Research Report

Open Access

Construction and Analysis of a Yeast cDNA Library from *Medicago truncatula*

Shuwen Li, Di Dong, Yinruizhi Li, Mengdi Wang, Liebao Han, Tiejun Zhang ✉

College of Grassland Science, Beijing Forestry University, Beijing, 100083, China

✉ Corresponding author email: zhangtiejun@bjfu.edu.cnLegume Genomics and Genetics, 2022, Vol.13, No.1 doi: [10.5376/lgg.2022.13.0001](https://doi.org/10.5376/lgg.2022.13.0001)

Received: 29 Dec., 2021

Accepted: 12 Jan., 2022

Published: 3 Feb., 2022

Copyright © 2022 Li et al., This article was first published in Molecular Plant Breeding in Chinese, and here was authorized to translate and publish the paper in English under the terms of Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:

Li S.W., Dong D., Li Y.R.Z., Wang M.D., Han L.B., and Zhang T.J., 2022, Construction and analysis of a yeast cDNA library from *Medicago truncatula*, Legume Genomics and Genetics, 13(1): 1-7 (doi: [10.5376/lgg.2022.13.0001](https://doi.org/10.5376/lgg.2022.13.0001))

Abstract Yeast two-hybrid and one-hybrid technologies are an efficient molecular biology technique for screening protein interactions or protein and DNA interactions, and are an important means for the interaction and regulation of biological macromolecules. In order to obtain a high-capacity *Medicago truncatula* gene library, it provides a basis for further digging the related genes of *Medicago truncatula* and improving the quality of *Medicago truncatula*. In this study, *Medicago truncatula* from different tissue sources was selected, and different hormone induction or stress treatments were performed on *Medicago truncatula*, using SMART technology to successfully construct a high-capacity *Medicago truncatula* yeast hybrid cDNA library. The library quality test results showed that the library titration number was 5×10^7 CFU/mL, the library capacity was 1.28×10^7 CFU, and the average insert fragment length was greater than 1 000 bp. All 24 clones were able to amplify bands, and the cDNA fragment recombination rate was 100%. The library has high quality and complete genetic information, which meets the requirements of yeast hybrid screening test, and can be applied to the research of gene expression regulation of *Medicago truncatula* and the screening test of interaction protein.

Keywords *Medicago truncatula*; Yeast hybridization cDNA library; SMART technology; Different organizational sources

Plant cDNA library refers to the clone set formed by the reverse transcription of all mRNAs of plant tissues at a specific growth and development period into cDNAs, and then connecting them with appropriate library vectors and transferring them into specific bacteria (Gubler and Hoffman, 1983). Compared with the classical cDNA library, the full-length cDNA library has certain advantages. For example, the classical cDNA library has some shortcomings, mainly in cloning fragments, and the full-length cDNA library can provide complete mRNA information and obtain the full-length cDNA sequence (Yan et al., 2006). Homogenized cDNA can increase the possibility of cloning low abundance mRNA (Li et al., 2018). Plant cDNA library can not only be used to screen regulatory genes in plant growth and development stages, but also play an important role in the prediction of interaction proteins and *in vitro* expression (Wang et al., 2014). At the same time, plant cDNA library plays an important role in discovering and studying new gene functions, and is favored by researchers (Yang et al., 2007). Therefore, high-capacity cDNA library is of great significance for further study of gene function.

Medicago sativa is known as 'King of Forage' because of its rich nutrition and good palatability (Cao et al., 2011). Compared with the related species of *Medicago sativa* and other plants in Leguminosae, *Medicago truncatula* has many genetic advantages, such as small genome, high efficiency of genetic transformation, short life cycle, self-pollination and so on (Blondon et al., 1994; Trieu et al., 1996; Bonnin et al., 1997), which make it a model plant of Leguminosae. *Medicago truncatula* also plays an important role in biological nitrogen fixation. At the same time, *Medicago truncatula* has genetic similarity with plants in Leguminosae such as soybean, so the study of *Medicago truncatula* is an important basis for studying other plants in Leguminosae.

