Animal &
Plant Health
Agency

# United Kingdom National List Technical Protocol for Official Examination of Distinctness, Uniformity and Stability (DUS)

## Perennial Ryegrass

## *Lolium perenne* L.

**May 2020**

## Contents

# Section A – General Information

## 1. Purpose

1.1 This protocol sets out the procedures for conducting tests and assessments in relation to official examinations of DUS, maintenance of reference stocks and verification of VCU submissions of varieties of perennial Ryegrass entered for National List (NL) trials and Plant Breeders' Rights (PBR).

## 2 Scope

2.1 These procedures apply to all varieties of perennial Ryegrass. Special procedures and responsibilities for genetically modified (GM) varieties are set out in sections A5 and A6.

2.2 Except where specified in this protocol or authorised by APHA varieties and seeds, only National List candidates, Plant Breeders' Rights candidates, candidates for foreign authorities and the reference varieties may be incorporated in the DUS tests.

## 3 Responsibilities

3.1 The growing tests and assessments in this protocol are carried out under the responsibility of the Secretary of State for Environment, Food and Rural Affairs, Scottish Ministers, the Welsh Ministers and the Minister for Agriculture and Rural Development in Northern Ireland (the National Authorities).

3.2 They are supervised, on behalf of the National Authorities, by officials of the Testing Authorities, that is, the Animal and Plant Health Agency (APHA), Scottish Government Agriculture and Rural Development Division (SGARD), the Department of Agriculture, Environment and Rural Affairs (DAERA) and the Welsh Government (WG).

3.3 This protocol is authorised by the Plant Variety and Seeds Committee (PVSC). It cannot be amended without their approval. Requests and suggestions for amendment of the protocol should be put in writing to APHA varieties and seeds, either directly or via the Test Centre.

3.4 The procedures are administered by:

Plant Varieties and Seeds
The Animal and Plant Health Agency
Eastbrook
Shaftesbury Road
Cambridge Tel No: 0208 026 5993
CB2 8DR Fax No: 0208 415 2504

3.5 Test Centre

The DUS growing tests and assessments in this protocol are co-ordinated and carried out by the:

Herbage DUS Test Centre
Agri-Food and Biosciences Institute (AFBI)
Plant Testing Station
Crossnacreevy
Belfast Tel no 02890 548000
BT6 9SH Fax no 02890 548001

3.6 The test centre is responsible for providing the appropriate facilities.

# 4 Non-compliance with the Protocol

4.1 Where the protocol uses the word "must" for any action then failure to carry out this action will result in non-compliance. Where non-compliance occurs or there are concerns regarding the validity of any data or tests this must be reported to APHA. Where this protocol uses the word "should" for any action this is the method to be followed unless there are clear reasons not to do so which can be justified by the test centre as technically sound.

# 5 Responsibility for GM Releases

5.1 GM release consent holders are responsible for gm releases. All parties involved in DUS work operating under a GM release consent must adhere to the instructions of the release consent holder where necessary, to comply with the relevant consent conditions. Where DUS protocol non-compliance occurs, this must be reported to the consent holder and the test centre who will notify APHA.

# 6 Procedures for GM Varieties

6.1 Applicants intending to enter gm candidates must consult APHA, well in advance of their application, about specific requirements under GM regulations.

6.2 The test centre must ensure that no test or trial sites are planted with GM candidates and/or varieties until APHA has given the specific clearances.

# 7 Associated Documents

The following documents are associated with this protocol

| Reference | Title |
|---|---|
| Perennial Ryegrass VCU Protocol | United Kingdom National List Trials: Protocol and Procedures for Examining the Value for Cultivation and Use (VCU). Perennial Ryegrass. |
| CPVO-TP 004/2 | Protocol for Distinctness, Uniformity and Stability tests *Lolium* ssp Ryegrass: Adopted on 19.03.2019 |
| UPOV TG/1/3 | General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonised Descriptions of New Varieties of Plants. 19.04.2002 |
| UPOV TGP/8/1 | Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability. 21.10.2010 |
| UPOV TGP/9/1 | Examining Distinctness. 11.04.2008 |
| UPOV TGP/10/1 | Examining Uniformity. 30.10.2008 |
| UPOV TC/33/7 | Combined Over-Years Criterion for Distinctness (COYD) and Uniformity (COYU). (Revision of document TC/30/4). 09.12.1997 |
| UPOV TG/4/8 | Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Perennial Ryegrass. 09.04.2008 |
| Commission Directive | 2003/90/EC as amended setting out implementing measures for the purposes of Article 7 of Council Directive 2002/53/EC (13 June 2002) as regards the characteristics to be covered as a minimum by the examination and the minimum conditions for examining certain varieties of agricultural plant species. |
| Council Regulation | 2100/94/EC of 27 July 1994 on Community Plant Variety Rights. |
| Plant Varieties and Seeds (2001) 14 1-14 | The potential for management of reference collections in herbage variety registration trials using a cyclic planting system for reference varieties |

# Section B – Application Requirements

## 1 Purpose

1.1 The purpose of this section is to identify the specific requirements for National List and Plant Breeders' Rights applications.

## 2 Scope

2.1 These procedures apply to all applications.

## 3 Responsibilities

3.1 The applicants are responsible for ensuring that these procedures are complied with.

## 4 Receipt of Applications

4.1 The latest date for receipt of applications for acceptance of a variety onto the National List or for Plant Breeders' Rights, which is set administratively by APHA, is 5 January. Applications received after this date may be considered for inclusion in the current year's tests and trials on a case by case basis.

4.2 The procedures for the submission of National List and Plant Breeders' Rights applications, technical questionnaires and for payment of administration fees are set out on the GOV web site at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/748414/pbr-fees.pdf

4.3 Applicants should notify APHA of special DUS characteristics which may require additional examinations. These claims should, in addition, be noted in the technical questionnaire accompanying the application.

## 5 Receipt of Seed

5.1 The latest date for receipt of seed is 5 February and is set administratively by APHA. Seed submissions received after this date will normally be refused. Instructions for the delivery of seed will be made available to applicants by APHA.

## 6 Seed Quality Requirements

6.1 The seed must satisfy the quality requirements for basic seed as laid down in Schedule 2 of the Seed Marketing Regulations 2011 and equivalent regulations made by Devolved Administrations.

6.2 The seed must not be chemically treated. Seed treatment, where appropriate, will be undertaken by the test centre. The chemicals applied and rates of application will be determined by the test centre.

# 7 Seed Quantity

7.1 Year 1

| | |
|---|---|
| Perennial Ryegrass - diploid | 2400 g* |
| Perennial Ryegrass - tetraploid | 3200 g" |

* comprises 1500g for DUS tests and 900g for VCU trials.

"comprises 2000g for DUS tests and 1200g for VCU trials.

The DUS and VCU seed must be supplied as one lot.

7.2 Year 2 and Further Year Submissions

A sample of 22g of diploid and 33g of tetraploid seed will be withdrawn from VCU submissions in year 2 and any further years to authenticate the submission. Applicants should refer to Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) Perennial Ryegrass for seed requirements.

