

## Isolation of a porcine male specific DNA sequence

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A 3.8 kb male specific fragment was observed following agarose gel electrophoresis of porcine DNA digested with *Sph* I. DNA was recovered from this region of a gel using DE-81 paper and ligated to *Sph* I digested pUC18. Plasmids carrying male specific sequences were identified by their differential hybridization to male and female genomic DNA labelled by random hexamer priming. One such plasmid (pDALY13) was found to contain a 3.8kb fragment which can be used to unambiguously differentiate between male and female porcine DNA, for example by Southern blot analysis (Fig. 1) or by slot blots (Fig. 2). Hybridization to female DNA was only observed after prolonged exposure of such blots. The number of copies of the repeat sequence on the Y chromosome is at least 200 fold higher than in the rest of the genome. Probes generated from pDALY13 are therefore ideal for sexing porcine embryos or for assessing techniques for separating X- and Y-bearing porcine sperm.

Note: Following the submission of this manuscript the characterisation of a similar porcine male specific sequence was reported, McGraw, R.A. *et al* Nucleic Acids Res 16: 10389 (1988). DNA sequence comparison shows 80% similarity between the two sequences indicating that they are members of the same repeat family.

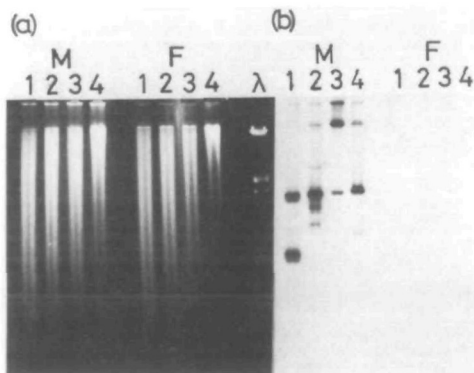


Fig. 1. Southern blot analysis: 2 $\mu$ g of digested genomic DNA were loaded per slot (M - male, F - female, 1.- *Bam* HI, 2.-*Eco* RI, 3.-*Hind* III, 4.- *Sph* I,  $\lambda$  - size markers). (a) Ethidium bromide stained gel. (b) Southern blot of gel in (a). Hybridization; 6x SSC, 5x Denhardt's, 0.05% SDS and 50 $\mu$ g/ml denatured salmon sperm DNA, 65 $^{\circ}$ C. Probe; isolated 3.8kb fragment labelled by random priming. The filter was washed in 2x SSC 65 $^{\circ}$ C and exposed for 2 hours at room temperature.

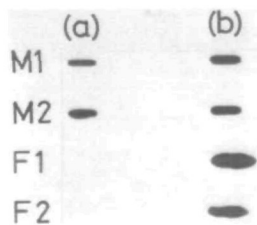


Fig. 2. Slot blots: Approximately 10 $\mu$ g of genomic DNA from two male and two female pigs were loaded per slot. Hybridization; as Fig. 1. Probes; (a) as Fig. 1., (b) a duplicate filter was probed with a porcine autosomal repeat sequence. The filters were exposed for 45min at room temperature.