Amplification of phage genes from lysates and environmental samples

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Abstract

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Guidelines

PCR reactions were performed with *Taq* DNA polymerase and dNTPs from New England Biolabs (Beverly, MA) or Invitrogen (Carlsbad, CA) and carried out with a PTC-100 or PTC-200 DNA Engine (MJ Research, Waltham, MA) or a Robocycler Gradient 96 (Stratagene, La Jolla, CA). Template amounts were: 10 ng genomic DNA for *Prochlorococcus* and *Synechococcus*, 1 µL filtrate for cyanophages, and 2 µL filtrate for environmental samples. PCR primers and amplification reaction conditions are shown in Suppl. Tables 3 and 4.

The *psbA* gene from all sources was amplified using primer pair *psbA*-F/R [62] and PCR protocol A (see Suppl. Tables 3 and 4). Four reactions were conducted with each template, and the products were pooled and analyzed by agarose gel electrophoresis. Primer *psbA*-R falls on the intron region in S-PM2 (see ref. [29]). Therefore for efficient amplification of phage *psbA* genes that may contain introns, and for increased sensitivity, we used the Pro-*psbA*-F/R primer set and protocol B in nested PCR reactions when no PCR product was visible from cyanophage lysates and environmental filtrates. To reduce the incidence of heteroduplex formation, amplification products from environmental samples were subjected to reconditioning PCR [63]: initial PCR products were diluted 1:10, then amplified using protocol A but for only 3 cycles.

The *psbD* gene from *Prochlorococcus*, *Synechococcus*, and cyanophages was amplified using primer pair *psbD*-54F/*psbD*-308R and protocol D. However when product yield was low or absent semi-nested PCR was carried out as follows. Amplification was first conducted using primer pair *psbD*-26F/*psbD*-308R and protocol C. Four reactions were conducted with each template, the products were pooled, diluted 1:10, and used as templates for a second round of amplification using primer pair *psbD*-54F/*psbD*-308R and protocol D. *psbD* from environmental samples was amplified using primer pair *psbD*-26F/*psbD*-308R and protocol C and subjected to reconditioning PCR as for *psbA* (see above).

In preparation for sequencing, PCR products were either purified directly using the QIAquick PCR

Purification Kit (Qiagen) or separated on an agarose gel and then purified using the QIAquick Gel Extraction Kit (Qiagen).

To confirm that the absence of *psbA* or *psbD* PCR products from phage was not simply due to a lack of amplifiable phage DNA, we screened phage lysates for known phage genes: *g20* (for myoviruses) and *DNApol* (for podoviruses). *g20* was amplified using primer pair g20-F/R and protocol E, and *DNApol* using primer pair DNApol-F/R and protocol F, both with 1µL filtrate from cyanophage isolates.

Supplementary Table 3. PCR conditions

PCR Protocol	Forward Primer	Reverse Primer	Primer Conc. (µM)	dNTP Conc. (µM)	MgCl2 Conc. (mM)	Units of Taq	Reaction Volume (µL)	Initial Denaturation	# of Cycles	Cycled Denaturation	Cycled Annealing	Cycled Extension	Final Extension
A	*psbA*-F	*psbA*-R	0.25	200	2.5	2.0	20.0	94°C, 5 min	35	94°C, 1 min	52°C, 1 min	72°C, 1.5 min	72°C, 10 min
B	Pro-*psbA*-F	Pro-*psbA*-R	0.25	80	5.0	2.5	50.0	92°C, 4 min	35	92°C, 1 min	50°C, 1 min	68°C, 1 min	68°C, 10 min
C	*psbD*-26F	*psbD*-308R	1.00	200	1.5	1.0	20.0	94°C, 5 min	35	94°C, 1 min	51°C, 1 min	72°C, 1 min	72°C, 10 min
D	*psbD*-54F	*psbD*-308R	1.00	200	1.5	1.0	20.0	94°C, 5 min	35	94°C, 1 min	51°C, 1 min	72°C, 1 min	72°C, 10 min
E	g20-F	g20-R	1.25	250	1.5	1.0	20.0	94°C, 3 min	35	94°C, 15 s	35°C, 1 min	73°C, 1 min	73°C, 10 min
F	DNApol-90F	DNApol-355R	4	200	0.5 mM for lysates 1.5 mM for extracted DNA	1.0	20.0	94°C, 4 min	35	94°C, 1 min	35°C, 1 min	72°C, 1 min	72°C, 10 min

Supplementary Table 4. PCR primers

Short Name	Full Name	Sequence	Source	Purpose
psbA-F	58-VDIDGIREP-66	5′-GTNGAYATHGAYGGNATHMGNGARCC-3′	Zeidner et al. 2003	*psbA* screening
psbA-R	331-MHERNAHNFP-340	5′-GGRAARTTRTGNGCRTTNCKYTCRTGCAT-3′	Zeidner et al. 2003	*psbA* screening
Pro-*psbA*-F	Pro-*psbA*-1F	5’-AACATCATYTCWGGTGCWGT-3’	Z. Johnson	*psbA* screening
Pro-*psbA*-R	Pro-*psbA*-1R	5’-TCGTGCATTACTTCCATACC-3’	Z. Johnson	*psbA* screening
psbD-26F	*psbD*-26Fa	5'-TTYGTNTTYRTNGGNTGGAGYGG-3'	J. A. Lee and D. Lindell	*psbD* screening
	psbD-26Fb	5'-TTYGTNTTYRTNGGNTGGTCNGG-3'		
psbD-54F	*psbD*-54Fa	5'-GTNACNAGYTGGTAYACNCAYGG-3'	J. A. Lee andD. Lindell	*psbD* screening
	psbD-54Fb	5'-GTNACNTCNTGGTAYACNCAYGG-3'		
psbD-308R	*psbD*-308Ra	5'-YTCYTGNGANACRAARTCRTANGC-3'	J. A. Lee and D. Lindell	*psbD* screening
	psbD-308Rb	5'-YTCYTGRCTNACRAARTCRTANGC-3'		
g20-F	CPS1.1	5’-GTAGWATWTTYTAYATTGAYGTWGG-3’	M.B. Sullivan	*g20* screening*
g20-R	CPS8.1	5’-ARTAYTTDCCDAYRWAWGGWTC-3’	M.B. Sullivan	*g20* screening**
DNApol-F	DNApol-90Fa	5’-GAYACIYTIRYIYTITCIMG-3’	D. Lindell	*DNApol* screening
	DNApol-90Fb	5’-GAYACIYTIRYIYTIAGYMG-3’		
DNApol-R	DNApol-355Ra	5’-GGIAYYTGIGCIARRTTIGG-3’	D. Lindell	*DNApol* screening
	DNApol-355Rb	5’-GGIAYRTTIGCIARRTTIGG-3’		

*Not found in P-SSM4

**Nt 115325 --> 115304 in P-SSM4

W = A or T

Y = C or T
R = A or G
D = not C
M = A or C
I = inosine (from adenosine, pairs with A, T, or C)

g20 amplicon ~594 bp (as found in proof "Results and Discussion" Environmental Microbiology 2008, Portal Protein Diversity and Phage Ecology).

Protocol