A PCR-based Technology for Rapid Screening of Genomic DNA Library

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We explored the feasibility of rapid screening of gDNA library with PCR technique. We adopted porcine α-1,3GT cDNA fragment as the probe, used primers synthesized by the specific sequence on cDNA to carry out α-1,3GT gene screening of porcine gDNA library by combining PCR and *in situ* plaque hybridization, and then performed enzymatic digestion, Southern blot, sequencing, and fluorescence *in situ* hybridization for location. After having finished one-time hybridization and one-time PCR, we obtained 7 positive monoclones with very strong signals, and each insert length of them was over 8 kb, including the third intron. Moreover, 3 tested clones among them contain the third and fourth exons according to sequencing results. FISH mapped the inserts of the 3 clones to pig chromosome 1q2.10-q2.11. PCR could be applicable to rapid screening of DNA library and this approach is much simpler compared to conventional *in situ* plaque hybridization alone.

Key Words: *genomic DNA library; polymerase chain reaction; hybridization*

Screening of DNA library is currently performed using *in situ* plaque hybridization. The disadvantage of this method is relatively trivial operation process and the risk of contacting radiation [1,2]. When carrying out research on xenotransplantation in transgenic pigs, the author had screened porcine genomic DNA (gDNA) library by combining PCR and *in situ* plaque hybridization, so as to quickly obtain the targeting guide sequence aiming at α-1,3 GT gene, and map it on pig's chromosome using FISH analysis.

MATERIALS AND METHODS

Porcine α-1,3GT cDNA fragment was kindly provided by Dr. Vanhove (France). Escherichia coli JM109 was kept in the National Key Lab of Chinese Medical Genetics. EcoRI enzyme, SacI enzyme and XhoII enzyme were bought from Biolabs Company; mouse anti-digoxin FITC-labeled, rabbit anti-mouse FITC-labeled, and goat anti-mouse FITC-labeled antibodies were from Sigma Company. α-^{32}P-dATP and dNTP were from Dupont Company and Sino-American Company, respectively. Sequencing kit and plasmid extraction kit were from PE Company and QIAGE Company, respectively, and gDNA library (λgt11, EMBL3SP6/T_7) was provided by Clontech Company. PCR primers and sequencing primers were synthesized by the National Key Lab of Chinese Medical Genetics. primer 1: 5' TCC GAG CTG GTT TAA CAA TGG 3', primer 2: 5' TCT TCT TCG TGG TAA CTG TGA GTC 3', primer 3: 5' GAC TCA CAG TTA CCA CGA AGA AGA 3', primer 4: 5' ATG ATT CCA ATA TGC CAG AA 3', primer 5: 5' GTA GAC ATT TGC TTG GCG CA 3'.

Escherichia coli JM109 were transformed with α-1,3GT cDNA plasmid; amplified and extracted

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plasmid DNA was digested with EcoR1 for 4 h and the products of enzyme digestion were separated by agarose gel electrophoresis followed by identification of the cDNA fragment probe applicable to screening α-1,3GT gDNA library by automatic sequencing on a PE377 sequencer.

α-^{32}P-labeled probe cDNA obtained by the random primer method was used for alternative screening with PCR in turn to obtain positive clones of gDNA fragment. Enzyme digestion of these positive clones with SacI, XhoI and EcoR1 were carried out and Southern blotting, sequencing, and FISH analysis were carried out. Finally, fragments over 8kb containing the 3rd and 4th exons and the 3rd intron as the targeting guide sequence were selected.

