3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Maize SSR Primer Set

Catalog Number **M4193**
Storage Temperature: –20 °C

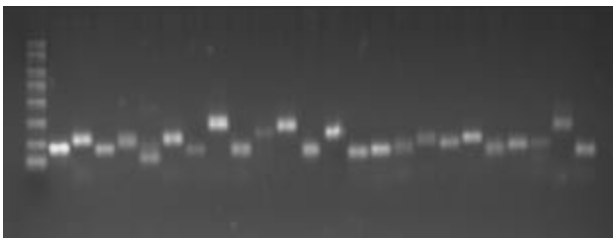
TECHNICAL BULLETIN

Product Description

SSR (simple sequence repeat) or SSLP (simple sequence length polymorphism) are PCR-based molecular markers widely used in genetic mapping, gene localization, and marker-facilitated breeding. More than a thousand SSR markers have been mapped in the Maize genome.

The Maize SSR Primer Set consists of 384 pairs of primers selected from the Maize Genetics and Genomic Database. The primer pairs were selected to cover the entire maize genome with an average of 20 cM units of map distance between two SSR markers. This primer set is useful for screening new maize populations for genetic mapping, genotyping, marker-trait association analysis, and physical mapping of BAC clones, etc. All primers pass sequence and PCR validation tests.

1. The figure below shows typical PCR results using the Maize SSR Primer Set. The first lane on the left is a PCR molecular weight marker. The rest are PCR products from 24 SSR primers. For detailed sequence information and polymorphism data on all 384 pairs of primers in the set, please consult the Maize Genetics and Genomic Database, Data Center SSRs at http://www.maizegdb.org/ and the Sigma-Aldrich website www.sigmaaldrich.com/maizessr



Components/Reagents

- Maize SSR Primer Set, Product Code M4193, Four 96-well plates containing lyophilized primer pairs
- PCR multiwell plates, 96-well, Product Code Z374903, four plates
- Sealing film, Product Code Z369683, 20 sheets

Materials required but not provided

- DNA template
- JumpStart™ RedTaq™ ReadyMix™, Product Code P1107, or equivalent
- Dedicated pipettes (free of amplicon contamination)
- Aerosol resistant pipette tips
- PCR plates, Product Code P6861
- Tris-EDTA (TE) Buffer, 100X, Product Code T9285
- Water, PCR reagent, Product Code W1754
- GenElute™ Plant Genomic DNA Miniprep kit, Product Code G2N70, or equivalent
- Ethidium bromide, Product Codes E7637
- PCR marker, Product Code P9577, or equivalent
- Agarose. Product Code A9539, or equivalent
- Horizontal gel electrophoresis unit, high throughput, Product Code Z373060, or equivalent

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The primers are shipped lyophilized. Prepare a 10 µM (10 pmole/µl) solution of each primer by adding 100 µl of 1x TE buffer to each well. After reconstitution, transfer 25 µl of the primers to the PCR plates provided and add 75 µl of 1x TE buffer to make a 2.5 µM working stock. Seal the original plates and the working stock plates with the sealing film provided, and store them at –20 °C. Use primers from the working stock plates for PCR. Before each use, briefly centrifuge the plates if any condensate forms on the plate seal.

Storage/Stability
The original lyophilized primers are stable at –20 °C for at least 2 years. The reconstituted primers are stable at –20 °C for at least one year. The primers can be frozen and thawed at least 10 times without compromising performance.

Procedure
DNA isolation
DNA template for SSR PCR may be prepared using any of several isolation methods including the CTAB extraction method and column-based genomic DNA isolation methods including Sigma's GenElute Plant Genomic DNA miniprep kit, Product Code G2N70. 5-20 µg, depending on tissue type, of high quality DNA is routinely obtained per extraction using this kit.

PCR
Though the SSR primers are compatible with a variety of PCR conditions, JumpStart RedTaq Ready Mix, Product Code P1107, developed for SSR applications, is recommended. This reagent is a 2X master mix containing all the components for PCR including an anti-Taq antibody for hot start and a red dye tracer for direct gel loading of PCR products. The hot start mechanism prevents non-target amplification and allows convenient assembly of a large number of samples at room temperature.

1. Make a PCR master mix for one 96-well primer plate (extra reagent is to account for loss when using a multi-channel pipette tray). Alternatively, the PCR master mix can be equally divided along a row or column of a PCR plate for dispensing.

