# **RESEARCH NOTE**

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# DNA barcode *trnH-psbA* is a promising candidate for efficient identification of forage legumes and grasses

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# Abstract

**Objective:** Grasslands are widespread ecosystems that fulfil many functions. Plant species richness (PSR) is known to have beneficial effects on such functions and monitoring PSR is crucial for tracking the effects of land use and agricultural management on these ecosystems. Unfortunately, traditional morphology-based methods are labor-intensive and cannot be adapted for high-throughput assessments. DNA barcoding could aid increasing the throughput of PSR assessments in grasslands. In this proof-of-concept work, we aimed at determining which of three plant DNA barcodes (*rbcLa, matK* and *trnH-psbA*) best discriminates 16 key grass and legume species common in temperate sub-alpine grasslands.

**Results:** Barcode *trnH-psbA* had a 100% correct assignment rate (CAR) in the five analyzed legumes, followed by *rbcLa* (93.3%) and *matK* (55.6%). Barcode *trnH-psbA* had a 100% CAR in the grasses *Cynosurus cristatus, Dactylis glomerata* and *Trisetum flavescens*. However, the closely related *Festuca, Lolium* and *Poa* species were not always correctly identified, which led to an overall CAR in grasses of 66.7%, 50.0% and 46.4% for *trnH-psbA, matK* and *rbcLa*, respectively. Barcode *trnH-psbA* is thus the most promising candidate for PSR assessments in permanent grasslands and could greatly support plant biodiversity monitoring on a larger scale.

Keywords: DNA barcoding, Forages, Grasslands, Species richness

# Introduction

Grasslands are some of the most widespread ecosystems on Earth, covering two-fifth of its land surface [1]. They provide roughage for ruminant livestock production and many other environmental services related to carbon sequestration, water flow regulation and soil stabilization [2, 3]. Plant species richness (PSR) is a component of biodiversity with major effects on the ecosystem functioning of grasslands. In experimental grassland plant communities, high levels of PSR stabilize yields and confer tolerance against environmental stressors [4]. Similar effects have been observed in semi-natural grasslands, which

\*Correspondence: roland.koelliker@usys.ethz.ch Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Universitaetstrasse 2, 8092 Zurich, Switzerland are composed of a limited number of species and are an important component of sustainable livestock production [5]. Assessing PSR is thus crucial for tracking its changes and effects on ecosystem services. However, such assessments have traditionally relied on morphology-based surveys that are labor-intensive and require trained taxonomists, limiting their use for surveying PSR over large scales and long time periods [3]. Furthermore, grasses and legumes (the two plant families of major economic relevance in temperate grasslands) can be taxonomically assessed with highest precision only when certain distinctive morphological characters are on display (e.g., flowering bodies and leaves). Still, some grass and legume species are difficult to distinguish from closely related species. A standardized, precise, high-throughput



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solution for PSR surveys in grasslands is therefore desirable for large-scale assessments of changes in PSR.

DNA barcoding is a methodology that has been successfully applied for standardizing and increasing the throughput of PSR surveys in ecological studies [6, 7]. DNA barcodes are organellar or nuclear loci that show a high degree of species-level conservation [8, 9]. By comparing newly sequenced DNA barcodes to reference databases, it is possible to assign an unknown biological sample to its correct taxonomy. An international effort is currently in place to maintain a well-curated, public reference database of DNA barcodes (The Barcode Of Life Datasystems database, BOLD [10]).

In animals, the DNA barcode of choice is the mitochondrial *COI* gene, which can reproducibly differentiate most of the major animal phyla [8]. In plants, in contrast, there is no single DNA barcode with comparable success [11]. Most plant DNA barcodes are located in the chloroplast genome, either within coding sequences (such as *rbcLa* and *matK*) or in intergenic regions (such as *trnHpsbA*) [11, 12], although some nuclear loci have also been used as DNA barcodes, e.g., the internal transcribed spacer of the ribosomal DNA (ITS) [13]. More than one barcode per plant individual are typically sequenced and used for taxonomical assignments [11, 12]. However, sequencing more than one DNA barcode per plant may not be technically feasible in higher throughput settings, particularly when analyzing mixed-species samples.

The aim of the present study was to determine the best DNA barcode sequences for forage species by screening the BOLD database for promising candidates and sequencing three DNA barcodes (*rbcLa, matK* and *trnH*-*psbA*) from multiple cultivars of 16 forage plant species that are common in sub-alpine grasslands.

# **Main text**

# Methods

# Plant material and DNA extraction

Seeds of 2–3 cultivars of 16 forage species (Alopecurus pratensis L., Arrhenaterum elatius L., Cynosurus cristatus L., Dactylis glomerata L., Festuca pratensis Huds., F. rubra L., Lolium perenne L., L. multiflorum Lam., Lotus corniculatus L., Medicago sativa L., Phleum pratense L., Poa pratensis L., Trifolium pratense L., T. repens L. and Trisetum flavescens L.), kindly provided by Agroscope, Zurich, Switzerland were used for the study (Table 1). Seeds were germinated and transferred into pot trays (77 wells, 50 cm × 32 cm, with compost as substrate). The species selected are predominant components of sub-alpine grasslands and hold great potential for multifunctional, species-rich agriculture [14, 15]. Plants were grown for 3 weeks after which DNA was extracted from three plants per species. For grasses, three leaf

fragments of ~1 cm and for legumes three young leaflets were harvested. The plant material was freeze-dried for 48 h and pulverized in a QIAGEN TissueLyser II (QIA-GEN, Hilden, Germany). DNA was extracted using the NucleoSpin<sup>®</sup> II kit (Macherey–Nagel, Düren, Germany) and its integrity visually inspected by agarose gel electrophoresis (1% w/v). DNA purity and concentration were determined with a NanoDrop<sup>TM</sup> spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

# DNA barcode amplification and sequencing

The BOLD database was screened for DNA barcode sequences of the selected species and close relatives; barcodes rbcLa, matK and trnH-psbA were selected as candidates because they reported the most available sequences. Those DNA barcodes are mainly located in the chloroplast genome and are not known to have paralogs that can interfere with taxonomic assignments, as is the case for some nuclear loci such as ITS [13]. Primer sequences for the three barcodes were obtained from BOLD [10] and were optimized for amplification in the target plant families (Additional file 1: Table S1). Each PCR reaction consisted of 15 ng of template DNA, 1×flexi buffer (Promega, Madison, WI, USA), 2 mM MgCl<sub>2</sub>, 200 µM dNTPs, each primer at 0.4 µM, 0.75 units of GoTaq<sup>®</sup> G2 Flexi DNA Polymerase (Promega, Madison, WI, USA) and water to a final volume of 30  $\mu$ L.

For *rbcLa*, PCR conditions were 5 min at 94 °C followed by 33 cycles of 40 s at 94 °C, 1 min at 55 °C and 40 s at 72 °C, followed by a final extension cycle of 10 min at 72 °C. For *matK* and *trnH-psbA*, a 5 min at 94 °C followed by 50 cycles of 40 s at 94 °C, 1 min at 54 °C and 40 s at 72 °C followed by a final extension cycle of 10 min at 72 °C were used. The integrity of the amplicons was visually inspected by agarose gel electrophoresis (1% w/v).

Amplicons were purified in a MultiScreen PCR96 filter plate (Merck, Darmstadt, Germany). Sequencing reactions were prepared with  $1 \times$  BigDye<sup>TM</sup> Terminator 3.1 Reaction Mix (ThermoFisher Scientific, Waltham, MA, USA),  $1 \times$  BigDye<sup>TM</sup> 3.1 Sequencing Buffer, forward or reverse primer at 0.16 µM and 800 ng of purified amplicon to a final volume of 5 µL. The same primers used for PCR were used for sequencing. Capillary electrophoresis was performed on a 3130 ABI (ThermoFisher Scientific, Waltham, MA, USA). The resulting traces were quality filtered and merged using GAP4 [16] with the default settings. All traces and sequences were uploaded to BOLD v4 (project code: SWFRG; http://www.boldsystems.org/ index.php/Public\_SearchTerms).

