

PCR Primers Used for Citrus Germplasm Characterization

Note that we expect to rename SSR markers on recent maps, adding a prefix with the developer's initials and using two digits for the primer number, so TAA1 will become JK-TAA01, CCT01 will become NB-CCT01, etc.

Table 1. Citrus primer sequences and information on detection methods used.

No.	Locus	F-Primer	R-Primer	Repeat Motif	PCR ¹ Protocol	Gel ² Method	Detection Method ³	Quality Score ⁴	Contributor Initials ⁵
1	TAA1	GACAACATCAACAACAGCAAGAGC	AAGAAGAAGAGCCCCATTAGC	TAA	-	dPAGE	L	0	N.B./J.K.
2	TAA45	GCACCTTTTATACCTGACTCGG	TTCAGCATTGAGTTGGTTACG	TAA	-	dPAGE	L	0	N.B./J.K.
3	TAA52	GATCTTGACTGAACCTTAAAG	ATGTATTGTGTTGATAACG	TAA	-	dPAGE	L	0	N.B./J.K.
4	CAC19	ACAACCTTCAACAAAACCTAGG	AAGACTTGGTGCGACAGG	CAC	-	dPAGE	L	0	N.B./J.K.
5	TAA15	GAAAGGGTTACTTGACCAGGC	CTTCCCAGCTGCACAAGC	TAA	1	dPAGE	L	4	N.B./J.K.
6	TAA27	GGATGAAAAATGCTCAAAATG	TAGTACCCACAGGGAAGAGAGC	TAA	2	dPAGE	L	4	N.B./J.K.
7	TAA41	AGGTCTACATTGGCATTGTC	ACATGCAGTGCTATAATGAATG	TAA	3	dPAGE	L	3	N.B./J.K.
8	CAC23	ATCACAATTACTAGCAGCGCC	TGCCATTGTAGCATGTTGG	CAC	4	dPAGE	L	5	N.B./J.K.
9	cAGG9	AATGCTGAAGATAATCCGCG	TGCCTTGCTCTCCACTCC	AGG	5	dPAGE	L	5	N.B./J.K.
10	TAA3	AGAGAAGAAACATTTGCGGAGC	GAGATGGGACTTGGTTCATCAGC	TAA	6	dPAGE	L	2	N.B./J.K.
11	CAC15	TAAATCTCCACTCTGCAAAAGC	GATAGGAAGCGTCGTAGACCC	CAC	7	dPAGE	L	5	N.B./J.K.
12	CAC33	GGTGATGCTGCTACTGATGC	CAATTGTGAATTTGTGATTCCG	CAC	8	dPAGE	L	5	N.B./J.K.
13	CAC39	AGAAGCCATCTCTCTGCTGC	AATTCAGTCCCATTCCATTCC	CAC	2	dPAGE	L	5	N.B./J.K.
14	TAA33	GGTACTGATAGTACTGCGGCG	GCTAATCGCTACGTCTTCGC	TAA	4	dPAGE	L	4	N.B./J.K.
15	CCT01	TCAACACCTCGAACAGAAGG	CCCACATGCTAGCACAAAGA	CCT	9	dPAGE	L	5	N.B.
16	GT03	GCCTTCTTGATTTACCGGAC	TGCTCCGAACTTCATCATTG	GT	10	dPAGE	L	4	N.B.
17	CT02	ACGGTGCCTTTTGTAGGTAAG	TGACTGTTGGATTGGGATG	CT	11	dPAGE	L	2	N.B.
18	AC01	TTTGACATCAACATAAAACAAGAAA	TTTTAAAATCCCTGACCAGA	AC	12	dPAGE	L	2	N.B.
19	CAG01	AACACTCGCACCAAATCCTC	TAAATGGCAACCCCAGCTTTG	CAG	13	dPAGE	L	4	N.B.
20	CAT01	GCTTTCGATCCCTCCACATA	GATCCCTACAATCCTTGGTCC	CAT	14	dPAGE	L	5	N.B.
21	ATC09	TTCTTATGTAATTGCTCTTTG	TGTGAGTGTGTTGTGCGTGTG	ATC	15	dPAGE	L	5	N.B.
22	AG14	AAAGGGAAAGCCCTAATCTCA	CTTCCTCTTGCGGAGTGTTT	AG	16	dPAGE	L	4	N.B.
23	CTT01	TCAGACATTGAGTTGCTCG	TAACCACTTAGGCTTCGGCA	CTT	17	dPAGE	L	5	N.B.
24	CT21	CGAACTCATTAAAAGCCGAAAC	CAACAACCACCACTCTCACG	CT	18	dPAGE	L	5	N.B.
26	CT19	CGCCAAGCTT ACCACTCACTAC	GCCACGATTTGTAG GGGATAG	CT	4	dPAGE	L	5	N.B.
27	CT19-02 ⁶	ACCACTCACTAC TTAATTACCCTTTT	AGGGTT GCCACGATTTGTAG	CT					C.F.

1 See Table 3 for codes for PCR conditions

2 dPAGE = denaturing PAGE; ndPAGE = non-denaturing PAGE; A=agarose

3 SS=Silver stain; R=radioactive label; L=Li-Cor fluorescent label; A=ABI fluorescent label

4 Quality score: 1-5 (best) based on intensity, stutter, band sharpness

5 Contributor's initials, see Table 4 for full information

6 Redesigned version of CT19 –works better for same locus, overlaps highlighted

Table 2. Variation detected with primers for SSRs and other markers.

