Assignment¹ of the glucose-6-phosphate dehydrogenase (G6PD) gene to tammar wallaby chromosome Xq by fluorescence in situ hybridization with a BAC clone

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¹ This localization resolves the inconsistency of a previous assignment to distal Xq by Spencer et al. (1991) using radioactive in situ hybridization, and an assignment to XqCEN by linkage analysis (McKenzie et al., 1996).

Rationale and significance

Comparative mapping, between marsupials and eutherians (divergence 180 mya), provides information about mammalian genome organization and evolution. G6PD is a key marker on the X chromosome in all mammals. It has been important for studies of X chromosome inactivation, being random in eutherians and paternal in marsupials. G6PD is a housekeeping X-linked gene that converts glucose into pentose sugars, whose bi-products prevent cellular oxidative stress. Numerous human variants exist and deficiencies cause severe anemia and newborn jaundice (Mehta et al., 2000).

Older radiation in situ hybridization results placed G6PD on the distal third of Xq (Spencer et al., 1991) in the model marsupial *Macropus eugenii* (tammar wallaby). However, genetic linkage was established between G6PD and ribosomal RNA genes on the short arm, suggesting that G6PD is close to the centromere (McKenzie et al., 1996). This study maps G6PD by in situ fluorescent hybridization to a proximal position on the long arm of the tammar X chromosome.

Fig. 1. FISH mapping of Me BAC G6PD to the long arm of the X chromosome just below the centromere (male metaphase).

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Materials and methods

A *Macropus eugenii* (Me) genomic male BAC DNA library was screened with a 670-bp Me G6PD probe. This probe was amplified from female cerebellum cDNA with the forward primer F 5'-ATCATCATGGGTGCATCG-3' and reverse primer R 5'-TTGACCTTCTCATCACGGAC-3'. Cycling parameters were an initial cycle at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s; 50 °C for 30 s; 72 °C for 1.5 min and a final extension of 72 °C

for 10 min. The PCR product obtained was 99 % homologous to the *Macropus robustus* G6PD mRNA (accession number MRU 13899). One positive BAC clone was isolated and demonstrated by PCR to contain exons 5 to 6 (including intron 5) and exon 12.

Probe name(s): Me BAC G6PD
Probe type: genomic DNA
Insert size: approximately 95 kb
Vector: pRazor BAC
Proof of authenticity: sequencing

Results

Mapping data:

Most precise location: proximal Xq (Fig. 1)

No. of male cells examined: 10

Number of male cells with specific signal:

1 (2), 2 (8), 3 (0), 4 (0) chromatids per cell

References

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