# Taxonomical assignments

Sequences of *matK*, *rbcLa* and *trnH-psbA* were down-loaded from BOLD v4 on May 23, 2019 [10]. Only

# Table 1 Size of barcode sequences obtained for 48 plants from 16 different species of forage grasses and legumes

beta     metk     tmtpspi       SWFEQU13-19     Abpecuang promises     Seg 01     559 01 <t< th=""><th rowspan="2">BOLD Process ID</th><th rowspan="2">Species</th><th rowspan="2">Cultivar</th><th colspan="3">Sequence size in bp [number of n's in sequence]</th></t<>	BOLD Process ID	Species	Cultivar	Sequence size in bp [number of n's in sequence]		
SNFRC013-19     Alspecture prateries     Nikol (Satssucht Steinach, DE)     0*     838 [8]     0*       SNFRC023-19     Alspecture prateries     Alspect (Agroscope, C-P)     0*     661 [0]     0*       SNFRC023-19     Anhenathenum elanus     Vance (Satssucht Steinach, DE)     549 [0]     448 [0]     512 [0]       SNFRC031-19     Anhenathenum elanus     Vance (Satssucht Steinach, DE)     549 [0]     451 [0]     512 [0]     0*       SNFRC031-19     Anhenathenum elanus     Valeatri (UE Ziverke, C2)     0*     625 [0]     0*     646 [0]     531 [0]     466 [0]     544 [0]     531 [0]     466 [0]     547 [0]     549 [0]				rbcLa	matK	trnH-psbA
SVFREG0.1-10     Alspecture pretoms:     Alopset (Agencoup, C1-)     657 [0]     185 [0]     679 [0]       SVFREG0.1-10     Attenanterum elatus     Vener (Agencoup, C1-)     550 [0]     610 [0]     62       SVFREG0.1-10     Attenanterum elatus     Vener (Sastzacht Steinach, D2)     549 [0]     613 [0]     62       SVFREG0.1-10     Attenanterum elatus     Venetar (D2 Points, C2)     64 [0]     531 [0]     631 [0]     64 [0]       SVFREG0.1-10     Cheats (Agencoup, C2)     570 [0]     631 [0]     544 [0]     544 [0]       SVFREG0.1-19     Datylis glomeats     Technolds (OSPA PRO, C7)     570 [0]     631 [0]     576 [0]       SVFREG0.1-19     Datylis glomeats     Technolds (OSPA PRO, C7)     540 [0]     580 [0]     581 [0]       SVFREG0.1-19     Datylis glomeats     Technolds (Seatuc, D1     541 [0]     576 [0]       SVFREG0.1-19     Datylis glomeats     Technold (Agazaca, Seatuc, D1     541 [0]     570 [0]       SVFREG0.1-19     Featuc pratemas     Paradis (Agacacap, C1+)     581 [0]     581 [0]     581 [0]       SVFREG0.1-19     Featuc pratemas	SWFRG013-19	Alopecurus pratensis	'Alko' (Saatszucht Steinach, DE)	O <sup>a</sup>	838 [0]	0 <sup>a</sup>
SMFRE00-1-9     Anherent protein     Moper (Mappeope, CH)     55 [0]     18 [0]     56 [0]       SMFRE01-1-9     Anherentheum elatus     Medar (DF Zvotice, C2)     550 [0]     610 [0]     0 <sup>+</sup> SMFRE01-1-9     Anherentheum elatus     Medar (DF Zvotice, C2)     0 <sup>+</sup> 82 [0]     9 <sup>+</sup> SMFRE03-1-9     Cynosuus cristaus     Caral (Mappeope, C)-     541 [0]     531 [0]     640 [0]       SMFRE04-1-9     Cynosuus cristaus     Tan'otkil (MSTARD, D)     570 [0]     870 [0]     569 [0]       SMFRE04-1-9     Dacyls glomeata     Baenace' (Bachan, D)     577 [0]     640 [0]     591 [0]       SMFRE00-1-9     Dacyls glomeata     Baenace' (Bachan, D)     577 [0]     861 [3]     561 [0]       SMFRE00-1-9     Dacyls glomeata     Back' (Agroscope, CH)     532 [0]     581 [0]     551 [0]       SMFRE00-1-9     Featura patensis     Pradel (Mappoope, CH)     532 [0]     581 [0]     531 [0]       SMFRE00-1-9     Featura patensis     Pradel (Mappoope, CH)     532 [0]     581 [0]     531 [0]       SMFRE00-1-9     Featura paten     Macal (Mappoo	SWFRG014-19	Alopecurus pratensis	'Alopex' (Agroscope, CH)	0 <sup>a</sup>	663 [0]	O <sup>a</sup>
SMFRE015-19     Anternatherom elatula     Monel (Sastrouch Seinach, DE)     549(0)     46(0)     512(0)       SMFRE031-19     Anternatherom elatula     Mediar (DLF Zvottoc, CZ)     0*     825(0)     0*       SMFRE031-19     Anternatherom elatula     Mediar (DLF Zvottoc, CZ)     0*     825(0)     0*       SMFRE031-19     Opensuurs cristatus     Terarl (HBL AT)     585(0)     510(0)     564(0)       SMFRE031-19     Dacylis glementa     Benecel (Barenbrug, NL)     577(0)     60(0)     519(0)       SMFRE031-19     Dacylis glementa     Benecel (Barenbrug, NL)     577(0)     580(0)     551(0)       SMFRE031-19     Dacylis glementa     Penadisiaf (Agroscope, CH)     344(0)     888 [7]     558(0)       SMFRE031-19     Festuca protensis     Pradisiaf (Agroscope, CH)     551(0)     560(0)     551(0)       SMFRE031-19     Festuca protensis     Pradisiaf (Agroscope, CH)     588(0)     541(0)     591(0)       SMFRE031-19     Festuca nubra     Pradisiaf (Agroscope, CH)     581(0)     541(0)     551(0)       SMFRE031-19     Lalium mubillorum	SWFRG029-19	Alopecurus pratensis	'Alopex' (Agroscope, CH)	552 [0]	185 [0]	569 [0]
SMPRE01:19     Anhenatheum elatus     Mediari (DLF Zivotice, CZ)     Spice     Spice     Spice       SMPRE03:1-9     Cinesul Agroscope, CH)     541 (0     513 00     666 (0)       SMPRE045-19     Cinesulari Scientals     Cirestal Agroscope, CH)     541 (0)     513 00     666 (0)       SMPRE046-19     Cinesulari Scientals     Cirestal Agroscope, CH)     546 (0)     569 (0)     569 (0)       SMPRE046-19     Dacybis glemeeta     Baceoxid (Barcebrug, NL)     577 (0)     846 (0)     569 (0)       SMPRE041-19     Dacybis glemeeta     Redar (Agroscope, CH)     534 (0)     868 (7)     568 (0)       SMPRE041-19     Fenuca protensis     Phandiski (Agroscope, CH)     591 (0)     888 (7)     571 (1)       SMPRE041-19     Fenuca nubra     Phandiski (Agroscope, CH)     591 (0)     848 (1)     571 (1)       SMPRE041-19     Fenuca nubra     Phandiski (Agroscope, CH)     581 (0)     541 (0)     571 (1)       SMPRE041-19     Colum muthiforum     Carbur (Agroscope, CH)     581 (0)     541 (0)     551 (0)       SMPRE041-19     Colum muthiforum     Carbur (Agroscop	SWFRG015-19	Arrhenatherum elatius	'Arone' (Saatszucht Steinach, DE)	549 [0]	436 [0]	512 [0]
SMPREG03-1-9     Anhenartherum etiniza     Mediatir (Dit Zhoating, C/I)     9 <sup>4</sup> 825 (n)     9 <sup>4</sup> SMPREG03-19     Cynosurus cristatus     'Enetal (Agrascop, C/I)     541 [0]     513 [0]     544 [0]       SMPREG04-19     Cynosurus cristatus     Tendi (HgL A7)     552 [0]     670 [0]     561 [0]	SWFRG016-19	Arrhenatherum elatius	'Median' (DLF Životice, CZ)	550 [0]	610 [0]	O <sup>a</sup>
SMFRG03-19     Cynosuns criterius     Cleral (Agroscope, CH)     541 [0]     513 [0]     646 [0]       SMFRG04-19     Cynosuns criterius     Tend (MBLF, AT)     538 [0]     331 [0]     564 [0]       SMFRG04-19     Cynosuns criterius     Baccold (StarAPAC, CZ)     539 [0]     870 [0]     560 [0]       SMFRG01-19     Dacrylis glomenta     Baccold (Barcohnug, NL)     577 [0]     660 [0]     561 [0]       SMFRG01-19     Dacrylis glomenta     Bacdu (Agroscope, CH)     534 [0]     861 [3]     561 [0]       SMFRG01-19     Petruca pratensis     'Pandali (Agroscope, CH)     544 [0]     888 [3]     570 [0]       SMFRG01-19     Fetruca rubra     'Pandali (Agroscope, CH)     559 [0]     888 [3]     594 [0]       SMFRG02-19     Fetruca rubra     'Pandali (Agroscope, CH)     581 [0]     884 [0]     551 [0]       SMFRG02-19     Lalum multiforum     'Carlui (Agroscope, CH)     571 [0]     881 [0]     894 [0]     567 [7]       SMFRG02-19     Lalum multiforum     'Carlui (Agroscope, CH)     571 [0]     881 [0]     581 [0]     567 [7]       SMFRG02-1	SWFRG031-19	Arrhenatherum elatius	'Median' (DLF Životice, CZ)	O <sup>a</sup>	825 [0]	O <sup>a</sup>
SVFRG045-19     Cynoxux cristotus     Land (HLE, AT)     S45 [0]     S11 [0]     S64 [0]       SVFRG046-19     Cynoxux cristotus     Redrovskil (OSAV PR0, CZ)     579 [0]     670 [0]     569 [0]       SVFRG01-19     Dacrylls glomentat     Breneuri (Ran, FR)     546 [0]     686 [13]     576 [0]       SVFRG01-19     Dacrylls glomentat     Breneuri (Ran, FR)     544 [0]     686 [13]     576 [0]       SVFRG01-19     Fetuca pratensis     Cosmoli (Sastzuch Steinach, DE)     544 [0]     588 [0]     553 [0]       SVFRG01-19     Fetuca pratensis     Pradel (Agroscope, CH)     552 [0]     581 [0]     571 [0]       SVFRG01-19     Fetuca rabra     Pradel (Agroscope, CH)     581 [0]     581 [0]     590 [0]     531 [0]       SVFRG02-19     Fetuca rabra     Pralosias (Chrweize, CH)     581 [0]     584 [0]     591 [0]       SVFRG02-19     Lalum mutiflorum     Casix (Agroscope, CH)     577 [0]     884 [8]     567 [7]       SVFRG02-19     Lalum mutiflorum     Casix (Agroscope, CH)     577 [0]     884 [8]     591 [0]       SVFRG02-19     Lalum mutifl	SWFRG030-19	Cynosurus cristatus	'Cresta' (Agroscope, CH)	541 [0]	513 [0]	466 [0]
