

TECHNICAL NOTE

Development and mapping of a public reference set of SSR markers in *Lolium perenne* L.

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Abstract

We report on the characterization and mapping of 76 simple sequence repeat (SSR) markers for *Lolium perenne*. These markers are publicly available or obtained either from genomic libraries enriched for SSR motifs or *L. perenne* expressed sequence tag (EST) clones. Four *L. perenne* mapping populations were used to map the SSR markers. A consensus linkage map of the four mapping populations containing 65 of the SSR markers is presented, together with primer information and a quality score indicating the usefulness of the SSR marker in different populations. The SSR markers identified all seven *L. perenne* linkage groups.

Keywords: consensus linkage map, reference set, ryegrass, SSR

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Simple sequence repeat (SSR) markers are the marker of choice for most genetic and breeding applications due to their widespread distribution, high levels of polymorphism, high reproducibility and codominant mode of inheritance. In *Lolium perenne*, only a limited number of SSRs are publicly available (Jones *et al.* 2001; Kubik *et al.* 2001; Warnke *et al.* 2004) and even fewer have been assigned to map positions. We report here on collaboration among four European institutions (DIAS in Denmark, DLF-Trifolium in Denmark, DvP in Belgium and INRA in France) for the development and mapping of a public reference set of SSR markers in *L. perenne*.

In the present paper, we used 48 public SSR markers (Jones *et al.* 2001; Kubik *et al.* 2001; Lauvergeat *et al.* 2005) and 28 new SSR markers developed by three institutes. SSR markers with the notation LpSSR# were developed by

DIAS from small-insert *L. perenne* genomic DNA libraries enriched for (CA)_n-, (CT)_n-, (AAC)_n- or (GAA)_n-repeats after one round of affinity capture using biotinylated oligonucleotides (Fischer & Bachmann 1998). In total, 480 clones were isolated from the libraries, and 204 clones were deemed positive after two rounds of colony screening (Westman & Kresovich 1998). Sequence information was obtained from 185 of the 204 positive clones. The level of redundancy across all four libraries was 40% (56 clones), and 137 (67%) clones contained SSR repeats with $n \geq 6$ with a majority of imperfect repeats. Primers flanking SSRs were designed using the OLIGO version 5.0 primer analysis software package (National Biosciences). In the present study, only 20 of these SSR markers have been used. Five SSR markers with the notation DLF# were developed by DLF-Trifolium from expressed sequence tag (EST) clones and primers designed with the OLIGO version 5.0 software package. SSR markers with the notation rye# and uni001 were developed from *L. perenne* genomic DNA libraries enriched for (GA)_n- and (GT)_n-repeats (Van de Wiel *et al.*

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1999). After sequencing of derived plasmids, forward and reverse primers flanking the SSR were developed using PRIMER 0.5 (Whitehead Institute for Biomedical Research).

Four different populations were used for map construction: (i) the P150/112 intraspecific reference population of 87 genotypes (Bert *et al.* 1999; <http://ukcrop.net/perl/ace/search/FoggDB>), (ii) 184 genotypes from the VrnA two-way pseudo-testcross population (Jensen *et al.* 2004), (iii) the SB2TC1 population containing 252 F₁ genotypes (Muylle 2003; Muylle *et al.* 2004) and (iv) pop8490 containing 147 genotypes (Barre *et al.* 2000).

Genomic DNA was isolated from the P150/112 and the VrnA populations according to Guidet *et al.* (1991). From the SB2TC1 and pop8490 populations, genomic DNA was prepared using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Marooif *et al.* 1984; Weising *et al.* 1991). Genomic DNA from each of the four populations was subsequently used for polymerase chain reactions (PCRs).

