Protocol

Analysis of Recombinant Clones

This protocol is for the Analysis of Thermo Scientific Recombinant Clones

Analyze 4-6 white colonies for the presence and orientation of the DNA insert using one of the following methods.

Restriction analysis. Isolate plasmid DNA from an overnight bacterial culture using a convenient plasmid miniprep method such as GeneJET™ Plasmid Miniprep Kit. Use FastDigest® restriction enzymes to digest DNA from recombinant clones in just 5 min.

Sequencing. Isolate plasmid DNA from an overnight bacterial culture using a reliable plasmid miniprep method such as GeneJET™ Plasmid Miniprep Kit. Sequence the insert using appropriate sequencing primers.

Colony PCR. Use the following protocol for colony screening by PCR.

1. Prepare enough PCR master mix for the number of colonies analyzed plus one extra. For each 20 µl of reaction, mix the following reagents (see table below):
2. Mix thoroughly, spin briefly and aliquot 20 µl of the mix into the PCR tubes on ice.
3. Pick up an individual colony with a sterile pipette tip and resuspend it in 20 µl of the PCR master mix. Make a short strike with the same tip over culture plate to save the clone for repropagation.
4. Perform PCR: 95°C, 3 min; 94°C, 30 s, 45°C*, 30 s, 72°C 1 min/kb; 30 cycles.
5. Analyze on a gel for the presence of the PCR product of the expected length.

** Depends on primer pair used (Tm-5).*

Component	*Taq* DNA Polymerase or DreamTaq™ DNA Polymerase	PCR Master Mix (2X)
10X Taq buffer	2 µl	-
dNTP Mix, 2mM each	2 µl	-
25 mM MgCl2	1.2 µl	-
M13/pUC Reverse Sequencing primer (#SO101)	0.6 µl (10 µM)	0.6 µl (10 µM)
M13/pUC Forward Sequencing primer (#SO100)	0.6 µl (10 µM)	0.6 µl (10 µM)
***Taq* DNA polymerase**	0.1 µl (0.5 u)	-
PCR Master Mix (2X)	-	10 µl
Water, nuclease-free	to 20 µl	to 20 µl
Total volume	**20 µl**	**20 µl**

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