

Product Information

Maize Polymorphic SSR Primers

Product Number: **M 8818**

Storage Temperature: -20°C

TECHNICAL BULLETIN

Product Description

SSR (simple sequence repeat) or SLP (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 Polymorphic SSR Primers consists of 480 pairs of primers selected from the maize genome database¹. The primer pairs selected can generate SSR markers that cover the entire maize genome and give high polymorphism among commonly used maize mapping populations¹. All primers have been extensively tested and thus are ideal 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. For detailed sequence information and polymorphism data on all 480 pairs of primers, please consult the Maize Genome database¹ and Sigma-Aldrich Website.²

Components/Reagents

- Five 96-well plates containing lyophilized primer pairs (Product Code P 6861)
- Five 96-well storage plates (Product Code Z37,490-3)
- 20 sheets of sealing films (Product Code Z36,968-3)

Materials required but not provided

- DNA template
- JumpStart RedTaq Ready Mix (Product Code P 1107) or equivalent
- Dedicated pipettes (free of amplicon contamination)
- Aerosol resistant pipette tips
- PCR plates (Product Code P 6861)

- Thermal cycler
- TE buffer
- PCR reagent water (Product Code W 1754)
- GenElute plant genomic DNA isolation kit (Product Code G2N-70) or equivalent
- Ethidium bromide (Product Codes E 7637, E 2515, E 4391, or E1510)
- PCR marker (Product Code P 2993) or equivalent
- Agarose (Product Code A 9539) or equivalent
- Horizontal gel electrophoresis unit, high throughput (Product Code Z37,306-0) or equivalent

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household or other uses. Consult the MSDS for information concerning hazards and safe handling practices.

Preparation Instructions

The primers are shipped lyophilized. Prepare a $10\ \mu\text{M}$ ($10\ \text{pmole}/\mu\text{l}$) solution of each primer by adding $100\ \mu\text{l}$ of TE buffer to each well. After reconstitution, transfer $25\ \mu\text{l}$ of the primers to the PCR plates provided and add $75\ \mu\text{l}$ of TE buffer to make $2.5\ \mu\text{M}$ 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 reactions. Before each usage, briefly centrifuge the plates if any condensate forms on the plate seal.

The reconstituted primer solution is sufficient for minimum of 100 PCR reactions.

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 isolated using any of several isolation methods including the CTAB extraction method³ and column-based genomic DNA isolation methods including Sigma's GenElute plant genomic DNA isolation kit (Product Code G2N-70). 5 to 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 P 1107), developed for SSR applications, is recommended. This reagent is a 2x master mix containing all 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 thus increases PCR product yield and allows convenient assembly of a large number of samples at room temperature.

1. Make 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)	Final conc.
JumpStart RedTaq Ready Mix (P 1107)	1100	1 x
Genomic DNA	x (2.2 – 5.5 µg total)	20 – 50 ng /reaction
PCR grade water	660 – x	
Total volume	1760	

2. Using a multi-channel pipette, add 4 µl primer solution (2.5 µM working stock) 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 is no bubble 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 Sigma Agarose (Product Code A9539). Optimal gel percentage may be different with other brands of agarose (see reference 1 for more details).*

References

1. Maize Genome Database. Public SSRs and SSR methods. <http://www.maizegdb.org/>
2. URL-link for information on the MaizeSSR primer set: www.sigmaldrich.com/maizessr
3. Lukowitz, W., et al., Positional cloning in Arabidopsis. Why it feels good to have a genome initiative working for you. *Plant Physiol.* **123**, 795-805 (2000).

Appendices

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

Maize SSR primer set (partial information. Please see www.sigmaaldrich.com/maizessr for the entire set)

Plate #	Position	Primer Name	Linkage group	Forward primer	Reverse primer
1	A1	umc2100	1	AAAGGCATTATGCTCACGTTGATT	TGACGTGCAAACAACCTTCATTAC
1	A2	umc1354	1	GATCAGCCCGTTCAGCAAGTT	GAGTGGAGGCGGAGGATCTG
1	A3	umc1282	1	TACTACTACACGACTCCCAACAGGA	GCGAGGGTTCTTTCCATAGAGAAT
1	A4	umc1292	1	GAAGTGGGGAACATGGTTAATGTC	TCACGGTTCAGACAGATACAGCTC
1	A5	umc1353	1	AGACAGGATCATCGAAAACACACA	ACCTCAGCCTCCTCGTCAACTACT
1	A6	umc1071	1	AGGAAGACACGAGAGACACCGTAG	GTGGTTGTTCGAGTTCGTCGTATT
1	A7	umc1160	1	CGTTTGATATGATGTGGAGATTTCG	AAGCTTGTGAATGTTCTGGATGTC
1	A8	umc1177	1	CGTGTACCGCTCCTCTATAGTCGT	AAGTGGCCGAATTCATCCTTTATT
1	A9	umc1222	1	CTCAGAACAGAAGCCATCAAAAGC	CGTCTTCGTGAGAGACATCCTGT
1	A10	umc1269	1	TATATTAGAGGCACCTCCCTCCGT	AGCTGCTTCAGCGACTTTGG
1	A11	umc1363	1	TGTTTAAGTGTTGGCAGAAAGCAA	TCTCCCTCCCCTGTACATGAATTA
1	A12	umc2012	1	CTTGCATTGAACGACGACCTG	CGTACGCTTGCAATGCTTCTCT

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