Primer sequence information was distributed among the different institutes involved in fingerprinting (DIAS, INRA and DvP). Each institute generated genotyping information for one or two mapping populations (DIAS fingerprinted the P150/112 population and the VrnA population; INRA fingerprinted the pop8490 population; DvP fingerprinted the SB2TC1 population). At each institute, the PCR and fragment separation conditions were optimized for the technology available in-house. At DIAS, PCR conditions were determined empirically for each primer pairs. The forward primer was fluorescently labelled for detection on a MegaBACE™ 1000 96 capillary electrophoresis system (Amersham Biosciences). Allele scoring was done using the software MEGABACE GENETIC PROFILER version 2.0 (Amersham Biosciences). At DvP, PCR amplifications were performed using the GeneAmp PCR Reagent Kit of Applied Biosystems. One of the primers was fluorescently labelled to allow fragment separation and detection on an ABI PRISM 377 DNA sequencer (Applied Biosystems). GENOTYPER version 2.5 (Applied Biosystems) was used to score the fingerprints. At INRA, PCR amplifications were performed using either nonlabelled primers with silver-nitrate staining colouration after electrophoresis on polyacrylamide gel (Tixier *et al.* 1997) or one M13-labelled tailed primers (Boutin-Ganache *et al.* 2001) followed by electrophoresis on a LI-COR 4200 IR² (Sciencetech).

Map construction was carried out for each population using the Haldane mapping function within the software package JOINMAP 3.0 (Van Ooijen & Voorrips 2001). Other marker information available for these populations at the different institute [e.g. amplified fragment length polymorphism (AFLP), restriction fragment length (RFLP), sequence tagged sites (STS)] was also used for the construction of the respective maps. For three populations (VrnA, SB2TC1 and pop8490), the BC1-type data classification was used. Separate maternal (_M) and paternal (_P) maps were

calculated. Determination of linkage groups (LG) was performed with LOD ratio thresholds of 5.0. For the P150/112 population, the HAP-type classification was used. The determination of LG was performed with LOD ratio thresholds of 5.0 with the exception of LG 1, LG 3 and LG 7, where LOD ratios of 9.0, 9.0 and 7.0 were used, respectively. LG from all four populations were subsequently joined using the 'Combine Groups for Map Integration' function within JOINMAP. For several LG, it was only possible to join a subset of the data from the four populations due to insufficient linkage. The combined map (Fig. 1) consists of seven LG, named according to the chromosome assignment in the reference population P150/112 (<http://ukcrop.net/perl/ace/search/FoggDB>). The following maps were used to generate the individual LG presented in Fig. 1:

LG 1: SB2TC1_M, SB2TC1_P, P150/112, pop8490_P and VrnA_P

LG 2: SB2TC1_M, SB2TC1_P, pop8490_M, pop8490_P, VrnA_P

LG 3: SB2TC1_M, SB2TC1_P, p150/112, pop8490_M, pop8490_P, VrnA_M

LG 4: SB2TC1_M, SB2TC1_P, p150/112, pop8490_M, pop8490_P, VrnA_M, VrnA_P

LG 5: pop8490_M

LG 6: p150/112

LG 7: SB2TC1_M, SB2TC1_P, P150/112, pop8490_M, pop8490_P, VrnA_M, VrnA_P

In Table 1, details regarding LG assignment of the 76 markers together with information on annealing temperature and a quality estimate are presented. For SSR markers that could not be included in the consensus map, LG assignment in Table 1 is derived from one or several of the separate maps. In total, 28 new and 48 previously published SSR markers were mapped in one or several of the four mapping populations. Five of the SSR primer pairs amplified two or three loci, whereas the remaining amplified only one locus. Ten SSR markers (13%) were polymorphic in all four populations tested, whereas 15 (20%) revealed polymorphism in three populations.

The consensus map length is 772 cm (Fig. 1). A total of 317 markers were mapped to the consensus map giving an average of one marker per 2.4 cm. An average number of 9.6 SSR markers mapped to each individual linkage group. LG7 has the best coverage of SSR markers (17) while we only identified one SSR marker on LG6. The largest gaps are found on LG5 and LG6 (25 and 26 cm, respectively), due to difficulties in obtaining common markers between the different populations in these LG. However, in Table 1, other SSR markers are listed which map to these LG but which could not be mapped in the consensus map (five extra SSR markers map to LG5 in at least one of the populations and five other SSR markers map to LG6 in at least one of the populations).

