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 12º 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
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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 16º 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
55º 2’
72º 2’
1 cycle

window 2
95º 2’
1 cycle

window 3

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95º 45”
55º 45”
72º 1:15
20 cycles

window 4
72º 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.