SVFREG06-19     Cynosura cristatus     Radrovski (OSPA PRO, C2)     529 [0]     870 [0]     507 [0]       SVFRG001-19     Dacryfis glomenta     Barexel (Barenbrug, NL)     577 [0]     640 [0]     519 [0]       SVFRG002-19     Dacryfis glomenta     Brennur (Ru, F, F)     544 [0]     866 [1]     561 [0]       SVFRG003-19     Partuca pratensis     Paradisi (Grascope, C+)     544 [0]     886 [1]     553 [0]       SVFRG001-19     Fetuca pratensis     Paradisi (Grascope, C+)     523 [0]     869 [0]     553 [0]       SVFRG001-19     Fetuca pratensis     Paradisi (Grascope, C+)     588 [0]     869 [0]     570 [0]       SVFRG003-19     Fetuca robra     Para Sola's (Schweizer, C+)     588 [0]     634 [0]     570 [0]       SVFRG003-19     Lolum multifleuum     Carbul (Agroscope, C+)     581 [0]     634 [0]     571 [0]       SVFRG003-19     Lolum multifleuum     Carbul (Agroscope, C+)     571 [0]     884 [8]     572 [0]       SVFRG003-19     Lolum multifleuum     Carbul (Agroscope, C+)     571 [0]     884 [8]     571 [0]       SVFRG003-19     Lolum multifl	SWFRG045-19	Cynosurus cristatus	'Lena' (HBLF, AT)	585 [0]	531 [0]	564 [0]
SVMFRG01-19     Dackyls glomeenta     Barescel (Barenbrug, NL)     57 (0)     64 (0)     51 (0)       SVMFRG07-19     Dackyls glomeenta     Breanu's (R2n, PLN)     534 (0)     866 (1)     56 (1)       SVMFRG07-19     Dackyls glomeenta     Bread (Agroscope, CH)     534 (0)     868 (7)     558 (0)       SVMFRG07-19     Festuca protensis     Panadel' (Agroscope, CH)     558 (0)     868 (3)     274 (21)       SVMFRG07-19     Festuca protensis     Panadel' (Agroscope, CH)     558 (0)     868 (3)     274 (21)       SVMFRG07-19     Festuca protensis     Panade' (Agroscope, CH)     588 (0)     867 (0)     571 (0)       SVMFRG07-19     Festuca rubra     Toloand' Gastascucht Steinach, DE     571 (0)     868 (3)     571 (0)       SVMFRG07-19     Lohum multiforum     Carbu' (Agroscope, CH)     571 (0)     884 (8)     677 (7)       SVMFRG07-19     Lohum multiforum     Carbu' (Agroscope, CH)     571 (0)     884 (3)     539 (5)       SVMFRG07-19     Lohum multiforum     Carbu' (Agroscope, CH)     571 (0)     884 (3)     591 (0)       SVMFRG07-19     Lohum mul	SWFRG046-19	Cynosurus cristatus	'Rožnovská' (OSEVA PRO, CZ)	529 [0]	870 [0]	569 [0]
SWFRG002-19     Dackyls glomerata     Brennus' (R2n, FR)     546 [0]     865 [13]     576 [0]       SWFRG017-19     Dackyls glomerata     Redd (Agroscope, CH)     534 [0]     866 [13]     56 [0]       SWFRG019-19     Festuca protensis     Pradel' (Agroscope, CH)     549 [0]     888 [7]     568 [0]       SWFRG019-19     Festuca protensis     Pradel' (Agroscope, CH)     559 [0]     888 [3]     274 [2]]       SWFRG0019-19     Festuca rubra     Telandel' (Satssuch's Steinach, DE)     558 [0]     543 [0]     570 [0]       SWFRG002-19     Festuca rubra     Telandel' Gatssuch's Steinach, DE)     558 [0]     543 [0]     571 [0]       SWFRG003-19     Lolum multiforum     Carbu' (Agroscope, CH)     571 [0]     584 [0]     551 [0]       SWFRG003-19     Lolum multiforum     Zater (Agroscope, CH)     571 [0]     584 [0]     551 [0]       SWFRG003-19     Lolum multiforum     Zater (Agroscope, CH)     571 [0]     584 [0]     591 [0]       SWFRG003-19     Lolum multiforum     Zater (Agroscope, CH)     581 [0]     633 [0]     614 [0]       SWFRG003-19     Lolum	SWFRG001-19	Dactylis glomerata	'Barexcel' (Barenbrug, NL)	577 [0]	640 [0]	519 [0]
SWFRG017-19     Dactylis glomerata     Reda' (Agroscope, CH)     534 [0]     866 [13]     561 [0]       SWFRG031-90     Festuca pratensis     Cosmolit (Sastaxuch Steinach, DE)     547 [0]     579 [0]     558 [0]       SWFRG031-91     Festuca pratensis     Phadolit (Agroscope, CH)     552 [0]     590 [0]     553 [0]       SWFRG031-91     Festuca rubra     Techn (DLF-Triffolum, DK)     559 [0]     886 [0]     677 [0]       SWFRG031-91     Festuca rubra     Pandolit (Schwakter, CH)     588 [0]     864 [0]     571 [0]       SWFRG031-91     Lolium rubifforum     Carbut (Agroscope, CH)     571 [0]     886 [0]     867 [7]       SWFRG031-91     Lolium rubifforum     Carbut (Agroscope, CH)     571 [0]     886 [0]     851 [0]     571 [0]       SWFRG031-91     Lolium prenne     Arrad (Agroscope, CH)     571 [0]     888 [0]     896 [0]     271 [0]       SWFRG031-91     Lolux coniculatus     Lotar (OSEVA UNL SK)     544 [0]     399 [0]     244 [0]       SWFRG031-91     Lotux coniculatus     Lotar (OSEVA UNL SK)     544 [0]     399 [0]     441 [0]	SWFRG002-19	Dactylis glomerata	'Brennus' (R2n, FR)	546 [0]	865 [13]	576 [0]
SWFRG003-19     Festuca protensis     Cosmolit' (Sastszucht Steinach, DE)     547 (0)     579 (0)     558 (0)       SWFRG019-19     Festuca protensis     Pradici's (Agroscope, CH)     552 (0)     886 (3)     274 (21)       SWFRG019-19     Festuca rubra     Pradici's (Agroscope, CH)     558 (0)     866 (3)     574 (0)       SWFRG027-19     Festuca rubra     Prah Sola's (Schwelzer, CH)     588 (0)     864 (3)     574 (0)       SWFRG021-19     Festuca rubra     Prah Sola's (Schwelzer, CH)     588 (0)     874 (0)     571 (0)       SWFRG021-19     Lolium multiflorum     Caribi' (Agroscope, CH)     571 (0)     884 (8)     551 (0)       SWFRG021-19     Lolium multiflorum     Caria' (Agroscope, CH)     571 (0)     884 (0)     533 (0)     541 (0)       SWFRG021-19     Lolium pereme     Avaria' (Agroscope, CH)     571 (0)     883 (3)     511 (0)       SWFRG021-19     Lolium pereme     Avaria' (Agroscope, CH)     582 (0)     399 (0)     294 (0)       SWFRG021-19     Lolium pereme     Avaria' (Agroscope, CH)     582 (0)     509 (0)     141 (0)       SWFRG0	SWFRG017-19	Dactylis glomerata	'Reda' (Agroscope, CH)	534 [0]	866 [13]	561 [0]
SMFRG004-19     Festuca pratensis     Prandisia' (Agroscope, CH)     549 (0)     888 [7]     566 (0)       SMFRG0019-19     Festuca rubra     Phadel' (Agroscope, CH)     552 (0)     560 (0)     533 (0)       SMFRG0019-19     Festuca rubra     Phadel' (Agroscope, CH)     558 (0)     866 (3)     274 (21)       SMFRG002-19     Lolum multiflourm     Vais' (Agroscope, CH)     558 (0)     543 (0)     551 (0)       SMFRG005-19     Lolum multiflourm     Carbia' (Agroscope, CH)     577 (0)     884 (8)     567 (7)       SMFRG005-19     Lolum multiflourm     Carbia' (Agroscope, CH)     577 (0)     883 (3)     539 (5)       SMFRG005-19     Lolum perenne     Avrai' (Agroscope, CH)     577 (0)     883 (3)     567 (7)       SMFRG005-19     Lolum perenne     Vareia' (Agroscope, CH)     577 (0)     883 (0)     561 (0)       SMFRG025-19     Lolum perenne     Vareia' (Agroscope, CH)     571 (0)     835 (0)     671 (5)       SMFRG025-19     Lolum perenne     Vareia' (Agroscope, CH)     580 (0)     350 (0)     576 (0)       SMFRG025-19     Lolur comiculatus	SWFRG003-19	Festuca pratensis	'Cosmolit' (Saatszucht Steinach, DE)	547 [0]	579 [0]	558 [0]
SMFRG019-19     Festuca nutra     "Fradel' (Agroscope, Ch)     SS2 [0]     S90 [0]     S53 [0]       SMFRG007-19     Festuca nutra     "Echo" (DLF-Trifolium, DK)     S58 [0]     869 [0]     270 [0]       SMFRG023-19     Festuca nutra     "Pandal' (Saatszucht's Steinach, DE)     S58 [0]     543 [0]     594 [0]       SMFRG021-19     Lolium multiflorum     "Axis' (Agroscope, Ch)     S18 [0]     874 [0]     S51 [0]       SMFRG01-19     Lolium multiflorum     Zeato" (Agroscope, Ch)     S71 [0]     884 [8]     S67 [7]       SMFRG01-19     Lolium multiflorum     Zeato" (Agroscope, Ch)     S71 [0]     884 [8]     S67 [15]       SMFRG021-19     Lolium perenne     'Arvella' (Agroscope, Ch)     S42 [0]     833 [0]     614 [0]       SMFRG021-19     Lolum perenne     'Arvella' (Agroscope, Ch)     S44 [0]     392 [0]     244 [0]       SMFRG031-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     S44 [0]     392 [0]     244 [0]       SMFRG032-19     Lotus comiculatus     Palor (CWR, VUR, VC, Z)     502 [0]     702 [0]     421 [0]       SMFRG032-19     Med	SWFRG004-19	Festuca pratensis	'Paradisia' (Agroscope, CH)	549 [0]	888 [7]	566 [0]
