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Variants of inflammatory mediators: alpha-1-protease inhibitors, cortisol binding globulin, interleukin-1-receptor antagonist and beta-2-adrenergic receptor genes in atopic asthmatic and non-asthmatic South Africans.

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5. Termination reaction

Add 4 µL of stop solution to each of the termination reactions, mix thoroughly and centrifuge briefly to separate the oil from the aqueous phase. Alternatively, remove 6 µL from each termination reaction and transfer to a fresh tube containing 3-4 µL of stop solution. Samples may be stored frozen until ready to load the sequencing gel. Immediately before loading, heat samples to 90°C for 10 min and load 3 µL/lane (all the samples should be loaded onto the gel within 5 min).

Plate pretreatment

The plates should be cleaned thoroughly with absolute ethanol and allowed to dry, following which the smaller of the two plates (i.e. the notched plate) should be siliconised (Sigmacote was used for the present study). The spacers are then placed along the edge of the large plate and the notched plate is then placed on top, such that all the edges are even. The plates are then taped together, taping the bottom edge last.

Gel preparation

Gel concentration (%)	Acrylamide/ bis-acrylamide	Urea (7 M)	20x glycerol tolerant gel buffer (GTB)	ddH_2O
6	5.7 g/0.3 g	42 g	4-5 mL*	~45 mL

*use 4 mL for faster gel migration.

Dissolve the above reagents, adjust the volume to 100 mL with ddH_2O, filter at 4°C (the colder the gel solution, the easier it is to pour) and degas for 3 min. When ready to pour, add 1 mL of 10% ammonium persulfate and 25 µL TEMED. Gels should be prepared 2-20 hrs prior to use (if kept overnight, store covered at 4°C).

The gels were pre-run at 90 W constant power for 30 min, until the gel temperature reached 48-50°C. Samples were then loaded and the gels were run for 1½-2 hrs at room temperature at 60 W (constant power) until the bromophenol blue dye front reached the bottom of the gel. The gel temperature was maintained at 48-50°C. The running buffer used was 0.5xGTB. After electrophoresis, the gels were blotted onto 3 MM Whatman filter paper, dried on a slab gel dryer at 80°C for 1-1½ hrs and autoradiographed for 24-72 hrs (depending on the age of the [α-^{33}P] ddNTP).

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