# Development and Characterization of Genomic SSR Markers for Switchgrass (*Panicum virgatum* L.)

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## Introduction

Genomic SSRs (Simple Sequence Repeats), or microsatellite markers have the advantages of being highly polymorphic and tending to be widely distributed throughout the genome. Little effort has been made to develop SSRs from enriched genomic libraries in switchgrass. The objective of this study was to develop and characterize a large set of genomic SSR markers for switchgrass.

## Materials and Methods

Four microsatellite-enriched libraries, including core sequences CA-, GA-, CAG- and AAG-libraries were constructed using genomic DNA of 'SL93 7x15', a switchgrass genotype selected in a southern lowland breeding population of Oklahoma State University. The genomic libraries constructed by Genetic Identification Services (GIS, CA), were designated as A, B, C and D.

After spreading out onto LB/X-GAL/IPTG plates and incubation, 764 recombinant clones of each library were picked at random for sequencing at Oklahoma State University Core Facility. The vector sequences were trimmed, and quality information about the base calls of the target genomic sequence were analyzed by Sequence Scanner v1.0 software (Applied Biosystems, CA, USA).

SSRs were detected and primers designed by SSR Locator V.1 software (Maia and Costa de Oliveira, 2008). All primers were checked to identify redundancy. All primer pairs were tested on a panel of two switchgrass genotypes as 'SL93 7x15' and 'NL94 16x13'. Polymorphisms of functional primer pairs were investigated on four switchgrass cultivars, 'Alamo', 'Kanlow', 'Blackwell' and 'Dacotah'.

## Results

The percentages of unique SSR clones in dinucleotide motif libraries A (CA/TG, 70.8%) and B (GA/TC, 76.0%) are higher than trinucleotide motif libraries C (CAG/CTG, 41.7%) and D (AAG/CTT, 55.0%).

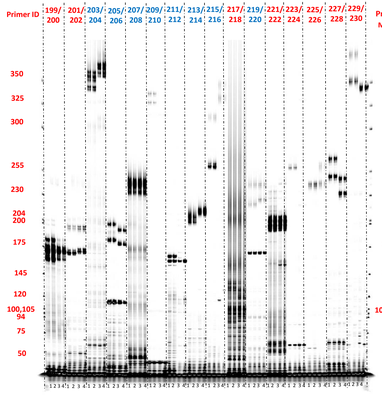


**Figure 1. SSR bands by 16 primer pairs in 2 switchgrass genotypes with 2 reps each**

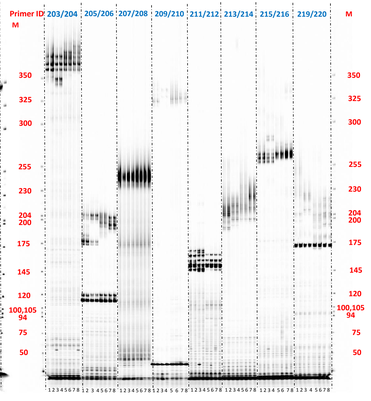


**Figure 2. SSR bands of 8 primer pairs in Alamo, Kanlow, Blackwell, Dacotah with 2 reps from left to right .**

In dinucleotide motif libraries A (78.3%) and B (82.1%), predominant motif of the SSR sequences was the expected type, while trinucleotide- motif libraries C and D, the proportions of the target motif sequence were 41.7% and 37.7%. All four libraries contained a high proportion of perfect repeat structure, ranging from 63.5% to 77.6%. Of the 1660 SSR primer pairs tested, a total of 1183 pairs (71.3%) amplified strong bands with expected fragment size in 'SL93 7x15' and/or 'NL94 16x13'. Major portion of designed SSR primer pairs amplified polymorphic bands (Figure 1 and 2, Table 1). Among 1760 SSR repeats identified, 73% were of perfect type and remaining were compound repeats (Table 2). Table 3 shows majority of the developed SSR markers are targeted repeats.

**Table 1 Efficacy of SSR primer pairs from four enriched libraries**

| Library | Motif | SSR Clones | Primer pairs tested | Functional Primer pairs |
|---|---|---|---|---|
| A | CA/TG | 708 | 532 | 338(63.5%) |
| B | GA/TC | 621 | 514 | 376(73.2%) |
| C | CAG/CTG | 621 | 279 | 220(78.9%) |
| D | AAG/CTT | 593 | 335 | 249(74.3%) |
| Total | | 2568 | 1660 | 1183 |

**Table 2 Frequency of repeat types of the libraries**

| Library | Perfect | | Compound | | | Total |
|---|---|---|---|---|---|---|
| | No. | Ave. repeat no. | No. | Ave. loci no | Ave. repeat no./loci | |
| A | 353 | 20.3 | 203 | 3.1 | 11.1 | 556 |
| B | 410 | 17.7 | 127 | 2.5 | 11.2 | 537 |
| C | 242 | 7.2 | 70 | 3.3 | 7.1 | 312 |
| D | 272 | 13.8 | 84 | 3.1 | 10.7 | 356 |
| Total | 1277 | 14.8 | 484 | 3.0 | 10.0 | 1761 |

**Table 3 Frequencies of motif types in the genomic SSRs isolated from four SSR-enriched libraries**

| Motif | Library A | Library B | Library C | Library D | Total |
|---|---|---|---|---|---|
| AC/GT | 514(51.9%) | 60(8.3%) | 95(20.1%) | 100(18.8%) | 769(28.3%) |
| CA/TG | 262(26.4%) | 38(5.3%) | 40(8.5%) | 52(9.8%) | 392(14.4%) |
| AG/CT | 79(8.0%) | 299(41.4%) | 36(7.6%) | 31(5.8%) | 445(16.4%) |
| GA/TC | 72(7.3%) | 294(40.7%) | 23(4.9%) | 25(4.7%) | 414(15.2%) |
| GCA/TGC | | | 104(22.0%) | 2(0.4%) | 106(3.9%) |
| CAG/CTG | | | 93(19.7%) | 1(0.2%) | 94(3.5%) |
| GAA/TTC | | 4(0.6%) | | 136(25.5%) | 140(5.1%) |
| AAG/CTT | | | | 65(12.2%) | 65(2.4%) |
| Others | 64(6.4%) | 28(3.7%) | 82(17.2%) | 121(22.7%) | 295(10.8%) |
| Total | 991 | 723 | 473 | 533 | 2720 |

## Cited Reference

Luciano Carlos da Maia, Dario Abel Palmieri, and Velci Queiroz de Souza et al. 2008. SSR Locator: tool for simple sequence repeat discovery integrated with primer design and PCR simulation. International Journal of Plant Genomics ,Volume 2008, Article ID 412696, doi:10.1155/2008/412696.