Table 1 Characterization of the *Lolium perenne* SSR loci

SSR	GenBank Accession no.	Repeat motif/ repeat class*	Primer sequences (5'–3')	Size in bpt	Distortion (%)‡	LG§	Quality index¶	T _a **	Reference
LpSSR006	AY919044	(CT) ₂₃ Perfect	F: CAATGGAGTCCCAACAG R: TACCTGGGCAAATCTTG	290 (260–290)	33	4	3 Polymorphic 1 Nonpolymorphic No amplification	58	DIAS
LpSSR011	AY919045	(CA) ₁₅ (CA) ₉ Interrupted	F: AAATGTTTCATCGTATCG R: CAGGTCCCTGCCTTAC	175 (142–188)	25	4	4 Polymorphic Nonpolymorphic No amplification	46	DIAS
LpSSR017	AY919046	(GA) ₂₆ (GA) ₂ (GA) ₂ Interrupted	F: TGAGCACCATGAAGGAG R: GGTGTGCCCGAGGTATT	220 (224–254)	0	7	2 Polymorphic 2 Nonpolymorphic No amplification	50	DIAS
LpSSR020	AY919047	(GA) ₅ (GA) ₂ (GA) ₁₆ Interrupted	F: GGGAAATACAGTTCCTGC R: GATGCTCCTGCCTACTTTTA	230 (225–297)	0	7	3 Polymorphic 1 Monomorphic No amplification	50	DIAS
LpSSR021	AY919048	(GA) ₂₁ Perfect	F: AACAGTCAATGGACAGATT R: TTTGTTTTCCCTTTTGG	300 (286–325)	100	2	2 Polymorphic 1 Monomorphic 1 No amplification	50	DIAS
LpSSR023	AY919049	(GT) ₄ (GT) ₁₉ (GA) ₂₃ Interrupted	F: ATGCACGGGTTTTATPCTATT R: CGCGAGGCTTAAGGTGT	300 (233–324)	25	4	4 Polymorphic Monomorphic No amplification	58	DIAS
LpSSR026	AY919050	(CT) ₂₅ Imperfect	F: GCAAAGTGTACAACCTCT R: ACTCAGCTATCTCATAGGA	220 (212–218)	0	5§	1 Polymorphic 2 Monomorphic 1 No amplification	52	DIAS
LpSSR027	AY919051	(CT) ₁₇ Perfect	F: CACCACCTTCTCCAAC R: AACCAAGCACTTAGGAACA	230 (241–291)	0	1§	1 Polymorphic 2 Monomorphic 1 No amplification	55	DIAS
LpSSR057	AY919052	(GA) ₂₁ Perfect	F: TAGCTCCAGAAACAAAGTC R: CATAGCAGTACAGCCAGTCA	180 (162–192)	33	1	3 Polymorphic 1 Monomorphic No amplification	50	DIAS
LpSSR058	AY919053	(GA) ₁₄ Imperfect	F: CGATGAACCTCAAGGGGATT R: GCACCGGTCTAGGGACAGAA	320 (322–350)	0	6	2 Polymorphic 2 Monomorphic No amplification	58	DIAS
