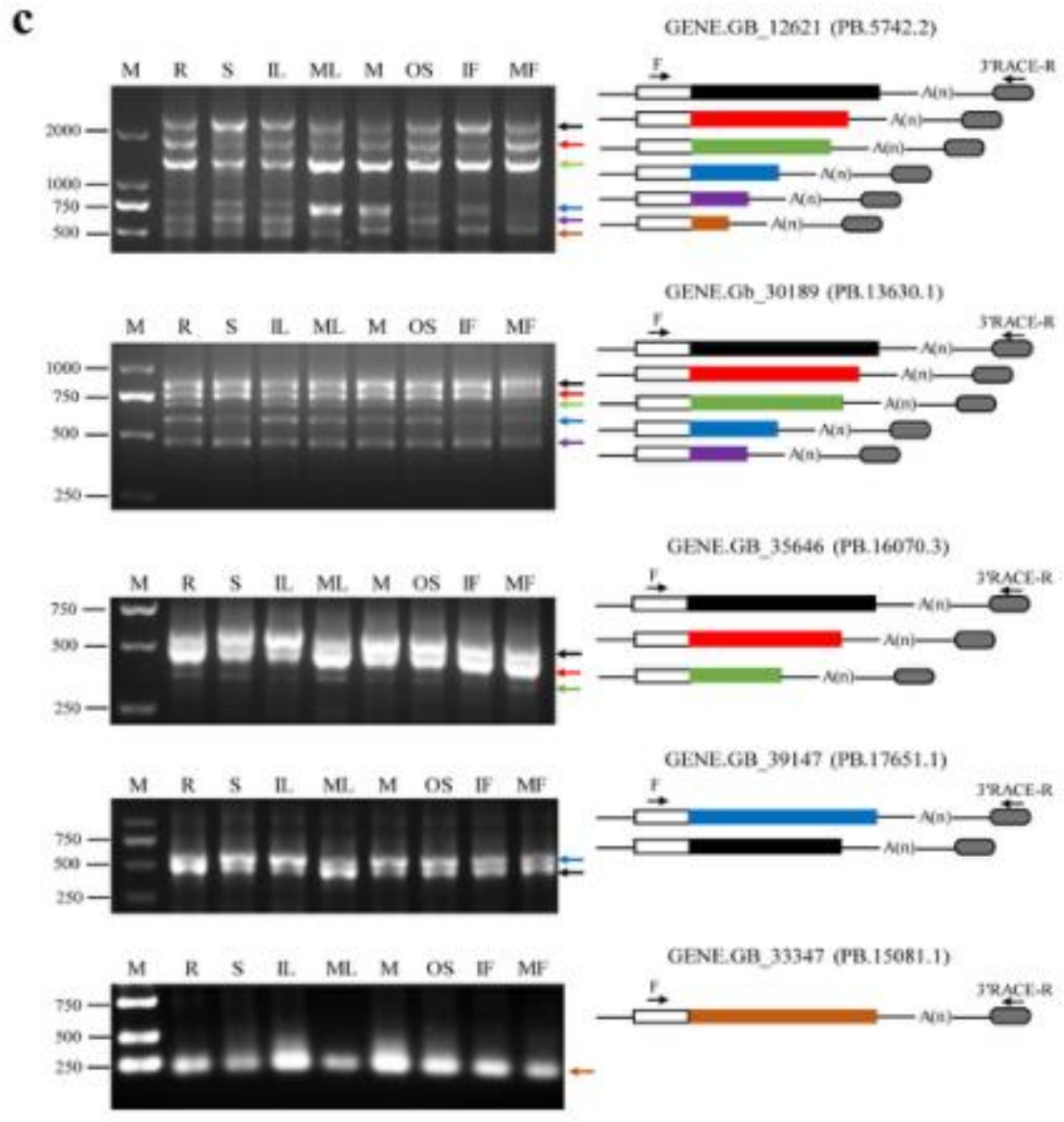

Figure 4 Validation of variable polyadenylation in different tissues of Ginkgo biloba

(Left: Electrophoresis bands amplified by 3' RACE in different tissues; Right: Schematic diagram of variable polyadenylation structures of different genes)

2. Gene expression level verification

When using RNA-seq to screen research objects, the results obtained by RNA-seq-based differential analysis generally need to be determined.

Quantitative level verification, in order to prove that the differences between the different samples studied are credible. The common verification methods are:

qRT-PCR verification: Verification of gene expression levels, based on comparison with internal reference genes (housekeeping genes: genes necessary to maintain cellular gene metabolic activities

Because, the expression level is relatively stable in various tissues and cells), the relative quantitative level of the target gene can be obtained, and the common internal reference genes GAPDH, β-Actin, 18S rRNA, etc. Note: Due to the different principles of qRT-PCR and transcriptome quantification, there will be differences in expression levels. It is generally recommended to check whether the gene expression trends are consistent.

Western Blot: Verify the expression of protein products of different genes at the translation level, apply specific antibodies, and mark changes in protein levels. common

Antibody types include rabbit anti, goat anti, mouse anti and so on.

