PRCE Genotyping Protocol
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(Hashimoto-Partyka MK, Lydon JP, and Iruela-ARispe ML. Genesis 2006)

For AB, AC, AD reactions:

Reagent	**Amount (for 30** μL reaction)
H2O	12.3μL
10x Buffer (Hotmaster)	3.0μL
dNTP (2.5 mM)	2.5μL
Primer A	2.0μL
Primer B	2.0μL
Primer C	2.0μL
Primer D	2.0μL
Taq (Hotmaster)	0.2μL

For EC, ED reactions:

Reagent	**Amount (for 30** μL reaction)
H2O	14.3μL
10x Buffer (Hotmaster)	3.0μL
dNTP 2.5 mM)	2.5μL
Primer E	2.0μL
Primer C	2.0μL
Primer D	2.0μL
Taq (Hotmaster)	0.2μL

Cycling conditions for both reactions:

1. 94°C for 2 min
2. 94°C for 20s
3. 59°C for 20s
4. 68°C for 40s
5. Repeat 2-4, 26x
6. 68°C for 5 min
7. 4°

Primer Sequences:
A – 5’ TGT GCA CTT TTG GAG GCA AG 3’
B – 5’ GTG GAG GCT TCT GGA CAG T 3’
C – 5’ TAA AGC GCA TGC TCC AGA C 3’
D – 5’ TGA TTT TGC CTT TGG CAG ATG 3’
E – 5’ GGT CTC TGG CCT GAT TTT CC 3’

Primers	Genotype
AB	PRCE, PR – ECKO(VEN), PRKO
AD	PRKO