LpSSR059	AY919054	(CT) ₂₀ Perfect	F: GATCGGATCGGTACAGGAGA R: GAAGCGCACCTTCGTGTTCT	200 (185–223)	50	5	4 Polymorphic Monomorphic No amplification	55	DIAS
LpSSR066	AY919055	(TG) ₂₄ Perfect	F: GCCAGTCCCATTCGAGATAA R: CCCCACTCCCAACCAAGCAA	270 (233–302)	0	7	2 Polymorphic 2 Monomorphic No amplification	60	DIAS
LpSSR071	AY919056	(CTT) ₁ (GTT) ₂ (CTT) ₂ Interrupted	F: GGAAGTGGGGCAGCAG R: GCAACAACGCAACACCCCTAA	360 (361–364)	0	2§	1 Polymorphic 2 Monomorphic 1 No amplification	64	DIAS
LpSSR076	AY919057	(CA) ₂₈ Perfect	F: CCCATACTTCGAGGCATAAAA R: AAATTCGCCCATCAGAGAAC	280 (262–283)	50	2	4 Polymorphic Monomorphic No amplification	52	DIAS
LpSSR082	AY919058	(CA) ₂₅ Perfect	F: CTAAACTAAATGTTTCATCGT R: CCTGCTTACTCTCTGTT	180 (143–191)	25	4	4 Polymorphic Monomorphic No amplification	54	DIAS
LpSSR085	AY919059	(CA) ₄₇ Imperfect	F: GCCAGATCCCTTGTAGAAG R: GCACCATTTAAAACCAAGA	240 (174–263)	0	1	2 Polymorphic 1 Monomorphic 1 No amplification	57	DIAS
LpSSR089a	AY919060	(TG)(TG) ₂ (TG) ₉ Interrupted	F: TGTGTTTCGGTGTTCCTTG R: CCAAAAATCGAGAAAATGGTTC	220 (117–117)	0	4	1 Polymorphic 3 Monomorphic No amplification	60	DIAS
LpSSR089b	AY919060	—	F: TGTGTTTCGGTGTTCCTTG R: CCAAAAATCGAGAAAATGGTTC	220 (98–98)	0	6§	1 Polymorphic 3 Monomorphic No amplification	60	DIAS
LpSSR091	AY919061	(TG) ₂₅ Imperfect	F: CACTCTCGGTCTCGCCTTAT R: TTCGCATGCATACAACACAT	190 (162–202)	0	7	1 Polymorphic 2 Monomorphic 1 No amplification	60	DIAS
LpSSR100	AY919062	(TG) ₈ (TG) ₂ Interrupted	F: AACTACTGTAGTTGGCATTTTC R: CGGCTCACTGAACATTTC	210 (217–223)	50	3	2 Polymorphic 2 Monomorphic No amplification	50	DIAS
LpSSR112	AY919063	(CA) ₂₀ Perfect	F: GACCCGAGACAGCCTA R: ACGCATATGGTCTTCAGAA	260 (234–264)	66	2	3 Polymorphic 1 Monomorphic No amplification	55	DIAS
DLF008	CX820348	(ACT) ₇ Perfect	F: CCGTGTCTTGATACTTGGAC R: GAACGAGCATCTTCCCTTCT	250 (243–268)	0	7	3 Polymorphic 1 Monomorphic No amplification	52	DLF