In order to obtain a high-capacity *Medicago truncatula* yeast hybrid cDNA library, and further dig the related genes of *Medicago truncatula*, *Medicago truncatula* from different tissue sources was selected, and different hormone induction or stress treatments were performed on *Medicago truncatula*, using SMART technology to successfully construct a high-capacity *Medicago truncatula* yeast hybrid cDNA library in this study. The library

can be used for screening protein interactions or protein and DNA interactions in various mechanisms, which provides a basis for further research on basic biology such as protein interaction or protein and DNA interactions.

1 Results and Analysis

1.1 Good mRNA isolation quality of *Medicago truncatula*

After 1% agarose gel electrophoresis detection (Figure 1), the results showed that the purified mRNA was a fuzzy drag band, and the brightest part of the drag band was more than 500 bp, indicating that the isolated and purified mRNA did not decompose and had good quality, which could be used as the initial sample of the library.

Figure 1 mRNA isolation of *Medicago truncatula*

1.2 Preliminary construction of cDNA primary library

5 μ L purified ds-cDNA was detected by 1% agarose gel electrophoresis, respectively. The results showed that ds-cDNA gel electrophoresis showed diffuse bands with a size between 250 and 2000 bp (Figure 2), indicating that the purified cDNA meet the requirements and could be used for the next experiment of library construction. 10 μ L transformed bacterial liquid was diluted, and the diluted concentration was 1/1 000 of the original concentration. Then 50 μ L diluted liquid was coated with LB plate (containing K⁺resistance). According to the method of “CFU/mL=colony number on the plate/50 μ L $\times$ 1 000 times $\times$ 1 $\times$ 10³ μ L, total clone number of library (CFU)=CFU/mL $\times$ total volume of library bacterial liquid (mL)”, the total clone number of primary library was 1.2 $\times$ 10⁷ CFU (Figure 3A). 24 clones were randomly selected from the clone library for colony PCR identification. The recombination rate and insert fragment length were detected by 1% agarose gel electrophoresis (Figure 3B). According to the “recombination rate=the number of successful recombination/the total number of clones $\times$ 100%”, the recombination rate of primary library was 100%, and the average insert fragment length was > 1 000 bp, which met the requirements of high-quality primary library.

Figure 2 cDNA synthesis and purification detection

Figure 3 Primary library capacity identification and detection of cDNA inserts in the library

Note: A: Primary library capacity identification; B: Primary library recombination rate test; 1~24: PCR identification of 24 clones

1.3 Preliminary construction of yeast hybrid cDNA library

The 10 μ L transformed bacterial solution was diluted 1 000 times, and 50 μ L was taken out and spread in LR medium, and placed in a constant temperature incubator for counting the next day (Figure 4A). According to the method of “CFU/mL=colony number on the plate/50 μ L $\times$ 1 000 times $\times$ 1 $\times$ 10³ μ L, total clone number of library (CFU)=CFU/mL $\times$ total volume of library bacterial liquid (mL)”, the total clone number of *Medicago truncatula* yeast hybrid cDNA library was 1.28 $\times$ 10⁷ CFU. 24 clones were randomly selected from the clone library for colony PCR identification. The recombination rate and insert fragment length were detected by agarose gel electrophoresis, the results showed that recombination rate was 100%, and the average insert fragment length was > 1 000 bp (Figure 4B), indicating that the yeast hybrid cDNA library met the construction requirements and could be used for subsequent yeast hybrid experiments of *Medicago truncatula*.