7.3 Shortfall in Seed Quantities

Where sufficient seed is unavailable in the first instance a further stock should be supplied in the following year which will be authenticated against the original submission. An additional charge may be applied.

# 8 Labelling Requirements, Including Provisions for GM Varieties

8.1 Applicants **must** clearly label their seed with the following information;-

- Applicant
- Breeder's reference number or name
- Type of seed (DUS only/combined submission of DUS and VCU for year 1 sowings).
- Quantity of seed
- Whether it is a parental line.

8.2 All packages of GM material must be clearly labelled as "GMO" or "Genetically Modified Organism".

# Section C – Growing Test Procedures

## 1 Purpose

1.1 The purpose of this section is to provide details of the procedures used in the growing tests for DUS analysis.

## 2 Scope

2.1 These procedures apply to all varieties of Perennial Ryegrass.

## 3 Responsibilities

3.1 The Test Centre is responsible for conducting these procedures.

3.2 The Test Centre will be responsible for ensuring that no material supplied to them is used for any other purpose than the conduct of these procedures or the release of reference samples for authorised purposes. (See Section E7)

## 4 Reference Varieties

4.1 The principles governing the selection of reference varieties are set out in Appendix 1.

4.2 Seed of reference varieties will be supplied by the Test Centre.

## 5 Design of Tests

5.1 The Test Centre is responsible for selecting a suitable site which should be on ground that has normally not had a ryegrass seed crop in the previous five years but may be less where the risk is negligible.

5.2 Field husbandry should follow best local practice for all operations and particularly as regards cultivation, drilling, fertiliser and spray application, use of irrigation, and control of pests and diseases.

5.3 From information given in the Technical Questionnaire the candidate variety may be grown in a single spaced plant test and compared with varieties which are in the same classification for the following characters, ploidy and utilisation type – forage or amenity.

5.4 The tests are carried out using a randomised block design, with a plot of each variety present in every block as follows

| | |
|---|---|
| No. of blocks | 6 |
| No. of plants per block | 10 |
| Hence, No. of plants per variety | 60 |
| Plant spacing | 75 cm (approx) |

The plots are arranged in the order of the sowing list in the first block. The plots are fully randomised within each of the other five blocks.

5.5 Seed is sown singly under glass into multipots in March/April. After establishment, the plants are moved outdoors for hardening off and transplanted in the field in July to provide single spaced plants according to a plan produced by the Test Centre. Varieties are coded by the Test Centre.

5.6 At the end of the second and third recording years in September/October, any candidate varieties with serious distinctness problems are sown out in close comparison plots. These are row plots grown alongside relevant problem varieties for examination during the subsequent years.

5.7 For glasshouse seedling tests, a trial with thirty established plants of each variety is replicated in time by being sown at weekly intervals for six weeks, so giving 180 plants per variety in total. The plants in each trial are fully randomised.

5.8 Recordings are taken on each trial after approximately 8-12 weeks, depending upon the growth stage. Characters recorded are those agreed with the applicant.

# 6 Records and Recording

6.1 All records and plot data should be in a form determined and validated by the Test Centre.

6.2 Characters, recording details and instructions are given in Section D. Any variant and abnormal plants or plants resulting from an adverse reaction to husbandry practice are recorded but excluded from the sample.

6.3 In the first recording year, characters, as indicated in Section D5.1, are measured on all varieties and the data analysed to assess uniformity of the candidate variety and to determine the most similar reference varieties. (For details see Section G).

6.4 In the second recording year, characters, as indicated in Section D5.1, are measured on all varieties and the data analysed and, together with those from the first year, used to assess distinctness and uniformity of the candidate variety. (For details see Section G).

6.5 In the third recording year, characters, as indicated in Section D5.1 are measured on all varieties and the data analysed and, together with those from the first and second year, used to assess distinctness and uniformity of the candidate variety. (For details see Section G).

6.6 If the Test Centre notices unusual or novel characters in candidate varieties a note may be made of these at any time and a photographic record made.

# 7 Communication with the Applicant

7.1 The Test Centre will notify the applicant or his agent of any DUS problems at the earliest practical opportunity through preliminary (1 year) and interim (2 year) reports. All such notifications must be copied to APHA.

7.2 If confidentiality considerations allow, the applicant should be informed which variety is similar to his own and be invited to submit any information which may help to distinguish them.

7.3 If DUS problems arise, applicants will be invited to visit the DUS tests by arrangement so that the material can be examined and discussions held with the Test Centre.

7.4 After each recording season the results are summarised and reported to the applicant and APHA by the Test Centre.

# Section D – Summary of DUS Characteristics to be Assessed, Method of Assessment and Standards Applied

## 1 Purpose

1.1 The purpose of this section is to summarise the characteristics to be assessed.

## 2 Scope

2.1 This section summarises characteristics, states of expression, method of observation and standards required for DUS assessment.

## 3 Responsibilities

3.1 The Test Centre is responsible for co-ordinating the procedures in this summary.

## 4 Organisation

4.1 The minimum duration of tests to assess characteristics should normally be three growing periods although varieties may be determined DUS after two years of tests. Shorter periods may be applied for assessment of additional characteristics. Additional growing periods may be approved by the UK National List and Seeds Committee.

## 5 DUS Characteristics to be Assessed

5.1 Routine Characteristics

The following table summarises the DUS characteristics to be routinely examined.

Note: * denotes a characteristic which must be examined according to Commission Directive 2003/90/EC, the CPVO protocol and/or UPOV Guidelines.
G denotes a grouping characteristic.
D denotes a characteristic used in the variety description.

CPVO character numbers will be determined when the protocol is agreed by the Administrative Council.

# Perennial Ryegrass Characteristics Routinely Recorded in DUS Tests

| CPVO TP/4/2 | UPOV TG/4/8 | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference required UPOV TC33/7 | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|
| 1D | 1* | **Plant: ploidy** | Single spaced plant test (DUS plot) | 60 | TQ declaration/ laboratory assessment | 2=Diploid<br>4=Tetraploid | Ploidy difference | Observation |
| 2D | 2 | **Plant: vegetative growth habit (without vernalization)** | DUS plot | 60 | Visually scored | 1=erect<br>3=semi-erect<br>5=medium<br>7=semi-prostrate<br>9=prostrate | COYD @1% | COYU @ 0.1% |
| 3D | 5 | **Leaf: intensity of green colour (without vernalization)** | DUS plot | 60 | Visually scored | 1=very light<br>3=light<br>5=medium<br>7=dark<br>9=very dark | COYD @1% | COYU @ 0.1% |
| 4D | 6 | **Plant: width (after vernalization)** | DUS plot | 60 | Measured | 1=very narrow<br>3=narrow<br>5=medium<br>7=wide<br>9=very wide | COYD @1% | COYU @ 0.1% |
| 5D | 7 | **Plant: vegetative growth habit (after vernalization)** | DUS plot | 60 | Visually scored | 1=erect<br>3=semi-erect<br>5=medium<br>7=semi-prostrate<br>9=prostrate | COYD @1% | COYU @ 0.1% |
| 6D | 8 | **Plant: height (after vernalization)** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=tall<br>9=very tall | COYD @1% | COYU @0.1% |
| 7D | | **Leaf: intensity of green colour (after vernalization)** | DUS plot | 60 | Visually scored | 1=very light<br>3=light<br>5=medium<br>7=dark<br>9=very dark | COYD @1% | COYU @0.1% |
| 9D | 10 | **Plant: tendency to form inflorescences (without vernalization)** | DUS plot | 60 | Visually scored | 1=absent or very weak<br>3=weak<br>5=medium<br>7=strong<br>9= very strong | COYD @1% | COYU @0.1% |
| 10D | 11* | **Plant: time of inflorescence emergence (after vernalization)** | DUS plot | 60 | Visually scored, and time recorded | 1=very early<br>3=early<br>5=medium<br>7=late<br>9=very late | COYD @1% | COYU @0.1% |
| 11D | 12 | **Plant: natural height at inflorescence emergence** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=tall<br>9=very tall | COYD @1% | COYU @0.1% |

