|  |  |
| --- | --- |
| **Multiplex 1** | **Volume (µl)** |
| RlCA1b5 F+R (10 µM) | 0,1 |
| Ga1a19 F+R (10 µM) | 0,2 |
| RlCA2a34 F+R (10 µM) | 0,2 |
| Rrid013A F+R (10 µM) | 0,2 |
| Res17 F+R (10 µM) | 0,2 |
| Rrid064A F+R (10 µM) | 0,2 |
| Type-it Microsatellite PCR Kit | 5 |
| Q-Solution | 2 |
| ddH2O | 0,9 |
| DNA | 1 |
|  |  |
| **Multiplex 2** | **Volume (µl)** |
| Re1Caga10 F+R (10 µM) | 0,2 |
| Ress22 F+R (10 µM) | 0,1 |
| RlCA18 F+R (10 µM) | 0,2 |
| Rrid082A F+R (10 µM) | 0,2 |
| Rrid169A F+R (10 µM) | 0,2 |
| RlCA1b6 F+R (10 µM) | 0,2 |
| Type-it Microsatellite PCR Kit | 5 |
| Q-Solution | 2 |
| ddH2O | 0,9 |
| DNA | 1 |
|  |  |
| **Multiplex 3** | **Volume (µl)** |
| RICA5 F+R (10 µM) | 0,1 |
| Ga1a23 F+R (10 µM) | 0,1 |
| Rrid059A F+R (10 µM) | 0,1 |
| RlCA1a27 F+R (10 µM) | 0,1 |
| Rrid135A F+R (10 µM) | 0,1 |
| Pper3.22 F+R (10 µM) | 0,1 |
| Type-it Microsatellite PCR Kit | 5 |
| ddH2O | 3,4 |
| DNA | 1 |

**Table S1.** The composition of three multiplex PCR mixtures for microsatellite amplification.

**Table S2.** Species-specific loci and alleles of three representatives of the *Pelophylax esculentus* complex.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Locus** | ***P. lessonae*** | ***P. ridibundus*** | **L genome in *P. esculentus*** | **R genome in *P. esculentus*** |
| GA1A19 | 96 | 103, 107, 109, 119, 121, 125, 140, 145, 147, 149, 151, 153, 155 | 96 | 103, 107, 119, 149, 151, 155, 157, 160 |
| Re1Caga10 | - | 91, 95, 104, 108, 110, 113, 115, 117, 119, 121, 123, 131, 147 | - | 95, 104, 108, 110, 113, 115, 121 |
| RlCA 18 | 169, 171, 175, 177, 179, 181, 185, 190, 202 | - | 169, 171, 173, 175, 177, 179, 181, 185, 187, 190, 202 | - |
| RlCA2a34 | 109, 127, 132, 134, 136, 140, 142, 145, 149, 151, 159, 174 | - | 109, 111, 127, 132, 134, 136, 138, 140, 142, 145, 147, 149, 151 | - |
| Rrid169A | - | 181, 183, 185, 187, 189, 193, 199, 201, 205, 207, 208 | - | 185, 187, 189, 208 |
| RlCA1b5 | 118, 120 | 129, 131, 133 | 118, 120 | 133 |
| Rrid064A | - | 207, 221, 223 | - | 221, 230 |
| Res22 | - | 81, 89, 109, 115, 117, 123, 129, 131 | - | 109, 129 |
| Rrid082A | - | 160, 162, 164, 166, 169, 171, 175, 177, 179,188, 190 | - | 175, 177 |
| Res17 | - | 156, 168 | - | 156, 168 |
| Rrid013A | 288, 294, 297, 300 | 283, 288, 291 | 283, 288, 291, 294, 297, 300 | 283, 288 |
| RlCA1b6 | 81, 83 | 81, 85, 87, 90, 92, 94, 96, 100 | 81, 83 | 81, 85, 94, 96 |
| RlCA 5 | 234, 245, 251, 253, 257, 259, 261 | - | 234, 245, 247, 253, 257, 259, 261, 263, 267 | - |
| Ga1a23 | 117, 121, 123, 125, 127, 129, 133, 138 | - | 109, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 138, 141 | - |
| Rrid059A | 101 | 128, 131, 133, 135, 136, 137, 139, 141, 143, 146 | 101, 133 | 133, 136 |
| RlCa1a27 | 89, 101, 105, 108, 112, 114 | - | 89, 101, 105, 106, 108, 110, 112, 114, 116 | - |
| Rrid135A | - | 172, 203, 206, 207, 221, 223, 226, 241, 256 | - | 203, 207, 221 |
| Pper3.22 | - | 343, 348, 354, 357 | - | 343, 354, 357 |

Obrázok, na ktorom je text, diagram

Obsah vygenerovaný umelou inteligenciou môže byť nesprávny.

**Figure S1.** Multivariate analyses of water frogs based on microsatellite data. A. Principal component analysis (PCA) analysis, B. Principal coordinates analysis (PCoA). RR – *Pelophylax ridibundus*, LR – *P. esculentus*, LL – *P. lessonae.*

Obrázok, na ktorom je diagram, náčrt, vývoj, rad

Obsah vygenerovaný umelou inteligenciou môže byť nesprávny.

**Figure S2.** A histogram showing the frequency of *Pelophylax esculentus* individuals in a particular erythrocyte size category. Blue dashed line indicates mean value.

Obrázok, na ktorom je text, detské kresby, pestrofarebnosť, diagram

Obsah vygenerovaný umelou inteligenciou môže byť nesprávny.

**Figure S3.** Multivariate principal component analysis (PCA) analysis of water frog males (A) and females (B) based on morphometric data. RR – *Pelophylax ridibundus*, LR – *P. esculentus*, LL – *P. lessonae.*