Genetics of *Penstemon pachyphyllus*

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Introduction

Primers were created for the *Penstemon* genus species by Dockter et al. 2013, and tested on species: *P. cyananthus*, *P. davidsonii*, *P. dissectus*, and *P. fruticosus*. *Penstemon pachyphyllus* was confirmed with some of these primers, and untested with others. Our research included running both the confirmed and untested primers on the *P. pachyphyllus* species.

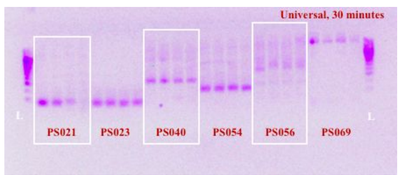
Primer testing provides insight on the genetics of *Penstemon* species, including *P. pachyphyllus*. New information on *P. pachyphyllus* can be used for further conservation efforts.

Results

The Universal PCR program showed that seven confirmed *P. pachyphyllus* and three untested *P. pachyphyllus* primers amplified the DNA.

Confirmed pachyphyllus	**Move to Gradient (✔ □) or** reason to discontinue
PS004	gel 1/2 didn't work, 1 band too high
PS012	✔ □
PS014	✔ □
PS017	✔ □
PS025	too many (high) bands
PS032	2 bands, but did not run
PS034	✔ □
PS035	✔ □
PS048	✔ □
PS075	✔ □
PS052	too many (high) bands
PS053	barely anything to see

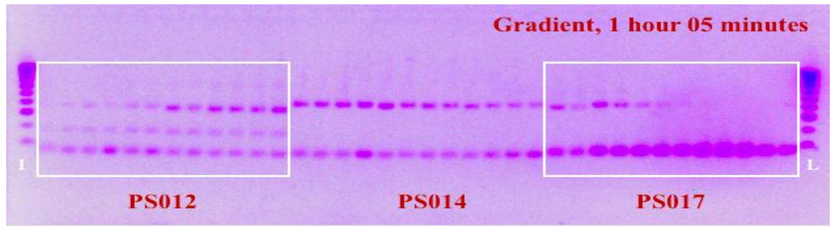
Untested pachyphyllus	**Move to Gradient (✔ □) or** reason to discontinue
PS003	smear and high
PS005	✔ □
PS021	bands too low
PS023	bands too low
PS040	✔ □
PS054	✔ □
PS056	no bands ever
PS069	too high

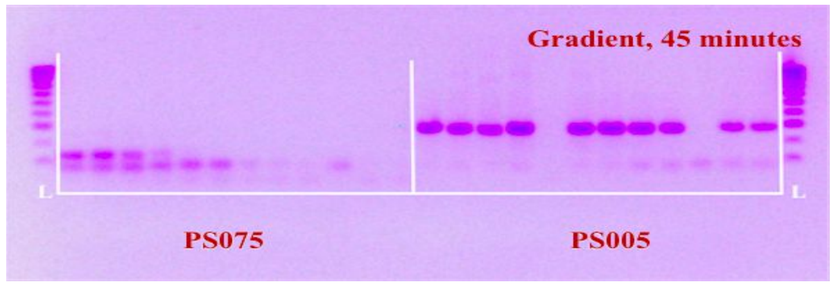


Objectives

- Run confirmed and untested primers on *P. pachyphyllus*
- Identify heterozygous primers that amplify with *P. pachyphyllus* using the Universal PCR program
- Find the best annealing temperature(s) for each primer that amplifies *P. pachyphyllus* (48-59℃) using the Gradient PCR program

Figures





PS012 - two band regions with seperate best annealing temperatures, same amplification range (53-59℃)
PS014 - band size between 400 and 500, best annealing temperature at 51-52℃
PS017 - better annealing at lower temperatures, best annealing temperature 50℃, higher temperatures did not amplify at all
PS075 - higher temperatures did not amplify at all, band size ~200
PS005 - discontinuity in temperatures amplified; best annealing temperatures 51℃, 53-55℃; thick bands bands may split apart in Beckman

Methods

DNA was systematically placed into a PCR plate and run with the confirmed and untested primers (over multiple plates) on the Universal program. The Universal program can be applied to any primer, and provides general insight as to whether the primer can amplify. Universal is not ideal for obtaining specific results, considering the low temperature (45℃) are prone to causing smearing.

The PCR products were transferred to a gel and run in an electrophoresis box at recorded intervals, to produce bands. The confirmed and untested primers that appeared heterozygous (2 bands) and/or amplified well were selected for retesting on the Gradient program.

The Gradient program runs primers at 12 temperatures (48-59℃) each. The product of the same primer at different intervals reveals best annealing temperature. All primers tested on this second set were run with the same DNA sample (RBS-1).

Conclusion

The Gradient program can give more specific amplification temperatures, band size range, and best annealing temperatures of primers than the Universal program. With these more definitive results, *P. pachyphyllus* can be tested against previous genetic DNA data of the other four (previously mentioned) *Penstemon* species. Having comparable information can provide insight to the *Penstemon* genus as a whole, allowing for broader conservation efforts.

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