| CPVO TP/4/2 | UPOV TG/4/8 | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|
| 12D | | **Plant: habit of growth at inflorescence emergence** | DUS plot | 60 | Computer derived (UK 11/10) | 1=erect<br>3=semi-erect<br>5=medium<br>7=semi-prostrate<br>9=very prostrate | COYD @1% | COYU @0.1% |
| 13D | 14* | **Flag Leaf: length** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |
| 14D | 15* | **Flag Leaf: width** | DUS plot | 60 | Measured | 1=very narrow<br>3=narrow<br>5=medium<br>7=broad<br>9=very broad | COYD @1% | COYU @0.1% |
| 15D | 16 | **Flag leaf: length/width ratio** | DUS plot | 60 | Computer derived (UK 14/15) | 1=very low<br>3=low<br>5=medium<br>7=high<br>9=very high | COYD @1% | COYU @0.1% |
| 16D | 17* | **Plant: length of longest stem, inflorescence included(when fully expanded)** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |
| 17D | 18 | **Plant: length of upper internode** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |
| 18D | 19 | **Inflorescence: length** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |
| 19D | 20 | **Inflorescence: number of spikelets** | DUS plot | 60 | Counted | 1=very few<br>3=few<br>5=medium<br>7=many<br>9=very many | COYD @1% | COYU @0.1% |
| 20D | 21 | **Inflorescence: density** | DUS plot | 60 | Computer derived (UK 24/31) | 1=very lax<br>3=lax<br>5=medium<br>7=dense<br>9=very dense | COYD @1% | COYU @0.1% |

| CPVO TP/4/2 | UPOV TG/4/8 | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|
| 21D | 22 | **Inflorescence: length of outer glume on basal spikelet** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |
| 22D | 23 | **Inflorescence: length of basal spikelet excluding awn** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |

## 5.2 UK Approved Additional Characteristics (Non CPVO approved)

The following table summarises UK approved characteristics which have been accepted by the NLSC for DUS assessment. These characteristics can be derived from the primary characteristics without incurring additional examination costs. *NB UK Character 11 (Plant: width at inflorescence emergence) is required to allow the calculation of CPVO Character 12 (Plant: habit of growth at inflorescence emergence).*

| CPVO TP/4/2 | UPOV TG/4/8 | UK | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference required UPOV TC33/7 | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|---|
| | | 60 | **Plant: natural height (after vernalization)** | DUS plot | 60 | Measured | 1=very short<br>3=short<br>5=medium<br>7=tall<br>9=very tall | COYD @1% | COYU @0.1% |
| | 13 | 11 | **Plant: width at inflorescence emergence** | DUS plot | 60 | Measured | 1=very narrow<br>3=narrow<br>5=medium<br>7=wide<br>9=very wide | COYD @1% | COYU @0.1% |
| | | 51 | **Plant: vegetative spring development** | DUS plot | 60 | Computer derived (UK 5-60) | 1=very little<br>3=little<br>5=medium<br>7=much<br>9=very much | COYD @1% | COYU @0.1% |
| | | 52 | **Plant: vegetative growth habit** | DUS plot | 60 | Computer derived (UK 5/70) | 3=prostrate<br>5=medium<br>7=erect | COYD @1% | COYU @0.1% |
| | | 54 | **Plant: vegetative attitude in spring** | DUS plot | 60 | Computer derived (UK 5/60) | 1=very prostrate<br>3=prostrate<br>5=medium<br>7=erect<br>9=very erect | COYD @1% | COYU @0.1% |

| CPVO TP/4/2 | UPOV TG/4/8 | UK | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference required UPOV TC33/7 | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|---|
| | | 71 | **Plant: vegetative shape in spring** | DUS plot | 60 | Computer derived (UK 60/70) | 1=very compact<br>3=compact<br>5=medium<br>7=spreading<br>9=very spreading | COYD @1% | COYU @0.1% |
| | | 103 | **Plant: seasonal width** | DUS plot | 60 | Computer derived (UK √ (11*70)) | 1=very narrow<br>3=narrow<br>5=medium<br>7=wide<br>9=very wide | COYD @1% | COYU @0.1% |
| | | 39 | **Flag leaf: size** | DUS plot | 60 | Computer derived (UK √(14*15)) | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | COYD @1% | COYU @0.1% |
| | | 101 | **Inflorescence: total basal spikelet length** | DUS plot | 60 | Computer derived (UK √ (24*35)) | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long | COYD @1% | COYU @0.1% |
| | | 102 | **Inflorescence: spikelet protuberance** | DUS plot | 60 | Computer derived (UK 35-34) | 1=very little<br>3=little<br>5=medium<br>7=much<br>9=very much | COYD @1% | COYU @0.1% |
| | | 107 | **Inflorescence: glume span** | DUS plot | 60 | Computer derived (UK 35/34) | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | COYD @1% | COYU @0.1% |
| | | 108 | **Plant: volume** | DUS plot | 60 | Computer derived (UK √ (11*10)) | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | COYD @1% | COYU @0.1% |
| | | 117 | **Inflorescence: total length of spikelets** | DUS plot | 60 | Computer derived (UK 31*35) | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | COYD @1% | COYU @0.1% |
| | | 118 | **Inflorescence: total length of glumes** | DUS plot | 60 | Computer derived (UK 31*34) | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | COYD @1% | COYU @0.1% |
| | | 25D | **Inflorescence: awns** | DUS plot | 60 | Observations | 0=absent<br>1=present | For description purposes only | |

| CPVO TP/4/1 | UPOV TG/4/8 | UK | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference required UPOV TC33/7 | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|---|
| | | 124D | **Seedling: fluorescence** | Laboratory test | 400 | Observations | 0=absent<br>1=present | For description purposes only | |

## 5.3 Approved Additional Characteristics

The following table summarises the additional characteristics which have been approved by the NLSC and can be examined at the request of the applicant where necessary to establish Distinctness. A fee may be charged for examination of these characteristics as advised by APHA.

