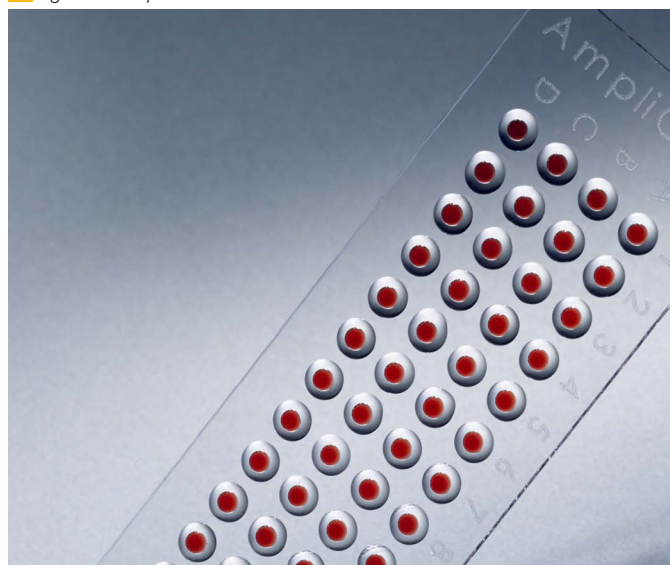


Singleplex or Multiplex PCR of human DNA on AmpliGrid AG480F

In the singleplex PCR system, one DNA fragment is amplified using human template and the GoTaq® PCR kit or a Qiagen Kit on the AmpliGrid slides.

In the multiplex PCR system, four DNA fragments are amplified using female human DNA template and six DNA fragments.

1 Figure 1: AmpliGrid AG480F



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, e.g. OAX04503)
- Qiagen Multiplex PCR Kit (Qiagen, #206143)
 - Nuclease-free water
 - 5x Q-Solution
 - 2x Multiplex PCR Master Mix
- GoTaq® PCR Kit
 - GoTaq® DNA Polymerase (5 U/μL)
 - 10x GoldSTAR® reaction buffer
- AdvaGold 0.1 % (Advalytix, OAX04227)
- Aliquot preparation: DNA positive control, 100 pg/μL, Aliquot dilution for storage: 1 μL (10 ng/μL stock DNA + 99 μL Nuclease-free water)
- AmpliSpeed slide cycler (Advalytix, e.g. OAX04101)

Singleplex PCR

- Primer pair: (372 bp)

(5): Left: 5'-TGGCCCCTGTGTTCAAGT -3'

Right: 5'-AGAATTGCTGAAGTGTGTTAGCC -3'

Primer mix 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'- AAACATCAAATAGTCCAAGATTCG-3'

(5): Left: 5'-TGGCCCCTGTGTTCAAGT -3'

Right: 5'-AGAATTGCTGAAGTGTGTTAGCC -3'

(6): Left: 5'-GGTGGATGCTTCTGCCTAAA -3'

Right: 5'- TTGGTTATGGGTGCCAAGAT-3'

Primer mix contains 2 μM of each forward and reverse primer.

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 A or B

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

GoTaq® Singleplex or Multiplex PCR**A** Table A: PCR setup with GoTaq® PCR Kit

Component	1 reaction
10x GoldSTAR® Buffer	0.1 µL
Primermix 2 pmol/µL each	0.1 µL
GoTaq® DNA-Polymerase 5 U/µL	0.02 µL
AdvaGold, 0,1%	0.1 µL
Nuclease-free water	0.68 µL
Total Volume	1 µL

Qiagen Singleplex or Multiplex PCR**B** Table B: PCR setup with Qiagen Multiplex PCR

Component	1 reaction
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
Nuclease-free water	0.24 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 amplification program (table C)

C Table C: amplification program

Temperature	Time	Cycle
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 4 µL of loading dye on top of each reaction site
- Mix loading dye and amplicon by carefully pipetting up and down
- Transfer 4 µL of the mix on a PAGE gel (8 %)
- Analyze samples by PAGE and silverstaining

2 Figure 2: AmpliSpeed slide cycler ASC200D

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