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Table 1 Continued

SSR	GenBank Accession no.	Repeat motif/ repeat class*	Primer sequences (5'-3')	Size in bp†	Distortion (%)‡	LG§	Quality index¶	T _a **	Reference
DLF013	CX820349	(GTT) ₆ (GCT) ₃ Interrupted	F: GTAGTCCAGCGGAGTCAAT R: ATAGCAAACCTTTGGCACACAT	190 (192–198)	100	3§	2 Polymorphic 2 Monomorphic No amplification	55	DLF
DLF020	CX820350	(CGA) ₅ Perfect	F: ATGACGACGAGGAGGAAT R: ATAGCGACGAGAAAAGGTAA	280 (357–389)	0	7	3 Polymorphic 1 Monomorphic No amplification	56	DLF
DLF025	CX820351	(CT) ₁₀ Perfect	F: CGCGAGAAAGCTAACAGA R: TCACGAGAGGGCAAGT	350 (344–365)	66	4	3 Polymorphic 1 Monomorphic No amplification	55	DLF
DLF027	CX820352	(TA) ₁₁ Interrupted	F: CGCTTTGTCAACTCATACC R: CAAACCCGTTCTTCTACATT	290 (286–317)	50	1	2 Polymorphic 2 Monomorphic No amplification	52	DLF
rye012	AY919064	(CA) ₂₃ Perfect	F: GGTCTAATTGTCTGCTTTC R: GAGTGATTGGAGGTGAGAA	198 (142–242)	33	4	3 Polymorphic Monomorphic 1 No amplification	51	DvP
rye014	AY919065	(CA) ₂₆ Perfect	F: CTGCTCTGTGTTGTGTGAC R: GCCTTTCATCGTTACTGTCT	227 (214–244)	0	6§	1 Polymorphic 1 Monomorphic 2 No amplification	51	DvP
uni001	AY919066	(AC) ₁₇ Perfect	F: AGCCACACTTTACCTAATGCTG R: CCCGAAAACCTACAAATTAAA	150 144–175	50	3	4 Polymorphic Monomorphic No amplification	55	DvP
B1A2	AJ872206	(GA) ₁₄ Perfect	F: GTGCAGCAGTTTGAATTGGA R: AGCATCGGAGCTATGAATG	218 (159–255)	75	3	4 Polymorphic Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B1A8	AJ872208	(TG) ₇ Perfect	F: GACTTTCAGGCATCGGCAT R: CCCAGCTCCATTCTTAATGC	295 (281–306)	33	6§	3 Polymorphic 1 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B1A10	AJ872210	(CA) ₁₂ Interrupted	F: GCGACAGGAGTGAACACTGA R: TAAGCGTAAGGCAGCAGTG	200 (190–200)	0	3	2 Polymorphic Monomorphic 2 No amplification	55	Lauvergeat <i>et al.</i> 2005
B1B3	AJ872214	(TG) ₇ Perfect	F: AGGTGCTCTGTGCTTTGGA R: TTTACCCCCAGGATCAAAT	217 (209–223)	0	3	4 Polymorphic Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B1B6	AJ872215	(CTT) ₄ (GT) ₂ Interrupted	F: GGAGTGCATCTTTCTTGCT R: GCAAACCCAGACACCCATTA	291 (270–305)	100	1	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B1C8	AJ872218	(CA) ₈ (CT) ₆ Interrupted	F: TTCTGGCCATGTTGATTGTC R: GTCTACGGGTTGGAGCAGTG	198 (198–203)	0	7	2 Polymorphic 2 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B1C9	AJ872219	(CT) ₈ Perfect	F: GAGCGATGCACAGGTTACT R: AAAGGAGCCGCTAATCAC	193 (188–220)	33	3	3 Polymorphic 1 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B2F1	AJ879807	(CA) ₅ Interrupted	F: CCAACCATATGCAACGATGA R: TCCATTGTCTTTGGGAGA	178 (179–182)	0	5	1 Polymorphic 3 Monomorphic No amplification	50	Lauvergeat <i>et al.</i> 2005
B2G6a	AJ872228	(TGA) ₈ Perfect	F: CCAACTAGACAAAGGGGATTG R: GGAGAGCACCAATTCATCCAT	180 (200–200)	0	1§	4 Polymorphic Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B2G6b	AJ872228	—	F: CCAACTAGACAAAGGGGATTG R: GGAGAGCACCAATTCATCCAT	180 (222–246)	0	7	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B3A1	AJ872232	(AC) ₅ (AG) ₅ Interrupted	F: CTGTGCTCCTTGTGGGAG R: ATATTCTGGATCGTGGCGTT	303 (301–303)	0	2§	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B3A3	AJ872234	(GT) ₈ Interrupted	F: GGGTGAAGTCTCTTTGTGA R: ATGGTGAAGCCCTGAAACTG	167 (162–164)	100	7	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B3B7	AJ872239	(TG) ₉ Interrupted	F: AGGCGACCAATACGCTGTCT R: ATCTCTGATGGCTTTGTGGC	286 (264–299)	0	1	2 Polymorphic 2 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B3B8	AJ872240	(TG) ₁₀ Perfect	F: TGTCAATGTCGCTGCTACG R: GAGAGTGGCGATCATCTTC	307 (306–310)	50	3	2 Polymorphic 2 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B3C5	AJ872242	(GT) ₈ Perfect	F: TGTCAATGTTACGAAAGTGCG R: TGTCCACATAAATGCACCTCA	125 (120–140)	0	7	2 Polymorphic 1 Monomorphic 1 No amplification	55	Lauvergeat <i>et al.</i> 2005