Screening procedures were briefly described as below. *In situ* plaque hybridization of the probe cDNA labeled with α-^{32}P-ATP and λgt11 MBL_3SP6/T_7 gDNA library was performed to obtain 15 positive clones. The 3rd intron of the insert fragment in the 15 positive clones were amplified by PCR using primers 1 and 2, and then PCR reaction products were separated by electrophoresis in 0.8% agarose gel to obtain the first 7 PCR positive monocolones. *In situ* plaque hybridization of probe cDNA labeled with α-^{32}P-ATP and phage colony plated by the first 7 PCR positive clones were carried out to verify PCR positive monocolones. PCR was used to rescreen any one positive monocolone selected randomly from each one of the second 7 hybridization signal plates to pick up the new 7 PCR positive monoclones (number 1-7). The 4 positive monoclones selected from monocolones 1-7 were analyzed using *in situ* plaque hybridization again to obtain numerous positive signal monoclones. The extracted λgt11B DNA of 4 positive monoclones selected from the previous step was digested with enzymes SacI, XholI and EcoRI to get 4 fragments. Southern blotting showed that each insert length was over 8 kb. The fragments over 8 kb containing the 3rd exon as the guide sequence were certified by sequencing.

After preparation of porcine peripheral blood cell chromosome slides, we used the above guide sequence as the probe and performed FISH location with porcine chromosome to certify the above fragment available.

