

2014-05-09 nytt kravsett

WE CLAIM:

- 5 1. A method of asymmetric PCR comprising:
 - providing a nucleic acid sample to be used as a target template
 - identifying target template(s)
 - performing a polymerase chain reaction, utilizing highly complementary primers wherein either the forward or the reverse primer concentration is decreased to unblock the PCR reaction
- 10 by initially promoting linear amplification which will progressively shift towards exponential amplification by the COMplementary-Primer-ASymmetric (COMPAS)-PCR;
 - identifying the amplified nucleotide target sequence(s);
2. The method of claim 1 wherein the highly complementary primers have a common overlapping DNA target sequence.
- 15 3. The method of claim 2 wherein the overlapping DNA target sequence is direct tandem repeats.
4. The method of any one of claims 3 wherein the direct tandem repeat target is in the 5S-rDNA region.
5. A method for detecting, identification or monitoring salmonid species comprising:
 - providing a nucleic acid sample from salmonid to be used as (a) target template(s);
 - performing a polymerase chain reaction (PCR) applying COMplementary-Primer-ASymmetric (COMPAS)-PCR according to any one of claims 1-4, to amplify a nucleic acid target sequence of the template (s), utilizing a set or several sets of highly complementary primer pair(s) capable of priming said target(s);
- 25 - identifying the amplified nucleotide target sequence(s);
 - determining the species.
6. The method of any one of claims 1-5 wherein the determination of the species is performed by a melting curve analysis of the PCR product or by electrophoresis analysis.
7. The method of any one of claims 1-5 wherein the forward primer is extended in the 3' end to
 - 30 favor priming to the target and not to the reverse primer.

8. The method of any one of claims 1-7 wherein the reverse primer is extended in the 3' end to favor priming to the target and not to the forward primer.
9. The method of any one of claims 1-8 wherein either the reverse or the forward primer has a
5 SNP at its 3' end.
10. The method of any one of claims 1-9 wherein the primers in the primer pair are oligonucleotides each having a length of about 12 to about 30, preferably about 20 bp.
11. The method of any one of any one of claims 1-10, wherein the complementary set of primers is selected from a set of primer pair (s) wherein the forward primer is selected from
10 Table 1 or a complementary sequence thereof and the reverse primer is selected from Table 2 or a complementary sequence thereof.
12. The method of claims 5-11, wherein said salmonid comprises *Salmo trutta*, *Salmo salar* and hybrids thereof.
13. Oligonucleotide primer pairs, selected from the oligonucleotides of Tables 1 and 2 or
15 oligonucleotides with complementary sequences or their functional equivalent sequences.
14. Kit for detecting and identification of salmonid species, comprising a collection of oligonucleotide primer pairs selected from Tables 1 and 2 or complementary sequences thereof, in any combinations, capable of detecting salmonid species by the method of any one of claims 1-12.
- 20 15. Use of a method of any one of claims 1-12.
16. Use of the oligonucleotide primer pairs of claim 13.
17. Use of the kit of claim 14.