Table 1 Continued

SSR	GenBank Accession no.	Repeat motif/ repeat class*	Primer sequences (5'–3')	Size in bpt	Distortion (%)‡	LG§	Quality index¶	T _a **	Reference
B3C10	AJ872244	(CTC) ₄ Perfect	F: CTACAACCTCCGTGCTGCTGA R: TGCATGGTTCCTCAAATGCT	145 (138–138)	0	7	1 Polymorphic 3 Monomorphic 3 No amplification	50	Lauvergeat <i>et al.</i> 2005
B3C11	AJ872245	(CATG) ₃ (TG) ₉ Interrupted	F: ATTCACTCGCTCGAAAATG R: AACACCAAGCTAGCCACCAC	145 (141–150)	0	7	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B3D2	AJ872247	(AT) ₄ Perfect	F: ATACGAGCGAATTGCCTCTC R: TCTCCCATCGCTTATGTTCC	135 (114–114)	100	1	1 Polymorphic 2 Monomorphic 1 No amplification	55	Lauvergeat <i>et al.</i> 2005
B3D4	AJ872249	(CG) ₄ Perfect	F: AAACCCATACCGACATACCG R: GCGCTCTGTGAGAGTGAGTG	112 (113–115)	0	6§	2 Polymorphic Monomorphic 2 No amplification	55	Lauvergeat <i>et al.</i> 2005
B3D12	AJ872251	(TC) ₇ Imperfect	F: GGGCATCACTGAGAAGAGGA R: TACAAAGGAAGTCGGGCATC	298 (291–302)	0	2	1 Polymorphic 2 Monomorphic 1 No amplification	50	Lauvergeat <i>et al.</i> 2005
B3E6	AJ872252	(AG) ₄ (GT) ₇ Interrupted	F: CTGTAACAACAGCCGCTGAG R: GTCTCGAGCACAGGAGTTCA	196 (199–301)	100	3	1 Polymorphic 2 Monomorphic 1 No amplification	55	Lauvergeat <i>et al.</i> 2005
B4C4	AJ872256	(GT) ₄ (TC) ₃ (TC) ₃ (CT) ₆ Interrupted	F: TGCATGCACCCCTTGTAGC R: GGAGACTTTGTGTGCAGC	152 (139–150)	0	7	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B4D7	AJ872260	(CT) ₁₀ Imperfect	F: CGGGAGCTCTCTCTCCTTCT R: TCCAGAACCCTTCTCGAGGTC	215 (223–223)	100	1	1 Polymorphic 3 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B4D9	AJ872261	(TC) ₁₀ (TC) ₄ Interrupted	F: GACGTCATACCTGCGTGCTA R: GCGAATCAAAGAGCATGTG	251 (218–222)	50	4	2 Polymorphic 2 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B5E1	AJ872265	(CA) ₃ (CA) ₅ Interrupted	F: AAACATCAACGGAAGGATGC R: TGATATGCATGTGTATGGAGG	207 (209–233)	50	5§	2 Polymorphic 2 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
B5G4	AJ872263	(CA) ₈ Imperfect	F: TGGAGTTGTTGGACCTTTTCC R: AGATGCTGGTTGGTTCCAG	300 (294–300)	0	7	3 Polymorphic 1 Monomorphic No amplification	55	Lauvergeat <i>et al.</i> 2005
LPSSRH 01A02		(CA) ₂₇ Perfect	F: AAAGACCCGATACGGAAGT R: AACCAAAGCCTCAAGACA	131 (146–150)	100	5§	1 Polymorphic 1 Monomorphic 2 No amplification	60	Jones <i>et al.</i> 2001
LPSSRH 01E10		(CA) ₁₀ Perfect	F: CGCAGCTTAATTTAGTC R: GCTTTGAGTATGTAAAAGTT	103 (109–109)	0	4	2 Polymorphic 2 Monomorphic No amplification	55	Jones <i>et al.</i> 2001
LPSSRH 01H06		(CA) ₉ Perfect	F: ATTGACTGGCTTCCGTTGTT R: CGCGATGTCAGATTCTTG	150 (141–149)	0	4	2 Polymorphic 2 Monomorphic No amplification	60	Jones <i>et al.</i> 2001
LPSSRH 02C11		(CA) ₄ (CA) ₄ Interrupted	F: TGGAAATACGATGAAAATF R: CATCACAAGATTAACAAGAG	198 (178–185)	100	3§	2 Polymorphic 2 Monomorphic No amplification	55	Jones <i>et al.</i> 2001
LP165		(CT) ₁₄ Perfect	F: CCATCACCTCCACTAT R: AGCTCGCAGTCTGTTG	(99–104)	33	7	3 Polymorphic 1 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
LP20a		(GA) ₁₆ (A) ₅ (GA) ₄ Interrupted	F: ACCGCTGTGCTAAATCTG R: ATGCGCTGTGTCGTGCCCT	(85–85)	66	4	3 Polymorphic 1 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
LP20b		—	F: ACCGCTGTGCTAAATCTG R: ATGCGCTGTGTCGTGCCCT	(82–90)	100	6§	1 Polymorphic 3 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
LP204a		(CT) ₂₀ Perfect	F: GAGCTTCTCTCGATCCT R: AGTGGATGTGACTACA	(102–102)	100	4	1 Polymorphic 1 Monomorphic 2 No amplification	55	Kubik <i>et al.</i> 2001
LP204b		—	F: GAGCTTCTCTCGATCCT R: AGTGGATGTGACTACA	(106–106)	100	5§	1 Polymorphic 1 Monomorphic 2 No amplification	55	Kubik <i>et al.</i> 2001
LP204c		—	F: GAGCTTCTCTCGATCCT R: AGTGGATGTGACTACA	(100–100)	0	7	1 Polymorphic 1 Monomorphic 2 No amplification	55	Kubik <i>et al.</i> 2001
LP8a		(CT) ₁₇ Perfect	F: TGACTTCTCTCGATCCT R: ATGTGACTACAAAACCA	(97–97)	0	4	2 Polymorphic 2 Monomorphic No amplification	50	Kubik <i>et al.</i> 2001
LP8b		—	F: TGACTTCTCTCGATCCT R: ATGTGACTACAAAACCA	(101–101)	0	5§	1 Polymorphic 3 Monomorphic No amplification	50	Kubik <i>et al.</i> 2001

