Probably terrible protocol for Phusion PCR, someone should double/triple check

Masp reactions Phusion PCA 20uL reaction

Core primers

Masp1

Overlap 18 long, 54 Tm, 56% GC

Calculated Tm of core "primer": 62 C, less than 20 long so annealing should be roughly equivalent to Tm

Actual Protocol MaSP 1 Core primers (60.1 by thermosci)

Mix and centrifuge components prior to use

Assembling on ice, add Phusion Polymerase last, transfer to Thermocycler heated to 98 C

12.8uL Nuclease-free water

4 uL 5X Phusion HF or GC buffer

0.4 uL 10 mM dNTPs

1uL 10 uM Forward Primer

1uL 10uM Reverse Primer

0.6 uL DMSO, (optional, if not used add 0.6 uL water)

0.2 uL Phusion DNA Polymerase

Step	Temp	Time
Initial Denaturation	98 C	30 seconds
35 Cycles Denaturation Annealing Elongation	 98 60 72	 10 sec 30 sec 10 sec
Final Extension	72	10 minutes
Hold	4-10	

MasP1 Peripheral P1 19 long, 54 Tm, 53 GC and P2 16 long, 58 Tm, 63 GC) Tm1 61 and Tm2 65

(58.8 and 63.1 by thermosci)

Mix and centrifuge components prior to use

Assembling on ice, add Phusion Polymerase last, transfer to Thermocycler heated to 98 C

To 11.3uL Nuclease-free water (subtract volume of template from 12.8)

4 uL 5X Phusion HF or GC buffer

0.4 uL 10 mM dNTPs

1uL 10 uM Forward Primer

1uL 10uM Reverse Primer

1.5 uL? Template DNA (Variable)

0.6 uL DMSO, (optional, if not used add 0.6 uL water)

0.2 uL Phusion DNA Polymerase

Step	Temp	Time
Initial Denaturation	98 C	30 seconds
35 Cycles Denaturation Annealing Elongation	 98 58.8 72	 10 sec 30 sec 10 sec
Final Extension	72	10 minutes
Hold	4-10	

Masp2

Core overlap 17 long, Tm 53, 59% GC by ApE, Tm 60 by phusion's calculator 59.6 by thermoscibi

Mix and centrifuge components prior to use

Assembling on ice, add Phusion Polymerase last, transfer to Thermocycler heated to 98 C

12.8uL Nuclease-free water

4 uL 5X Phusion HF or GC buffer

0.4 uL 10 mM dNTPs

1uL 10 uM Forward Primer

1uL 10uM Reverse Primer

0.6 uL DMSO, (optional, if not used add 0.6 uL water)

0.2 uL Phusion DNA Polymerase

Step	Temp	Time
Initial Denaturation	98 C	30 seconds

35 Cycles		
Denaturation	98	10 sec
Annealing	60	30 sec
Elongation	72	10 sec
Final Extension	72	10 minutes
Hold	4-10	

MaSP2 Peripheral Primers Tm1 52 and GC 59 Tm2 53 GC 60 or 59 and 60

By thermoscibio 57.6 and 57.0

Mix and centrifuge components prior to use

Assembling on ice, add Phusion Polymerase last, transfer to Thermocycler heated to 98 C

To 11.3uL Nuclease-free water (subtract volume of template from 12.8)

4 uL 5X Phusion HF or GC buffer

0.4 uL 10 mM dNTPs

1uL 10 uM Forward Primer

1uL 10uM Reverse Primer

1.5 uL? Template DNA (Variable)

0.6 uL DMSO, (optional, if not used add 0.6 uL water)

0.2 uL Phusion DNA Polymerase

Step	Temp	Time
Initial Denaturation	98 C	30 seconds
35 Cycles		
Denaturation	98	10 sec
Annealing	57	30 sec
Elongation	72	10 sec
Final Extension	72	10 minutes
Hold	4-10	