SWFRG007-19     Festuca rubra     Fecho' (DLF-Trifolium, DK)     559 [0]     886 [3]     274 [21]       SWFRG025-19     Festuca rubra     Phan Sola' (Schweizer, CH)     588 [0]     869 [0]     570 [0]       SWFRG025-19     Festuca rubra     Phalan' (Saatszucht Steinach, DE)     588 [0]     874 [0]     551 [0]       SWFRG025-19     Lolium multiflorum     'Carbu' (Agroscope, CH)     571 [0]     888 [3]     539 [5]       SWFRG025-19     Lolium perenne     'Carbu' (Agroscope, CH)     577 [0]     888 [3]     539 [5]       SWFRG025-19     Lolium perenne     'Arvella' (Agroscope, CH)     542 [0]     833 [0]     641 [0]       SWFRG025-19     Lolium perenne     'Lipresso' (Euro Grass, DE)     488 [0]     359 [0]     641 [0]       SWFRG025-19     Lotus conriculatus     'Lotar' (OSEVA UN), SK)     548 [0]     399 [0]     414 [0]       SWFRG025-19     Medicago sativa     'Catera' (Saatszuch' Steinach, DE)     526 [0]     432 [0]     438 [3]       SWFRG025-19     Medicago sativa     'Catera' (Saatszuch' Steinach, DE)     526 [0]     432 [0]     488 [3]       SWFRG025-19	SWFRG019-19	Festuca pratensis	'Pradel' (Agroscope, CH)	552 [0]	590 [0]	553 [0]
SWFRG008-19     Festuca rubra     Pran Sola' (Schweizer, CH)     588 (0)     869 (0)     570 (0)       SWFRG005-19     Lolum multiflorum     Yakis (Agroscope, CH)     581 (0)     543 (0)     551 (0)       SWFRG005-19     Lolum multiflorum     'Carbu' (Agroscope, CH)     577 (0)     884 (8)     567 (7)       SWFRG001-19     Lolum multiflorum     'Carbu' (Agroscope, CH)     571 (0)     884 (8)     531 (0)       SWFRG021-19     Lolum prenne     'Arae' (Agroscope, CH)     571 (0)     883 (3)     539 (5)       SWFRG021-19     Lolum prenne     'Arae' (Agroscope, CH)     582 (0)     481 (0)     567 (15)       SWFRG021-19     Lotus comiculatus     'Lotar' (OSEVA UN, SK)     544 (0)     399 (0)     244 (0)       SWFRG032-19     Lotus comiculatus     'Lotar' (OSEVA UN, SK)     548 (0)     390 (0)     412 (0)       SWFRG032-19     Medicago sativa     'Catera' (Satszucht Steinach, DE)     526 (0)     433 (0)     488 (3)       SWFRG032-19     Medicago sativa     'Catera' (Satszucht Steinach, DE)     526 (0)     432 (0)     481 (0)       SWFRG032-19     Medi	SWFRG007-19	Festuca rubra	'Echo' (DLF-Trifolium, DK)	559 [0]	886 [3]	274 [21]
SWFRG023-19     Festuca nubra     'Roland' (Sastszucht Steinach, DE)     558 (0)     543 (0)     594 (0)       SWFRG005-19     Lolium multiflorum     'Axid' (Agroscope, CH)     577 (0)     884 (8)     567 (7)       SWFRG01-19     Lolium multiflorum     'Zebra' (Agroscope, CH)     577 (0)     884 (8)     551 (0)       SWFRG01-19     Lolium perenne     'Arara' (Agroscope, CH)     577 (0)     883 (3)     539 (5)       SWFRG021-19     Lolium perenne     'Arara' (Agroscope, CH)     547 (0)     883 (0)     614 (0)       SWFRG021-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     544 (0)     899 (0)     241 (0)       SWFRG021-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     548 (0)     509 (0)     414 (0)       SWFRG021-19     Medicago sativa     'Lotar' (OSEVA UNI, SK)     580 (0)     410 (0)     268 (14)       SWFRG032-19     Medicago sativa     'Lotar' (CRE, VAIRY, CZ)     500 (0)     702 (0)     438 (3)       SWFRG032-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     520 (0)     571 (0)     580 (0)       SWFRG032-19     <	SWFRG008-19	Festuca rubra	'Pran Solas' (Schweizer, CH)	588 [0]	869 [0]	570 [0]
SWFRG005-19     Lolium multiflorum     'Axis' (Agroscope, CH)     581 [0]     874 [0]     551 [0]       SWFRG006-19     Lolium multiflorum     'Carlbu' (Agroscope, CH)     577 [0]     884 [8]     567 [7]       SWFRG001-19     Lolium multiflorum     'Zebra' (Agroscope, CH)     571 [0]     886 [0]     531 [0]       SWFRG010-19     Lolium perenne     'Arara' (Agroscope, CH)     582 [0]     481 [0]     597 [7]       SWFRG021-19     Lolium perenne     'Lipresso' (Euro Grass, DE)     488 [0]     835 [0]     614 [0]       SWFRG021-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     548 [0]     599 [0]     414 [0]       SWFRG031-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     580 [0]     410 [0]     268 [14]       SWFRG031-19     Medicago sativa     'Arateni's (Barenbrug, NL)     580 [0]     410 [0]     286 [0]       SWFRG031-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     526 [0]     435 [0]     244 [0]       SWFRG031-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     581 [0]     64 [1]     27 [5]       SWFRG041-19	SWFRG023-19	Festuca rubra	'Roland' (Saatszucht Steinach, DE)	558 [0]	543 [0]	594 [0]
SWFRG006-19     Lolum multiflorum     'Caribu' (Agroscope, CH)     577 [0]     884 [8]     567 [7]       SWFRG021-19     Lolum multiflorum     'Zebra' (Agroscope, CH)     571 [0]     586 [0]     551 [0]       SWFRG01-19     Lolum perenne     'Arrai' (Agroscope, CH)     547 [0]     883 [3]     539 [5]       SWFRG02-19     Lolum perenne     'Arrai' (Agroscope, CH)     582 [0]     481 [0]     567 [15]       SWFRG02-19     Lolum perenne     'Lipresso' (Euro Grass, DE)     488 [0]     835 [0]     614 [0]       SWFRG02-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     548 [0]     509 [0]     414 [0]       SWFRG039-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     548 [0]     509 [0]     412 [0]       SWFRG032-19     Medicago sativa     'Lateri' (Satershrug, NL)     580 [0]     435 [0]     438 [3]       SWFRG032-19     Medicago sativa     'Sateri' (Agroscope, CH)     550 [0]     576 [0]     287 [0]       SWFRG032-19     Medicago sativa     'Sateri' (Satesucht Steinach, DE)     526 [0]     435 [0]     448 [3]       SWFRG041-19     Onobryc	SWFRG005-19	Lolium multiflorum	'Axis' (Agroscope, CH)	581 [0]	874 [0]	551 [0]
SWFRG021-19     Lolum multiflorum     'Zebra' (Agroscope, CH)     57 (D)     586 (D)     55 (D)       SWFRG009-19     Lolum perenne     'Anari (Agroscope, CH)     547 (D)     883 (3)     539 (5)       SWFRG021-19     Lolum perenne     'Anari (Agroscope, CH)     582 (D)     481 (D)     567 (T)       SWFRG025-19     Lolum perenne     'Lipresso' (Euro Grass, DE)     488 (D)     359 (D)     414 (D)       SWFRG024-19     Lotus corniculatus     'Lotar' (OSEVA UNL SK)     544 (D)     399 (D)     294 (D)       SWFRG023-19     Lotus corniculatus     'Lotar' (OSEVA UNL SK)     548 (D)     509 (D)     414 (D)       SWFRG032-19     Medicago sativa     'Catera' (Saatracuch' Steinach, DE)     526 (D)     435 (D)     488 [3]       SWFRG032-19     Medicago sativa     'Catera' (Saatracuch' Steinach, DE)     526 (D)     432 (D)     445 [2]       SWFRG032-19     Onobrychis viciifolia     'Perdix '(Agroscope, CH)     582 (D)     627 (D)     284 (D)       SWFRG032-19     Onobrychis viciifolia     'Perdix '(Agroscope, CL)     543 (D)     694 (D)     287 (D)       SWFRG042-19 <td>SWFRG006-19</td> <td>Lolium multiflorum</td> <td>'Caribu' (Agroscope, CH)</td> <td>577 [0]</td> <td>884 [8]</td> <td>567 [7]</td>	SWFRG006-19	Lolium multiflorum	'Caribu' (Agroscope, CH)	577 [0]	884 [8]	567 [7]
SWFRG009-19     Lolium perenne     'Arara' (Agroscope, CH)     547 (0)     888 [3]     539 [5]       SWFRG010-19     Lolium perenne     'Arvella' (Agroscope, CH)     582 (0)     481 (0)     567 [15]       SWFRG025-19     Lolium perenne     'Lipresso' (Euro Grass, DE)     488 (0)     835 (0)     614 (0)       SWFRG039-19     Lotus corniculatus     'Lotar' (OSEVA UNI, SK)     544 (0)     399 (0)     294 (0)       SWFRG039-19     Lotus corniculatus     'Lotar' (OSEVA UNI, SK)     548 (0)     500 (0)     414 (0)       SWFRG037-19     Medicago sativa     'Artemis' (Barenbrug, NL)     580 (0)     410 (0)     268 (14)       SWFRG038-19     Medicago sativa     'Sandtit' (Barenbrug, NL)     580 (0)     435 (0)     438 (3)       SWFRG032-19     Medicago sativa     'Sandtit' (Agroscope, CH)     550 (0)     576 (0)     289 (0)       SWFRG032-19     Onobrychis vicifolia     'Perly' (Agroscope, CH)     582 (0)     694 (10)     287 (0)       SWFRG041-19     Onobrychis vicifolia     'Verly' (Agroscope, CH)     584 (0)     544 (0)     544 (0)       SWFRG032-19	SWFRG021-19	Lolium multiflorum	'Zebra' (Agroscope, CH)	571 [0]	586 [0]	551 [0]