| CPVO TP/4/2 | UPOV TG/4/8 | UK | Character | Sample source (Material examined) | Number of plants or sample size for assessment | Method of assessment and recording | States of expression | D Method Minimum difference required UPOV TC33/7 | U Method UPOV UPOV TC33/7 |
|---|---|---|---|---|---|---|---|---|---|
| | | 22 | **Plant: tendency to form inflorescences in the aftermath** | DUS plot | 60 | Visually scored | 1=very little<br>3=little<br>5=medium<br>7=much<br>9=very much | COYD @1% | COYU @0.1% |
| | | 121 | **Seedling: tiller Number** | Glasshouse trial | 180 | Measured | 1=none<br>3=small<br>5=medium<br>7=large<br>9=very large | ANOVA (t-test) @1% [1] | F test @ 1%[2] |
| | | 122 | **Seedling: habit of growth** | Glasshouse trial | 180 | Visually scored | 1=prostrate<br>3=semi-prostrate<br>5=medium<br>7=semi-erect<br>9=erect | ANOVA (t-test) @1% [1] | F test @ 1%[2] |
| | | 123 | **Seedling: width of vegetative leaf** | Glasshouse trial | 180 | Measured | 1=very narrow<br>3=narrow<br>5=medium<br>7=wide<br>9=very wide | ANOVA (t-test) @1% [1] | F test @ 1%[2] |

Note
[1] Variety means are compared using trial X variety interaction as an estimate of error variance.
[2] The within trial variance of the candidate variety, averaged over trials, is compared with that of a control variety.

Electrophoresis

In *Lolium perenne* L., the composition of seed proteins can be used as an additional characteristic for establishing distinctness, uniformity and stability on request of the applicant under the following conditions:

- in case of distinctness assessment with COYD, if the difference is significant on a level between 1% and 5% for at least one of the CPVO characteristics mentioned in chapter 5.

- in case of distinctness assessment on the basis of notes, if the difference is at least 1 note in 2 out of 3 years.

Distinctness between two varieties must not be established on the basis of seed protein polymorphisms alone. If electrophoresis is used for testing of distinctness, the same population standard and the same acceptance probability as for other characteristics should be applied for the assessment of uniformity.

Electrophoresis characteristics with a lack of uniformity shall not be taken into account for the assessment of distinctness.

## 5.4 New Additional DUS Characteristics

Applicants can suggest new additional characters on the Technical Questionnaire for testing DUS or after notification by the DUS Test Centre of distinctness problems. For procedures see Section F.

# Section E – Reference Seed Stock Maintenance and VCU Seed Stock Authentication Procedures

## 1 Purpose

1.1 This section sets out the procedures for reference seed stock maintenance and VCU seed stock authentication.

## 2 Scope

2.1 These procedures apply to all reference collection varieties and VCU seed submissions where the VCU seed has not been taken from the same bulk as the seed used for the DUS test.

## 3 Responsibilities

3.1 The Test Centre is responsible for conducting these procedures.

## 4 Procedures for Reference Seed Stock Maintenance

4.1 The seed sample submitted with the successful or pending application is considered to be the definitive stock of the variety. Subject to meeting the required quality standards a small portion of the seed is sown for observations and measurements. The remainder is dried and stored under controlled and monitored refrigerated conditions as part of the official reference collection.

4.2 If during the normal tests there is any evidence that a seed stock is deteriorating in storage, or that stocks are less than 100 grams, a request should be made to the maintainer asking for a replacement stock (1500g diploid varieties; 2000g) of the variety. This replacement stock must be authenticated against the definitive reference sample. Plots will be established from any replacement reference seed sample to be authenticated and compared with the definitive stock over a maximum of two recording seasons.

4.3 If the replacement seed sample meets the required standard of no significant (P=0.01) differences in the first year of test using a within-year block by varieties analysis of variance of plot means or no significant (P=0.01) differences over two years in a COYD with MJRA analysis (see associated document UPOV TC/33/7 for details) it will be accepted as representing the variety. It may then be accepted as definitive and substituted for the existing definitive stock in the reference collection.

4.4 A replacement sample or additional replacement sample will be considered sufficiently uniform after one year of test, if the standard deviations of the primary measured characters are not significantly greater at the 1% (P=0.01) significance level than that of the mean standard deviations of the control varieties. A replacement sample or additional replacement sample will be considered sufficiently uniform after two years of test if for all primary measured characters, the combined over years uniformity (COYU) is not significantly greater at the 1% (P=0.01) significance level than that of the reference varieties.

4.5 In the event of the replacement sample not meeting the required acceptance standards, an additional replacement sample is requested. Plots will be established from any additional replacement seed sample and compared over a maximum *of* two recording seasons. If the additional replacement sample does not meet the acceptance criteria set out in 4.3, the variety will be deleted from the reference collection.

# 5 Procedures for VCU Seed Stock Authentication

5.1 Evidence will be requested from the breeder of the relationship between the VCU seed sample and the definitive DUS seed sample. Plots will be established from any VCU seed sample to be authenticated and compared visually with the definitive stock over the recording season.

5.2 The plots must be examined from establishment, through flowering to maturity.

5.3 If the new seed sample cannot be visually distinguished from the reference stock it will be accepted as representing the variety.

5.4 If the VCU seed sample can be visually distinguishable from the definitive stock in the authentication plots then it will not be accepted as representing the candidate variety.

# 6 Procedures for the Inclusion of New Common Knowledge Varieties into the Reference Collection

6.1 When a new variety enters into common knowledge such that it must be included in the reference collection, a request will be sent by the Test Centre to the Testing Authority which has added this variety to its National list for the supply of at least 50g of seed of the definitive sample. This seed will then be used to validate a larger sample of seed from the breeder. (The amount of seed requested will be 1500g for diploid varieties and 2000g for tetraploid (see B7.1.)) The standards for this validation will be as for VCU seed stock authentication (see E6).

# 7 Release of Reference Samples for Authorised Purposes

7.1 A maximum of 50g of seed of reference samples can be supplied by the Test Centre, on request, to UK, EU and UPOV DUS Testing Authorities and UK, EU and OECD Seed Certification Agencies, provided the recipient is notified in writing that this material, or any material derived from it, must not be supplied to a Third party or used for any other purpose than as a reference for official DUS testing or seed certification.

7.2 Provision of reference samples, other than in 7.1, to any other parties must be authorised by APHA.

# Section F – Procedures for Assessment of New Additional DUS Characters

## 1 Purpose

1.1 This Section sets out the procedures for assessment of new additional DUS characters for varieties of Perennial Ryegrass entered for National List trials and PBR.

## 2 Scope

2.1 These procedures apply to applications where new additional DUS characteristics which have not been approved by the NLSC are requested for use for determinations of DUS.

## 3 Responsibilities

3.1 The Test Centre is responsible for liaising with the applicant to produce a proposed procedure for the conduct of new tests. This procedure must ensure that Distinctness, Uniformity and Stability will be assessed.

3.2 All new additional characteristics must be authorised by the National List and Seeds Committee.

## 4 Reference Varieties

4.1 The reference varieties will include only those varieties from which the candidate variety is not distinct, as well as other appropriate varieties for control purposes.

4.2 Seed of reference varieties will be supplied by the Test Centre.

## 5 Procedures

5.1 Details of the proposed special test or assessments will be submitted to the NLSC to consider the feasibility of setting up a test acceptable to the UK Authorities. The applicant will be advised by APHA of arrangements and costs.

