Jan 21, 2016

Screening Recombinant Clones by PCR

DOI
https://dx.doi.org/10.17504/protocols.io.c48yzv

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Protocol Citation: Matthew Sullivan 2016. Screening Recombinant Clones by PCR. **protocols.io** https://dx.doi.org/10.17504/protocols.io.c48yzv

Protocol status: Working

Created: June 19, 2015

Last Modified: November 13, 2017

Protocol Integer ID: 896

Keywords: recombinant clones by pcr, screening recombinant clone, pcr

Guidelines

This protocol describes conditions using either ABI reagents or PGC Scientific Reagents. The reactions have been tested on the gradient Eppendorf, the Gene Techne and the ABI 9700 thermal cyclers and all machines use the same cycling conditions.

When testing plasmid DNA preps, use 0.5 µL of DNA as the template in place of the bacterial colony.

Recommened vector primers:

	Primer	Sequence (5' to 3')	Fragment Size
	pUC 18 Forward	TGT AAA ACG ACG GCC AGT	109 bp^12
	pUC 18 Reverse	TCA CAC AGG AAA CAG CTA TGA C	

Thermal Cycling Protocol:

	95°C	5 minutes	1 cycle
	95°C 55°C 70°C	30 seconds 30 seconds 30 seconds*	35 cycles
	70°C	7 minutes	1 cycle
	4-10°C		Hold cycle

*increase to 1 minute if insert is great than 1 Kb

Reagent catalog numbers

ABI Reagents:
- dNTP mix N808-0007
- AmpliTaq Gold N808-0247

PGC Scientific Reagents
- dNTP mix 62-6113-40
- Taq DNA polymerase (w/10x Buffer containing $MgCl_2$) 62-6086-02

In pBlueScript these primers bind at nt 599 and nt 833 = 234 bp amplicon.
In pGEM-t Easy the primers bind at nt 2975 and nt 197 = 242 bp amplicon.

1 Transfer the transformed clones to a stock LB-Amp or other appropriate plated media.

2 Grow for 24 hr at 37°C.
24:00:00

3 Prepare forward and reverse primers for the vector used for transformation at 100 ng/µl in DDH_2O. See table in guidelines for recommended primers to use with pUC18 vector.

Note

When choosing primers, find ones that flank the multiple cloning region of your vector

4 Label thin-walled tubes.

Note

Include a vector control and a known plasmid control when first starting out. The vector control will yield the smallest fragment in comparison to your transformed clones and the plasmid control will yield a fragment that is the size of the vector control plus the size of the insert.

5 Prepare 1 ml of Master Mix:

	ABI Reagents			PGC Scientific Reagents			Final Concentration
	DDH_2O	690 µl		DDH_2O	833.5 µl		-----
	10x Buffer II	100 µl		10x Buffer w/$MgCl_2$	100 µl		1x Buffer
	dNTP mix	80 µl		dNTP mix	20 µl		200 µM each
	25mM $MgCl_2$	80 µl					2mM
	Primer F	20 µl		Primer F	20 µl		0.33 µM
	Primer R	20 µl		Primer R	20 µl		0.33 µM
	AmpliTaq Gold	10 µl		Taq Polymerase	6.5 µl		1U/20 µl rxn.

Note

This protocol describes conditions using either ABi reagents or PGC Scientifics reagents.

6 Vortex and spin Master Mix and dispense 20 µl per thin-walled tube.

7 Cap all tubes.

8 Set a 10 µl pipetter to 1 µl.

Note

You do not need to use barrier tips.

9 Place tranformant plate on template and touch the pipette tip to the appropriate colony.

10 Pipette up and down in the appropriate PCR tube.

11 Re-cap tube, change tip and repeat steps 9-10 until all colonies have been added.

Note

The colony is your DNA template.

Note

When testing plasmid DNA preps, use 0.5 µl of DNA as the template in place of the bacterial colony.

12 Perform PCR using the thermal cycling protocol:

	95°C	5 minutes	1 cycle
	95°C 55°C 70°C	30 seconds 30 seconds 30 seconds*	35 cycles
	70°C	7 minutes	1 cycle

	4-10°C		Hold cycle

*increase to 1 minute if insert is great than 1 Kb

13 Remove tubes at end of thermal cycling.

14 Add 2 µl 10x gel loading buffer to each tube.

2 µL

15 Spin to bring contents to bottom of tube and load 10 µl into lane of a 1% agarose gel in 0.5x TBE buffer.

Note

Be sure to have a DNA ladder in the first and last lane of each gel so that an accurate assessment of the size of the inserts can be determined.