Figure 4 Construction and identification of yeast hybridization cDNA library

Note: A: Yeast hybridization cDNA library capacity identification; B: Identification of recombination rate of yeast hybridization cDNA library; 1~24: PCR identification of 24 clones

1.4 Y187 competent cells transformed with cDNA library

The clone number of SD/-Leu medium with dilution of 1:10 000 was 500. According to the calculation formula, the cell density was more than 3 $\times$ 10⁷ cells/mL, and the library titer was 5 $\times$ 10⁷ CFU/mL (Figure 5A). 24 single colonies were randomly selected from SD/-Leu medium for PCR detection after expanded culture (Figure 5B). Among them, 23 clones could amplify bands, and the library recombination rate was 96%, the average insert fragment of the library was more than 1 000 bp.

Figure 5 Identification of Y187 competent cells transformed with cDNA library

Note: A: Library titer identification; B: Yeast clone identification; 1~24: PCR identification of 24 clones

2 Discussion

High-capacity cDNA library is the basis for discovering and studying new gene functions and screening protein interactions or protein and DNA interactions by yeast two-hybrid and one-hybrid technologies. There are several important indexes to evaluate library quality, including the representativeness of cDNA library and the integrity of recombinant sequences (Chen, 2011, China Agriculture Press, pp.142-148; Gao et al., 2014). The representativeness of the library can be reflected by the library capacity, which mainly refers to the integrity of the cDNA types contained in the library, indicating that the integrity of the genetic information (mRNA) expressed in

the source tissue is an important indicator of library quality (Zhu et al., 2018, Jiangsu Agricultural Sciences, 46(9): 47-50). Clarke and Carbon (1976) defined the library volume as follows: the total number of clones of independent recombinants in the cDNA library, that is library titer concentration. When the library titer concentration $\geq 1.7 \times 10^5$ CFU/mL, this library is called an effective library. When the library titer concentration $\geq 1 \times 10^6$ CFU/mL, this library meets the screening requirements of low abundance mRNA. The size of cDNA inserts is also an important indicator affecting the quality of the library. The length of plant cDNA is between 0.5~3 kb (Wang et al., 2012), which is generally ≥ 1 kb (Wang et al., 2009). Inserted fragments in the library, too small or too large, will affect the quality of the library. Only when the inserted cDNA fragment is close to the full-length cDNA fragment, the genetic information reflected in the library will be more complete. In this study, SMRT cDNA technology was used to successfully construct a high-capacity yeast hybrid cDNA library from *Medicago truncatula*. The constructed cDNA library contained abundant cDNA clones and complete genetic information. The calculated library titer concentration was about 5×10^7 CFU/mL, the average length of inserted fragments in the library was more than 1 000 bp, and the recombination rate of cDNA fragments was 100%, which met the requirements of cDNA library integrity and high quality, and could be used for subsequent yeast hybrid experiments.

Yeast hybrid system mainly includes yeast two-hybrid technology and yeast one-hybrid technology. Yeast two-hybrid technology is not only applied to the screening of interaction proteins, but also plays an important role in the study of the interaction between antigens and antibodies in cells, the screening of drug action sites, the interaction between drugs and proteins, and the linkage map of genome proteins (Dyer et al., 2010; Zheng et al., 2013). Yeast one-hybrid technology was developed based on the yeast two-hybrid technology, which was mainly used to study the protein and DNA interactions (Li and Herskowitz, 1993). Yeast hybrid system is widely used because of its simple, efficient, and high sensitivity. It has become the most commonly used technology to study gene function (Li et al., 2016). The high-capacity yeast hybrid cDNA library from *Medicago truncatula* constructed in this study can not only be used for yeast two-hybrid screening for interaction proteins of *Medicago truncatula*, but also be effective for yeast one-hybrid screening for protein and DNA interaction of *Medicago truncatula*.

