

LM-PCR protocol

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This is a protocol for performing LM-PCR for ChIP material. You should spec your ChIP, have positive and negative controls for QPCR enrichment measurement, etc...

Start with ~ 1/2 an IP (~1ng. If you have product in the 5-10 ng range (larger IP's, typically from cell lines) do fewer PCR cycles (15 instead of 20)).

Blunt-End w/ T4 DNA Pol (IVGN)

Rxn vol: 50 ul

10X T4 Buffer	5 ul
10mM dNTP	2 ul
5 U/ul T4 DNA Pol	1 ul
water + sample to 50 ul	

Incubate 12° C 15 minutes. (According to manufacturer's notes, incubating at 37 can lead to 1bp overhangs).

Qiaquick Cleanup with ERC (Identical for all reactions)

- Add 1/10 volume 3M Na Acetate, pH 5.2
- Add 300 ul ERC buffer
- Apply to column
- Spin 1' full speed
- Discard flow-through
- Apply 700 ul PE buffer
- spin 1' full speed
- Discard flow-through
- spin 1' full speed
- Place into new eppendorf
- Allow to air dry, 1'.
- Apply 30 ul EB or water
- spin 1' full speed
- Keep eluate

Ligate:

Make Annealed Oligo Linker (6.7uM):

100 uM OJW102 linker	6.7 ul
100 uM OJW103 linker	6.7 ul
water to 100 ul	

Heat to 95, allow to slowly cool by removing block from incubator and allowing entire heating block to cool.

Ligation Rxn vol: 50 ul

10X T4 ligase buffer	5 ul
6.7 uM annealed linker	6.7 ul
High Conc T4 (NEB)	1 ul
water + sample to 50 ul	

Place at room temp for 1 hour, overnight at 16° C.

Qiaquick cleanup, as before. Elute 30 ul.

PCR:

Reaction vol: 50 ul

10X PCR buffer (no MgCl)	5
25 mM MgCl	4
10 mM dNTP	2
Taq 5U/ul (Promega)	1
OJW102 linker (10uM)	5

water and sample to 50 ul (entire Ligated sample).

It is imperative that you DO NOT USE a hotstart Taq, since the directional linker must be filled in before the primer will bind.

PCR conditions:

window 1
55° 2'
72° 2'
1 cycle

window 2
95° 2'
1 cycle

window 3

95° 45''
55° 45''
72° 1:15
20 cycles

window 4
72° 5'
1 cycle

Qiaquick cleanup as before, elute 30 ul.

Spec via nanodrop. First round may not produce measureable yield.

Repeat PCR w/ 50 ng of first round PCR product and 15-20 cycles to generate 2nd round product.