SWFRG010-19     Lolium perenne     'Arvella' (Agroscope, CH)     582 (0)     481 (0)     567 (15)       SWFRG022-19     Lolium perenne     'Lipresso' (Euro Grass, DE)     488 (0)     835 (0)     614 (0)       SWFRG022-19     Lotus comiculatus     'Lotar' (OSEVA UN, SK)     544 (0)     399 (0)     294 (0)       SWFRG040-19     Lotus comiculatus     'Lotar' (OSEVA UN, SK)     548 (0)     509 (0)     414 (0)       SWFRG040-19     Lotus comiculatus     'Lotar' (OSEVA UN, SK)     548 (0)     399 (0)     432 (0)       SWFRG037-19     Medicago sativa     'Artemis' (Barenbrug, NL)     580 (0)     410 (0)     268 [14]       SWFRG038-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     526 (0)     435 (0)     438 (3)       SWFRG043-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     580 (0)     627 (0)     284 (0)       SWFRG044-19     Onobrychis viciifolia     'Višňovský' (Agrogen, CZ)     543 (0)     694 (10)     287 (0)       SWFRG044-19     Onobrychis viciifolia     'Višňovský' (Agrogen, CZ)     543 (0)     64 (0)     548 (0)       SWFRG044	SWFRG009-19	Lolium perenne	'Arara' (Agroscope, CH)	547 [0]	883 [3]	539 [5]
SWFRG025-19     Lolium prenne     'Lipresso' (Euro Grass, DE)     488 [0]     835 [0]     614 [0]       SWFRG024-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     544 [0]     399 [0]     294 [0]       SWFRG039-19     Lotus comiculatus     'Lotar' (OSEVA UNI, SK)     548 [0]     509 [0]     414 [0]       SWFRG022-19     Medicago sativa     'Lotar' (OSEVA UNI, SK)     548 [0]     509 [0]     412 [0]       SWFRG022-19     Medicago sativa     'Catera' (Sastzuch' Steinach, DE)     526 [0]     435 [0]     438 [3]       SWFRG038-19     Medicago sativa     'Sanditi' (Barenbrug, NL)     548 [0]     432 [0]     445 [2]       SWFRG038-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG04-19     Onobrychis viciifolia     'Veñovský' (Agrogen, CZ)     543 [0]     694 [10]     287 [0]       SWFRG04-19     Phleum pratense     'Tiller' (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG01-19     Pha pratensis     'Likoli' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFRG02-19     <	SWFRG010-19	Lolium perenne	'Arvella' (Agroscope, CH)	582 [0]	481 [0]	567 [15]
SWFRG024-19     Lotus corniculatus     'Lotar' (OSEVA UNI, SK)     544 [0]     399 [0]     294 [0]       SWFRG039-19     Lotus corniculatus     'Lotar' (OSEVA UNI, SK)     548 [0]     509 [0]     414 [0]       SWFRG039-19     Lotus corniculatus     'Polom' (CVRV, VURV, CZ)     502 [0]     702 [0]     412 [0]       SWFRG037-19     Medicago sativa     'Artemis' (Barenbrug, NL)     580 [0]     410 [0]     268 [14]       SWFRG038-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     526 [0]     433 [0]     438 [3]       SWFRG038-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG032-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG047-19     Phleum pratense     'Anjo' (LVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG012-19     Pone pratensis     'Likolio' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFRG020-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG020-19     Trifolum prat	SWFRG025-19	Lolium perenne	'Lipresso' (Euro Grass, DE)	488 [0]	835 [0]	614 [0]
SWFRG039-19     Lotus coniculatus     'Lotar' (OSEVA UNI, SN)     548 [0]     509 [0]     414 [0]       SWFRG040-19     Lotus coniculatus     'Polom' (CVRV, VÜRV, CZ)     502 [0]     702 [0]     412 [0]       SWFRG022-19     Medicago sativa     'Artemis' (Barenbrug, NL)     580 [0]     410 [0]     268 [14]       SWFRG038-19     Medicago sativa     'Catera' (Saatszuch' Steinach, DE)     526 [0]     432 [0]     445 [2]       SWFRG038-19     Medicago sativa     'Sanditi' (Barenbrug, NL)     580 [0]     576 [0]     289 [0]       SWFRG038-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG043-19     Onobrychis viciifolia     'Višňovský (Agrogen, CZ)     543 [0]     694 [10]     287 [0]       SWFRG047-19     Phileum pratense     'Tile' (DLF-Tirifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG012-19     Poa pratensis     'Likolio' (DSV, DE)     470 [0]     865 [0]     576 [0]       SWFRG020-19     Tirifolum pratense     'Dipomat' (DSV, DE)     541 [0]     480 [0]     676 [0]       SWFRG03-19	SWFRG024-19	Lotus corniculatus	'Lotar' (OSEVA UNI, SK)	544 [0]	399 [0]	294 [0]
SWFRG040-19     Lotus corniculatus     'Polom' (CVRV, VÚRV, CZ)     502 [0]     702 [0]     412 [0]       SWFRG022-19     Medicago sativa     'Artemis' (Barenbrug, NL)     580 [0]     410 [0]     268 [14]       SWFRG037-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     526 [0]     435 [0]     438 [3]       SWFRG038-19     Medicago sativa     'Sanditi' (Barenbrug, NL)     548 [0]     432 [0]     445 [2]       SWFRG038-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG032-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     582 [0]     694 [10]     287 [0]       SWFRG032-19     Onobrychis viciifolia     'Perdix' (Agroscope, CL)     543 [0]     694 [10]     287 [0]       SWFRG032-19     Phleum pratense     'Anjci (LVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG047-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG011-19     Poa pratensis     'Tomory (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG027-19     P	SWFRG039-19	Lotus corniculatus	'Lotar' (OSEVA UNI, SK)	548 [0]	509 [0]	414 [0]
SWFRG022-19     Medicago sativa     'Artemis' (Barenbrug, NL)     580 [0]     410 [0]     268 [14]       SWFRG037-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     526 [0]     435 [0]     438 [3]       SWFRG038-19     Medicago sativa     'Sanditi' (Barenbrug, NL)     548 [0]     432 [0]     445 [2]       SWFRG028-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG033-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG032-19     Phleum pratense     'Anjo' (LVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG032-19     Phleum pratense     'Tille'' (DLF-Tirfolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFRG02-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG03-19     Pridoium pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG02-19     Poa pratensis	SWFRG040-19	Lotus corniculatus	'Polom' (CVRV, VÚRV, CZ)	502 [0]	702 [0]	412 [0]
SWFRG037-19     Medicago sativa     'Catera' (Saatszucht Steinach, DE)     526 [0]     435 [0]     438 [3]       SWFRG038-19     Medicago sativa     'Sanditi' (Barenbrug, NL)     548 [0]     432 [0]     445 [2]       SWFRG038-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG043-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG032-19     Phleum pratense     'Anjo' (ILVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG037-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG047-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG047-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG047-19     Poa pratensis     'Nixe' (Saatszucht Steinach, DE)     571 [0]     868 [0]     576 [0]       SWFRG027-19     Poa pratensis     'Diplomat' (DSV, DE)     64 [0]     549 [0]     0 <sup>a</sup> SWFRG035-19     Tirfolium pratense     'D	SWFRG022-19	Medicago sativa	'Artemis' (Barenbrug, NL)	580 [0]	410 [0]	268 [14]
SWFRG038-19     Medicago sativa     'Sanditi' (Barenbrug, NL)     548 [0]     432 [0]     445 [2]       SWFRG028-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG043-19     Onobrychis viciifolia     'Perdix' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG043-19     Onobrychis viciifolia     'Višňovský' (Agrogen, CZ)     543 [0]     694 [10]     287 [0]       SWFRG032-19     Phleum pratense     'Anjo' (LVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG048-19     Phleum pratense     'Tiller' (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG048-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     868 [0]     576 [0]       SWFRG022-19     Propa pratensis     'Likollo' (DSV, DE)     574 [0]     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG02-19     Trifolium pratense     'Bonu's (Selgen, CZ)     549 [0]     0 <sup>a</sup> 410 [0]     SWFRG035-19     Tifiolium ratense	SWFRG037-19	Medicago sativa	'Catera' (Saatszucht Steinach, DE)	526 [0]	435 [0]	438 [3]