A

ORF	Start	Stop	Intron	Size (nt)	Size (aa)
E6	1	423	N/A	423	140
E7	423	755	N/A	333	110
E1	742	2604	N/A	1863	620
E2	2534	3685	N/A	1152	383
L2	3745	5361	N/A	1617	538
L1	5372	6901	N/A	1530	509
E1^E4	742	3419	758-3138	297	98
E8^E2	1094	3685	1126-3138	579	192
E1^M1	742	3231	1195-3138	546	181
E1^M2	742	3231	1126-3138	477	158

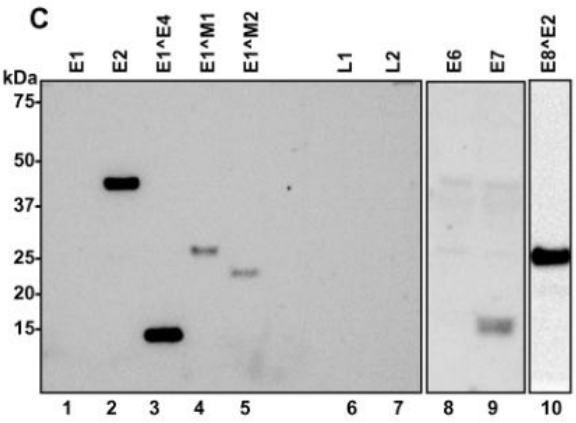

Figure 5 Protein expression of different genes of mouse papillomavirus[2]

(Left: Nucleotide and protein sizes of different genes; Right: Western Blot results)

3. Gene mapping verification

After using RNA-seq to obtain the target molecule, it is generally necessary to qualitatively verify its position in the cell. The first purpose is to prove

This is to prove that the RNA molecule is stably expressed and exists in the cell, and the second is to determine the experimental means needed for subsequent overexpression/knockout experiments (RNA analysis

The position of the progeny in the nucleus/cytoplasm is different, and the means of overexpression/knockout required may also be different).

Fluorescence in situ hybridization: using a known sequence and specific single-stranded nucleic acid as a probe, labeled with biotin or fluorescein, at a certain temperature

Under the condition of ion concentration and base complementary pairing rule, the sequence is hybridized in situ, and the fluorescence method is used to display, and finally the DNA/RNA is crawled in the cell.

The original position of slices and tissue sections (paraffin section or frozen section) is marked to determine the position of the gene in the cell.

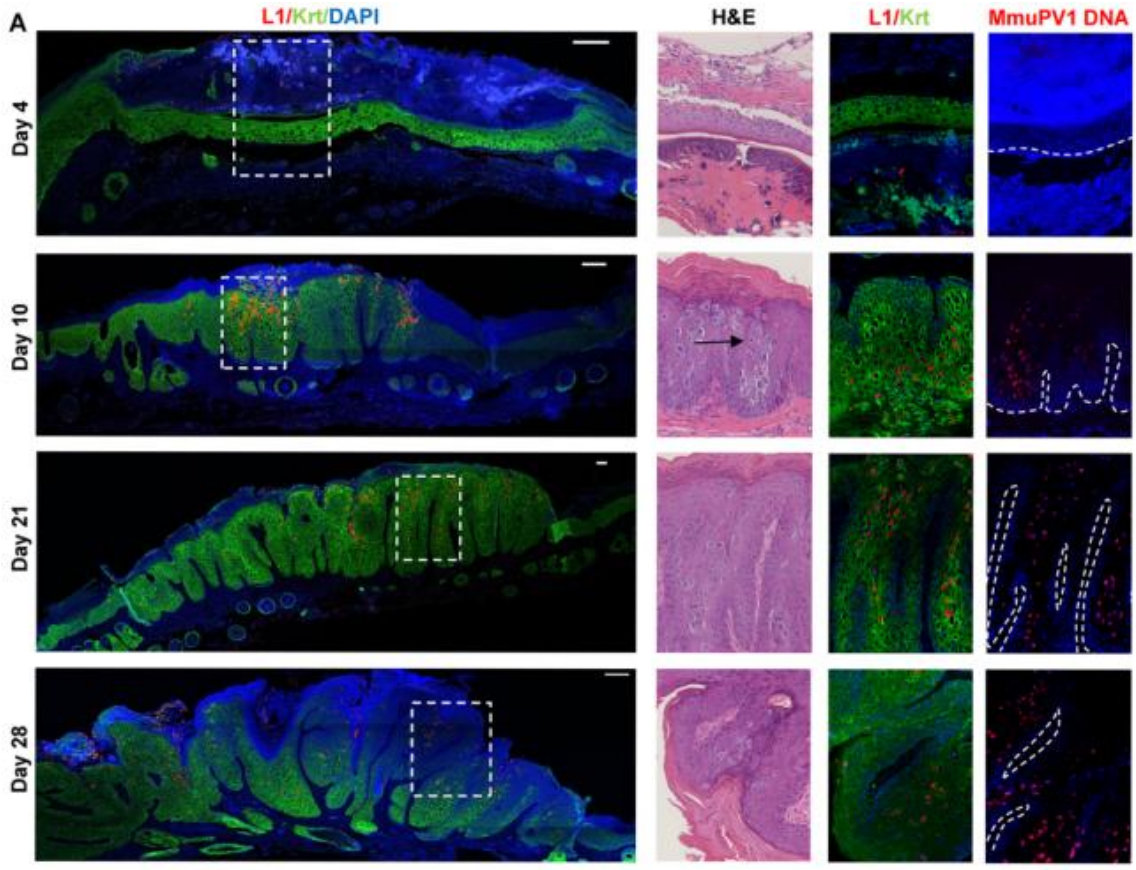

Figure 6 Cellular localization of mouse papillomavirus L1 protein[2]

Subcellular localization: Through transfection of recombinant expression plasmid vector, the expression position of the target gene in the cell is detected by fluorescence microscopy, and then its function is analyzed.

References:

[1] Ye J, Cheng S, Zhou X, et al. A global survey of full-length transcriptome of Ginkgo biloba reveals transcript
variants involved in flavonoid biosynthesis[J]. Industrial Crops and Products, 2019.

[2] Xue X, Majerciak V, Uberoi A, et al. The full transcription map of mouse papillomavirus type 1
(MmuPV1) in mouse wart tissues.[J]. PLOS Pathogens, 2017, 13(11)