MICROSATELLITE PRIMER REDESIGN FOR LION GENETIC ANALYSIS

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Background

For the lion (*Panthera leo*), genetic research has heavily relied on genomic information originally derived from the domestic cat (*Felis catus*). Domestic cat microsatellites are the primary marker used for nuclear analysis in lions. However, non-specific matching from genomic differences between the lion and domestic cat (10.8 MY divergence) can result in inconsistent amplification creating issues for downstream analysis. To increase specificity and reliability, particularly in the use of samples that may contain degraded DNA, the primers of seventeen microsatellites were redesigned to be closer to the target repeat (miniSTR) using African lion genomic sequences found by both molecular and bioinformatic methods.

Methods

PCR

PCR OPTIMIZATION
WITH KAPA2G ROBUST PCR KIT
USING A TEMPERATURE GRADIENT
(FIGURE 1)

↓

VISUALIZE PCR PRODUCT ON AGAROSE GEL

↓

TOPO® TA CLONING®

↓

SEQUENCING
CAPILLARY ELECTROPHORESIS
WITH ABI 3130

→

ePCR

USING SRA DATA FROM NCBI & CLC GENOMICS WORKBENCH

MAPPED READ TO REFERENCE
USING TIGER GENOME AS REFERENCE

↓

CONSENSUS SEQUENCE CREATED

↓

FIND BINDING SITES & CREATE FRAGMENTS

↓

80-200 BP miniSTRs DESIGNED
USING CLC DESIGN PRIMERS TOOL

Future Directions

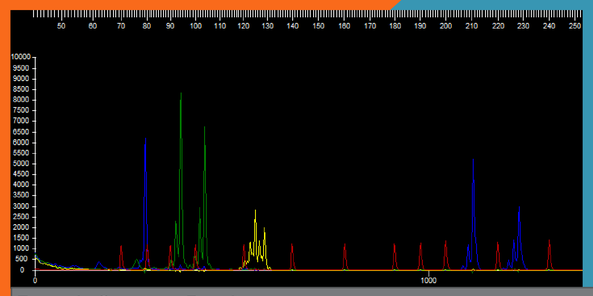
For lions, miniSTRs benefit conservation genetic research and forensics by providing methodology that offers greater amplification success with degraded samples, such as from non-invasive sampling, forensic samples, or historical samples from museums. By using miniSTRs that are based off previously used microsatellites, new data can be combined with or compared directly to previous genetic knowledge.

I am using these miniSTRs in a study comparing modern and historic lion DNA providing baseline genetic data to assist in the development of long-term population management policies.

Candidate microsatellites were chosen based on how many studies used the marker, level of heterozygosity, number of alleles, and allelic richness as determined by the previous studies. These miniSTRs were tested on DNA from 25 lions. DNA was extracted from various sample types (tissue, bone, hide) of different ages (up to 100-years-old). All samples show 100% amplification success.

Original Locus	FCA Size*	Chrom.	Justification	MiniSTR	Leo Size**	H_{OBS}	A	N	Mix
FCA006[1,7,8]	186	D3	Used in 3 studies, 10 alleles, unique chromosome	Leo006	80-130	0.75	11	28	1
FCA008[1,2,3,4,7,8]	142	A1	Used in 6 studies, ave Ho >0.66, 6 alleles, unique chromosome	Leo008	111-133	0.82	7	28	2
FCA026[2,3,4,5,6,7,9]	--	D3	Used in 7 studies, ave Ho >0.69, 9 alleles, no linkage with FCA006	--	--	--	--	--	--
FCA031[2,3,4,8]	247	E3	Used in 4 studies, Ho >0.70, 9 alleles, unique chromosome	Leo031	186-200	0.44	8	27	3
FCA045[3,4,5,6,7,8]	131	D4	Used in 6 studies, ave Ho >0.48, 5 alleles, unique chromosome	Leo045	80-110	0.29	11	28	1
FCA077[1,3,4,5,6,7,8]	146	C2	Used in 7 studies, ave Ho >0.665, ave 7 alleles, unique chromosome	Leo077	98-112	0.89	7	28	2
FCA085[1,2,7,8,9]	136	E2	Used in 5 studies, Ho >0.60, 6 alleles, no known linkage with FCA096 or FCA098	Leo085	72-94	0.64	8	28	2
FCA091[1,7,8,9]	136	B4	Used in 4 studies, 11 alleles, unique chromosome	--	--	--	--	--	--
FCA096[2,5,6,7]	--	E2	Used in 4 studies, Ho >0.74, 5 alleles, no known linkage with FCA085 or FCA098	--	--	--	--	--	--
FCA098[1,7]	117	A2	Used in 2 studies, 8 alleles, no known linkage with FCA085 or FCA096	Leo098	92-112	0.68	7	28	4
FCA126[1-9]	139	B1	Used in 9 studies, ave Ho >0.64, ave 8 alleles, unique chromosome	Leo126	87-149	0.75	8	28	1
FCA224[1,2,7,9]	160	A3	Used in 4 studies, Ho >0.82, 10 alleles, unique chromosome	Leo224	78-96	0.61	6	28	3
FCA230[1,2,7,8]	94	B3	Used in 4 studies, Ho >0.72, 10 alleles, no known linkage with FCA391	Leo230	76-90	0.86	7	28	4
FCA247[1,7,8,9]	147	C1	Used in 4 studies, 8 alleles, unique chromosome	Leo247	114-132	0.82	9	28	3
FCA281[1,7]	232	E1	Used in 2 studies, 12 alleles, unique chromosome	Leo281	107-247	0.68	7	28	1
FCA391[1,2,3,7]	222	B3	Used in 3 studies, ave Ho >0.765, 8 alleles, no known linkage with FCA230	Leo391	170-198	0.71	7	28	2
FCA506[2,3,4]	216	F2	Used in 3 studies, Ho >0.83, 14 alleles, unique chromosome	Leo506	170-227	0.79	10	28	4

Superscript: Study (*Journal*) Location, Number of Lions – **1**: Antunes et al 2008 (*PLOS Genetics*) Range, 357; **2**: Miller et al 2014 (*Journal of Heredity*) RSA, Zimbabwe, 361; **3**: Spong et al 2002 (*Evolutionary Biology*) Tanzania, 70; **4**: Tende et al 2014 (*PLOS One*) Nigeria, 18; **5**: Lyke et al 2013 (*Molecular Ecology*) Namibia, 90; **6**: Dubach et al 2013 (*Conservation Genetics*) Range, 480; **7**: Driscoll et al 2002 (*Genome Research*) Range, 60; **8**: Morandin et al (*Conservation Genetics*) 2014 Zimbabwe, 157; **9**: Bertola et al 2015 (*PLoS One*) Range, 48.



Example Genotype Results:
Electropherogram in STRand for multiplex PCR of Mix 1 (Leo006, Leo045, Leo126, and Leo281).

Example Primer Optimization
Optimal parameters were chosen as 60ºC for Buffer A and 54ºC for Buffer GC for brightness and clarity of bands and a distinct reduction of quality following these bands. PCR product using these parameters were used for cloning FCA224.

Primer pairs were optimized for individual and multiplex PCR and genotyping.