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MICROSATELLITE LETTERS

# Development of 27 novel cross-species microsatellite markers for the endangered *Hucho bleekeri* using next-generation sequencing technology

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**Abstract** In this study, we developed 27 polymorphic microsatellite loci in *Hucho bleekeri*, a glacial relict and freshwater resident salmonid fish in China. The number of alleles varied from 2 to 8 for each primer set. The observed and expected heterozygosity ranged from 0.250 to 0.906 and 0.508 to 0.845, respectively. The polymorphic information content ranged from 0.371 to 0.808. These microsatellite loci should be useful to study population genetics, paternity identification, speciation and adaptive evolution of this lineage.

**Keywords** *Hucho bleekeri* · Microsatellite · Endangered species

*Hucho bleekeri*, a glacial relict and freshwater resident salmonid fish, is endemic to the Yangtze River drainage in China. It became a critically endangered species mainly due to over-fishing and habitat destruction since 1960s (Hu et al. 2008). A series of measures have been taken to protect this species such as listing it into China Red Data Book to ban on fishing and establishing a national nature reserve (Wang 1998). And recently it has been performed successfully controlled breeding, which has great significance to the species preservation. To better address the conservation status of the endangered species, a management programme based on genetics is being devised to define the efficiency of these conservation measures (Zhu et al. 2005). Despite the increasing demand for preservation and management plans, no suitable high resolution molecular markers have been available for this species. In current study, we report a set of polymorphic microsatellite loci for *H. bleekeri* that can serve as effective genetic markers for conducting further preservation and management in this endangered fish.

We used the microsatellite makers isolated from closely related species *Brachymystax lenok*, that had been constructed DNA Library by Ion PGM$^{TM}$ sequencer with Ion 318 chip kit v2 (Life tech LTD) in previous study (unpublished). 72 primer pairs were designed using Primer Premier 5.0 based on the flanking sequence of the repeat motifs. The polymorphisms of the loci were analyzed using a population of 24 wild individuals collected from Taibai River in Baoji City, Shaanxi Province, China. Total genomic DNA of *H. bleekeri* was extracted from tissue using the rapid salt-extraction method (Salah and Iciar 1997). The PCR amplification was carried out in a 25 μL volume that contained 1× PCR buffer, 50–100 ng genomic DNA, 0.25 μM of each primer, 200 μM dNTPs, 0.5 mM $MgCl_2$, and 0.25 U Taq DNA polymerase (TaKaRa). The PCR cycle comprised an initial denaturation at 94 °C for 5 min, followed by 35 cycles of at 94 °C for 30 s, at the appropriate temperature (Table 1) for 30 s and extension at 72 °C for 60 s, and then 72 °C for 10 min. The PCR products were separated on 12 % non-denaturing polyacrylamide gel and visualized by silver staining. A 20-bp DNA ladder molecular weight marker (TaKaRa) was used as the standard to determine the size of the alleles.

Among these primer sets synthesized, only 27 sets showed clearly polymorphism (Table 1 and Appendix).

Ke Wang and Shuhuan Zhang have contributed equally to this work.

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**Table 1** Characterization of 27 polymorphic microsatellite loci in *H. bleekeri*

