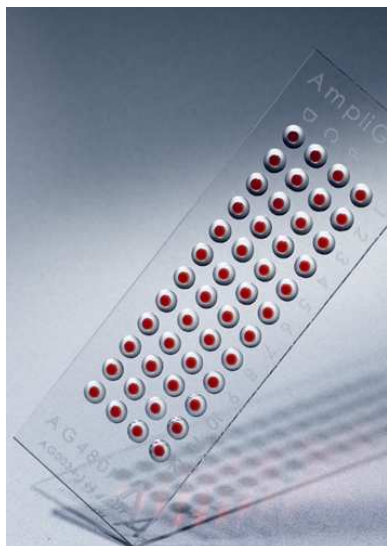


Singleplex or Multiplex PCR of human DNA on AmpliGrid AG480F

In the singleplex PCR system, one DNA fragment is amplified using human template and the multiplex PCR kit from Qiagen or Promega GoTaq[®] DNA Polymerase together with AmpliGrid slides.

In the multiplex PCR system, four DNA fragments are amplified using female human DNA template and six DNA fragments are amplified using male human template and the multiplex PCR kit from Qiagen or Promega GoTaq[®] DNA Polymerase together with AmpliGrid slides.

1 AmpliGrid AG480F



- AdvaGold 0.1 % (Advalytix)
- Aliquot preparation: DNA positive control, 100 pg/μl
Aliquot dilution for storage: 1 μl (10 ng/μl stock DNA + 99μl PCR-clean water)
- AmpliSpeed slide cycler (Advalytix)

Singleplex PCR:

- Primer pairs: (372bp)
(5): Left: 5'-TGGCCCCTGTGTTCAAGT -3'
Right: 5'-AGAATTGCTGAAGTGTGTTAGCC -3'
PCR Primermix contains 2 μM of each forward and reverse primer.

Multiplex PCR:

- Primer pairs: 1 (108bp), 2 (157 bp), 3 (209bp), 4 (240bp), 5 (372bp), 6 (574bp)
(1): Left: 5'-ATACTA ACCATGCGGGTTGC -3'
Right: 5'-AGAGGGACAACAAACGTGCT -3'
(2): Left: 5'- GTGAGGATTCTGGGCACACT-3'
Right: 5'-TGTTTATTCTGGCACTCCAATG -3'
(3): Left: 5'-GATAGCAAATGCACCACGG -3'
Right: 5'-TTTTCCCGCCTAAAGCATC -3'
(4): Left: 5'- AGGCATTGTGGAGATAACGC-3'
Right: 5'- AAACATCAAAATAGTCCAAGATTCG-3'
(5): Left: 5'-TGGCCCCTGTGTTCAAGT -3'
Right: 5'-AGAATTGCTGAAGTGTGTTAGCC -3'
(6): Left: 5'-GGTGGATGCTTCTGCCTAAA -3'
Right: 5'- TTGGTTATGGGTGCCAAGAT-3'

PCR Primermix contains 2 μM of each forward and reverse primer.

Material:

- Template: male DNA (Promega, 9948 Male DNA 10 ng/μl), female DNA (Promega, 9947A Female DNA 10 ng/μl)
- AmpliGrid AG480F incl. sealing solution (Advalytix)
- Qiagen Multiplex PCR Kit (Qiagen, #206143)
 - PCR clean water
 - 5x Q-Solution
 - 2x Multiplex PCR Master Mix
- Promega GoTaq[®] DNA Polymerase (Promega)
 - GoTaq[®] DNA Polymerase (0.8 U/μl)
 - 10x GoldSTAR[®] reaction buffer

Protocol:

DNA TEMPLATE

- Deposit 1 μl DNA solution (e.g., 100 pg/μl) on reaction sites and let air-dry at room temperature or at 37°C.

MASTER MIX

- Prepare master mix in a fresh PCR-clean tube according to table 1 or 2

Make sure to prepare enough master mix for all the reactions, taking pipetting errors into account (dead volume of electronic multipipet about 10 μl)

Qiagen Singleplex or Multiplex PCR:

2 *AmpliSpeed
ASC200D*



A *Table 1: PCR setup with Qiagen Taq reagents*

Reagent	1 Spot
2x Qiagen Multiplex PCR Master Mix	0.5 µl
Primermix 2 pmol/µl each	0.1 µl
Q-Solution, 5x	0.06 µl
AdvaGold, 0.1%	0.1 µl
ddH ₂ O	0.24 µl
Total volume:	1 µl

Promega Singleplex or Multiplex PCR:

B *Table 2: PCR setup with Promega GoTaq® reagents*

Reagent	1 Spot
10x GoldSTAR® reaction buffer	0.1 µl
Primermix 2 pmol/µl each	0.1 µl
GoTaq® DNA-Polymerase 0.8 U/µl	0.1 µl
AdvaGold, 0.1%	0.1 µl
ddH ₂ O	0.6 µl
Total volume:	1 µl

- Mix gently and spin down shortly
- Distribute 1 µl of master mix on each reaction site previously spotted with DNA
- Immediately cover with 5 µl sealing solution
- Transfer AmpliGrid onto the thermal cycler
- Run PCR programme (table 3)

C *Table 3: PCR programme*

Temperature	Time	Cycles
95 °C	10 min	
94 °C	30 sec	
63°C	60 sec	35
72°C	60 sec	
72°C	10 min	
Ambient	hold	

Analysis:

- Add 4µl of loading dye on top of each reaction site
- Analyse Samples by PAGE and silverstaining

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