PROTOCOL 4.

Microsatellite (SSR) analysis of DNAs isolated from Dalmatian sage herbarium samples

Things to be done before the start:

- **Dilute DNA samples down to the concentration of 1ng/µl.**
- **Dilute SSR primers down to final concentration of 10 µM**
- **Thaw 10 x PCR Buffer and dNTPs in fridge at temperature of 4 – 8 °C.**
- **Add 10 ml QX Wash Buffer to a reservoir of the QX Cartridge Stand and cover with 2 ml mineral oil.**
- **Place QIAxcel gel cartridge into the QX Cartridge Stand, remove the purge cap seal from the back of QIAxcel gel cartridge, protected QIAxcel gel cartridge with the cover and incubate for at least 20 minutes..**
- **Equilibrate QX DNA Size Marker (25 - 500 bp) and Alignment Marker (15/600 bp) to room temperature (both are stored at -20 °C).**
- **Load 15 µl QX Alignment Marker into each tube of a 12-tube strips and cover with a drop of mineral oil.**
- **Place 12-tube strips containing 15 µl of QX Alignment Marker in the MARKER 1 position of buffer tray.**
- **Fill the WP and WI positions of the buffer tray with 8 ml QX Wash Buffer and add 2 ml mineral oil.**
- **Fill the BUFFER position of the buffer tray with 18 ml QX Separation Buffer and add 4 ml mineral oil**

STEPS INVOLVED IN SSR ANALYSIS:

PART 1. PCR AMPLIFICATION OF TWO SSR LOCI
PART 2. CAPILLARY ELECTROPHORESIS USING AN QIAXCEL® ADVANCED SYSTEM
PART 3. CAPILLARY ELECTROPHORESIS USING AN ABI 3730XL® ANALYZER
PART 4. SSRs FRAGMENT SCORING USING GENEMAPPER® 4.0

PART 1. PCR AMPLIFICATION OF TWO SSR LOCI

1) DNA samples

DNA samples were isolated from: 1) fresh leaf of plant grown in Zagreb Botanical Garden, 2) leaf from Herbarium Croaticum (ZA) collected on the Island of Biševo (Cro) in 1970, 3) leaf from Herbarium Croaticum (ZA) collected near city of Šibenik (Cro) in 1995, 4) leaf from Herbarium Croaticum (ZA) collected on the Island of Krk (Cro) in 1981, 5) leaf from Herbarium Croaticum (ZA) collected on the Island of Šipan (Cro) in 1979,

2) PCR amplification

- PCR mix (fluorescently lebelled SSR primers developed by (Radosavljević et al. 2010, 2011):

36,50 µl H2O
10,00 µl 10 x PCR Buffer (TaKaRa®)
8,00 µl dNTP (TaKaRa®)
5,00 µl SSR primer SoUZ001F (FAM-5'-CGAACCGGACCAGAGTCTAA-3') 10 µM
5,00 µl SSR primer SoUZ001R (5'- CTTGCGCCATCTCTCTTCTC -3') 10 µM

5,00 µl SSR primer SoUZ019F (VIC-5'- CAAAGCTCCTCGAAGACGAA -3') 10 µM
5,00 µl SSR primer SoUZ019R (5'- CACGAGCAAGCGTAATAGCA -3') 10 µM
0,50 µl TaqHS polimerase (TaKaRa®)
75,00 µl divide in five 0.2 ml PCR tubes (15 µl of PCR mix in each tube) and add 5 µl of DNA (c = 1 ng/µl) to get final volume of 20 µl (each tube contains different DNA sample!)

- PCR profile

94 °C 5 min;
94 °C 45 sec, 60 °C 30 sec, 72 °C 90 sec;
94 °C 45 sec, 59 °C 30 sec, 72 °C 90 sec;
94 °C 45 sec, 58 °C 30 sec, 72 °C 90 sec;
94 °C 45 sec, 57 °C 30 sec, 72 °C 90 sec;
94 °C 45 sec, 56 °C 30 sec, 72 °C 90 sec;
94 °C 45 sec, 55 °C 30 sec, 72 °C 90 sec (25 cycles);
72 °C 8 min;
12 °C (forever)

- PCR primer list

Name	PCR primer sequence (5'-3')	Dye	SSR motive	Expected length (pb)
SoUZ001	F: cgaaccggaccagagtctaa R: cttgcgccatctctcttctc	FAM	$(AG)_{15}$	141 - 221
SoUZ019	F: caaagctcctcgaagacgaa R: cacgagcaagcgtaatagca	VIC	$(AGA)_{16}$	132 - 174