No.	Locus	Alleles/ Accessions ¹	Flying Dragon	B.H. citron	Dancy Mandarin	Star Ruby	Contributor Initials ²
1	TAA1	0	-	-	-	-	N.B./J.K.
2	TAA45	0	-	-	-	-	N.B./J.K.
3	TAA52	0	-	-	-	-	N.B./J.K.
4	CAC19	0	-	-	-	-	N.B./J.K.
5	TAA15	22/375	-	161	179, 194	-	N.B./J.K.
6	TAA27	10/375	-	209	209	-	N.B./J.K.
7	TAA41	33/375	-	130	134, 143	-	N.B./J.K.
8	CAC23	9/375	-	243, 249	246, 249	-	N.B./J.K.
9	cAGG9	7/375	-	102	115	-	N.B./J.K.
10	TAA3	10/375	-	149	143	-	N.B./J.K.
11	CAC15	4/375	-	157	157	-	N.B./J.K.
12	CAC33	6/375	-	141, 147	141	-	N.B./J.K.
13	CAC39	8/375	-	168	162	-	N.B./J.K.
14	TAA33	8/375	-	112	112	-	N.B./J.K.
15	CCT01	7/375	-	158	158	-	N.B.
16	GT03	18/375	-	149, 167	149, 173	-	N.B.
17	CT02	10/375	-	100, 142	136, 140	-	N.B.
18	AC01	11/375	-	160	138	-	N.B.
19	CAG01	6/375	-	123, 126	123, 126	-	N.B.
20	CAT01	13/375	-	136	146	-	N.B.
21	ATC09	8/375	-	175, 184	175, 184	-	N.B.
22	AG14	20/375	-	123	145, 151	-	N.B.
23	CTT01	10/375	-	136	148	-	N.B.
24	CT21	12/375	-	145	143	-	N.B.
26	CT19	15/375	-	153	145, 149	-	N.B.
27							

1 Number of alleles detected /number of accessions tested

2 Contributor's initials, see Table 4 for full information

Table 3. PCR conditions used for various citrus primers

Code	Mg+2 mM	Primer Conc. uM	Final template Conc. (ng/ul)	Polymerase (source, units/ul in reaction)	Cycling Protocol ¹
1	4	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@55, 60"@72), 7'@72
2	3	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@45, 60"@72), 7'@72
3	4	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@45, 60"@72), 7'@72
4	2	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@45, 60"@72), 7'@72
5	2	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@55, 60"@72), 7'@72
6	3	0.0375	0.5	Promega, 0.1	5'@95, 40x:(60"@95, 30"@55, 60"@72), 7'@72
7	2	0.05	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@55, 60"@72), 7'@72
8	3	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@63.5, 60"@72), 7'@72
9	3	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@59, 60"@72), 7'@72
10	3	0.025	0.5	Promega, 0.1	5'@95, 36x:(60"@95, 30"@50, 60"@72), 7'@72
11	3	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@57, 60"@72), 7'@72
12	4	0.0375	0.5	Promega, 0.1	5'@95, Touchdown 13x: 27x:(60"@95, 30"@55, 60"@72), 7'@72
13	4	0.025	0.5	Promega, 0.1	5'@95, Touchdown 13x: 30x:(60"@95, 30"@63, 60"@72), 7'@72
14	2	0.025	0.5	Promega, 0.1	5'@95, Touchdown 13x: 27x:(60"@95, 30"@56, 60"@72), 7'@72
15	6	0.0375	0.5	Promega, 0.1	5'@95, 45x:(60"@95, 30"@47, 60"@72), 7'@72
16	3	0.0375	0.5	Promega, 0.1	5'@95, 40x:(60"@95, 30"@50, 60"@72), 7'@72
17	2	0.025	0.5	Promega, 0.1	5'@95, 42x:(60"@95, 30"@47, 60"@72), 7'@72
18	2	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@46, 60"@72), 7'@72
19	3	0.025	0.5	Promega, 0.1	5'@95, 38x:(60"@95, 30"@58, 60"@72), 7'@72

¹Initial denaturation time @ temp., cycles x:(denaturing time @ temp., annealing time @ temp., extension time @ temp.), final extension time @ temp. Touchdown nnx: initial annealing temp. was nn degrees higher than listed and was reduced 1 degree per cycle until the specified annealing temp. was reached, total cycles was the sum of touchdown and standard PCR.

Table 4. Developer and contributor information

Initials of contributor	Name of contributor	Primer developer if different from contributor	Address of Contributor	Current e-mail address of contributor	Reference
N.B./J.K.	Noelle Anglin-Barkley	James Kijas	National Clonal Germplasm Repository for Citrus and Dates; 1060 Martin Luther King Blvd, Riverside, CA 92507. U.S.A.	Elle.Barkley@ARS.USDA.GOV	Theor. Appl. Genet. 94:701
N.B.	Noelle Anglin-Barkley		National Clonal Germplasm Repository for Citrus and Dates; 1060 Martin Luther King Blvd, Riverside, CA 92507. U.S.A.	Elle.Barkley@ARS.USDA.GOV	Theor. Appl. Genet. 112:1519-1531.