5.2 The NLSC will consider the results of the commissioned test or trial when reaching its recommendation on the granting of Plant Breeders' Rights and/or National Listing.

5.3 Where the test for a character is approved by the NLSC it should be subsequently listed in Section D5.1 or 5.2 as appropriate.

# Section G – Procedures for DUS Decisions

## 1 Purpose

1.1 This section sets out the procedures for assessing DUS decisions on varieties of Perennial Ryegrass.

## 2 Scope

2.1 These procedures apply to all varieties of Perennial Ryegrass entered for National List and Plant Breeders' Rights tests and those being tested for Foreign Authorities.

## 3 Responsibilities

3.1 The Test Centre is responsible for applying the criteria for DUS, set out in this procedure.

3.2 The Test Centre is responsible for producing the DUS reports in accordance with these procedures and for ensuring that they are in accordance with the UPOV Guidelines.

## 4 Reference Varieties

4.1 Appendix 1 sets out which varieties are considered as reference varieties for these procedures.

4.2 A system of cyclic planting of reference varieties in two years out of every three years is used, with the data for the missing year compensated for by the use of historic data from two earlier years. Please see associated document Plant Varieties and Seeds (2001) 14,1-14 for details.

## 5 Distinctness

5.1 In accordance with associated document UPOV TG1/3 varieties can be considered distinct where they have a different expression in a grouping character e.g. ploidy and utilisation type.

5.2 The standard applied for distinctness over two years of test is a significant difference at 1% (P = 0.01) significance level in at least one character in a combined over years distinctness (COYD) with Modified Joint Regression (MJRA) analysis. Please see associated document UPOV TC/33/7 for details.

5.3 The standard applied over three years of test is a significant difference at the 1% (P = 0.01) in at least one character in a combined over years distinctness (COYD) with Modified Joint Regression (MJRA) analysis. Please see associated document UPOV TC/33/7 for details.

5.4 A two-tier system is used for assessing distinctness. This determines the characteristics for which a variety must also be uniform. The varieties are examined first for distinctness using only the primary measured characters. The varieties which are not distinct are then re-examined using secondary computer-derived characteristics. A variety must be uniform in all primary measured characters. However, if a secondary computer-derived characteristic is necessary for distinctness in a variety, then the variety must also be uniform in that characteristic.

5.5 Where varieties are grown in close proximity under the same conditions, and a direct comparison can be made, observations can be made on differences not revealed by single spaced plant trials and further observations on single spaced plant trials or special tests can be undertaken.

# 6 Uniformity

6.1 Uniformity is assessed for all characteristics used to establish Distinctness.

6.2 Any outlier plants (off-types) are identified by the analysis and decisions taken by the Test Centre on whether they should be excluded or not from the calculation of variety means and standard deviations. Off-type plants in the field are identified by visual assessment and are marked for a decision on omission for recording depending upon incidence across replicates.

6.3 After the variants have been excluded, the characteristics listed in Section D5 are used to assess the uniformity of the remaining plants, according to the methods of Combined Over Years Uniformity (COYU) analysis described in associated document UPOV TC/33/7.

6.4 A variety can be considered sufficiently uniform after two years of tests if, for all primary measured characters and any secondary computer-derived characters necessary for distinctness, the combined over years uniformity (COYU) is not significantly greater than that of the reference varieties at the 1% (P=0.01) significance level. In all cases an examination of data from individual years is carried out to investigate the COYU result should this reveal potential uniformity problems.

6.5 A variety is considered sufficiently uniform after three years of tests when, for all primary measured characters and any secondary computer-derived characters required for distinctness, the combined over years uniformity (COYU) is not significantly greater than that of the reference varieties at the 0.1% (P=0.001) significance level. In all cases an examination of the data from individual years is carried out to investigate the COYU result should this reveal potential uniformity problems.

# 7 Stability

7.1 A variety is considered sufficiently stable when there is no evidence to indicate that it lacks uniformity or fails to conform to the essential characteristics of its description in different submissions or in different tests.

# 8 DUS Report and Variety Description

8.1 Upon completion of the DUS examination the DUS Summary report will be submitted to APHA by the specified date. This report will specify all non-routine characteristics for establishing distinctness.

8.2 The final DUS report, including the full variety description, will be submitted to APHA by the specified date. The characteristics to be used in the description are identified in Section D.

# Appendix 1 – Reference Collection Varieties

## 1 National Listing

1.1. The DUS reference collection, for NL purposes, for any given category of plant variety comprises the following at the time when the application for the candidate is made:

1.2 All other candidate varieties already in DUS test in the UK, or entering testing at the same time as the candidate, including those being tested for other Member States.

1.3 All varieties on the UK National List and varieties on the EC Common Catalogue whose seed is known to be certified or marketed in the UK.

1.4 Varieties nominated by the authorities concerned where tests are done for other Member States.

1.5 Any varieties nominated by the applicant as being comparable i.e. known to be similar.

1.6 Any other varieties considered to be comparable i.e. known to be similar by the appropriate Test Centre or DUS Centre Group.

## 2 Plant Breeders Rights

2.1 The DUS reference collection, for PBR purposes, for any given category of plant variety comprises the following at the time when the application for the candidate is made:

2.2 All other candidate varieties already in DUS tests in the UK, or entering DUS testing at the same time as the candidate, including those being tested for other Member States or the Community Plant Variety office (CPVO).

2.3 Varieties protected in the UK, EC or in a UPOV Member State, which are known to be similar to the candidate variety.

2.4 Other available comparable varieties in common knowledge.

# Appendix 2 – Electrophoresis

The following section contains a characteristic derived by using protein electrophoresis and a description of the method to be used.

## 1 Introduction

In *Lolium perenne* L., the composition of seed proteins can be used as additional characteristic for establishing distinctness, uniformity and stability on request of the applicant under the following conditions:

- in case of distinctness assessment with COYD, if the difference is significant on a level between 1 % and 5 % for at least one of the characteristics mentioned in SECTION 5.1,
- in case of distinctness assessment on the basis of notes, if the difference is at least 1 note in 2 out of 3 years.

Distinctness between two varieties must not be established on the basis of seed protein polymorphisms alone.

## 2 Characteristics Derived by Using Electrophoresis

The composition of seed proteins is determined by SDS-polyacrylamide-gel-electrophoresis (SDS-PAGE). The bands are numbered according to their position in the gel. For the purpose of description the bands are combined in groups. The characteristic is described for the following band ranges.

| Band range | Description of the banding pattern |
|---|---|
| O | 1 – 20 |
| P | 21 – 40 |
| R | 61 – 90 |
| U | 141 – 154 |
| V | 155 – 190 |

# 3 Description of the SDS-PAGE Method for the Detection of Seed Protein Polymorphism in Lolium Perenne

## 3.1 Number of Seeds per Test

A bulked sample of 1.5 g seed material is analysed per variety. This weight equals an amount of approximately 1000 seeds.

