Protocol-at-a-Glance

LD-Insert Screening Amplimer Sets
Protocol-at-a-Glance
(PT1579-2)

This Protocol-at-a-Glance is provided for your convenience, but is not intended for first-time users. Please read the User Manual before using this abbreviated protocol.

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cDNA Insert Screening

Uses:

λ Libraries

(λgt10, λgt11, λACT, & λTriplEx™)

Plasmid Libraries

(pACT, pACT2, pGAD 10, pGAD GH, pGAD GL, pB42 AD, & pTriplEx)

Template Prep:

λ Libraries — *E. coli*: Pick plaque and transfer to 25 µl deionized H_2O. Vortex. Use 5 µl per reaction.

Plasmid Libraries — *E. coli*: Pick colony and transfer to 25 µl deionized H_2O. Boil samples 5 min. Use 5 µl per reaction.

Yeast: See User Manual, Section IV.D. Use 1–5 µl per reaction.

PCR Set-up:

1. Thaw all components on ice. Mix each component thoroughly before use.
2. Prepare PCR Master Mix (prepare enough mix for all PCR reactions +1):

	1 rxn	(n+1)rxn	Expt.
PCR-grade deionized H_2O	36 µl	36(n+1) µl	___ µl
10X Advantage® 2 PCR Buffer	5 µl	5(n+1) µl	___ µl
5' LD Amplimer	1 µl	1(n+1) µl	___ µl
3' LD Amplimer	1 µl	1(n+1) µl	___ µl
50X dNTP Mix (10 mM each)	1 µl	1(n+1) µl	___ µl
Advantage 2 Polymerase Mix (50X)*	1 µl	1(n+1) µl	___ µl

* If you are not using Advantage 2 Polymerase Mix, use the recipe above if Mg^{++} is included in the 10X reaction buffer, or use the recipe for the genomic PCR Master Mix if Mg^{++} is not included in the buffer.

3. Mix components by vortexing, and spin briefly to collect contents at bottom of tube.
4. For each PCR reaction, combine 45 µl of PCR Master Mix and 5 µl of template DNA (use 1–5 µl of yeast-isolated plasmid). Use 5 µl of deionized H_2O as a negative control.
5. Spin tubes briefly to collect contents.
6. Add 1–2 drops of mineral oil to each tube to prevent evaporation.

PCR:

7. Commence thermal cycling in a Applied Biosystems DNA Thermal Cycler Model 480. These are general guidelines—optimal parameters may vary with different thermal cyclers, templates, and other experimental variables.

Target Size	Cycle Parameters	Target Size	Cycle Parameters
< 5 kb:	• 94°C for 1 min • 25–35 cycles 94°C 30 sec[a] 68°C 3 min • 68°C for 3 min[b] • Soak at 15°C	5–9 kb:	• 94°C for 1 min • 25–35 cycles 94°C 30 sec[a] 68°C 6 min • 68°C for 6 min[b] • Soak at 15°C

[a] Use shortest possible denaturation time.
[b] Optional: may reduce background.

Analysis:

8. Transfer 5 µl of the PCR product to a fresh tube, and add 1 µl of stop/loading buffer.
9. Electrophorese samples on a suitable agarose/EtBr gel. (We recommend 1.5% agarose for inserts of 0.3–1.5 kb, 1.2% agarose for inserts of 0.5–10 kb, and 0.8% agarose for inserts >5 kb.)

Genomic & Cosmid Insert Screening

Uses:

λ Libraries
(EMBL3 & EMBL3 SP6/T7 [also known as λGEM® II])

Cosmid Libraries
(pWE15)

Template Prep:

λ Libraries: *E. coli*: Pick plaque and transfer to 25 µl deionized H_2O. Vortex. Use 5 µl/PCR rxn.

Cosmid Libraries: *E. coli*: Standard alkaline lysis method. Resuspend DNA to ~8 ng/µl (5-fold more dilute than standard miniprep DNA). Use 5 µl/PCR rxn.

Set-up PCR:

1. Thaw all components on ice. Mix each component thoroughly before use.
2. Prepare PCR Master Mix (prepare enough mix for all PCR reactions +1):

	1 rxn	(n+1)rxn	Expt.
PCR-grade deionized H_2O	33.8 µl	33.8(n+1) µl	___ µl
10X *Tth* PCR reaction buffer	5 µl	5(n+1) µl	___ µl
25 mM $Mg(OAc)_2$	2.2 µl	2.2(n+1) µl	___ µl
5' LD Amplimer	1 µl	1(n+1) µl	___ µl
3' LD Amplimer	1 µl	1(n+1) µl	___ µl
50X dNTP Mix (10 mM each)	1 µl	1(n+1) µl	___ µl
Advantage *Tth* Polymerase Mix (50X)*	1 µl	1(n+1) µl	___ µl

* If you are not using Advantage *Tth* Polymerase Mix, use the recipe above if Mg^{++} is not included in the 10X reaction buffer, or use the recipe for the cDNA PCR Master Mix if Mg^{++} is included in the buffer.

3. Mix components by vortexing, and spin briefly to collect contents at bottom of tube.
4. For each PCR reaction, combine 45 µl of PCR Master Mix and 5 µl of template DNA. Use 5 µl of deionized H_2O as a negative control.
5. Spin tubes briefly to collect contents.
6. Add 1–2 drops of mineral oil to each tube to prevent evaporation.

PCR:

7. Commence thermal cycling in a Applied Biosystems DNA Thermal Cycler Model 480. These are general guidelines—optimal parameters may vary with different thermal cyclers, templates, and other experimental variables.

Target Size	Cycle Parameters	Target Size	Cycle Parameters
< 5 kb:	• 94°C for 1 min • 25–35 cycles 94°C 30 sec[a] 68°C 3 min • 68°C for 3 min[b] • Soak at 15°C	10–20 kb:	• 94°C for 1 min • 25–35 cycles 94°C 30 sec[a] 68°C 12 min • 68°C for 12 min[b] • Soak at 15°C
5–9 kb:	• 94°C for 1 min • 25–35 cycles 94°C 30 sec[a] 68°C 6 min • 68°C for 6min[b] • Soak at 15°C	20–40 kb:	• 94°C for 1 min • 25–35 cycles 94°C 15 sec[a] 68°C 22 min • 68°C for 22 min[b] • Soak at 15°C

[a] Use shortest possible denaturation time.
[b] Optional: may reduce background.

Analysis:

8. Transfer 5 µl of the PCR product to a fresh tube and add 1 µl of stop/loading buffer.
9. Electrophorese samples on a suitable agarose/EtBr gel. (We recommend 1.5% agarose for inserts of 0.3–1.5 kb, 1.2% agarose for inserts of 0.5–10 kb, and 0.8% agarose for inserts >5 kb.)

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