

APPENDIX S5. Sequence data quality for *rbcL*, Round 2 optimization with the KAPA3G Plant PCR Kit.

Reaction variables and PCR program	Q20 values		
	<i>Linum</i>	<i>Anethum</i>	<i>Senna</i>
1.5 mM MgCl ₂ , + PE, TD 58 to 48 (#3)	504	870	430
1.5 mM MgCl ₂ , – PE, TD 58 to 48 (#3)	817	836	791
2.0 mM MgCl ₂ , + PE, TD 58 to 48 (#3)	732	772	773
2.0 mM MgCl ₂ , – PE, TD 58 to 48 (#3)	378	854*	544
1.5 mM MgCl ₂ , + PE, TD 48 to 58 (#4)	798	799	830
1.5 mM MgCl ₂ , – PE, TD 48 to 58 (#4)	652	801*	166
2.0 mM MgCl ₂ , + PE, TD 48 to 58 (#4)	730	803	422
2.0 mM MgCl ₂ , – PE, TD 48 to 58 (#4)	716	607	663

Note: Q20 values are shown for *Linum usitatissimum*, *Anethum graveolens*, and *Senna* sp. with two

different MgCl₂ concentrations, the presence (+ PE) or absence (– PE) of Plant Enhancer, and two different touchdown programs (annealing temperature decreased from 58°C to 48°C, or increased from 48°C to 58°C over the first 11 cycles of a 40-cycle PCR program). Values in bold are considered high-quality data. Asterisks indicate trace files with minimal background noise.