It has become the first choice for researchers to study the function of unknown genes and screen protein interactions or protein and DNA interactions by constructing plant cDNA library, and some achievements have been made. cDNA library is widely studied in *Zoysia japonica*. Teng et al. (2019) successfully constructed a full-length cDNA library of *Zoysia japonica* senescence leaves, and 6 genes involved in cell growth and senescence were screened from the library. In order to reveal the stress resistance mechanism of *Zoysia japonica*, Wang et al. (2012) successfully constructed a cDNA library of *Zoysia japonica* induced by low temperature and drought using Gateway technology. In addition, cDNA libraries are also widely used in maize, soybean, cotton, and other crops (Arpat et al., 2004; Zhang et al., 2005; Li et al., 2010), which laid the foundation for the study of functional genes in plants. However, the information about the high-capacity *Medicago truncatula* yeast hybrid cDNA library is still unclear, and the relevant researchers cannot study the related functional genes of *Medicago truncatula* by yeast hybrid technology. Therefore, it is particularly important to construct a high-capacity *Medicago truncatula* yeast hybrid cDNA library.

In this study, *Medicago truncatula* from different tissue sources was selected, and different hormone induction or stress treatments were performed on *Medicago truncatula*, using SMART technology to successfully construct a high-capacity *Medicago truncatula* yeast hybrid cDNA library. And the library plasmid was successfully transformed into Y187 yeast competent cells. The constructed library is an open library for free use by national researchers, which can provide reference for national researchers in *Medicago truncatula* research. The library is of high quality and contains complete genetic information, which can be used in gene expression regulation research and screening of protein interactions. It can provide experimental basis for further gene mining of *Medicago truncatula* by yeast hybrid, and also provide basis for further research on basic biology such as protein interactions or protein and DNA interactions.

3 Materials and Methods

3.1 Experimental materials

The plant material used in this study is *Medicago truncatula* R108, cultivated in laboratory light incubator. CloneMiner II cDNA library construction kit, FastTrack MAG mRNA isolation kit, rapid gel extraction and PCR purification kit, plasmid DNA purification kit were purchased from Invitrogen (USA). *E. coli* DH10B, DNA ligase, DNA polymerase, T4 DNA Ligase and Y187 strain were purchased from TaKaRa (Japan). Pharmaceuticals such as agar, ethanol and isoamyl alcohol were purchased from Sigma (USA).

3.2 Extraction of total RNA and purification of mRNA from *Medicago truncatula*

A total of 0.1 g of *Medicago truncatula* from different tissue sources with good growth and uniformity was weighed and fully ground in liquid nitrogen to powder. The total RNA was extracted by Trizol method, and then the extracted mRNA was separated and purified. The integrity of RNA was detected by 1% agarose gel electrophoresis.

3.3 Construction and detection of cDNA primary library

The total RNA extracted from *Medicago truncatula* was used as the template to synthesize the first strand cDNA by reverse transcription, and then the synthesized first strand cDNA was used as template to synthesize the second strand cDNA under the action of *E. coli* DNA Ligase (10 U/ μ L), *E. coli* DNA Polymerase I (10 U/ μ L), *E. coli* RNaseH (2 U/ μ L) and T4 DNA Polymerase. The obtained cDNA was connected with the three-frame attB1 recombinant joint, and the cDNA was collected after fractionation by cDNA. The collected cDNA was recombined with BP and transformed into *E. coli* DH10B cells. After transformation, it was put into SOC medium and cultured in a shaking bed of 225~250 r/min at 37 °C for 1 h. A total of 10 μ L bacterial solution was taken to determine the storage capacity, recombination rate and inserted fragment length. The remaining culture was added with glycerol until the final concentration was 20% and stored at -80 °C.

3.4 Construction and preservation of a yeast cDNA library

The primary library plasmid was extracted and diluted to 300 ng/ μ L. The plasmid was combined with the vector pGADT7-DEST for LR recombination. After recombination, the recombinant plasmid was transformed into *E. coli* DH10B competent cells, and then the yeast two-hybrid cDNA library was obtained on SOC medium. 10 μ L of transformed bacterial liquid was used for library quality identification. The remaining bacterial liquid was added with glycerol until the final concentration was 20% and stored at -80°C.

