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Long Range PCR (LR-PCR)

Note: The following protocol was adapted from Takara.Mirus.Bio (Madison, WI) literature accompanying Takara LA Taq. It has been successfully used by the Bradeen Lab to amplify unique fragments of up to 22kb in length from genomic DNA of the wild potato *Solanum bulbocastanum*. Other commercially-available Taq preparations may work just as well as Takara LA Taq and mention of Takara.Mirus.Bio and Takara products in this protocol reflects our research experiences but should not be interpreted as endorsement to the exclusion of other products.

1. The following components are mixed in a thin-walled, 200ul PCR tube on ice.

CsCl-purified template DNA*	5ul (=100ng)
10X LA Buffer (supplied by Takara.Mirus.Bio)	5
dNTP mix (supplied by Takara.Mirus.Bio)	8 (=400uM final concentration)
Primer 1**	1 (=0.2uM final concentration)
Primer 2	1 (=0.2uM final concentration)
Takara LA Taq (Takara.Mirus.Bio)	0.5 (=2.5U)
ddH ₂ O	29.5
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TOTAL	50.0ul

*template DNA purity is a major technical consideration for LR-PCR; in our experience “standard” DNA preps (e.g. cTAB + chloroform extraction without CsCl-purification) yield unsatisfactory results

**primers should be approximately 32-35bp in length with >50% GC content and a matched melting temperature of approximately 65-70C.

2. Use the following thermocycler conditions with a “hot start” (i.e. begin thermocycler program, keeping reaction tube on ice until the thermocycler is >90C; quickly and carefully place reaction tube in the thermocycler and close the lid):

1 Cycle:	94C for 1min
14 Cycles:	94C for 10sec 60C for 10min 72C for 15min
16 Cycles:	94C for 10sec 60C for 10min 72C for 15min + 15sec extra/cycle
1 Cycle:	72C for 10min
HOLD:	4C

Note: Adjust melting temperature to reflect calculated primer melting temperatures and primer specificity. Long annealing and extension times aid amplification of long fragments; times can be adjusted to reflect length of LR-PCR product (long times for long fragments, shorter times for short fragments)

3. Visualize the LR-PCR via standard gel or CHEF gel electrophoresis and ethidium bromide staining.

Additional References & Resources:

- Cheng S, Chang SY, Gravitt P, Respass R (1994) Long PCR. *Nature* 369:684-685
Mundy J, Mayer R, Chua NH (1995) Cloning genomic sequences using long-range PCR. *Plant mol biol report* 13:156-163
Sambrook J, Russell DW (2001) *Molecular Cloning: a Laboratory Manual*, Third edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
Takara.Mirus.Bio website: <http://www.takaramirusbio.com/index.htm>