## 3.2 Equipment

Swing mill
Vortex-mixer
Rocking platform shaker
Table centrifuge (min. 6.000 RPM) Cryostat
Power supply with a capacity of at least 400 V and 150 mA providing constant current and constant voltage output

Any suitable vertical electrophoresis system can be used, provided that the gels can be kept at a constant temperature. A minimum running length of the gels of 10 cm is necessary. A gel thickness of no more than 1.5 mm is recommended.

## 3.3 Chemicals

All chemicals should be of "Analytical Reagent grade" or better.

### 3.3.1 Chemicals for Extraction of Seed Proteins

| **Chemical** | **Abbreviation** | **Formula** |
|---|---|---|
| Glycerol | | C3H8O3 |
| 2-Mercaptoethanol | ß-ME | C2H6OS |
| Sodium dodecyl sulphate | SDS | C12H25NaO4S |
| Hydrochloric acid | HCl | HCl |
| Tris-(hydroxymethyl)-aminomethane | Tris | C4H11NaO11S |

### 3.3.2 Chemicals for Electrophoresis

| Chemical | Abbreviation | Formula |
|---|---|---|
| 40% Acrylamide solution(w/v) | AA | $C_3H_5NO$ |
| Ammonium peroxodisulfate | APS, AP | (NH4)2S2O8 |
| 2% Bisacrylamide solution (w/v) | BIS | C7H10N2O2 |
| Bromphenol blue | | C19H10Br4O5S |
| Ethanol | EtOH | $C_2H_6O$ |
| Glycine | | C2H5NO2 |
| Dodecyl sulfate Sodium salt | SDS | C12H25NaO4S |
| Hydrochloric acid | | HCl |
| Sucrose | | C12H22O11 |
| NNN'N'Tetramethylethylenendiamine | TEMED | C6H16N2 |

### 3.3.3 Chemicals for Staining of Proteins

| Chemical | Abbreviation | Formula |
|---|---|---|
| Coomassie blue G250 | | $C_{47}H_{48}N_3O_7S2$ x Na |
| Coomassie blue R250 | | C45H44N3O7S2 x Na |
| Glacial acetic acid | | C2H4O2 |
| Glycerol | | C3H8O3 |
| Methanol | MeOH | $CH_3OH$ |
| Trichloro acetic acid | TCA | CHCl3O2 |

## 3.4 Solutions

### 3.4.1 Extraction Solutions

| No. | Solution | Ingredients | Amount | Remark |
|---|---|---|---|---|
| 3.4.1.1 | Extraction buffer | TRIS<br>Distilled water | 12.1g<br>Add 100 ml | Adjust to pH 6.8 with HCl |
| 3.4.1.2 | Extraction solution A | SDS<br>Extraction buffer (3.4.1.1)<br>Distilled water<br>Glycerol | 4.0 g<br>12.5 ml<br>24.0 ml<br>20.0 ml | Prepare daily<br>Warming to 30°C - 40°C to dissolve SDS if necessary |
| 3.4.1.3 | Extraction solution B | Extraction buffer A (3.4.1.2)<br>Distilled water | 27.0 ml<br>17.0 ml | Prepare daily |
| 3.4.1.4 | Extraction solution C | Extraction buffer B (3.4.1.3)<br>Mercaptoethanol | 22.0 ml<br>1.5 ml | Prepare daily |

## 3.4.2 **Electrophoresis Buffers and Gel Preparation Solutions**

| No. | Solution | Ingredients | Amount | Remark |
|---|---|---|---|---|
| 3.4.2.1 | Resolving gel buffer | TRIS<br>Distilled water | 75 g<br>add 1000 ml | Adjust to pH 8.9 with HCl, at 8°C stable for 4 months |
| 3.4.2.2 | Stacking gel buffer | TRIS<br>Bromphenol blue<br>Distilled water | 16 g<br>100 mg<br>add 1000 ml | Adjust to pH 6.7 with HCl, at 8°C stable for 4 months |
| 3.4.2.3 | Resolving gel preparing solution | Resolving gel buffer (3.4.2.1)<br>40 % Acrylamide solution (3.4.2.5)<br>2 % Bisacrylamide solution (3.4.2.6)<br>10 % SDS-Solution | 60 ml<br>33 ml<br>8.5 ml<br>1 ml | Prepare daily |
| 3.4.2.4 | Stacking gel preparing solution | Stacking gel buffer(3.4.2.2)<br>40% Acrylamide solution (3.4.2.5)<br>2% BIS solution (3.4.2.6)<br>Distilled water<br>Sucrose<br>10% SDS (3.4.2.8) | 280 ml<br>45 ml<br>73 ml<br>150 ml<br>80 g<br>6 ml | At 8°C stable for 1 month |
| 3.4.2.5 | Acrylamide solution | Acrylamide<br>Distilled water | 40 g<br>add 100 ml | It is strongly recommended to use a ready to use solution |
| 3.4.2.6 | BIS solution | Bisacrylamide<br>Distilled water | 2 g<br>add 100 ml | It is strongly recommended to use a ready to use solution |
| 3.4.2.7 | APS-solution | Ammoniumperoxodisulfate<br>Distilled water | 1 g add<br>50 ml | Prepare daily. |
| 3.4.2.8 | SDS-solution | SDS<br>Distilled water | 10 g<br>add 100 ml | Stable for month. |
| 3.4.2.9 | Ethanol 20% | Ethanol<br>Distilled water | 20 ml<br>add 100 ml | At room temperature stable for 6 month. |
| 3.4.2.10 | Electrophoresis buffer Stock solution | TRIS<br>SDS<br>Glycine<br>Distilled water | 103 g<br>20 g<br>70 g<br>add 1000 ml | Stable for month. |
| 3.4.2.11 | Electrophoresis buffer | Electrophoresis buffer Stock solution (3.4.2.10)<br>Distilled water | 50 ml<br>add 1000 ml | Prepare daily. |

### 3.4.3 **Staining Solutions**

| No. | Solution | Ingredients | Amount | Remark |
|---|---|---|---|---|
| 3.4.3.1 | Coomassie Blue Stock solution | Coomassie Blue G 250<br>Coomassie Blue R 250<br>Tap water | 0.25 g<br>0.75 g<br>add 100ml | Stirred for at least 1 h; Shaken very well before use |
| 3.4.3.2 | Staining solution | TCA<br>Acetic acid 80%<br>Tap water<br>Methanol<br>Stock solution (3.4.3.1) | 240 ml<br>520 ml<br>3100 ml<br>600 ml<br>90 ml | |
| 3.4.3.3 | Glycerol solution | Glycerol<br>Tap water | 50 g<br>add 1000 ml | |

## 3.5 Procedure

### 3.5.1 **Preparation of Samples**

1.5 g seeds are ground for 2 minutes by 2000 RPM with a swing mill and 3 steel balls. The grist is stored in a 5 ml glass tube.

### 13.5.2 **Extraction of Samples**

0.08 g well mixed grist is weighed in 2 ml reaction tubes and mixed with 1 ml extraction solution C (3.4.1.4) by using a vortex mixer. The samples are left for 1 hour at room temperature, suspended by using a vortex mixer and heated for 20 minutes in a water bath by a temperature of 75°C. After cooling in a water bath, the tubes are centrifuged at 10,000 x g for 10 minutes at 4°C.

