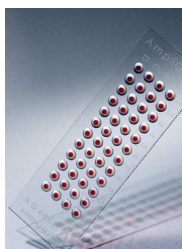


On-Slide DNA Sequencing using AmpliGrid AG480F

This protocol describes the sequencing procedure after PCR of human DNA directly on the AmpliGrid slide.

1 AmpliGrid AG480F



2 AmpliSpeed ASC200D



Material:

- PCR product from AmpliGrid amplification on AmpliGrid AG480F incl. sealing solution (Advalytix)
- Electronic multistep pipette
- AmpliSpeed slide cycler (Advalytix)

Exonuclease:

- ExoSAP-IT® (USB)

Sequencing:

- BigDye® v1.1 Terminator ready reaction mix (Applied Biosystems)
- Sequencing Primer, 3 pmol/μl
- PCR-clean water

Protocol:

On-slide Exonuclease clean-up of PCR products

The amplification primer and unconsumed dNTPs are removed from the PCR reaction by an exonuclease step without removing the PCR solution from the AmpliGrid slide. The enzyme is pipetted through the sealing solution directly in the aqueous phase.

- Add 0.4 μl of ExoSAP-IT® to the PCR reaction by penetrating the sealing solution with a 2.5 μl pipette tip
- Run the temperature protocol on the AmpliSpeed slide cycler as shown in table 1

A Table 1: Exonuclease step temperature profile

Temperature	Time
37°C	15 – 60 min
80°C	15 min

On-slide BigDye® Sequencing

The BigDye® sequencing reaction is carried out directly on the AmpliGrid slide without removing of the aqueous phase.

- For each AmpliGrid reaction site prepare a mixture of reagents as shown in table 2. After the exonuclease step the volume per reaction site is 1.4 μl
- For the sequencing reaction add 0.6 μl of the BigDye®/primer mixture to each reaction site to a final volume of 2 μl

AmpliGrid slides are designed to hold 1 μl. Anyway it is possible to increase the volume of the aqueous phase. Droplet merging as a result may occur if not handled carefully. We do recommend to leave the slides on the cycler during pipetting.

B Table 2: BigDye® reaction mix setup

Component	Volume
BigDye® mix	0.5 μl
Sequencing primer, 3 pmol/μl	0.1 μl
Total volume	0.6 μl

- Run the sequencing protocol on the AmpliGrid slide cycler as shown in table 3

C Table 3: Sequencing reaction on AmpliSpeed slide cycler

Temperature	Time	Cycles
95°C	2 min	
95°C	30 sec	
annealing temperature	30 sec	30
59°C	4 min	

- Recover sample by adding 5 μl PCR-clean water pipetted on top of sealing solution or recovering 2 μl from underneath the sealing solution

Analysis:

- Transfer samples to MTP
- Clean-up sample using Sephadex G-50 column or any other Sephadex based clean-up kit
- Analyse sample with capillary electrophoresis (ABI PRISM®) according to manufacturer manual. If CE signals are too low, increase time for sample take-up in ABI software

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