SWFRG028-19     Onobrychis vicilifolia     'Perdix' (Agroscope, CH)     550 [0]     576 [0]     289 [0]       SWFRG043-19     Onobrychis vicilifolia     'Perly' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG044-19     Onobrychis vicilifolia     'Višňovský' (Agrogen, CZ)     543 [0]     694 [10]     287 [0]       SWFRG032-19     Phleum pratense     'Anjo' (ILVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG047-19     Phleum pratense     'Tirler' (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG048-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFRG027-19     Poa pratensis     'Nixe' (Saatszucht Steinach, DE)     571 [0]     868 [0]     576 [0]       SWFRG026-19     Trifolium pratense     'Diplomat' (DSV, DE)     549 [0]     0 <sup>a</sup> 410 [0]       SWFRG035-19     Trifolium retense     'Diplomat' (DSV, DE)     564 [0]     556 [0]     485 [0]       SWFRG036-19     Trifolium retense<	SWFRG038-19	Medicago sativa	'Sanditi' (Barenbrug, NL)	548 [0]	432 [0]	445 [2]
SWFRG043-19     Onobrychis viciifolia     'Perly' (Agroscope, CH)     582 [0]     627 [0]     284 [0]       SWFRG044-19     Onobrychis viciifolia     'Višňovský' (Agrogen, CZ)     543 [0]     694 [10]     287 [0]       SWFRG032-19     Phleum pratense     'Anjo' (LVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFRG047-19     Phleum pratense     'Tiller' (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG048-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     868 [0]     576 [0]       SWFRG027-19     Poa pratensis     'Nixe' (Saatszucht Steinach, DE)     571 [0]     868 [0]     576 [0]       SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG027-19     Poa pratensis     'Diplomat' (DSV, DE)     544 [0]     430 [0]     440 [0]       SWFRG026-19     Trifolium pratense     'Diplomat' (DSV, DE)     564 [0]     556 [0]     485 [0]       SWFRG026-19     Trifolium repens	SWFRG028-19	Onobrychis viciifolia	'Perdix' (Agroscope, CH)	550 [0]	576 [0]	289 [0]
SWFR6044-19     Onobychis viciifolia     'Višňovský' (Agrogen, CZ)     543 [0]     69 [10]     287 [0]       SWFR6032-19     Phleum pratense     'Anjo' (ILVO, BE)     540 [0]     0 <sup>a</sup> 0 <sup>a</sup> SWFR6047-19     Phleum pratense     'Tiller' (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFR6048-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFR6011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFR6012-19     Poa pratensis     'Nixe' (Saatszucht Steinach, DE)     571 [0]     868 [0]     576 [0]       SWFR6020-19     Trifolium pratense     'Bonus' (Selgen, CZ)     549 [0]     0 <sup>a</sup> 410 [0]       SWFR6035-19     Trifolium pratense     'Diplomat' (DSV, DE)     564 [0]     556 [0]     485 [0]       SWFR6036-19     Trifolium pratense     'Pavo' (Agroscope, CH)     514 [0]     430 [0]     448 [0]       SWFR6026-19     Trifolium repens     'Beaumont' (CW 090; Barenbrug, NL)     550 [0]     431 [0]     471 [0]       SWFR6042-19     Trifolium repens	SWFRG043-19	Onobrychis viciifolia	'Perly' (Agroscope, CH)	582 [0]	627 [0]	284 [0]
SWFR6032-19   Phleum pratense   'Anjo' (LVO, BE)   540 [0]   0 <sup>a</sup> 0 <sup>a</sup> SWFR6047-19   Phleum pratense   'Tiller' (DLF-Trifolium, DK)   576 [0]   527 [5]   584 [0]     SWFR6048-19   Phleum pratense   'Toro' (CRA-FLC, IT)   0 <sup>a</sup> 516 [1]   0 <sup>a</sup> SWFR6011-19   Poa pratensis   'Likollo' (DSV, DE)   470 [0]   865 [0]   540 [0]     SWFR6012-19   Poa pratensis   'Nixe' (Saatszucht Steinach, DE)   571 [0]   868 [0]   576 [0]     SWFR6027-19   Poa pratensis   'Tormy' (DLF-Trifolium, DK)   0 <sup>a</sup> 489 [0]   0 <sup>a</sup> SWFR6020-19   Trifolium pratense   'Bonus' (Selgen, CZ)   549 [0]   0 <sup>a</sup> 410 [0]     SWFR6035-19   Trifolium pratense   'Diplomat' (DSV, DE)   564 [0]   556 [0]   485 [0]     SWFR6036-19   Trifolium pratense   'Pavo' (Agroscope, CH)   514 [0]   430 [0]   448 [0]     SWFR6041-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   550 [0]   481 [0]   471 [0]     SWFR6042-19   Trifolium repens   'Bombus' (Agroscope, CH)   579 [0]   481 [0]   477 [0] <t< td=""><td>SWFRG044-19</td><td>Onobrychis viciifolia</td><td>'Višňovský' (Agrogen, CZ)</td><td>543 [0]</td><td>694 [10]</td><td>287 [0]</td></t<>	SWFRG044-19	Onobrychis viciifolia	'Višňovský' (Agrogen, CZ)	543 [0]	694 [10]	287 [0]
WFRG047-19     Phleum pratense     'Tiller' (DLF-Trifolium, DK)     576 [0]     527 [5]     584 [0]       SWFRG048-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFRG012-19     Poa pratensis     'Nixe' (Saatszucht Steinach, DE)     571 [0]     868 [0]     576 [0]       SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG027-19     Trifolium pratense     'Bonus' (Selgen, CZ)     549 [0]     0 <sup>a</sup> 410 [0]       SWFRG035-19     Trifolium pratense     'Diplomat' (DSV	SWFRG032-19	Phleum pratense	'Anjo' (ILVO, BE)	540 [0]	0 <sup>a</sup>	0 <sup>a</sup>
SWFRG048-19     Phleum pratense     'Toro' (CRA-FLC, IT)     0 <sup>a</sup> 516 [1]     0 <sup>a</sup> SWFRG011-19     Poa pratensis     'Likollo' (DSV, DE)     470 [0]     865 [0]     540 [0]       SWFRG012-19     Poa pratensis     'Nixe' (Saatszucht Steinach, DE)     571 [0]     868 [0]     576 [0]       SWFRG027-19     Poa pratensis     'Tormy' (DLF-Trifolium, DK)     0 <sup>a</sup> 489 [0]     0 <sup>a</sup> SWFRG020-19     Trifolium pratense     'Bonus' (Selgen, CZ)     549 [0]     0 <sup>a</sup> 410 [0]       SWFRG035-19     Trifolium pratense     'Diplomat' (DSV, DE)     564 [0]     556 [0]     485 [0]       SWFRG036-19     Trifolium pratense     'Diplomat' (DSV, DE)     564 [0]     556 [0]     488 [0]       SWFRG036-19     Trifolium repens     'Beaumont' (CW 090; Barenbrug, NL)     550 [0]     419 [0]     448 [0]       SWFRG041-19     Trifolium repens     'Bombus' (Agroscope, CH)     579 [0]     481 [0]     471 [0]       SWFRG042-19     Trifolium repens     'Bombus' (Agroscope, CH)     579 [0]     481 [0]     471 [0]       SWFRG038-19     Trisetum flavescens <td>SWFRG047-19</td> <td>, Phleum pratense</td> <td>'Tiller' (DLF-Trifolium, DK)</td> <td>576 [0]</td> <td>527 [5]</td> <td>584 [0]</td>	SWFRG047-19	, Phleum pratense	'Tiller' (DLF-Trifolium, DK)	576 [0]	527 [5]	584 [0]
SWFRG011-19   Poa pratensis   'Likollo' (DSV, DE)   470 [0]   865 [0]   540 [0]     SWFRG012-19   Poa pratensis   'Nixe' (Saatszucht Steinach, DE)   571 [0]   868 [0]   576 [0]     SWFRG027-19   Poa pratensis   'Tommy' (DLF-Trifolium, DK)   0 <sup>a</sup> 489 [0]   0 <sup>a</sup> SWFRG020-19   Trifolium pratense   'Bonus' (Selgen, CZ)   549 [0]   0 <sup>a</sup> 410 [0]     SWFRG035-19   Trifolium pratense   'Diplomat' (DSV, DE)   564 [0]   556 [0]   485 [0]     SWFRG036-19   Trifolium pratense   'Pavo' (Agroscope, CH)   514 [0]   430 [0]   448 [0]     SWFRG041-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   550 [0]   419 [0]   448 [0]     SWFRG041-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   550 [0]   481 [0]   471 [0]     SWFRG042-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   579 [0]   481 [0]   447 [0]     SWFRG042-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   570 [0]   580 [6]   570 [0]     SWFRG042-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   571 [0]	SWFRG048-19	Phleum pratense	'Toro' (CRA-FLC, IT)	0 <sup>a</sup>	516[1]	0 <sup>a</sup>
SWFRG012-19Poa pratensis'Nixe' (Saatszucht Steinach, DE)571 [0]868 [0]576 [0]SWFRG027-19Poa pratensis'Tommy' (DLF-Trifolium, DK)0a489 [0]0aSWFRG020-19Trifolium pratense'Bonus' (Selgen, CZ)549 [0]0a410 [0]SWFRG035-19Trifolium pratense'Diplomat' (DSV, DE)564 [0]556 [0]485 [0]SWFRG036-19Trifolium pratense'Pavo' (Agroscope, CH)514 [0]430 [0]496 [0]SWFRG026-19Trifolium repens'Beaumont' (CW 090; Barenbrug, NL)550 [0]419 [0]448 [0]SWFRG041-19Trifolium repens'Bombus' (Agroscope, CH)579 [0]481 [0]471 [0]SWFRG042-19Trifolium repens'Hebe' (Svalöf-Weibull, SE)571 [0]444 [0]447 [0]SWFRG033-19Trisetum flavescens'Gunther' (HBLFA, AT)504 [0]859 [6]570 [0]SWFRG033-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]886 [4]571 [0]SWFRG034-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]887 [4]568 [0]SWFRG034-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]887 [4]568 [0]SWFRG034-19Trisetum flavescens'Trisett51' (Saatszucht Steinach, DE)558 [0]887 [4]568 [0]Total sequences'Trisett51' (Saatszucht Steinach, DE)558 [0]887 [4]568 [0]	SWFRG011-19	Poa pratensis	'Likollo' (DSV, DE)	470 [0]	865 [0]	540 [0]
