Assaying meiotic exchanges within the intron1-through-3 interval of the Xp/Yp pseudoautosomal gene *SHOX*

General approach:
To improve fidelity of the assay, DNA is first digested with *Kpn* I restriction enzyme which cleaves outside the test interval. To selectively amplify recombinant molecules, aliquots of this DNA are then amplified with two allele-specific primers in repulsion phase directed to SNP sites that are heterozygous in the semen donor. PCR products from this 1° PCR are then digested with S1 nuclease and reamplified using repulsion phase allele-specific primers directed to two internal heterozygous SNP sites thereby increasing the specificity of single molecule recombinant recovery. 2° PCR reactions showing a recombinant are then re-amplified using two internal universal primers. Exchange breakpoints in 3° PCR products are then mapped by dotblot hybridisation with ASOs.

Specific details of SNP sites, allele-specific primer (ASP) combinations, universal primers and PCR cycling conditions used for this interval are shown below. The 3' end of primer sites are shown with respect to the Genbank entry U82668. Some ASPs include a synthetic 5' extension indicated in blue lower case; this extension was added to improve PCR efficiency.

Combination 1:

1° PCR:

SNPs: +701T (42851 bp) and +9697G (51847 bp)

ASPs:	AS701T	5' GGGGATCCTCGGCCTCT 3'
	AD9697G	5' gtctacgtagtcagctctggATTCATGACCAAGGGTCC 3'
plus	ADAP	5' gtctacgtagtcagctctgg 3'

PCR cycling:

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 1 cycle  of 96°C 2 min
 5 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
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2° PCR:

SNPs: +1096A (43246 bp) and +8965G (51115 bp)

ASPs: AS1096A 5' GTAACCTGTGTGGGGGA 3'

AS8965G 5' AACCTGATCCTGTTTCCC 3'

PCR cycling:

```
 1 cycle  of 96°C 2 min
 5 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 57°C 30 sec, 61°C 11 min
11 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min
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3° PCR:

Primers:

To type SNPs +2378 to +7982 inclusive:

UD 5' AGGACCACGTAGACAATGAC 3' (43851 bp)

s14 5' TCAGCAACAGCTTCTATCGG 3' (50422 bp)

PCR cycling:

```
1 cycle  of 96°C 2 min
5 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 9 min
7 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 9 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 9 min
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Combination 2:

1° PCR:

SNPs: +1096G (43246 bp) and +8965C (51115 bp)

ASPs: AS1096G 5' GTAACCTGTGTGGGGGG 3'

AS8965C 5' AACCTGATCCTGTTTCCG 3'

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 54°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 53°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 52°C 30 sec, 61°C 11 min

2° PCR:

SNPs: +2784A (44934 bp) and UF (50741 bp)

ASPs: AS2784A 5' CGTCCTAAGTCAAGGTTA 3'

UF 5' GTCTTGGAGTGATTCACGA 3'

NOTE: UF is a universal primer not an ASP.

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 54°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 53°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 52°C 30 sec, 61°C 11 min

3° PCR:

Primers:

To type SNPs +3571 to +7982 inclusive:

UE 5' ACGACAACTGGGCGGCATAC 3' (45099 bp)

s14 5' TCAGCAACAGCTTCTATCGG 3' (50422 bp)

AND to type +2900:

OX-C 5' TTGGCGAAAGCTGTTGGGTC 3' (44830 bp)

UB 5' CAGGCTTCCACGGTAACCTC 3' (45906 bp)

PCR cycling:
UE to s14:
1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 9 min
7 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 9 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 9 min

OX-C to UB:
1 cycle of 96°C 2 min
4 cycles of 96°C 20 sec, 60°C 30 sec, 65°C 1 min
6 cycles of 96°C 20 sec, 59°C 30 sec, 65°C 1 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 65°C 1 min

Combination 3:

1° PCR:

SNPs: +1096G (43246 bp) and +9697G (51847 bp)

ASPs: AS1096G 5' GTAACCTGTGTGGGGGG 3'

AD9697G 5' gtctacgtagtcagctctggATTCATGACCAAGGGTCC 3'

plus ADAP 5' gtctacgtagtcagctctgg 3'

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 54°C 30 sec, 61°C 11 min

10 cycles of 96°C 20 sec, 53°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 52°C 30 sec, 61°C 11 min

2° PCR:

SNPs: +2900G (45050 bp) and +9177C (51327 bp)

ASPs: AS2900G 5' TAAGAGGCTCAGAGAGAG 3'

AS9177C 5' CCACAGCCACCCTTACG 3'

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 57°C 30 sec, 61°C 11 min
11 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min

3° PCR:

Primers:

To type SNPs +3571 to +7982 inclusive:

