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| **Species** | **Sequences (5’-3’)** | **Minimum number of mismatches** |
|  | **Forward primer (20bp)** |  |
| *Pseudacris maculata* | CGTAGCACACATCTGCCGTG | - |
| *Pseudacris triseriata* | TGTAGCACATATYTGCCGTG | 2 |
| *Pseudacris crucifer* | TGTRGCTCACATTTGCCGTG | 3 |
| *Dryophytes versicolor* | *NA* | *NA* |
| *Lithobates sylvaticus* | TATCGCACATATCTGYCGAG | 5 |
| *Lithobates septentrionalis* | TATTGCTCACATCTGTCGTG | 5 |
| *Lithobates catesbeianus* | TATYGCTCACATCTGCCGTG | 4 |
| *Lithobates clamitans* | TATCGCTCACATCTGCCGTG | 4 |
| *Lithobates palustris* | CATCGCCCACATTTGCCGCG | 5 |
| *Lithobates pipiens* | CATCGCCCACATTTGCCGCG | 5 |
| *Anaxyrus americanus* | *NA* | *NA* |
|  | **Reverse primer (20bp)** |  |
| *Pseudacris maculata* | CCGACGAAGGCTGTTGCTAT | **-** |
| *Pseudacris triseriata* | CCAACGAAGGCTGTTGCTAT | 1 |
| *Pseudacris crucifer* | *NA* | *NA* |
| *Dryophytes versicolor* | CCMACAAAGGCTGTGGCTAT | 3 |
| *Lithobates sylvaticus* | CCTACAAAGGCTGTWGCTAT | 2 |
| *Lithobates septentrionalis* | CCTACAAAAGCTGTAGCTAT | 4 |
| *Lithobates catesbeianus* | CCTACAAAAGCTGTAGCTAT | 4 |
| *Lithobates clamitans* | CCTACAAAAGCTGTGGCTAT | 4 |
| *Lithobates palustris* | CCGACGAAGGCTGTGGCCAT | 2 |
| *Lithobates pipiens* | CCTACRAAGGCTGTGGCTAT | 2 |
| *Anaxyrus americanus* | CCTACAAATGCAGTTGCTAT | 4 |
|  | **Probe (22bp)** |  |
| *Pseudacris maculata* | ACGCAACCTGCACGCAAACGGA | - |
| *Pseudacris triseriata* | ACGTAACCTACACGCAAACGGA | 2 |
| *Pseudacris crucifer* | RCGYAATCTYCACGCTAACGGAG | 3 |
| *Dryophytes versicolor* | *NA* | *NA* |
| *Lithobates sylvaticus* | ACGAAACCTCCACGCCAAYGGC | 4 |
| *Lithobates septentrionalis* | ACGAAACCTCCATGCCAACGGA | 4 |
| *Lithobates catesbeianus* | ACGAAATCTCCATGCCAACGGA | 5 |
| *Lithobates clamitans* | ACGAAATCTCCATGCTAACGGA | 5 |
| *Lithobates palustris* | ACGCAACCTTCATGCTAACGGC | 4 |
| *Lithobates pipiens* | ACGTAACCTCCACGCTAACGGC | 4 |
| *Anaxyrus americanus* | *NA* | *NA* |