3.5 Preparation of Y187 yeast competent cells

A small amount of Y187 yeast strains were taken and streaked on YPDA medium, and the medium was inverted cultured at 30°C for 3~5 d. Single yeast colonies were picked and cultured in YPDA medium for several times until OD_{600} reached 0.4~0.5. Separated the final bacterial liquid, centrifuged, then removed the supernatant. Then, resuspended with sterile deionized water, centrifuged, then added with TE/LiAc, and resuspended again. Finally, the cells were suspended with TE/LiAc solution, this cell suspension was Y187 yeast competent cells.

3.6 cDNA library transformed Y187 yeast competent cells

The 5 μ L cDNA library plasmid was transformed into the prepared Y187 yeast competent cells, and 10 μ L of the transformed cell stock solution was diluted 10, 100, 1 000, and 10 000 times and spread on SD/-Leu plates. After inverted culture at 30 °C for 3~5 d, the transformants were collected, the cell density was calculated, and the library titration number was determined. The 5 μ L cDNA library plasmid was transformed into the prepared Y187 yeast competent cells, and 10 μ L of the transformed cell solution was diluted 10, 100, 1 000, and 10 000 times and spread on SD/-Leu plates. After inverted culture at 30°C for 3~5 d, the transformants were collected, the cell density was calculated, and the library titer number was determined. 24 clones were randomly selected and performed PCR identification.

Authors' contributions

LSW is the experimental designer and executive of this study, participates in data collation and the writing of the first draft of the paper. WMD participated in data processing and some experiments. DD and LYRZ participated in the part of experiment. ZTJ is the main person in charge of the project, guiding the experimental design, data statistics, paper writing and revision. All authors read and approved the final manuscript.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (31772656).