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Table 1 Continued

SSR	GenBank Accession no.	Repeat motif/ repeat class*	Primer sequences (5'–3')	Size in bp†	Distortion (%)‡	LG§	Quality index¶	T _a **	Reference
LP8c		—	F: TGACTTCTCTCGATCOCT R: ATGTGACTACAAAACCA	(95–95)	0	7	1 Polymorphic 3 Monomorphic No amplification	50	Kubik <i>et al.</i> 2001
M10138		(CA) ₁₃ Imperfect	F: TAGAGGATCAGTTGCATC R: TAGTTCGGAGTTAGCTGA	(306–322)	0	3	2 Polymorphic 2 Monomorphic No amplification	50	Kubik <i>et al.</i> 2001
M144		(CT) ₁₆ Perfect	F: CAGAAGGAGGTCGTCGA R: CTGAAACCTAGGCTATCTGAG	(125–127)	0	4	2 Polymorphic 2 Monomorphic No amplification	60	Kubik <i>et al.</i> 2001
M15185		(GA) ₅ (GA) ₁₇ Interrupted	F: GGTCTGGTAGACATGCCTAC R: TACCAGCACAGCAGGTTTC	(142–198)	100	2	1 Polymorphic 1 Monomorphic 2 No amplification	55	Kubik <i>et al.</i> 2001
M16B		(GA) ₂₈ Imperfect	F: TGCTGTGGCTCTTGTGAC R: AGCCGAGGCTCAGCTCGA	(137–178)	50	1	2 Polymorphic Monomorphic 2 No amplification	65	Kubik <i>et al.</i> 2001
M4136		(GA) ₂₇ Imperfect	F: AGAGACCATCCCAAGCC R: TCTGGAAGATTTCCCTTG	(183–207)	25	2	4 Polymorphic Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
M4213		(GT) ₉ Imperfect	F: CACCTCCCCTGCATGGCATGT R: TACAACGACATGTCAAGGT	161–168	50	1	2 Polymorphic 2 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
PR3		(CA) ₂₂ Perfect	F: GTATAGTACCCATTTCCGT R: GCCGCCCTGCCATGCTG	(183–185)	0	2	1 Polymorphic 1 Monomorphic 2 No amplification	60	Kubik <i>et al.</i> 2001
PR8		(GT) ₄₂ Imperfect	F: AGGGTTCGTCGATTC R: GCCGTCCGACCCCTG	(119–134)	50	1	2 Polymorphic 1 Monomorphic 1 No amplification	60	Kubik <i>et al.</i> 2001
PR14		(GT) ₁₂ Imperfect	F: CCTTTTCGCTTCGTA R: CACCAACATTCGCGAGTG	(131–151)	50	4	2 Polymorphic 2 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
PR24		(GT) ₁₆ Imperfect	F: TGCTGTGATGCTGAATG R: GTATAGTACCCATTCGTTGTC	(148–150)	0	2	2 Polymorphic 2 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
PR25		(GT) ₁₅ Interrupted	F: AGGGTTCGTCGATTC R: CCTGCATACATTCATCCA	(98–119)	50	1	2 Polymorphic 2 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
PR37		(GT) ₁₈ Perfect	F: TCTGCATTCGTTGTCTCACTG R: GAGCCGTCGCACCCCTG	(100–124)	50	1	2 Polymorphic 2 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
PR39		(CA) ₁₇ Perfect	F: CATTCATCCACGTTAGAC R: CTTCACGACTGCTTC	(97–151)	0	1	3 Polymorphic 1 Monomorphic No amplification	55	Kubik <i>et al.</i> 2001
PRE		(CA) ₁₂ Perfect	F: CATTCATCCACGTTAGAC R: GTTAGGTTGCTCTGCAT	(98–134)	66	1	3 Polymorphic 1 Monomorphic No amplification	50	Kubik <i>et al.</i> 2001
PRG		(CA) ₁₃ Perfect	F: GCCGAGTGTATCAAGGT R: CTTTTCGCCCTTCGTA	(140–158)	0	4	1 Polymorphic 3 Monomorphic No amplification	50	Kubik <i>et al.</i> 2001