| Locus | Repeat motifs | Primer sequence (5′–3′) | Tm (°C) | Size range (bp) | Na | PIC | Ho | He | *P*-HWE | Accession no. |
|---|---|---|---|---|---|---|---|---|---|---|
| BLT2 | (AC)11 | F: ATGACGCCACTATGAGACCG | 60 | 230–310 | 4 | 0.681 | 0.656 | 0.742 | 0.018 | KM051898 |
| | | R: CAGGAAGAAGAACCAGAACCA | | | | | | | | |
| BLT4 | (TG)11 | F: TGAACAGACACTCACACAGGC | 55 | 185–215 | 4 | 0.696 | 0.750 | 0.744 | 0.036 | KM051900 |
| | | R: GTGTTTCAGCTGCTGCGTT | | | | | | | | |
| BLT20 | (GT)17 | F: CCGTACTGCCTAGCAACACA | 56 | 205–255 | 4 | 0.677 | 0.719 | 0.738 | 0.316 | KM051916 |
| | | R: GGCTGTTTTCACAGAAAGGC | | | | | | | | |
| BLT19 | (CA)15 | F: GTTCCTCTCTGTCCCCTTCC | 55 | 133–185 | 5 | 0.718 | 0.818 | 0.792 | 0.009* | KM051915 |
| | | R: AAACACCATGGAACTCGACC | | | | | | | | |
| BLT6 | (GT)13 | F: CCCAGGTCTGGTGTCCAGTA | 55 | 105–123 | 4 | 0.696 | 0.708 | 0.760 | 0.024 | KM051902 |
| | | R: GTACCGCGCTCAGCTCAT | | | | | | | | |
| BLT27 | (TC)11 | F: GGGCAAGGTGTTATGGCTAA | 58 | 135–153 | 4 | 0.691 | 0.719 | 0.751 | 0.501 | KM051923 |
| | | R: ATGAAACAGGTCCATAGCGG | | | | | | | | |
| BLT28 | (GT)18 | F: CCCTACCAAGCACCAATACC | 56 | 155–200 | 5 | 0.746 | 0.563 | 0.794 | 0.003* | KM051924 |
| | | R: TGATGTCAGGTTGCTTATTCAGA | | | | | | | | |
| BLT25 | (TG)14 | F: AATGAAACCAGCTCATTGCC | 56 | 160–200 | 3 | 0.534 | 0.300 | 0.647 | 0.008* | KM051921 |
| | | R: CAAGTCCTTCCAAATGGTCC | | | | | | | | |
| BLT30 | (TG)14 | F: CCGCTCACTTTGTTGACGTA | 56 | 165–200 | 5 | 0.740 | 0.531 | 0.788 | 0.001* | KM051926 |
| | | R: CCCTGTCCAACCTCTCTCAG | | | | | | | | |
| BLT16 | (CA)17 | F: ATCCAGTCAATAACCGCTGG | 55 | 183–205 | 4 | 0.692 | 0.625 | 0.764 | 0.019 | KM051912 |
| | | R: CCTCGAGAAACTCGGTTGTT | | | | | | | | |
| BLT31 | (AG)12 | F: TGGATGGGTGTTACAAGCAA | 56 | 90–100 | 3 | 0.505 | 0.833 | 0.594 | 0.142 | KM051927 |
| | | R: CAGATCTTGAGACAAAGAGCCA | | | | | | | | |
| Hbl1-101 | (CA)13 | F: TGTAATGTCACACACGCACG | 55 | 190–240 | 4 | 0.673 | 0.625 | 0.742 | 0.220 | KM385538 |
| | | R: CCAGACCAGAGGGACTTCAA | | | | | | | | |
| Hbl2-2 | (TTA)10 | F: TCTAGTGCTGCATGTCTGCC | 57 | 140–174 | 5 | 0.741 | 0.625 | 0.789 | 0.199 | KM385539 |
| | | R: AGGCTCACATTGGCTCGTAT | | | | | | | | |
| Hbl3-5 | (TAA)11 | F: TTGAAGTTGCCTTCTGGTCC | 58 | 152–185 | 4 | 0.703 | 0.531 | 0.761 | 0.009* | KM385540 |
| | | R: GGCCACACATGCAAAACAT | | | | | | | | |
| Hbl4-10 | (ATC)12 | F: GACAACAGCTACAGGGCACA | 56 | 215–240 | 5 | 0.737 | 0.688 | 0.784 | 0.194 | KM385541 |
| | | R: GACCTGGCTCTGGGTGATAG | | | | | | | | |
| Hbl5-11 | (TAT)11 | F: TATGTGCCCAAAATGCTGTC | 58 | 140–210 | 4 | 0.676 | 0.594 | 0.739 | 0.005* | KM385542 |
| | | R: AATGGGATGTATGGGACACG | | | | | | | | |
| Hbl6-240 | (AC)22 | F: GGGGAATGCAGTTGAAATGT | 55 | 140–180 | 6 | 0.808 | 0.531 | 0.845 | 0.001* | KM385543 |
| | | R: ATGGCATGTGTGGTGTGTTT | | | | | | | | |
| Hbl7-290 | (GT)14 | F: ACTCTGATCGCTACCTGGGG | 56 | 100–160 | 6 | 0.781 | 0.689 | 0.821 | 0.134 | KM385544 |
| | | R: TCTGTTCGGACTGCTACATCA | | | | | | | | |
| Hbl8-163 | (GT)15 | F: GACTGGTGAGTCACAGGCAA | 60 | 83–105 | 8 | 0.800 | 0.750 | 0.833 | 0.001* | KM385545 |
| | | R: GCCTAGAGTGAGACCGATGC | | | | | | | | |
| Hbl9-643 | (TG)14 | F: GGCACAGCATGTCCCTTTTA | 55 | 100–125 | 4 | 0.702 | 0.818 | 0.766 | 0.031 | KM385546 |
| | | R: CTGCAGCATGTTCTGGATGT | | | | | | | | |
| Hbl10-16 | (TAA)10 | F: TTCCTCTCCTCCTCCTCCAT | 56 | 155–185 | 5 | 0.749 | 0.719 | 0.796 | 0.063 | KM385547 |
| | | R: GACTTCGGGGATGGCTCTAT | | | | | | | | |
| Hbl11-21 | (TCAT)10 | F: CATTAATCCATCCAACCATGC | 56 | 125–200 | 5 | 0.741 | 0.625 | 0.789 | 0.495 | KM385548 |
| | | R: ACATCCCTGCCTTCGAAAC | | | | | | | | |
| Hbl12-23 | (AGTT)13 | F: AATGCTTATTCACGCGAGGT | 56 | 180–220 | 2 | 0.371 | 0.250 | 0.508 | 0.036 | KM385549 |
| | | R: ACACACAGCTTGGGACACAG | | | | | | | | |
| Hbl13-25 | (TGAG)13 | F: TTTCACTGTGACCTGCTGCT | 56 | 88–135 | 5 | 0.748 | 0.688 | 0.796 | 0.074 | KM385550 |
| | | R: CTCTCAACCAACACACACCG | | | | | | | | |

