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. $MgCl_2$ (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 x 10^4) or ES-derived hematopoietic cells (3-5 x 10^5) in 75 µl elution solution. See the manufacturer's protocol (http://www.5prime.com/media/3415/perfectpure_dna_cultured_cell_manual_5prime_1064553_122010.pdf).
2. 20 µl PCR reactions contained 5.5 µl template (82.5 ng or less), 10 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, 1 µM primers (25 mers), 200 µM 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

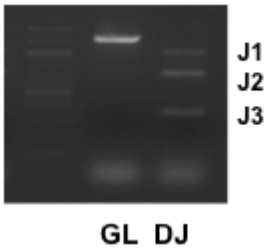

Figure 1.DNA PCR assays of germline (GL) or D_H-J_H rearranged *Igh* chain (DJ) genes were performed with mouse splenocytes. A PCR experiment using a primer D_HL(5') and J3(3') can detect three types of D_H-J_H rearrangement (J1, J2, and J3) (Schlissel *et al.*, 1991). All of three bands are present 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

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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.