

SOP for High-sensitivity Detection of Recombinant Adenovirus DNA by PCR

1. Scope

- 1.1 This procedure describes a method for high-sensitivity detection of the DNA of recombinant adenovirus by PCR.
- 1.2 For the procedure for preparation of DNA (crude extract of DNA) from recombinant adenovirus, see **Virus Bank SOP-AdV-001**.
- 1.3 For detailed instructions about use of the thermal cycler (MJ Research, PTC-200), see _____, and for densitometry (ATTO, AE-6920M-03), see _____.

2. Principles

- 2.1 Recombinant adenovirus is constructed by replacing the E1 gene in the genome of adenovirus type 5 (Ad5) with an expression unit. This vector has a foreign gene (different from the genomic DNA sequence of host cells and the DNA sequence of wild-type adenovirus). The recombinant adenovirus DNA can be detected by using corresponding primers at both end of the cloning site of the gene (cDNA) to be expressed and amplifying the cDNA by the polymerase chain reaction (PCR), with subsequent agarose gel electrophoresis for detection of products of PCR of the targeted size.

3. Reagents

- 3.1 TaKaRa LA Taq (5 U/microL; Takara Shuzo), 10x LA PCR buffer (Mg²⁺-free), 25 mM MgCl₂, 2.5 mM dNTP mixture (#RR002A; Takara Shuzo), molecular weight markers (1 kb Ladder; #15615-016; GIBCO-BRL), 50x TAE buffer (#24710-030; GIBCO BRL), 10 mg/ml ethidium bromide solution (#15585-011; GIBCO BRL), agarose (#A6013; Sigma).

4. Sequence of primers for PCR

4.1 Dissolve primers PAXCAF1 and PAXCAR1 for amplification of cDNA in sterile water to give a final concentration of 10 μM of each primer.

Sequences of primers are:

PAXCAF1: 5'-GGCTTCTGGCGTGTGACCGGC-3'

PAXCAR1: 5'-CAGAGGGGAAAAAGATCTCAGTGG-3'

5. Procedure

5.1 Perform PCR as follows. Pipette 49 μL of PCR mixture (the components are shown below; prepare without template DNA) into a 200- μL reaction tube. Add 1 μL of the solution of template genomic DNA (50 ng/ μL). Use a programmed thermal cycler for PCR. Wear gloves and keep reagents on ice all through the entire procedure.

PCR mixture (per sample)

10x LA PCR buffer	5.0 μL
Template genomic DNA (50 ng/ μL)	1.0 μL
PAXCAF1 (10 μM)	1.0 μL
PAXCAR1 (10 μM)	1.0 μL
dNTP mixture (2.5 mM each dNTP)	4.0 μL
TaKaRa LA Taq (5 U/ μL)	0.25 μL
Sterile water	37.75 μL

	50.0 μL

Program for thermal cycler for PCR

94°C, 1 min \rightarrow 94°C, 30sec \rightarrow 88°C, 30 sec \rightarrow 72°C, 3 min \rightarrow 72°C, 7 min \rightarrow 4°C, forever



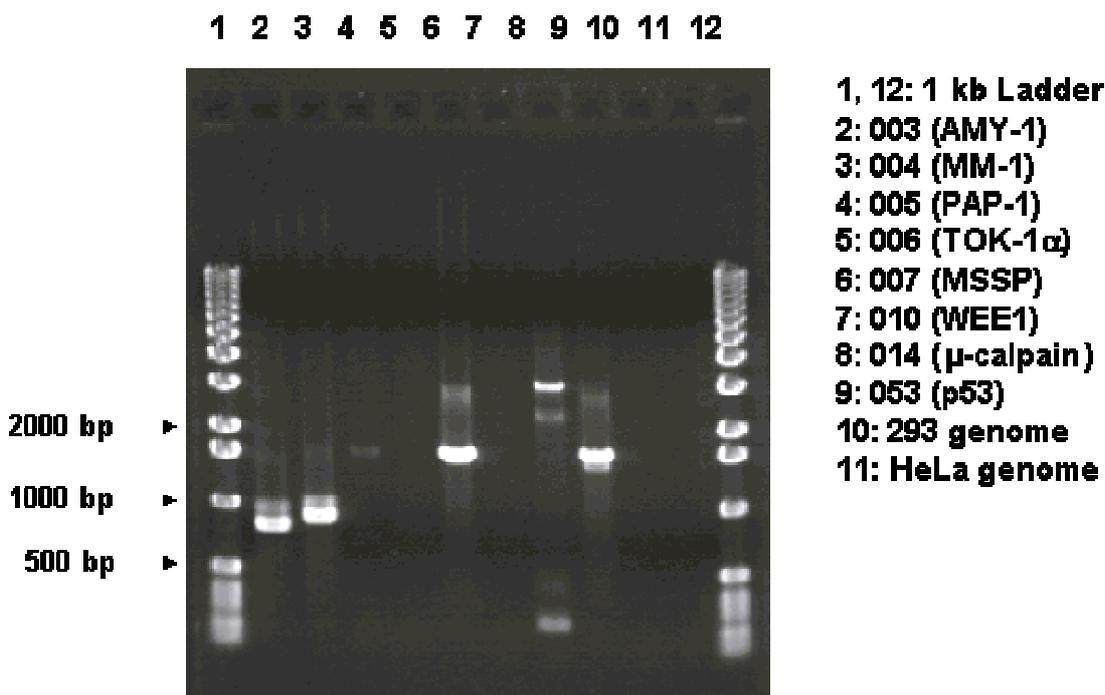
Total of 30 cycles

5.2 The products of PCR are analyzed by agarose gel electrophoresis. Suspend agarose (1% w/v) in 1x TAE buffer (diluted 50x TAE (GIBCO BRL) with

Milli-Q water (Milipore)). Heat the suspension to dissolve agarose completely and allow to cool but not to gel. Pour the solution into a Mupid-2 gel maker set (#EM-2; Advance) using 35 mL for a large plate (107 x 60 mm) or 18 mL for a small plate (52 x 60 mm). Place a comb (#COMB25; Advance; large plate, 25-well; small plate, 12-well) and leave to cool and harden. For electrophoresis, Mix 5 μ L of PCR product, with 1 μ L of 6x gel-loading buffer (0.25% bromophenol blue/0.25% xylene cyanol/30% glycerol). Load 5 μ L of this mixture on the gel and fractionate it by electrophoresis in 1x TAE Buffer, for 30 min, at 100 volts.

5.3 Soak the gel in a solution of ethidium bromide (2 μ g/mL) in Milli-Q water for 5 min at room temperature. Then wash the gel with tap water for 5 min. Visualize bands of DNA under UV (312 nm) light, save the live picture with a densitograph (AE-6920M-03; ATTO) and store as a PICT file.

5.4 Example of detection of products of PCR



Detection of recombinant adenovirus DNA by PCR using TaKaRa LA Taq

(annealing temperature: 68°C)