**Table 1** continued

| Locus | Repeat motifs | Primer sequence (5′–3′) | Tm (°C) | Size range (bp) | Na | PIC | Ho | He | *P*-HWE | Accession no. |
|---|---|---|---|---|---|---|---|---|---|---|
| Hbl14-29 | (TGTA)10 | F: GAGGTGCACGTGTTCAAAGA | 55 | 178–190 | 4 | 0.697 | 0.594 | 0.756 | 0.047 | KM385551 |
| | | R: TGGTTAAGACCAAGACCAACG | | | | | | | | |
| Hbl15-31 | (GTTA)10 | F: GAGCTGGCTTGGTTGGTTAG | 57 | 120–200 | 5 | 0.727 | 0.906 | 0.778 | 0.010 | KM385552 |
| | | R: GCACCAGTCTTCTTTTTCGG | | | | | | | | |
| Hbl16-128 | (GT)13 | F: AGCCTGTCAGCACTGATGATT | 52 | 100–120 | 4 | 0.696 | 0.594 | 0.756 | 0.093 | KM385553 |
| | | R: TGGTCTGGCCTATGAACACA | | | | | | | | |

*Tm* annealing temperature, *Size range (bp)* allele range, *Na* observed number of alleles, *PIC* polymorphic information content, *Ho* observed heterozygosity, *He* expected heterozygosity, *P-HWE* probability of Hardy-Weinberg equilibrium