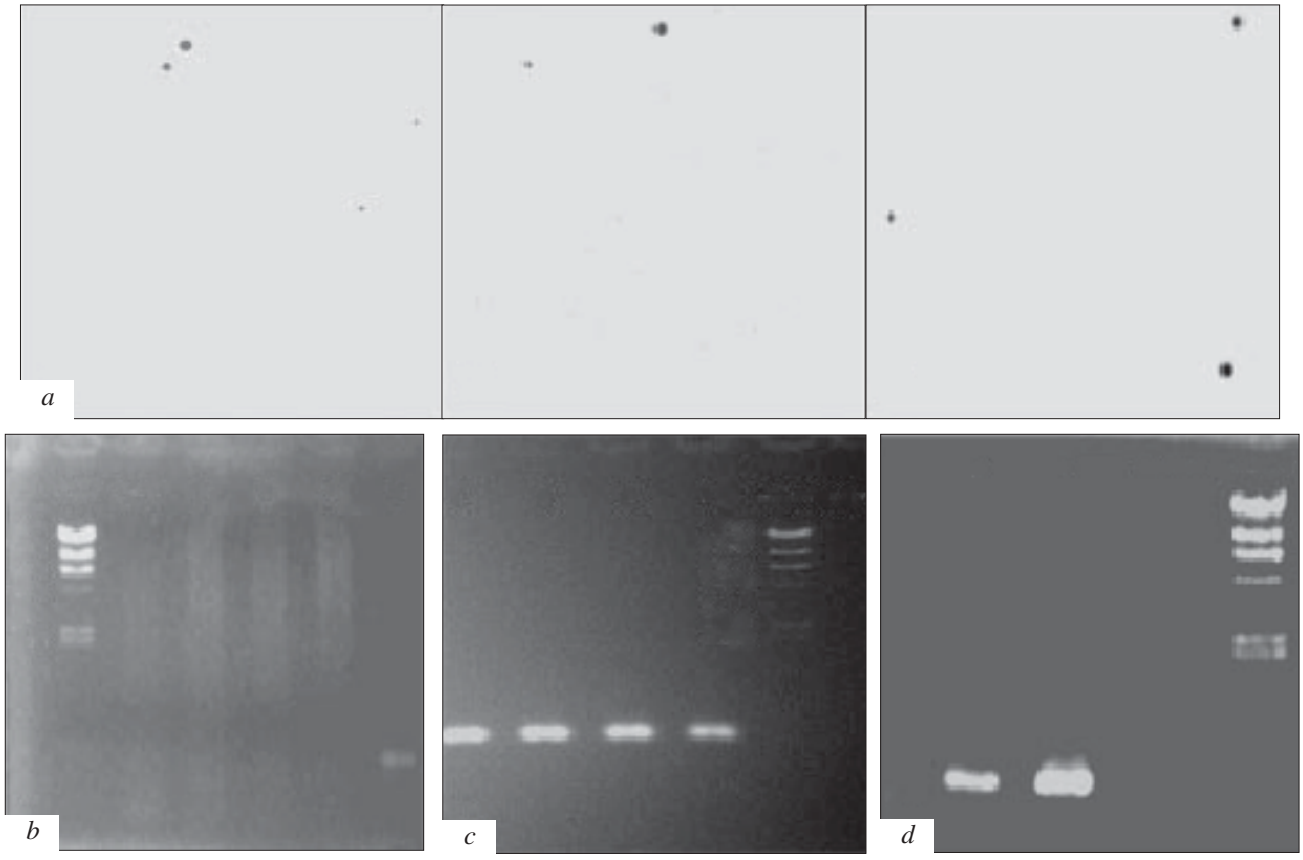

Fig. 1. The first plaque hybridization of cDNA probe labeled with α-^{32}P-ATP screened the porcine gDNA library (*a*: 9 positive signals of 15 positive colonies are shown) and the first PCR-screening of the first 15 positive plaque hybridization colonies (*b-d*). There were 7 positive signals. *b*: 1 positive signals; *c*: 4 positive signals, *d*: 2 positive signals.

RESULTS

The first radiolabeled probe cDNA screening revealed 15 positive clones (Fig. 1 showed only 3 of *in situ* hybridization films and 9 positive clone signals). The first PCR screened 15 positive probe cDNA hybridization clones, and obtained 7 positive signals (~550 b.p. specific band, Fig. 1, *b*). The second probe cDNA hybridization screened 7 PCR positive clone plates and obtained more positive monoclones (Fig. 2, *a* showed only 3 of *in situ* hybridization films). The second PCR amplified 7 monoclones (any monoclone of each positive hybridization signal plate) selected from the second probe cDNA hybridization screening positive signals and showed the same specific band of ~550 b.p. in the 7 clones with stronger signals (Fig. 2, *b*). We conducted *in situ* hybridization of the radiolabeled probe cDNA with the plates plated by 4 monoclones of the second positive PCR signals and obtained numerous positive monoclones (Fig. 3, *a* showed only one of hybridization films). Then, we extracted 4 positive monoclone λgt11 DNA to conduct enzyme digestion and certified through Southern Blot that each insert length of them is over 8kb (Fig. 3, *b-c*). Moreover, 3 selected clones among them contained the third and fourth exons and third intron according to the sequencing results, and FISH located the insert fragment of the 3 clones to pig chromosome 1q2.10-q2.11 (Fig. 3, *c*, *d*). Hence, the obtained fragments could be used as the targeting guide sequence of porcine α-1,3GT gene.

The present research has applied the technology to the screening for gDNA library for the first time. Since there are no introns and other repeated sequence in cDNA, and that the literature has reported the third intron sequence of porcine α-1,3 GT gene, the author has selected α-1,3GT cDNA fragment as the probe in this study and screened porcine gDNA library by making use of known cDNA sequence and partial intron sequence with powerful specificity as well as the primer synthesized by specific sequences on cDNA, and by combining *in situ* plaque hybridization and PCR. Then, the authors used PCR to further screen and appraise the positive clones obtained by hybridization of radioactivity probe. The experiment proves that only one-time hybridization and one-time PCR are necessary for obtaining positive monoclones. In this study, the author has also conducted sequencing for 3 positive clones among them, compared the sequence with the Genebank Database by GCG software, found a complete concord result, and clarified the juncture of the third intron and the