*Perfect, stretch of perfect repeats; Imperfect, stretch of repeats where single bases are different, i.e. CACACAGACACACA; Interrupted, stretch of repeats there are interrupted by several bases, i.e. CACACATGCTGACACACACA. †Size in bp, size of cloned allele in bp, the size range of alleles obtained in this study is shown in brackets. ‡Distortion %, of populations, in which the marker has been found polymorphic, where a significant χ^2 was found. §LG, linkage group, loci not included on the integrated map are followed by a 'd'. ¶Quality index, indicates number of populations out of four where the SSR was polymorphic, monomorphic or did not amplify. **T_a, annealing temperature.

We have established an SSR reference marker set covering all seven *L. perenne* LG. Hereby, we have established a foundation within the public domain for comparisons between mapping populations and wild populations. It is a general experience that SSR markers may occasionally not be reproducible when implemented in different laboratories due to differences in setup, equipment, etc. There-

fore, we have provided information on the quality of the markers. Markers that are polymorphic in all four populations should be easy to implement, although difficulties can be encountered with markers that are less polymorphic. Some LG are still not adequately covered, but we intend to develop additional SSR markers especially for these regions, with the perspective to establish bin maps.

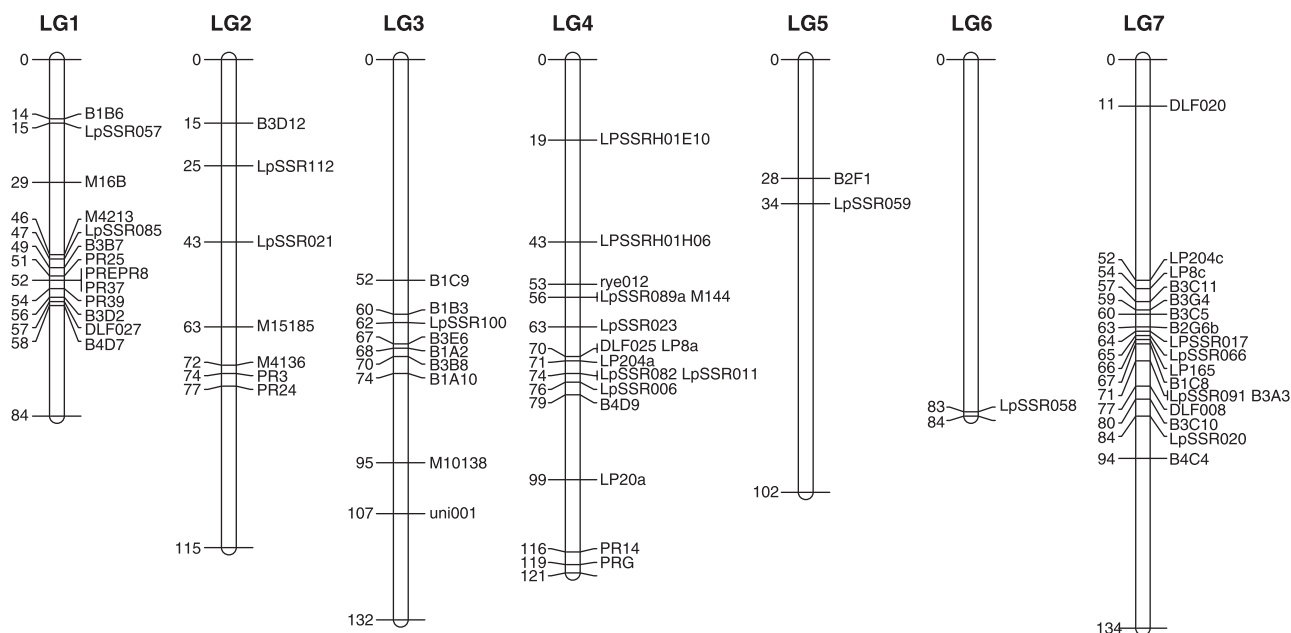


Fig. 1 Consensus linkage map based on data from four mapping populations. All non-SSR markers used in map construction have been omitted from the map.

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