* $P < 0.01$, significant departures from Hardy-Weinberg equilibrium

The polymorphic parameters, including the number of alleles (Na), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), and Hardy–Weinberg equilibrium (HWE) test, for each locus were performed using Popgen32 software. And the polymorphic information content (PIC) was calculated using PIC_CALC (Cheng et al. 2013). The number of alleles per locus varied from 2 to 8. $H_O$ and $H_E$ were 0.250–0.906 and 0.508–0.845, respectively (Table 1). The polymorphic information content (PIC) was 0.371–0.808 (Table 1). Eight loci (BLT19, BLT28, BLT25, BLT30, Hbl3-5, Hbl5-11, Hbl6-240, Hbl8-163) deviated significantly from the Hardy–Weinberg equilibrium ($P < 0.01$; Table 1). This is the first time to develop polymorphic microsatellite markers for *H. bleekeri* and the markers are expected to be useful for further studies of genetic diversity and paternity identification.

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## Appendix: Sequences of *H. bleekeri*

| Accession no. | Locus | Sequence |
|---|---|---|
| KM051898 | BLT2 | ATGACGCCACTATGAGACCGGTCAAACATACACATGTACACACACACACACACACACACAGAGAGA GACAGACAGACAGATATTAATCAATCAGCCGATAGATCAAAGACAAGACTCACTTCTCCCTGACTGTC TTACAGTAGAGTGGTTCTGGTTCTTCTTCCTG |
| KM051900 | BLT4 | TGAACAGACACTCACACAGGCTGATAAGAAGTACAGAAGATGAGAAGGTGTCCACACTTCATAGACT GAAAGGTCAGGAGTAAACAAAACAGTGATTTCTTGTGTGTGTGTGTGTGTGTGTGTGTACTCACAGGCT GGGGCTGCTCTCCAACGCAGCAGCTGAAACAC |
| KM051916 | BLT20 | CCGTACTGCCTAGCAACACAGCAACTAGTAGAAAAAGTGTAAACATATATGTGATGTATTACGTGGTC ACTGTGGAATAACAGCTTGAAGTTTAGGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTGTG CGTGTGTACTTAAAGAGGTGATACCTGCAGTAACCTGTAACCAAATAGTTAACCACTTAACCTCTGCC TTTCTGTGAAAACAGCC |
| KM051915 | BLT19 | GTTCCTCTCTGTCCCCTTCCCTTTGCATCTCTCTTCACACACACACACACACACACACACACACACACGC ACGCACGCACGCACACGAGACACACTCGTCGTCTCTTCTTCAGCTTGCGCTGACTTGAATGTACACT CTTGTTCAAATTAGGTCGAGTTCCATGGTGTTT |
| KM051902 | BLT6 | CCCAGGTCTGGTGTCCAGTATGTCTGTCGTGTGTGTGTGTGTGTGTGTGTGTGTGCGTGTGTACATAC CTGTGTCTGCTAGGTCTCTCAGTGATGAGCTGAGCGCGGTAC |
| KM051923 | BLT27 | GGGCAAGGTGTTATGGCTAAACTAAGCATAGTTTACAAATGTATCTCTCTCTCTCTCTCTCTCTCTACA ACACGTGTCATATTGTGTTGGTCCGCTATGGACCTGTTTCAT |
| KM051924 | BLT28 | CCCTACCAAGCACCAATACCTGATATAAAAGATATCAATGTTTGCTAAGCGAATTATGCTAATTGTAAT GCTTATATTCTATGTGTGTGTGCGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGACGAGCATG ATTTTGTGTGCTATACTCTGTACTCTGAATAAGCAACCTGACATCA |
| KM051921 | BLT25 | AATGAAACCAGCTCATTGCCACACTTTGTCGGTTAAAACACCTGTGTGTGTGTGTGTGTGTGTGTGTG TGTAAACACTACACATTTTACACAGTGAACATATCAAGCAGCAACATACCAAACAATAAAACAGTTAA TTACAAAATACCTCCCAGAGTAACCTAGGACCATTTGGAAGGACTTG |