SWFRG027-19Poa pratensis'Tommy' (DLF-Trifolium, DK)0 <sup>a</sup> 489 [0]0 <sup>a</sup> SWFRG020-19Trifolium pratense'Bonus' (Selgen, CZ)549 [0]0 <sup>a</sup> 410 [0]SWFRG035-19Trifolium pratense'Diplomat' (DSV, DE)564 [0]556 [0]485 [0]SWFRG036-19Trifolium pratense'Pavo' (Agroscope, CH)514 [0]430 [0]496 [0]SWFRG026-19Trifolium repens'Beaumont' (CW 090; Barenbrug, NL)550 [0]411 [0]448 [0]SWFRG041-19Trifolium repens'Bombus' (Agroscope, CH)579 [0]481 [0]471 [0]SWFRG042-19Trifolium repens'Bombus' (Agroscope, CH)579 [0]481 [0]447 [0]SWFRG042-19Trifolium repens'Hebe' (Svalöf-Weibull, SE)571 [0]444 [0]447 [0]SWFRG033-19Trisetum flavescens'Gunther' (HBLFA, AT)504 [0]859 [6]570 [0]SWFRG034-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]886 [4]571 [0]SWFRG034-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]887 [4]568 [0]SWFRG034-19Trisetum flavescens'Trisett51' (Saatszucht Steinach, DE)558 [0]887 [4]568 [0]Total sequences43 (89 58%)46 (95 83%)41 (85 42%)	SWERG012-19	Poa pratensis	'Nixe' (Saatszucht Steinach, DF)	571 [0]	868 [0]	576 [0]
SWFRG020-19Trifolium pratense'Bonus' (Selgen, CZ)549 [0]0 <sup>a</sup> 410 [0]SWFRG035-19Trifolium pratense'Diplomat' (DSV, DE)564 [0]556 [0]485 [0]SWFRG036-19Trifolium pratense'Pavo' (Agroscope, CH)514 [0]430 [0]496 [0]SWFRG026-19Trifolium repens'Beaumont' (CW 090; Barenbrug, NL)550 [0]419 [0]448 [0]SWFRG041-19Trifolium repens'Bombus' (Agroscope, CH)579 [0]481 [0]471 [0]SWFRG042-19Trifolium repens'Bebe' (Svalöf-Weibull, SE)571 [0]444 [0]447 [0]SWFRG018-19Trisetum flavescens'Gunther' (HBLFA, AT)504 [0]859 [6]570 [0]SWFRG033-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]586 [4]571 [0]SWFRG034-19Trisetum flavescens'Gunther' (HBLFA, AT)575 [0]887 [4]568 [0]Total sequences'Trisett51' (Saatszucht Steinach, DE)558 [0]887 [4]568 [0]	SWFRG027-19	Poa pratensis	'Tommy' (DI F-Trifolium, DK)	0 <sup>a</sup>	489 [0]	0ª
SWFRG035-19   Trifolium pratense   'Diplomat' (DSV, DE)   564 [0]   556 [0]   485 [0]     SWFRG036-19   Trifolium pratense   'Pavo' (Agroscope, CH)   514 [0]   430 [0]   496 [0]     SWFRG026-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   550 [0]   419 [0]   448 [0]     SWFRG041-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   579 [0]   481 [0]   471 [0]     SWFRG042-19   Trifolium repens   'Bombus' (Agroscope, CH)   579 [0]   481 [0]   447 [0]     SWFRG018-19   Trifolium repens   'Bombus' (Agroscope, CH)   571 [0]   444 [0]   447 [0]     SWFRG033-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   504 [0]   859 [6]   570 [0]     SWFRG033-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   575 [0]   586 [4]   571 [0]     SWFRG034-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   575 [0]   887 [4]   568 [0]     SWFRG034-19   Trisetum flavescens   'Trisett51' (Saatszucht Steinach, DE)   558 [0]   887 [4]   568 [0]     Total sequences   43 (89.58%)   46 (95.83%)   41 (85.42%)	SWFRG020-19	Trifolium pratense	'Bonus' (Selgen, CZ)	549 [0]	0 <sup>a</sup>	410 [0]
SWFRG036-19   Trifolium pratense   'Pavo' (Agroscope, CH)   514 [0]   430 [0]   496 [0]     SWFRG026-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   550 [0]   419 [0]   448 [0]     SWFRG041-19   Trifolium repens   'Beaumont' (CW 090; Barenbrug, NL)   570 [0]   481 [0]   471 [0]     SWFRG042-19   Trifolium repens   'Bombus' (Agroscope, CH)   579 [0]   481 [0]   447 [0]     SWFRG042-19   Trifolium repens   'Hebe' (Svalöf-Weibull, SE)   571 [0]   444 [0]   447 [0]     SWFRG018-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   504 [0]   859 [6]   570 [0]     SWFRG033-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   575 [0]   586 [4]   571 [0]     SWFRG034-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   575 [0]   887 [4]   568 [0]     SWFRG034-19   Trisetum flavescens   'Trisett51' (Saatszucht Steinach, DE)   558 [0]   887 [4]   568 [0]     Total sequences   43 (89.58%)   46 (95.83%)   41 (85.42%)	SWFRG035-19	Trifolium pratense	'Diplomat' (DSV, DE)	564 [0]	556 [0]	485 [0]
SWFRG026-19     Trifolium repens     'Beaumont' (CW 090; Barenbrug, NL)     550 [0]     419 [0]     448 [0]       SWFRG041-19     Trifolium repens     'Bombus' (Agroscope, CH)     579 [0]     481 [0]     471 [0]       SWFRG042-19     Trifolium repens     'Bombus' (Agroscope, CH)     579 [0]     444 [0]     447 [0]       SWFRG042-19     Trifolium repens     'Hebe' (Svalöf-Weibull, SE)     571 [0]     444 [0]     447 [0]       SWFRG018-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     504 [0]     859 [6]     570 [0]       SWFRG033-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     586 [4]     571 [0]       SWFRG034-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     586 [4]     571 [0]       SWFRG034-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     887 [4]     568 [0]       SWFRG034-19     Trisetum flavescens     'Trisett51' (Saatszucht Steinach, DE)     558 [0]     887 [4]     568 [0]       Total sequences     43 (89.58%)     46 (95.83%)     41 (85.42%)	SWERG036-19	Trifolium pratense	'Pavo' (Agroscope, CH)	514 [0]	430 [0]	496 [0]
SWFRG041-19     Trifolium repens     'Bombus' (Agroscope, CH)     579 [0]     481 [0]     471 [0]       SWFRG042-19     Trifolium repens     'Hebe' (Svalöf-Weibull, SE)     571 [0]     444 [0]     447 [0]       SWFRG018-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     504 [0]     859 [6]     570 [0]       SWFRG033-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     586 [4]     571 [0]       SWFRG034-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     586 [4]     571 [0]       SWFRG034-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     887 [4]     568 [0]       Total sequences     'Trisett51' (Saatszucht Steinach, DE)     558 [0]     887 [4]     568 [0]	SWFRG026-19	Trifolium repens	'Beaumont' (CW 090: Barenbrug, NL)	550 [0]	419[0]	448 [0]
SWFRG042-19   Trifolium repens   'Hebe' (Svalöf-Weibull, SE)   571 [0]   444 [0]   447 [0]     SWFRG018-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   504 [0]   859 [6]   570 [0]     SWFRG033-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   575 [0]   586 [4]   571 [0]     SWFRG034-19   Trisetum flavescens   'Gunther' (HBLFA, AT)   575 [0]   588 [4]   571 [0]     SWFRG034-19   Trisetum flavescens   'Trisett51' (Saatszucht Steinach, DE)   558 [0]   887 [4]   568 [0]     Total sequences   43 (89.58%)   46 (95.83%)   41 (85.42%)	SWFRG041-19	Trifolium repens	'Bombus' (Agroscope (H)	579 [0]	481 [0]	471 [0]
SWFRG018-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     504 [0]     859 [6]     570 [0]       SWFRG033-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     586 [4]     571 [0]       SWFRG034-19     Trisetum flavescens     'Trisett51' (Saatszucht Steinach, DE)     558 [0]     887 [4]     568 [0]       Total sequences     43 (89.58%)     46 (95.83%)     41 (85.42%)	SWFRG042-19	Trifolium repens	'Hebe' (Svalöf-Weibull SE)	571 [0]	444 [0]	447 [0]
SWFRG033-19     Trisetum flavescens     'Gunther' (HBLFA, AT)     575 [0]     586 [4]     571 [0]       SWFRG034-19     Trisetum flavescens     'Trisett51' (Saatszucht Steinach, DE)     558 [0]     887 [4]     568 [0]       Total sequences     43 (89.58%)     46 (95.83%)     41 (85.42%)	SWERG018-19	Trisetum flavescens	'Gunther' (HBI FALAT)	504 [0]	859 [6]	570 [0]
SWFRG034-19     Trisetum flavescens     'Trisett51' (Saatszucht Steinach, DE)     558 [0]     887 [4]     568 [0]       Total sequences     43 (89.58%)     46 (95.83%)     41 (85.42%)	SWERG033-19	Trisetum flavescens	'Gunther' (HBLFA, AT)	575 [0]	586 [4]	571 [0]
Total sequences     43 (89.58%)     46 (95.83%)     41 (85.42%)	SWFRG034-19	Trisetum flavescens	'Trisett51' (Saatszucht Steinach DF)	558 [0]	887 [4]	568 [0]
		Total sequences		43 (89 58%)	46 (95 83%)	41 (85 42%)