15 µl of the clarified supernatant is diluted with 70 µl extraction solution B (3.4.1.3). The sample is stored frozen until SDS-PAGE.

### 3.5.3 **Preparation of Gels**

#### 3.5.3.1 **Preparation of the Gels for SDS-PAGE**

The SDS-PAGE is a discontinuous electrophoresis and each gel consists of resolving gel and stacking gel. Clean and dry gel cassettes are assembled, according to the design of the equipment used.

The resolving gel solution is composed as described in 3.4.2.3 and polymerisation is started by addition of:

100 µl TEMED
5 ml APS solution (1.3.4.2.7).

The gels are carefully poured, avoiding the formation of bubbles. The gel should be poured to a height which leaves room for a 20 mm layer of stacking gel. The gel surface is carefully overlapped with 20% ethanol solution (3.4.2.9) using a syringe. The gel polymerises at room temperature for at least one hour. When the polymerisation is finished, the ethanol solution is removed. The gel surface is rinsed with distilled water and dried with filter paper.

To make the stacking gels the following is mixed under slow stirring:

15 ml stacking gel preparing solution (3.4.2.4)
60 µl TEMED
375 µl APS-solution (3.4.2.7)

The gels are carefully poured, avoiding the formation of bubbles. The height of the stacking gel should be about 2 cm. The well-forming combs are inserted into the liquid gel. The gels are allowed to polymerize at room temperature for about 1 hour. The combs should be removed carefully out of the gel.

### 3.5.4 **Sample Loading**

The wells of the gel are carefully rinsed using electrophoresis buffer (3.4.2.11).
For separation of the seed proteins each well is filled with 5 µl extract (see 3.5.2) using a multiple syringe.

### 3.5.5 **Electrophoresis**

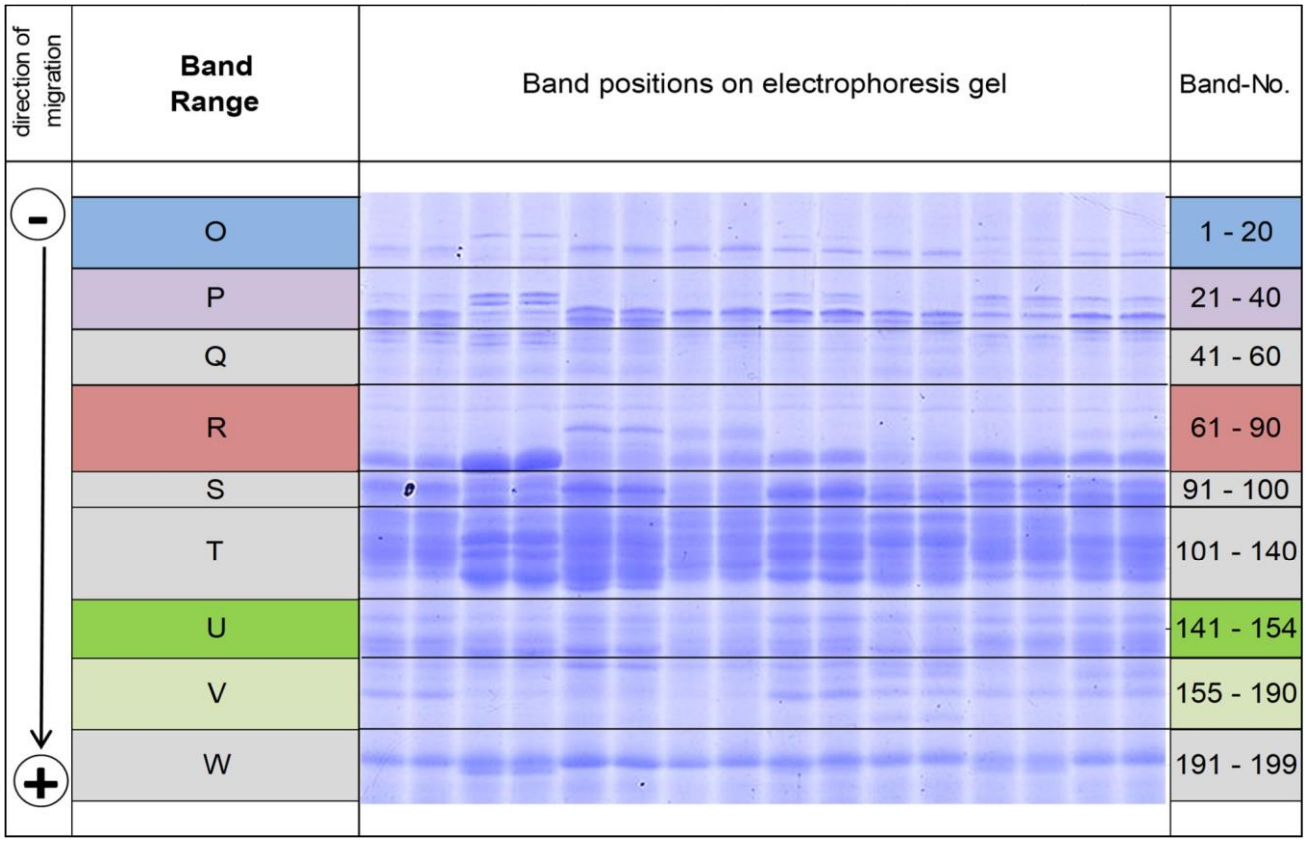
If a vertical dual slab gel instrument for example “Multigel Modell BSA” (Biometra) is used, the conditions for the electrophoresis are the following:

| | |
|---|---|
| Electrophoresis buffer: | Solution (1.3.4.2.11), fill up chamber |
| Voltage: | 120 V (for 20 minutes), then 230 V |
| Current: | 120 mA |
| Temperature: | 5°C to 15°C |
| Running distance: | when bromophenol blue runs out at the end of the gel, proceed electrophoresis for another 40 minutes before end of run |

### 3.5.6 **Staining**

2 gels from the SDS-PAGE are marked, eg by cutting the gels corner. Then the gels are transferred in a staining container filled with 300 ml staining solution (3.4.3.2) and incubated on a rocking platform shaker for 3 hours. The gels remain in the staining solution over night without shaking. For de-staining, the gels are incubated on the shaker in tap water for 2 x 30 min. Finally the gels are incubated on the shaker in 5 % glycerol solution .3.4.3.3) for 5 min. After this incubation the gels are dried between two layers of cellophane soaked in 5 % glycerol solution (3.4.3.3) at room temperature.