| KM051926 | BLT30 | CCGCTCACTTTGTTGACGTAGATGTGTACGTTATTTGTTTCAAAATTATATCACAGGTCAACTGTATTGC TGCTTAAGTGAGCAGGTGAAGGCTGAGTTTGTGTGTCTGTGTGTGTGTGTGTGTGTGTGTGCTT GTCTGGGTGAAGGTCTGATCTGCTCTGAGAGAGGTTGGACAGGG |
| KM051912 | BLT16 | ATCCAGTCAATAACCGCTGGCCAGGGGAATAAAGAGATGGAGTTAGGGAAAGTGTAGAAAGGAAGG GAGTAGCACACATGCGCACACACACACACACACACACACACACACACACACACTCTGACGTACACACA ATGCTATTGATTGCAGACTATCACAAAAAGGGTTGCCGGGGCAACAACCGAGTTTCTCGAGG |
| KM051927 | BLT31 | TGGATGGGTGTTACAAGCAATGTGGCAGAAGCTCCACATAGACCCAATCTAAAGAGAGAGAGAGAG AGAGAGAGAGTCCTTAGTTCTGGCTCTTTGTCTCAAGATCTG |
| KM385538 | Hbl1-101 | TGTAATGTCACACACGCACGCACGCACGCACACGCATGCACACACACACACACACACACACACACG AGTGTTGTCATTATCCATTACTTATTAGCTTACCTAACTTCGTTGCTAAAGTATAAAAGAATGAGATACC AAACTCGCTCGCAGGATTGGTTAACTCTTGAAGTCCCTCTGGTCTGG |
| KM385539 | Hbl2-2 | TCTAGTGCTGCATGTCTGCCTGGAGCAGGGTTTCCGGACATTTGACCTGCAATTTTATTTATCTGCTTA GTTATTATTATTATTATTATTATTATTATTATTATTTCAAAAGCCGGCTAAGCCAGCTAACAGAAACCCTAGCC TGCATACAGTGGCCCCATACGAGCCAATGTGAGCCT |
| KM385540 | Hbl3-5 | TTGAAGTTGCCTTCTGGTCCCAGCAATGGCATTGATAAAAGGTAACGTATTGTTTGTCCTTTTCTCTCA TTTATTAATAATAATAATAATAATAATAATAATAATAAAAATTTATGTCATGTAGCAGATGCTTTTATCCCA AAGAGACTAAATGTTTTGCATGTGTGGCC |
| KM385541 | Hbl4-10 | GACAACAGCTACAGGGCACAATAACGTTCTTTATTGATAAGGTTACAAACTCTTGACAAAGGCTTCAT CATATGCTTCATGCTTGGATTGATTGCTAATGAGCTAATGGTGAGTACTGACAATCAAGTTCAGGAATT CTCCTGAATTGAAATCTGCTCGAGCCTCAATTGTCAACTAAATGTATCATCATCATCATCATCATCA TCATCATCATCAATAAGCTTCTATCACCCAGAGCCAGGTC |
| KM385542 | Hbl5-11 | TATGTGCCCAAAATGCTGTCCAATTTCAGCATGCTCCTAATGATGAAATATCTGTGGATTAGAATTATAT ATTATTATTATTATTATTATTATTATTATTATATATAACAGTGCTTGCATAATAAGTAATGGTAGCCTTAG TATCGTGTCCCATACATCCCATT |
| KM385543 | Hbl6-240 | GGGGAATGCAGTTGAAATGTGTACACACACACACACACACACACACACACACACACACACACACAC TAGCAGGAGGAGCAGAGGGCGATGCAGTACACTTGTGAATACACAAACACAGGATTAGGCTTTAGGC TACATGTACAGACAGGCATATTCACAAAACACACCACACATGCCAT |
| KM385544 | Hbl7-290 | ACTCTGATCGCTACCTGGGGTGAGGCAGCAGTCAAAGTGTGTGTGTGTGTGTGTGTGTGTGTGCT GCTTGCTACCCGGCGGCGGTGATGTAGCAGTCCGAACAGA |
| KM385545 | Hbl8-163 | GACTGGTGAGTCACAGGCAAGTCACCATGTTGCCATGGGAGTGTGTGTGTGTGTGTGTGTGTGTG TGTGAGTATGCATGCATCGGTCTCACTCTAGGC |
| KM385546 | Hbl9-643 | GGCACAGCATGTCCCTTTTAGATGTGACATTGACACTCTTCGGTGTGTGTGTTTGTGTGTGTGTGTGT GTGTGTGTGTGTGTACTTGGTTAGTGTCTCTTTCCTCTCTTCACATCCAGAACATGCTGCAG |
| KM385547 | Hbl10-16 | TTCCTCTCCTCCTCCTCCATGTCCAGCTTGGCTTCCAGCTCCTTCCTCTCCTCCTCGATCTTCGCCTGC ATCTCCACCATCTTGTCAGGAGAAACCTTCTTCTTCTTCCCTTTAATAATAATAATAATAATAATAATAAT AATAGTGATTATCATTTATTTGTCTTATAGAGCCATCCCCGAAGTC |