<sup>a</sup> Repeatedly unsuccessful PCR

Barcode	Species-level as	Species-level assignment rate			Genus-level assignment rate		
	Overall (%)	Grasses (%)	Legumes (%)	Overall (%)	Grasses (%)	Legumes (%)	
rbcLa	62.8	46.4	93.3	69.8	53.6	100.0	
matK	51.1	48.4	57.1	73.3	61.3	100.00	
trnH-psbA	78.0	65.4	100.0	90.2	84.6	100.0	

Table 2 Species- and genus-level assignment success by barcode

sequences from the Poaceae and Fabaceae families with no contaminants and longer than 200 bp were included. In total, 6232 *rbcLa*, 11,971 *matK* and 1236 *trnH-psbA* sequences were present in the downloaded fasta files, which also include the plants from the BOLD project SWFRG (Additional file 1: Table S2). The taxonomical identifiers of the BOLD fasta files were reformatted to remove spaces and rearrange their informative fields in a consistent manner (*fasta\_name\_reformat.py* script from https://github.com/mloera/forage-barcoding).

Each barcode-specific fasta file was then used to make a blast database and the SWFRG sequences were queried in their corresponding database with blastn using the flag *outfmt*=6 (i.e., tabular format). The resulting blast output tables were parsed with the *blastn\_matcher.R* script from the above-mentioned GitHub repository. The script removes self-hits and corrects some misspellings in the taxonomy of queries and hits. The script then compares the taxonomy of the queries and hits at the species- and genuslevel. A "match" was called when the taxonomy of a query sequence is equal to the taxonomy of the highest scoring hit or hits (Additional file 1: Table S3). A "taxonomical assignment rate" for each barcode was then calculated as the ratio between the sum of its correct taxonomical assignments and the total number of query sequences.

# **Results and discussion**

# PCR and sequencing results

The primer sequences of *trnH-psbA* and *matK* were adapted to allow for amplification within the target species, while the primer sequences of *rbcLa* did not need any modification (Additional file 1: Table S1). From the 48 processed specimens, 130 sequences were obtained (46 for *matK*, 43 for *rbcLa* and 41 for *trnH-psbA-*) after repeating and optimizing failed amplifications. The size of the sequences ranged from 470 to 588 bp for *rbcLa*, 185 to 888 bp for *matK* and 268 to 614 bp for *trnH-psbA* (Table 1).

# Taxonomical assignments

Barcode *trnH-psbA* had a 100% correct assignment rate (CAR) in legumes, followed by *rbcLa* (93.3%) *matK* (57.1%; Table 2). The highest CAR for grasses was 65.4% with *trnH-psbA*, followed by *matK* (48.4%) and *rbcLa* (46.4%). Overall, genus-level CARs were 69.8%, 73.3%

and 90.2% for *rbcLa, matK* and *trnH-psbA*, respectively. Legumes had also the highest assignment rate on the genus level (100% correct assignments for all barcodes; Table 2), while correct assignments for grass genera were 53.6%, 61.3% and 84.6% for barcodes *rbcLa, matK* and *trnH-psbA*, respectively.

The low CARs for grass DNA barcodes could be due to various factors. Some grass species, such as *Poa* spp., are notoriously hard to discriminate morphologically and their phylogeny is subject to controversy [17, 18]. This could have resulted in misidentified reference sequences. Another factor is the high genetic similarity between some grass taxa. For example, the genetic similarity of some species of the *Festuca-Lolium* complex is reported to be > 90%, as calculated from transcriptomic data of orthologous genes [19]. This may result in a higher proportion of incorrect taxonomic assignments for such grass species [20].

Barcode *trnH-psbA* makes for a good candidate for large-scale DNA barcoding of forage legumes and some grasses, such as *C. cristatus*, *D. glomerata* and *T. flavescens* (Table 3). However, further work is needed to

Table 3 Species-leveltaxonomicassignmentsuccessby family, query species and barcode sequence

Family	Query species	matK	rbcLa	trnH-psbA
Poaceae	Alopecurus pratensis	0/3	0/1	0/1
	Arrhenatherum elatius	1/3	0/2	1/1
	Cynosurus cristatus	3/3	2/3	3/3
	Dactylis glomerata	3/3	2/3	3/3
	Festuca pratensis	2/3	0/3	1/3
	Festuca rubra	1/3	3/3	2/3
	Lolium multiflorum	0/3	2/3	1/3
	Lolium perenne	2/3	0/3	2/3
	Phleum pratense	1/2	1/2	1/1
	Poa pratensis	0/3	1/2	0/2
	Trisetum flavescens	2/3	2/3	3/3
Fabaceae	Lotus corniculatus	1/3	3/3	3/3
	Medicago sativa	2/3	3/3	3/3
	Onobrychis viciifolia	2/3	3/3	3/3
	Trifolium pratense	2/2	2/3	3/3
	Trifolium repens	1/3	3/3	3/3

Italics indicate 100% taxonomic assignment success

produce reference sequences in more forage species and cultivars. Overall, our results provide the basic tools to implement DNA barcoding in forage species (i.e., family-specific primer pairs and a standard bioinformatic workflow for taxonomic assignments) and can help in choosing an appropriate DNA barcode for high-throughput applications. Such high-throughput applications could greatly enhance the biodiversitymonitoring protocols that are used to study the ecology of grasslands, its dynamics and its interplay with agriculture.

# Limitations

This is exploratory work focused on the most common forage plant species from sub-alpine temperate grasslands; further work is needed to address other forage species from different kinds of grasslands.

As a proof of concept, three specimens per species were analyzed.

# Supplementary information

Supplementary information accompanies this paper at https://doi. org/10.1186/s13104-020-4897-5.

Additional file 1: Table S1. PCR primers used in this study. Table S2. Overview of the Barcoding of Life Datasystems (BOLD, [10]) reference barcode sequences used for taxonomical assignments. Table S3. Highest scoring blastn hits for the plant specimens of the BOLD project "SWFRG".

### Abbreviations

BOLD: Barcode of Life Datasystems; CAR: correct assignment rate; PSR: plant species richness.

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### Authors' contributions

RK and BS conceived the study and provided insights on experimental design and data analysis. ML did the laboratory and bioinformatic analyses. All authors read and approved the final manuscript.

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This work was funded by the Swiss Federal Office of Agriculture (FOAG). The funding body assisted in conceiving the study and in designing the experiments.

### Availability of data and materials

The datasets generated and/or analysed during the current study are available in the following GitHub repository: https://github.com/mloera/forage-barco ding. Sequencing trace files are found in BOLD (http://www.boldsystems.org/ index.php/Public\_SearchTerms) using the search term "SWFRG" and are also available on https://doi.org/10.5281/zenodo.3597069.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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