

G6PD primers:

I've recently found that the Forward and Reverse G6PD primers from Saunders, Hammer, and Nachman (Genetics 162:1849-1861) work much better in amplifying the primary G6PD target from genomic. The resulting fragment (5,271 bp) is almost identical in size to that from the Verrelli et al. (2002) study. The PCR fragment starts in intron 2 with nucleotide 202 upstream of exon 3 and ends in the 3' UTR with nucleotide 893 downstream from the stop codon after exon 13.

Saunders et al 2002:

13125F GTT TAT GTC TTC TGG GTC AGG GAT GG
18396R AGT GTT GCT GGA AGT CAT CTT GGG T

Long-Template enzyme system PCR Conditions:

initial 94° 1 min
94° 12 sec
65° 30 sec
68° 6 min
X 38

Re-amplifications were done from this fragment using three sets of primers with the following PCR conditions:

initial 94° 1 min
94° 12 sec
62° 25 sec
72° 3 min
X 35

Frag I:

13125F (from above)
15031R CTC CAC GAT GAT GCG GTTC C

Frag II:

14465F ACC ACA AGG TGG CAG CGT TG
16592R TGC CTT GCT GGG CCT CGA AGG

Frag III:

15830F CCA GGG ACG TGA TGC AGA AC
18370R GGG CAG GGA CAT GGA CAG TAA GAG

Sequencing primers:

Frag I:

13857F CTG AAA TCT GGC CTC TGT CC
13971R GTT CAG CCC CAT CTT AGC AG

Frag II:

14492F AAA CAC CGC CTT TCC GCT CTG
15143F GTG CAG AAC CTC ATG GTG CTG
15830F CCA GGG ACG TGA TGC AGA AC

Frag III:

16519F TTT GCA GCC GTC GTC CTC TAT G
17141F GCA TAC CTG TGG GCT ATG GG
17793F GTC TGT CCC AGA GCT TAT TGG