| | | |
|---|---|---|
| KM385548 | Hbl11-21 | CATTAATCCATCCAACCATGCTATCATTCATTCATTCATTCATTCATTCATTCATTCATTGGCCTAC ACAATAAAAATGCATCACTATGATTCTGACCATTGGTGCGGTTCTGAAGGCGAGGGTTTCGAAGGCA GGGATGT |
| KM385549 | Hbl12-23 | AATGCTTATTCACGCGAGGTATGCTACATAATTACAGCAGTGCCACATGACAAGCATAATAACATCAGC CATTATGAACTGTGGATCCAAATAAACAAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTA GTTAGTTAGTTTGTTAATTGTGTGCTCTCTGACTCGTAGTTTAATTAATTATTAATATATTATCATGTCATT CCCTACATGCCTCATCTGTGTCCCAAGCTGTGTGT |
| KM385550 | Hbl13-25 | TTTCACTGTGACCTGCTGCTGGTGACTATCAGGCAGTAAGGCAAGCTGTTGCTGAGTGAGTGAGTGA GTGAGTGAGTGAGTGAGTGAGTGAGTGAGTGAGTGAGTGGTGGGGAGCGCCAGCGGTGTGTGTTGG TTGAGAG |
| KM385551 | Hbl14-29 | GAGGTGCACGTGTTCAAAGATGGAGCACTGTGTATGTATGTATGTATGTATGTATGTATGTATGTATGTA TCAGTGCGTCTCACTCTGCCGCCTCAAAGAGCAGACAGAGTTTATTGATAGGTCTCTGTAGGACGTTG GTCTTGGTCTTAACCA |
| KM385552 | Hbl15-31 | GAGCTGGCTTGGTTGGTTAGTTGGTTAGTTGGTTGGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGT TAGTTAGCTAGCTGTGGCATGCTAGTTTGCTAATCCCCCACATGCCTTCAGGTAAAGCCAGCTACAGA AACTGAAAGATGCCGAAAAAGAAGACTGGTGC |
| KM385553 | Hbl16-128 | AGCGAGACAGAGCGATAGAGAAATAGAAAGAGAGAGAGGGGCAATCAGAGATATAAAATATAGACA AGCCTGTCAGCACTGATGATTGATGAGAGGGTGTGTGTGTGTGTGTGTGTGTGTGCTACAAAGAC AGTCTGTGTGTTAGCAGCACAGACAGACCAGTCTGTGTTCATAGGCCAGACCA |

The orange parts are primers
The highlighted parts are repetitive sequences

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