Reagent	Volume (µl)	Amount/reaction
JumpStart RedTaq Ready Mix (P1107)	1100	1x
Genomic DNA	*x* (2.2-5.5 µg total)	20-50 ng
PCR grade water	660-*x*	
Total volume	1760	

2. Using a multi-channel pipette, add 4 µl primer solution (2.5 µM working solution) to each well of a 96-well PCR plate. Verify primer presence in each well after addition.

3. Add 16 µl PCR master mix to each well of the PCR plate using a multi-channel pipette. Cover the plate and make sure there are no bubbles at the bottom of each well.

4. Cycling parameters:

Step	Temperature	Time	Cycles
Denaturation	94 °C	5 min	1
Denaturation	94 °C	30 sec	35
Annealing	55 °C	1 min	
Extension	72 °C	1 min 30 sec	
Final Extension	72 °C	10 min	1
Hold	4 °C		1

5. Evaluate PCR product by loading 5-10 µl of the reaction on a 2% agarose gel containing 0.5 µg/ml ethidium bromide.

Note: We recommend using agarose, Product Code A9539. Optimal gel percentage may be different with other brands of agarose.

Appendices

I. Example for PCR set up in a 96-well plate – For screening new mapping populations. P1 = parental line 1, P2= parental line 2, and F1 = the hybrid between P1 and P2.

DNA template	P1	P2	F1	P1	P2	F1	P1	P2	F1	P1	P2	F1
Primer row 1	A1	A1	A1	A2	A2	A2	A3	A3	A3	A4	A4	A4
Primer row 2	B1	B1	B1	B2	B2	B2	B3	B3	B3	B4	B4	B4
Primer row 3	C1	C1	C1	C2	C2	C2	C3	C3	C3	C4	C4	C4
Primer row 4	D1	D1	D1	D2	D2	D2	D3	D3	D3	D4	D4	D4
Primer row 5	E1	E1	E1	E2	E2	E2	E3	E3	E3	E4	E4	E4
Primer row 6	F1	F1	F1	F2	F2	F2	F3	F3	F3	F4	F4	F4
Primer row 7	G1	G1	G1	G2	G2	G2	G3	G3	G3	G4	G4	G4
Primer row 8	H1	H1	H1	H2	H2	H2	H3	H3	H3	H4	H4	H4

II. Maize SSR primer set - Note: Partial information, please see *www.sigmaaldrich.com/maizessr* for more information

SSR Name	Plate location (plate/ position)	Linkage group (chrom.)	Map location (IBMn)	Primer Sequence
p-umc1354	1/A1	1	0	GATCAGCCCGTTCAGCAAGTT // GAGTGGAGGCGGAGGATCTG
p-umc1566	1/A2	1	16.5	ATCTCGTCTACCTAACCCACCCTC // CAGGTGAAGAATCTGGTGAGGTC
p-umc1292	1/A3	1	32.08	GAAGTGGGGAACATGGTTAATGTC // TCACGGTTCAGACAGATACAGCTC
p-bnlg1124	1/A4	1	41.67	TCTTCATCTCTCTATCAAACTGACA // TGGCACATCCACAAGAACAT
p-bnlg1179	1/A5	1	64.14	GCGATTCAGTCCGCAGTAGT // GTACTGAACAAACCGTGGGC
p-bnlg1014	1/A6	1	82.8	CACGCTGTTTCAGACAGGAA // CGCCTGTGATTGCACTACAC
p-umc1685	1/A7	1	103	TAGTTTGAGGGATCAAGAACCACC // GCTCAAAGGCAAGGCAGTATTTTA
p-umc2225	1/A8	1	124.7	TCGGCTGACATAATAAAACCATAGC // ATGCGAATTTTACCGGGTTTTT
p-bnlg1429	1/A9	1	143.5	CTCCTCGCAAGGATCTTCAC // AGCACCGTTTCTCGTGAGAT
p-umc2226	1/A10	1	165.8	TGCTGTGCAGTTCTTGCTTCTTAC // AGCTTCACGCTCTTCTAGACCAAA
p-bnlg109	1/A11	1	188.58	GCCAGCTGATGTCTGATGAACAGCACA // GATCGGGCCAGATTTCTCAAGTCGTCA
p-umc1073	1/A12	1	208.5	CACCAACGCCAATTAGCATCC // GTGGGCGTGTTCTCCTACTACTCA

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