Protocols for PCR-based screening of genomic libraries

Yuji Yasukochi

Abstract

Citation: Yuji Yasukochi Protocols for PCR-based screening of genomic libraries. **protocols.io** dx.doi.org/10.17504/protocols.io.mvdc626
Published: 29 Jan 2018

Protocol

Preparation of DNA pools

Step 1.

- Add 50 µl LB containing 7.5% glycerol and an antibiotic for selection to each well of 384-well plates.
- Pick up white colonies with toothpicks.
- Put each toothpick into the same position of triplicated 384-well plates.
- Incubate the 384-well plates overnight at 37°C.
- Store one of the triplicated plates at less than –70°C.
- For row DNA pools: Assemble cultures in the same row of another plate into a microtube. Isolate BAC, fosmid or plasmid-DNA from the assembled culture by a standard alkali lysis method and dissolve it with 200 µl TE.
- For column DNA pools: Assemble cultures in the same column of the remaining plate into a microtube. Isolate DNA from the assembled culture by a standard alkali lysis method and dissolve it with 200 µl TE.
- For plate DNA pools: Assemble 20 µl aliquots of column or row DNA pools of the same plate.
- Dilute all DNA pools with 19-fold volume of PCR buffer each to prepare working solutions ready for PCR-screening.

PCR screening

Step 2.

- Prepare an appropriate amount (7 µl x plates) of PCR reaction mix solution containing 0.5 µM primers. Add *Taq* polymerase (0.5U/sample) prior to use and mix well.
- Transfer 7 µl of reaction into each microplate well for PCR amplification.
- Add 5 µl of plate DNA working solutions into each well, and perform amplification reaction.
- Load 5 µl of completed reactions on an agarose gel and run electorophoresis to detect which plates contain positive clones containing PCR amplicon of interest.
- Perform secondary PCR reactions using row and column DNA working solutions of positive plates in the same way as described above.
- If a single signal is detected from both of row and column pools, pick up the candidate clone. If multiple signals are detected from any of row and column pools, pick up all the candidate clones.
- Perform PCR reactions against 1 µl of individual overnight cultures to confirm which candidate clone harbors the target sequence.

References

Step 3.

1)Yasukochi Y. PCR-based screening for bacterial artificial chromosome libraries. Methods in Molecular Biology 2002; 192: 401-410.

2)Yoshido A, Sahara K, Yasukochi Y. 2014. Silk Moths (Lepidoptera) In “Protocols for Cytogenetic Mapping of Arthropod Genomes” edited by Sharakhov, I. V., pp. 219–256. CRC Press, Boca Raton, FL.