UE 5' ACGACAACTGGGCGGCATAC 3' (45099 bp)

s14 5' TCAGCAACAGCTTCTATCGG 3' (50422 bp)

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 9 min
7 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 9 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 9 min

Combination 4:

1° PCR:

SNPs: +701T (42851 bp) and +9177T (51327 bp)

ASPs: AS701T 5' GGGGATCCTCGGCCTCT 3'

AS9177T2 5' CCACAGCCACCCTTACA 3'

PCR cycling:

```
 1 cycle  of 96°C 2 min
 5 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 57°C 30 sec, 61°C 11 min
11 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min
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2° PCR:

SNPs: +1096A (43246 bp) and +7982C (50132 bp)

ASPs: AS1096A 5' GTAACCTGTGTGGGGGA 3'

AD7982C 5' gtctacgtagtcagctctggGCATTCCCATGTTTGTTG 3'

plus ADAP 5' gtctacgtagtcagctctgg 3'

PCR cycling:

```
 1 cycle  of 96°C 2 min
 5 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 57°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min
```

3° PCR:

Primers:

To type SNPs +3571 to +7445 inclusive:

UE 5' ACGACAACTGGGCGGCATAC 3' (45099 bp)

UG 5' TTCATGCGACCCCTGCAATC 3' (50086 bp)

AND to type +2900:

OX-C 5' TTGGCGAAAGCTGTTGGGTC 3' (44830 bp)

UB 5' CAGGCTTCCACGGTAACCTC 3' (45906 bp)

PCR cycling:

UE to UG:

1 cycle of 96°C 2 min
8 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 9 min
8 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 9 min
9 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 9 min

OX-C to UB:

1 cycle of 96°C 2 min
4 cycles of 96°C 20 sec, 60°C 30 sec, 65°C 1 min
6 cycles of 96°C 20 sec, 59°C 30 sec, 65°C 1 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 65°C 1 min

Combination 5:

1° PCR:

SNPs: +701T (42851 bp) and +9697G (51847 bp)

ASPs: AS701T 5' GGGGATCCTCGGCCTCT 3'

AD9697G 5' gtctacgtagtcagctctggATTCATGACCAAGGGTCC 3'

plus ADAP 5' gtctacgtagtcagctctgg 3'

PCR cycling:

```
 1 cycle  of 96°C 2 min
 5 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
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2° PCR:

SNPs: +1096A (43246 bp) and +9177C (51327 bp)

ASPs: AS1096A 5' GTAACCTGTGTGGGGGA 3'

AS9177C 5' CCACAGCCACCCTTACG 3'

PCR cycling:

```
 1 cycle  of 96°C 2 min
 6 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 57°C 30 sec, 61°C 11 min
11 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min
```

3° PCR:

Primers:

To type SNPs +2784 to +7982 inclusive:

OX-C 5' TTGGCGAAAGCTGTTGGGTC 3' (44830 bp)

s14 5' TCAGCAACAGCTTCTATCGG 3' (50422 bp)

PCR cycling:

```
1 cycle  of 96°C 2 min
5 cycles of 96°C 20 sec, 60°C 30 sec, 64°C 9 min
7 cycles of 96°C 20 sec, 59°C 30 sec, 64°C 9 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 64°C 9 min
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Combination 6:

1° PCR:

SNPs: +1096G (43246 bp) and +9177T (51327 bp)

ASPs: AS1096G 5' GTAACCTGTGTGGGGGG 3'

AS9177T2 5' CCACAGCCACCCTTACA 3'

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min
9 cycles of 96°C 20 sec, 55°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 54°C 30 sec, 61°C 11 min

2° PCR:

SNPs: +2900G (45050 bp) and +7982C (50132 bp)

ASPs: AS2900G 5' TAAGAGGCTCAGAGAGAG 3'

AD7982C 5' gtctacgtagtcagctctggGCATTCCCATGTTTGTTG 3'

plus ADAP 5' gtctacgtagtcagctctgg 3'

PCR cycling:

1 cycle of 96°C 2 min
6 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 11 min
10 cycles of 96°C 20 sec, 57°C 30 sec, 61°C 11 min
11 cycles of 96°C 20 sec, 56°C 30 sec, 61°C 11 min

3° PCR:

Primers:

To type SNPs +3571 to +7445 inclusive:

UE 5' ACGACAACTGGGCGGCATAC 3' (45099 bp)

UG 5' TTCATGCGACCCCTGCAATC 3' (50086 bp)

PCR cycling:

1 cycle of 96°C 2 min
5 cycles of 96°C 20 sec, 60°C 30 sec, 61°C 9 min
7 cycles of 96°C 20 sec, 59°C 30 sec, 61°C 9 min
8 cycles of 96°C 20 sec, 58°C 30 sec, 61°C 9 min