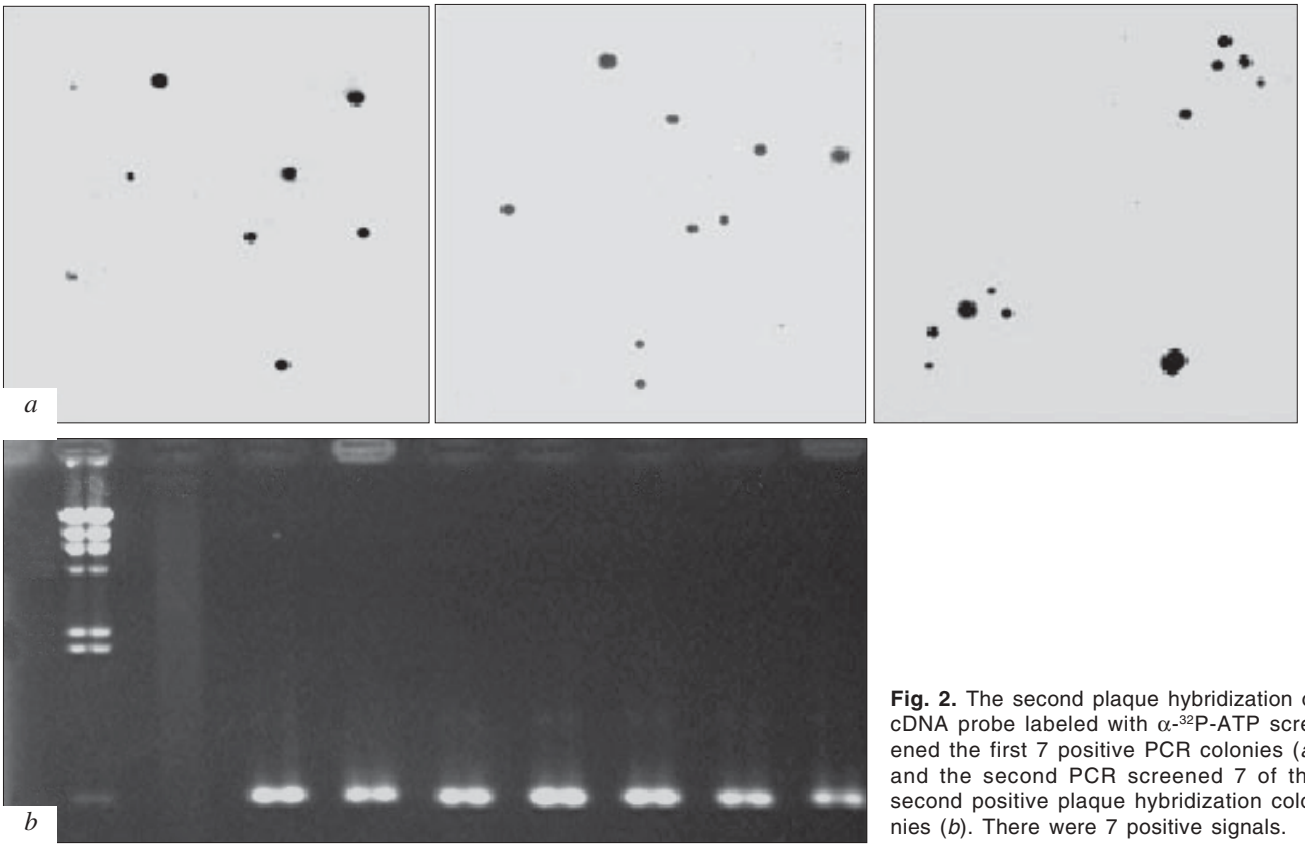

Fig. 2. The second plaque hybridization of cDNA probe labeled with α-^{32}P-ATP screened the first 7 positive PCR colonies (*a*) and the second PCR screened 7 of the second positive plaque hybridization colonies (*b*). There were 7 positive signals.

