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Short Technical Manual: Running ready-to-use GMA™ gels for SSCP in ORIGINS at 9°C

1. *Prepare 30 mM TAE buffer *(Fig. 1)*
2. *Fill the apparatus with 1.9 l 30 mM TAE buffer 2-3 mm above the upper electrode*
3. *Switch the ORIGINS ON by ON/OFF button. Set the Temperature to 9°C. Set Pump Delay to 12 min. Set the Time. Every value has to be confirmed by pressing SET button.*
4. *Prepare the samples:*
   A) Add 3 µl PCR product to 7 µl formamide-NaOH stock solution. Mix well.*
   B) Heat the samples at 95°C for 5-6 min and quickly place the tubes in ice-cold water for 3 min.
5. *When samples are ready, take the gel *(Fig. 2) place it in the ORIGINS by Elchrom and fix it with the catamaran frame *(Fig. 3, 4)*
6. *Switch the Pump and the Time OFF. Load 9 µl of the samples quickly and close the lid*
7. *Start Power Supply and Time on ORIGINS ON*
8. *When electrophoresis is completed, take the gel out using the forceps *(Fig. 5) and the needle*
9. *Place the gel on the Peel-IT™ and detach it from the plastic backing with nylon string, for faster staining or blotting *(Fig. 6)*
10. *Stain the gel in the Easy Staining tray on the shaker *(Fig. 7)*

***Additional information on the page 2**

1. Dilute 50 ml of 40xTAE stock solution (PIN 3031) to 2 l with dH_2O. *(Fig. 1a)*

40x CONC. TAE BUFFER: (P / N 3031)

Components	*Amount for 1 liter*
Tris (hydroxymethyl) aminomethane	*145.37*
$Na_2EDTA \cdot 2\ H_2O$	*11.16 g*
Acetic Acid (glacial)	*34.4 ml*

Fig. 1a

2. The level is marked on the left side of the ORIGINS. *(Fig. 1b)*

Fig. 1b

3. Set temperature to 9°C.

1. Move the cursor to set temperature value
2. With up and down arrows choose the temperature of 9°C.
3. Press SET button to confirm settings.

Settings are saved for subsequent runs!

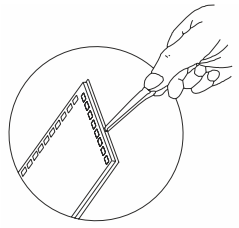

Fig.2

4. Mix 1 ml formamide and 10 µl 1M NaOH just before use. A few grains of bromphenolblue may be added for better visualisation.

A) Ratio of PCR product and denaturating stock solution can be changed depending on the DNA concentration (e.g 1µl PCR + 9 µl stock solution)

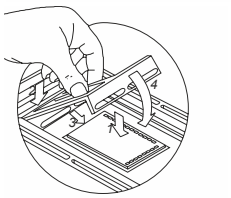

Fig. 3

5. Cut the alu bag on one side. Take the plastic bag with the gel out, and cut the plastic bag on 3 sides. Peel the plastic off. Grip the gel with forceps at the plastic backing overhang *(Fig. 2).* Place the gel in the ORIGINS and fix it with catamaran frame *(Fig 3).*

Catamaran frame should be placed on the plastic backing overhang, not on the gel *(Fig. 3, 4).* A needle can be used to move the gel. Rinse the sample wells with pipettor if air bubbles are present.

Correct Position of the catamaran

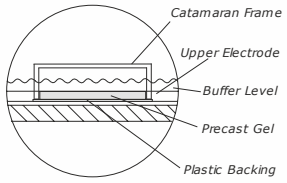

Fig. 4

6. Switch the Pump and Time OFF before loading the gel. For loading Wide Mini gels a Multichannel pipettor is recommended.

7. Recommended voltage is 72V 6 V/cm; cm = Distance between the electrodes. Amperage is always set to maximum. Running time depends on PCR fragment size:

Size in bp	150-200	200-250	250-350	350-450
Time in h	10	12	15	17

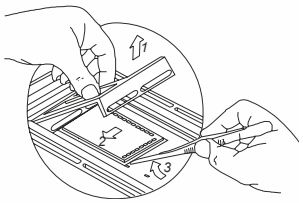

Fig. 5

Start the Power supply (72V) and then start Time ON on ORIGINS

8. To remove the gel use a needle to lift the edge of the gel. To avoid damage to the electrodes always move the gel parallel to the long axis of the apparatus to avoid damage of electrode wires.

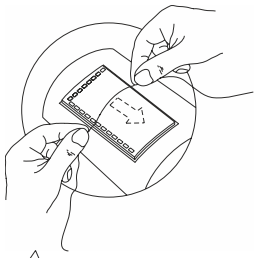

Fig. 6

9. Nylon string is provided in every box of gels. Instead of Peel-IT™ *(Fig. 6)*, any 1 or 2 l glass bottle can be used. Bottle need to be fixed to stay in place.

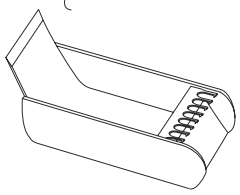

Fig. 7

10. Stain with SYBR Green II or SYBR Gold (1:10000) diluted in 30 mM TAE buffer for 20-30 min. It is recommended to protect the gel from the light during staining. Cover the Easy Staining tray *(Fig 7)* with the cardboard box or aluminum foil. Destain in distilled water for approximately 30 min is recommended when the gel is overstained.

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