Screening Recombinant Clones by PCR

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Abstract

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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	30 seconds	
55°C	30 seconds	35 cycles
70°C	30 seconds*	
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.

Protocol

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

Step 2.
Grow for 24 hr at 37°C.

DURATION
24:00:00

Step 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.

NOTES
VERVE Team 19 Jun 2015
When choosing primers, find ones that flank the multiple cloning region of your vector

Step 4.
Label thin-walled tubes.

NOTES
VERVE Team 19 Jun 2015
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.

Step 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.

NOTES
VERVE Team 29 Jul 2015
This protocol describes conditions using either ABi reagents or PGC Scientifics reagents.

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

Step 7.
Cap all tubes.

Step 8.
Set a 10 µl pipetter to 1 µl.

NOTES
VERVE Team 19 Jun 2015
You do not need to use barrier tips.

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

Step 10.
Pipette up and down in the appropriate PCR tube.

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

NOTES
VERVE Team 19 Jun 2015
The colony is your DNA template.
VERVE Team 29 Jul 2015
When testing plasmid DNA preps, use 0.5 µl of DNA as the template in place of the bacterial colony.

Step 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

Step 13.
Remove tubes at end of thermal cycling.

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

AMOUNT
2 µl Additional info:

Step 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.

NOTES
VERVE Team 19 Jun 2015
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.