

POKROČILÉ PRAKTIKUM Z BIOCHEMIE

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Úloha E: Identifikace jednotlivých druhů Václavek ze vzorku půdy

Náš vzorek: I

- Uveďte koncentraci a na základě naměřených dat zhodnoťte čistotu izolované DNA:

Nano-fotometr: $c = \underline{6,0 \text{ ng}/\mu\text{l}}$

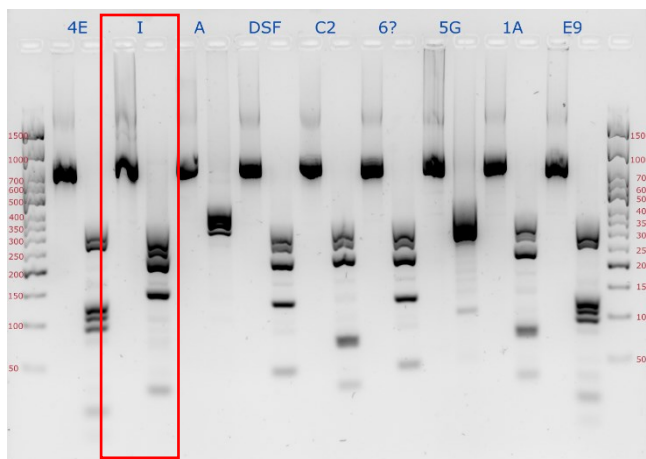
$$A_{260}/A_{280} = 2$$

DNA je nejspíš znečištěna RNA nebo organickými látkami

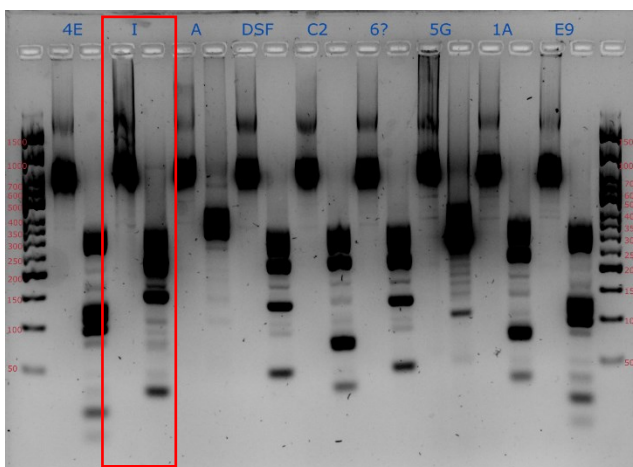
Qubit: $c = \underline{1,5 \text{ ng}/\mu\text{l}}$

Ředení vzorku 2 μl + 4 μl voda

- Na základě délky amplifikátu a výsledku restrikční analýzy určete, jaký druh václavky obsahoval váš vzorek půdy, výsledek zdůvodněte:



Jedná se nejspíše o *A. ostoyae* C2a, jelikož je na gelu vidět bandy pod 300 bp pak pod 250 bp, pod 150 pod 100 a pod 50 bp.



- Porovnejte separaci DNA fragmentů pomocí agarozové elektroforózy a Fragment Analyzeru:
- Na základě sekvencí jednotlivých druhů václavků z NCBI databáze zkuste navrhnout dvojice specifických primerů a TaqMan sondu pro detekci Vámi identifikovaného druhu václavky:
 - Gene bank: MW717692.1
 - OLIGO

	start	len	tm	gc%	any	3'	seq
LEFT PRIMER	59	20	59.87	55.00	5.00	2.00	aactctggtggaggctcgta
RIGHT PRIMER	163	20	60.92	55.00	4.00	3.00	tcggcaggaaccagctacta
HYB OLIGO	83	22	65.09	45.45	6.00	4.00	ttctgacgtgcaaatcgatcgt
 - 481 gctcctctga aatgcattag cagaaaccgc tatgcctgaa taggggaag ccagagga
 - 541 ctctggtgga ggctcgtagc gattctgacg tgcaaatcga tcgtcgaatt tgggtatagg
 - 601 ggcgaaagac taatcgaacc atctagtagc tggttctgc cgaagtttca

Primery vykazují v Primer Blast také vysokou nespecifitu, nutno obojí znovu navrhnout!