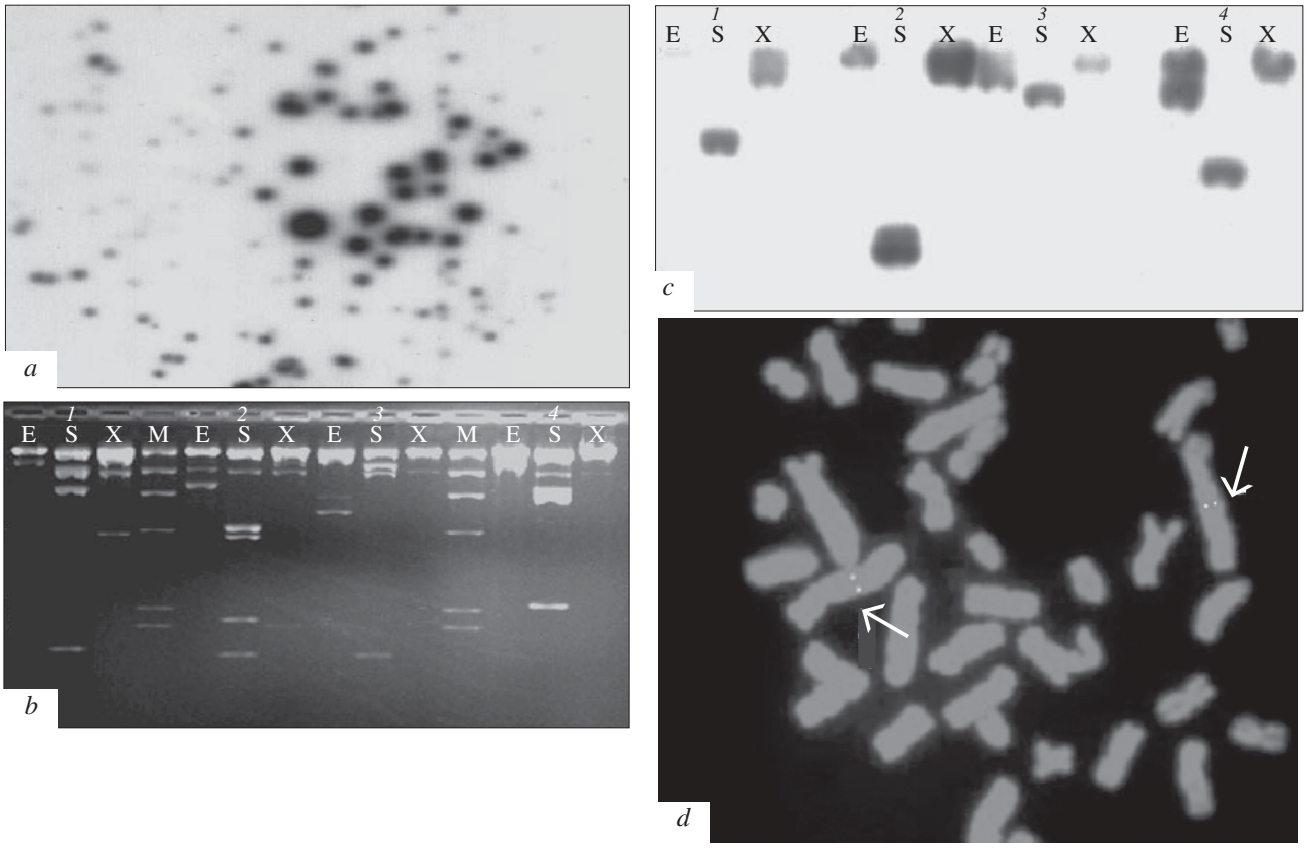

Fig. 3. The third plaque hybridization of cDNA probe labeled with α-^{32}P-ATP screened 4 of the second positive PCR colonies (*a*), EcoRI, SacI, and XhoII enzymes digested 4 of the third positive hybridization colonies (*b*), Southern blot confirmed the third 4 positive digested insert fragments (*c*), and FISH chromosome location (*d*). *b, c*: digestion with EcoRI (E), SacI (S), and XhoII (X), M: molecular weight marker; *d*: arrows show the position of the insert 1q2.10-q2.11.

third and fourth exons. The sequencing proved that the positive precision rate is 100%. Experiments showed that the strategy of screening of library by combining PCR technique and *in situ* plaque hybridization with cDNA fragment as the probe is simple, convenient, feasible, and highly credible. Of course, when PCR is adopted for screening, it is necessary to optimize PCR conditions for the known fragment of DNA sequence. The author thinks that as long as there is a known fragment of reliable sequence (only several hundred b.p.), it could be possible to adopt PCR directly for screening, and then carry out identification by sequencing, so as to avoid the danger of contacting isotope in repeated *in situ* hybridization, and greatly reduce the appearance of false positive clones. The establishment of this method has obvious actual application value, and it has been applied in our laboratory. Therefore, we think that screening of gDNA library with combining cDNA as the probe and PCR is an effective strategy.

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