Reference

- Arpat A.B., Waugh M., Sullivan J.P., Gonzales M., Frisch D., Main D., Wood T., Leslie A., Wing R.A., and Wilkins T.A., 2004, Functional genomics of cell elongation in developing cotton fibers, *Plant Molecular Biology*, 54(6): 911-929
<https://doi.org/10.1007/s11103-004-0392-y>
 PMid:15604659
- Blondon F., Marie D., Brown S., and Kondorosi A., 1994, Genome size and base composition in *Medicago sativa* and *M. truncatula* species, *Genome*, 37(2): 264-270
<https://doi.org/10.1139/g94-037>
 PMid:18470076
- Bonnin I., Prosperi J.M., and Olivieri I., 1997, Comparison of quantitative genetic parameters between two natural populations of a selfing plant species, *Medicago truncatula* Gaertn., *Theoretical and Applied Genetics*, 94(5): 641-651
<https://doi.org/10.1007/s001220050461>
- Cao H., Zhang H.L., Gai Q.H., Chen H., and Zhao M.L., 2011, Introduction test and production performance comprehensive evaluation of 22 alfalfa varieties, *Caoye Xuebao (Acta Prataculturae Sinica)*, 20(6): 219-229
- Clarke L., and Carbon J., 1976, A colony bank containing synthetic Col EI hybrid plasmids representative of the entire *E. coli* genome, *Cell*, 9(1): 91-99
[https://doi.org/10.1016/0092-8674\(76\)90055-6](https://doi.org/10.1016/0092-8674(76)90055-6)
- Dyer M.D., Neff C., Dufford M., Rivera C.G., Shattuck D., Bassaganya-Riera J., Murali T.M., and Sobral B.W., 2010, The human-bacterial pathogen protein interaction networks of *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*, *PLoS One*, 5(8): e12089
<https://doi.org/10.1371/journal.pone.0012089>
 PMid:20711500 PMCID:PMC2918508
- Gao X.H., Jia R., Wang M.S., Zhu D.K., Chen S., Lin M., Yin Z.Q., Wang Y., Chen X.Y., and Cheng A.C., 2014, Construction and identification of a cDNA library for use in the yeast two-hybrid system from duck embryonic fibroblast cells post-infected with duck enteritis virus, *Molecular Biology Reports*, 41(1): 467-475
<https://doi.org/10.1007/s11033-013-2881-z>
 PMid:24293127
- Gubler U., and Hoffman B.J., 1983, A simple and very efficient method for generating cDNA libraries, *Gene*, 25(2-3): 263-269
[https://doi.org/10.1016/0378-1119\(83\)90230-5](https://doi.org/10.1016/0378-1119(83)90230-5)
- Li C., Yan X.H., Zhou X.A., Sha A.H., Shan Z.H., Zhou R., and Wei W.H., 2010, Construction of a full-length homogenized cDNA library of soybean seeds at different developmental stages, *Zhongguo Nongye Kexue (Chinese Agricultural Sciences)*, 43(3): 462-467
- Li H., 2016, Construction of yeast hybrid cDNA library of *Diospyros kaki* Thunb. and screening of DkPDC2 promoter library, Thesis for M.S., Huazhong Agricultural University, Supervisor: Luo Z.R., pp.17-19
- Li J., and Herskowitz I., 1993, Isolation of ORC6, a component of the yeast origin recognition complex by a one-hybrid system, *Science*, 262(5141): 1870-1874
<https://doi.org/10.1126/science.8266075>
 PMid:8266075
- Li Z.Z., Wu P.Q., Cheng Y.X., Song H.X., Li M.L., Hou L.P., and Xu X.Y., 2018, Construction and evaluation of a homogenized cDNA library of male sterile flower buds in Chinese cabbage, *Zhiwu Shengli Xuebao (Journal of Plant Physiology)*, 54(2): 291-296
- Teng K., Tan P.H., Fan X.F., Yue Y.S., Jiang H.Y., Wand u J.Y., 2019, Construction of full-length cDNA library of senescent leaves of *Zoysia japonica* and screening verification by yeast single hybridization, *Caodi Xuebao (Acta Prata Sinica)*, 27(6): 1486-1493
- Trieu A.T., and Harrison M.J., 1996, Rapid transformation of *Medicago truncatula*: regeneration via shoot organogenesis, *Plant Cell Reports*, 16(1-2): 6-11
<https://doi.org/10.1007/BF01275439>
 PMid:24178644
- Wang L.J., Jin Z.P., Wang N.F., Li X.F., and Liu G.S., 2009, Construction of *Leymus chinensis* leaf cDNA library and analysis of some expressed sequence tags, *Caoye Xuebao (Acta Prataculturae Sinica)*, 18(1): 65-71
- Wang Z.T., 2014, Construction and EST analysis of cDNA library of disease-free leaves, Fujian Agriculture and Forestry University, Thesis for M.S., Supervisor: Zheng Y.S., pp. 17-19
- Wang Z., Zong J.Q., Guo H.L., and Liu J.X., 2012, Construction of low temperature and drought induced cDNA library of *Zoysia japonica* by gateway technology and its quality identification, *Caoye Kexue (Grass Science)*, 29(6): 911-917

- Yan H.J., Huang X.Q., Cheng Z.Q., 2006, CDNA library construction strategy and its analysis and research progress, Yunnan Nongye Daxue Xuebao (Journal of Yunnan Agricultural University), (1): 1-6
- Yang C.J., and Wang J., 2007, Construction strategy and application of cDNA library, Shengwu Jishu Tongbao (Biotechnology Bulletin), (1): 5-9
- Zhang Z.X., Zhang F.D., Tang W.H., Tang W.H., Pi Y.J., and Zheng Y.L., 2005, Construction and characterization of normalized cDNA library of maize inbred MO17 from multiple tissues and developmental stages, Molecular Biology, 39(2): 198-206
<https://doi.org/10.1007/s11008-005-0026-8>
- Zheng L.S., Li X.N., Sun C.T., Liu H.Y., Liu X., He M., and Lü W.F., 2013, Research progress of yeast two-hybrid technology and its application, Zhongguo Xumu Shouyi (Chinese Journal of Animal Husbandry and Veterinary Medicine), 40(9): 105-108