

*Bonafide BDI™ – Pure PRG; A Novel Alternative to Ryegrass Seedling Root Fluorescence Test*

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Adventitious presence (unintended presence) of annual ryegrass in perennial ryegrass seed lots causes significant economic losses to grass seed industry. International Seed Testing Association (ISTA) recommends the usage of SRF (Seedling Root Fluorescence) and/or grow –out test procedures to estimate the levels of annual rye presence in perennial seed lots. However, both SRF and grow-out test are labor intensive and time consuming. Furthermore, the SRF test produces inaccurate results and is environmentally influenced. Increasing numbers of perennial seed lots are rejected each year due to the inaccuracy of the SRF test. Thus, there is a clear need for a better testing procedure that could meet the diagnostic needs for ryegrass in an efficacious, rapid & cost effective manner. Towards this end we have developed a high throughput quantitative PCR (Q-PCR) based diagnostic tool that effectively detects the presence of annual rye grass seed contamination in a perennial rye grass lots. The DNA test is designed using an insertion/deletion (In-Del) site in a ryegrass gene involved in regulating the vernalization response of ryegrass. This new DNA test is more sensitive, accurate and cost effective in detecting annual and intermediate type contamination in perennial ryegrass with a high sensitivity of 0.04% in a sample size of 5000 seeds. Use of larger sample sizes (12.5-fold higher compared to SRF test) provided additional accuracy in detecting the level of contamination. This method has been validated on 68 perennial, 26 annual and 14 intermediate ryegrass varieties with consistent results.