

DNA PCR Assays for Igh Rearrangement

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[Abstract] This protocol is used for the detection of immunoglobulin heavy (H) chain rearrangements. This PCR-based assay enables detection of D_H-J_H recombination in cultured hematopoietic cells (Schlissel *et al.*, 1991; Satoh *et al.*, 2013) [e.g. ES-derived cells (Satoh *et al.*, 2013)].

Materials and Reagents

1. Mouse spleen cells or ES-derived hematopoietic cells
2. DNA extraction Kit: PerfectPure DNA Cultured Cell Kit (5 PRIME, catalog number: 2302000)
3. 10x PCR Buffer with KCl (Life Technologies, Applied biosystems®)
4. MgCl₂ (Life Technologies, Applied biosystems®)
5. Taq DNA polymerase (Life Technologies, Applied Biosystems®, catalog number: 4338856)
6. dNTPs (Life Technologies, Applied Biosystems®)
7. Primers (FASMAC)

The sequence of primers are as follows.

 - a. D_HL(5'), GGAATTCG(AorC)TTTTTGT(CorG)AAGGGATCTACTACTGTG
 - b. Mu0(5'), CCGCATGCCAAGGCTAGCCTGAAAGATTACC
 - c. J3(3'), GTCTAGATTCTCACAAGAGTCCGATAGACCCTGG
8. Agarose (UltraPure™ Agarose) (Life Technologies, Invitrogen™, catalog number: 16500-100)
9. Ethidium bromide (Wako Pure Chemical Industries, catalog number: 315-90051)

Equipment

1. PCR Thermal Cycler (Veriti Thermal Cycler) (Life Technologies, Applied Biosystems®)
2. Centrifuges (TOMY SEIKO, model: MX-150)
3. Electrophoresis apparatus (ADVANCE, Mupid-exU)

Procedure

1. Genomic DNA was prepared for PCR by lysing mouse spleen cells ($1-4 \times 10^4$) or ES-derived hematopoietic cells ($3-5 \times 10^5$) in 75 μ l elution solution. See the manufacturer's protocol (http://www.5prime.com/media/3415/perfectpure_dna_cultured_cell_manual_5prime_1064553_122010.pdf).
2. 20 μ l PCR reactions contained 5.5 μ l template (82.5 ng or less), 10 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, 1 μ M primers (25 mers), 200 μ M dNTPs and 1 U Taq DNA polymerase.
3. PCR program
 - a. 94 °C – 2 min
 - b. 94 °C – 1 min
 - c. 60 °C – 1 min
 - d. 72 °C – 1.75 min
 - e. Repeat steps b-d, 35x
 - f. 72 °C – 10 min
4. Half of each PCR products were electrophoresed on 1% agarose gels, and their amounts were evaluated by staining with ethidium bromide.
5. D_H - J_H recombination was detected as amplified fragments of 1,033 bp, 716 bp and 333 bp using a primer $D_HL(5')$ and $J3(3')$. Germline alleles were detected as an amplified fragment of 1,259 bp using a primer $Mu0(5')$ and $J3(3')$.

Results

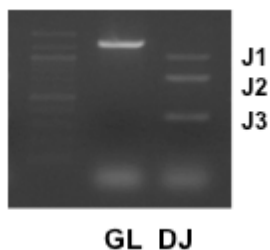


Figure 1. DNA PCR assays of germline (GL) or D_H - J_H rearranged *Igh* chain (DJ) genes were performed with mouse splenocytes. A PCR experiment using a primer $D_HL(5')$ and $J3(3')$ can detect three types of D_H - J_H rearrangement (J1, J2, and J3) (Schlissel *et al.*, 1991). All of three bands are present with successful D_H - J_H rearrangement. However, a J1 band is sometimes undetected in the ethidium bromide-based DNA-band visualization when the amount of template DNA is very small. The size marker was loaded in the left lane.

Acknowledgments

This protocol was adapted from a previously published paper by Schlissel *et al.* (1991). The representative data shown in the protocol was adapted from Satoh *et al.* (2013).

References

1. Satoh, Y., Yokota, T., Sudo, T., Kondo, M., Lai, A., Kincade, P. W., Kouro, T., Iida, R., Kokame, K., Miyata, T., Habuchi, Y., Matsui, K., Tanaka, H., Matsumura, I., Oritani, K., Kohwi-Shigematsu, T. and Kanakura, Y. (2013). [The Satb1 protein directs hematopoietic stem cell differentiation toward lymphoid lineages.](#) *Immunity* 38(6): 1105-1115.
2. Schlissel, M. S., Corcoran, L. M. and Baltimore, D. (1991). [Virus-transformed pre-B cells show ordered activation but not inactivation of immunoglobulin gene rearrangement and transcription.](#) *J Exp Med* 173(3): 711-720.