Southern blotting probes for HBV genotype D

PLOS One

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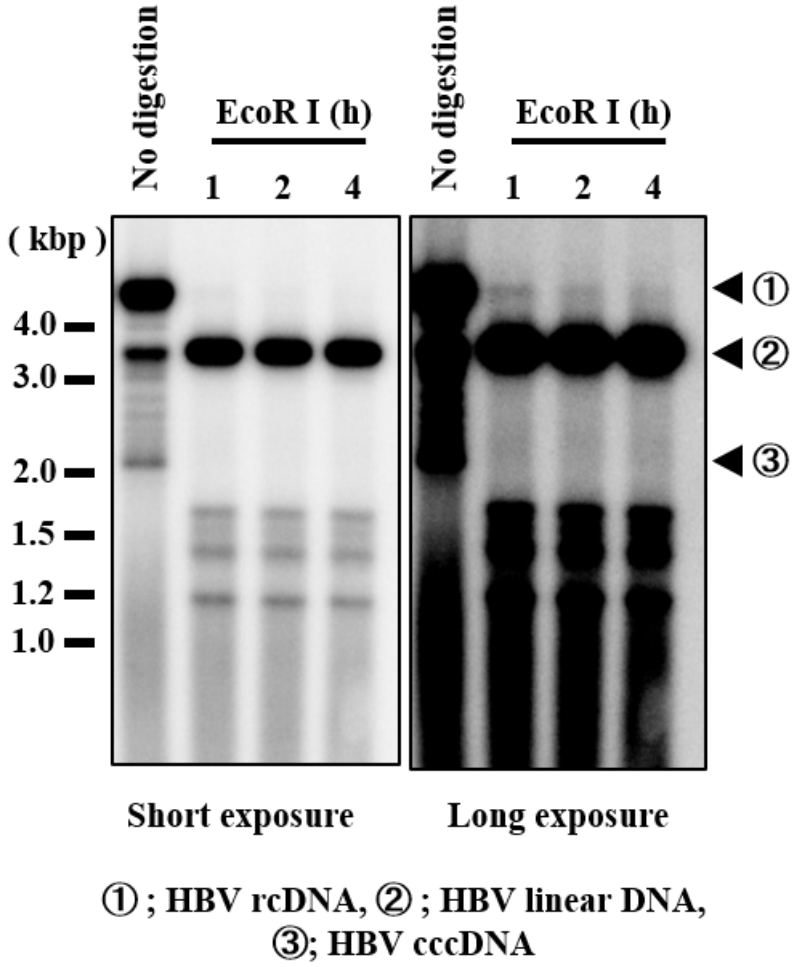
ABSTRACT

Southern blotting probes for HBV genotype D.
HBV cccDNA, rcDNA, and dslDNA can be detected by these probes.



Southern blotting data(HIRT DNA of Hep38.7 stable cell line).

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0212233

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Takeuchi F, Ikeda S, Tsukamoto Y, Iwasawa Y, Qihao C, Otakaki Y, Ryota O, Yao W, Narita R, Makoto H, Watashi K, Wakita T, Takeuchi K, Chayama K, Kogure A, Kato H, Fujita T (2019) Screening for inhibitor of episomal DNA identified dicumarol as a hepatitis B virus inhibitor. PLoS ONE 14(2): e0212233. doi: 10.1371/journal.pone.0212233

PROTOCOL STATUS

Working

MATERIALS TEXT

Primers sets

ccc1 fwd	GCAGAATCTTTCCACCAGCAA
ccc1 rev	TGGCCTGAGGATGAGTGTTTC
ccc2 fwd	GGAATTCCACAACCTTTCACCA
ccc2 rev	GAATTTTGGCCAAGACACACG
ccc3 fwd	CCAACCTCCAATCACTCACCA
ccc3 rev	GGCCCACTCCCATAGGAATTT
ccc4 fwd	GCCATTTGTTCAGTGGTTCGT
ccc4 rev	ACCCAAAAGACCCACAATTCG
ccc5 fwd	GCCCCATTTACACAATGTGGTT
ccc5 rev	ATGTTTGCTCCAGACCTGCTG
ccc6 fwd	CGGGACTGATAACTCTGTTGTCC
ccc6 rev	CGTTCACGGTGGTCTCCAT
ccc7 fwd	CGAATGTTGCCCAAGGTCTTA
ccc7 rev	GGCAAAAACGAGAGTAACTCCA
ccc8 fwd	TGAGCATTGTTCACCTCACCA
ccc8 rev	ACCTGCCTCGTCGTCTAACAA
ccc9 fwd	CCGCGTCGCAGAAGATCTCA
ccc9 rev	CCCGCCTTCCATAGAGTGTG

SAFETY WARNINGS

For the template, virus genome is used.
Please be careful for using virus genome.

BEFORE STARTING

Prepare the template DNA.
Hep38.7 (HBV genotype D stable cell line) supernatant is best template.
HIRT DNA and whole DNA are not goot for the template (genome DNA induce the unspecific amplification).

PCR reaction

1 Prepare the 9 tubes (ccc1-9 probes) and mix the reagent (DO NOT mix other primer set.For example, ccc1 fwd and ccc3 rev.

Ex taq	0.5 ul
10 X buffer	10 ul
dNTPmix	8 ul
Template (Supernatant DNA)	5 ul
Primer (3 uM)	17 ul X 2 (Forward and Reverse)
DNase free water	42.5 ul
Total	100 ul

2 Amplify cycle 2-4 50 times

94 ℃	1 min (Hot start)
94℃	10 sec

55	30 sec
72	24 sec
72	2 min
4	Pause

3 PCR product is purified by ethanol purification and gel extraction.