# 4 Interpretation of the Gels




Range Q, S, T and W are not analysed

Band range - O

| Type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bands | 4 8 12 | 8 | 8 9 | 8 12 | 4 8 10 | 4 8 | 2 8 | 2 8 12 | 2 8 14 | 8 14 | 8 18 | 8 12 16 | 4 8 10 20 | 8 10 | 6 7 | 2 8 9 | 8 20 | 6 10 | 1 6 16 | 7 8 14 | 8 16 18 | 8 10 20 | 8 10 12 | 8 14 18 | 1 8 10 14 | 7 8 9 14 | 9 10 14 | 9 10 16 |
| 1 | | | | | | | | | | | | | | | | | | | - | | | | | | -- | | | |
| 2 | | | | | | | -- | -- | -- | | | | | | | -- | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | -- | | | | -- | -- | | | | | | | -- | | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | | -- | | | -- | -- | | | | | | | | | |
| 7 | | | | | | | | | | | | | | | -- | | | | | -- | | | | | | -- | | |
| 8 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | | -- | -- | | | -- | -- | -- | -- | -- | -- | -- | | |
| 9 | | | -- | | | | | | | | | | | | | -- | | | | | | | | | | -- | -- | -- |
| 10 | | | | | -- | | | | | | | | -- | -- | | | | -- | | | | -- | -- | | -- | | -- | -- |
| 11 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | -- | | | -- | | | | -- | | | | -- | | | | | | | | | | | -- | | | | | |
| 13 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 14 | | | | | | | | | -- | -- | | | | | | | | | | -- | | | | -- | -- | -- | -- | |
| 15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | -- | | | | | | | -- | | -- | | | | | | | -- |
| 17 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 18 | | | | | | | | | | | -- | | | | | | | | | | -- | | | -- | | | | |
| 19 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | | -- | | | | -- | | | | | -- | | | | | | |

| Example Variety | Lilora | Tetramax | Fornido | (none) | Eugenius | Diwan | Bellamini | Henrietta | Traffic | Hugo 1 | Expert | Expert | Arsenal | Dressaro | Parcour | Phoenix | Option | Mezquita | Canberra | Ragtime | Twymax | (none) | (none) | Boccacio | Melpaula | Maiko | Resista | Tornado |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| Type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 22 | | | | | | | | | | | | | 28 | | 26 | 24 | | 26 | | 28 |
| | 28 | 28 | 28 | | 28 | | | | | 30 | | 28 | 28 | 32 | 29 | 30 | 30 | 26 | 28 | | 30 |
| Bands | 30 | 30 | 30 | 28 | 32 | 30 | | 34 | 28 | 32 | | 32 | 34 | 33 | 34 | 32 | 32 | 32 | 34 | 24 | 32 |
| | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 30 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 |
| | 38 | 38 | 40 | 38 | 38 | 40 | 40 | 40 | 36 | 40 | 38 | 40 | 40 | 40 | 39 | 38 | 40 | 40 | 40 | 40 | 40 |
| 22 | -- | | | | | | | | | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | | | | | | -- | | | -- | |
| 25 | | | | | | | | | | | | | | | | | | | | | |
| 26 | | | | | | | | | | | | | | | | -- | | -- | -- | | |
| 27 | | | | | | | | | | | | | | | | | | | | | |
| 28 | -- | -- | -- | -- | -- | | | | -- | | | -- | -- | -- | | | | | -- | | -- |
| 29 | | | | | | | | | | | | | | | -- | | | | | | |
| 30 | -- | -- | -- | | | -- | | | -- | -- | | | | | | -- | -- | | | | -- |
| 31 | | | | | | | | | | | | | | | | | | | | | |
| 32 | | | | | -- | | | | | -- | | -- | | -- | | -- | -- | -- | | | -- |
| 33 | | | | | | | | | | | | | | -- | | | | | | | |
| 34 | | | | | | | | -- | | | | | -- | | -- | | | | -- | | |
| 35 | | | | | | | | | | | | | | | | | | | | | |
| 36 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 37 | | | | | | | | | | | | | | | | | | | | | |
| 38 | -- | -- | | -- | -- | | | | | | -- | | | | | -- | | | | | |
| 39 | | | | | | | | | | | | | | | -- | | | | | | |
| 40 | | | -- | | | -- | -- | -- | | -- | | -- | -- | -- | | | -- | -- | -- | -- | -- |
| Example Variety | Lilora | Henrietta | Rokade | Kelvin | Aut | Abosan 1 | Virtuose | Bocardi | Sabor | Defender | Dasher 3 | (none) | Kilrea | Indra | (none) | Melpaula | Resista | (none) | Maritim | Bargizmo | Sanova |

Band range - R

| Type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 64 | | | | | | | 62 | 64 | 64 | 64 | | | | 64 | 64 | | 64 | | | |
| | | 64 | | | 64 | | 64 | 70 | | | | 64 | | | 64 | 66 | 72 | 70 | 62 | 64 | 64 | 72 | 72 | | 68 | 64 | 64 | 64 |
| Bands | 64 | 68 | 64 | 64 | 70 | 64 | 70 | 72 | 64 | 64 | 64 | 76 | 64 | | 70 | 70 | 78 | 72 | 64 | 72 | 72 | 74 | 76 | 64 | 70 | 76 | 70 | 76 |
| | 70 | 70 | 66 | 68 | 76 | 72 | 78 | 78 | 72 | 76 | 76 | 82 | 80 | 64 | 78 | 78 | 80 | 76 | 70 | 80 | 76 | 78 | 80 | 80 | 78 | 80 | 74 | 80 |
| | 82 | 82 | 82 | 82 | 82 | 84 | 82 | 82 | 82 | 82 | 84 | 84 | 82 | 82 | 82 | 82 | 82 | 82 | 82 | 84 | 84 | 84 | 82 | 84 | 82 | 82 | 80 | 84 |
| 62 | | | | | | | | | | | | | | | -- | | | | -- | | | | | | | | | |
| 64 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| 66 | | | -- | | | | | | | | | | | | | -- | | | | | | | | | | | | |
| 68 | | -- | | -- | | | | | | | | | | | | | | | | | | | | | -- | | | |
| 70 | -- | -- | | | -- | | -- | -- | | | | | | | -- | -- | | -- | -- | | | | | | -- | | -- | |
| 72 | | | | | | -- | | -- | -- | | | | | | | | -- | -- | | -- | -- | -- | -- | | | | | |
| 74 | | | | | | | | | | | | | | | | | | | | | | -- | | | | | -- | |
| 76 | | | | | -- | | | | | -- | -- | -- | | | | | | -- | | | -- | | -- | | | -- | | -- |
| 78 | | | | | | | -- | -- | | | | | | | -- | -- | -- | | | | | -- | | | -- | | | |
| 80 | | | | | | | | | | | | | -- | | | | -- | | | -- | | | -- | -- | | -- | -- | -- |
| 82 | -- | -- | -- | -- | -- | | -- | -- | -- | -- | | -- | -- | -- | -- | -- | -- | -- | -- | | | | -- | | -- | -- | | |
| 84 | | | | | | -- | | | | | -- | -- | | | | | | | | -- | -- | -- | | -- | | | | -- |
| Example Variety | Lilora | (none) | Aberdart | Baraudi | Twymax | Cleopatra | Astonhockey | (none) | Henrietta | Dexter 1 | Virtuose | Colorado | Cleancut | Traffic | Barnhem | Probat | Arusi | Maggie | Liszt 1 | Eiffel | Ventoux | Alfonso | Chouss | Defender | Channi | Barsaxo | Eurosport | (none) |

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