Sonda detekuje:

- Multiple organisms | 60 leaves
- Diaphora mendica genome assembly, chromosome: 17
 - Diaphora mendica genome assembly, chromosome: 3
 - Alcis repandata genome assembly, chromosome: 15
 - Alcis repandata genome assembly, chromosome: 30
 - Alcis repandata genome assembly, chromosome: W
 - Alcis repandata genome assembly, chromosome: 25
 - Alcis repandata genome assembly, chromosome: 19
 - Alcis repandata genome assembly, chromosome: 18
 - Podoscypha sp. 2 HJL-2023a isolate 140721-10 large subunit ribosomal RNA gene, partial sequence
 - Podoscypha sp. 1 HJL-2023a isolate 140719-19 large subunit ribosomal RNA gene, partial sequence
 - Podoscypha sp. 1 HJL-2023a isolate 20190729-69 large subunit ribosomal RNA gene, partial sequence
 - Podoscypha petalodes isolate 140721-15 large subunit ribosomal RNA gene, partial sequence
 - Podoscypha petalodes isolate 140721-14 large subunit ribosomal RNA gene, partial sequence
 - Thalpophila matura genome assembly, chromosome: 23
 - Conomelus anceps genome assembly, chromosome: 6
 - Cyldromonium dirinariae FA0004 gene for LSU rRNA, partial sequence
 - Cyldromonium dirinariae FA0006 gene for LSU rRNA, partial sequence
 - Cyldromonium lichenicola CBS:415.70A gene for LSU rRNA, partial sequence
 - Cyldromonium lichenicola CBS:188.70 gene for LSU rRNA, partial sequence
 - Cyldromonium dirinariae FA0005 gene for 28S rRNA, partial sequence
 - Aegus chrysomelinus SK001 gene for 28S ribosomal RNA, partial sequence
 - Dionchus sp. 104 gene for 28S rRNA, partial sequence
 - Serpula himantioides TUF12779 gene for large subunit ribosomal RNA
 - Serpula himantioides SH265 gene for large subunit ribosomal RNA
 - Serpula himantioides WD2185 gene for large subunit ribosomal RNA
 - Pratylenchus sp. D17V large subunit ribosomal RNA gene, partial sequence
 - Pratylenchus sp. 4455 large subunit ribosomal RNA gene, partial sequence
 - Pratylenchus sp. Df2 large subunit ribosomal RNA gene, partial sequence
 - Pseudosperma sp. AM-2023a voucher CAG 5/3.63 large subunit ribosomal RNA gene, partial sequence
 - Steinernema sp. NBAIRS58 large subunit ribosomal RNA gene, partial sequence
 - Orthosia incerta genome assembly, chromosome: 17
 - Lebertia obscura voucher HYDCA42 28S ribosomal RNA gene, partial sequence
 - Lebertia porosa aggr. sp. D TE-2023a voucher HYDCA75 28S ribosomal RNA gene, partial sequence
 - Lebertia porosa voucher HYDCA459 28S ribosomal RNA gene, partial sequence
 - Lebertia porosa aggr. sp. A TE-2023a voucher HYDCA269 28S ribosomal RNA gene, partial sequence
 - Lebertia porosa voucher HYDCA270 28S ribosomal RNA gene, partial sequence
 - Lebertia insignis voucher HYDCA292 28S ribosomal RNA gene, partial sequence
 - Lebertia gibbosa voucher HYDCA463 28S ribosomal RNA gene, partial sequence
 - Lebertia porosa aggr. sp. D TE-2023a voucher HYDCA10 28S ribosomal RNA gene, partial sequence
 - Pratylenchus sp. AK1 large subunit ribosomal RNA gene, partial sequence
 - |cl|Query_38561