PART 2. CAPILLARY ELECTROPHORESIS USING AN QIAXCEL® ADVANCED SYSTEM AND QIAXCEL SCREENGEL SOFTWARE

1) Switch on the QIAxcel instrument.
2) Switch on the computer and launch the QIAxcel ScreenGel Software.
3) Install the QIAxcel gel cartridge.
4) Load the buffer tray containing the QX Aligment marker, QX Wash Buffer and QX Separation Buffer into the buffer tray holder.
5) Load the 12-tube strips (without caps) or 96-well plate containing QX DNA Size Marker (into a tube) and PCR products (in all other tubes) onto the sample tray holder.
6) Open "Process Profile" screen
   - The system automatically detects the inserted cartridge (DNA High Res.)
   - Select a process profile (Default High Res v2.0)
   - Select additional steps such as analysis and report.
7) Open "Run Parameters" tab
   - Click "Preview" and select rows to be processed, size marker position and method (OL700).
8) In "Analysis" leave default settings.
9) Open "Marker" tab
   - Specifies the DNA marker (25 - 500 bp v2.0 /20 mg per µl/) and Alignment marker (QX 15 bp – 600bp).
10) Open "Report/Export" tab
   - Specifies the report and/or export parameters according your wishes.
11) In "Sample Selection" and "Sample Information" tabs leave default settings.
12) Open "Run Check" tab

- Confirm that samples and markers have been loaded correctly
- Click "Run" to start the run.

13) When the electrophoresis is finished a report is automatically generated according to the settings in selected process profile (e.g. a pdf file)
14) Select the environment icon "Analysis".
15) Activate an experiment by right click the experiment and select "Activate" from the context menu.
16) Analyse your samples using gel image or electropherogram view
    - If this experiment was modified the system ask you whether the changes should be saved or not.
17) Click "Yes" to save or "No" to discard the changes
18) Select the environment icon "Process" and click "Load position" in the status information bar.
19) Remove QIAxcel gel cartridge from QIAxcel instrument and put it into the QX Cartridge Stand protected with the cover or in its original packaging for long term storage.
20) Remove the buffer tray from QIAxcel instrument.
21) Switch off QIAxcel instrument.

References:

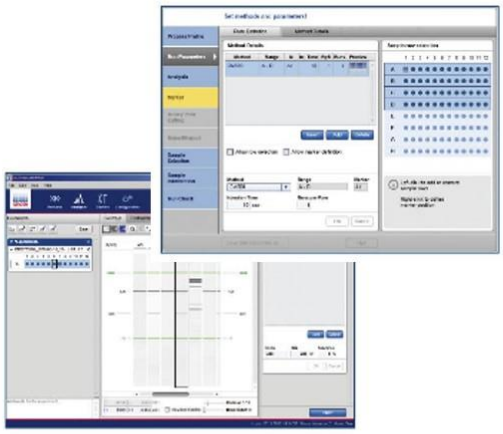
1) QIAxcel DNA Handbook
2) Radosavljević et al 2010. Isolation and characterization of polymorphic microsatellites loci from common sage (*Salvia officinalis* L.; Lamiaceae). Molecular Ecology Resources Database.
3) Radosavljevic et al. 2011. New microsatellite markers for *S officinalis* (Lamiaceae) and cross-amplification in closely related species. American Journal of Botany 98(10): e316-e318.
4) Radosavljevic et al 2012. Development of new microsatellite markers for Salvia officinalis L. and its potential use in conservation-genetic studies of narrow endemic *Salvia brachyodon* Vandas. International Journal of Molecular Sciences 13: 12082-12093.

Laboratory equipment and accessories used for SSR analysis

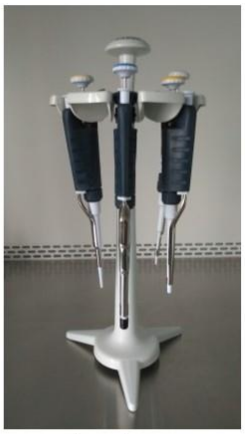
QIAxcel® Advance System

QIAxcel gel cartridge, Cartridge Stand and Chemicals

ProFlex® PCR System



QIAxcel® ScreenGel Software



Micropipette Set