PanPlex, a novel STR kit for big cat's DNA profiling: Implications in population management and wildlife forensics

PanPlex, 대형고양이과 동물의 DNA 정보수집을 위한 새로운 STR 키트: 야생동물 법의학과 개체군 관리에의 적용

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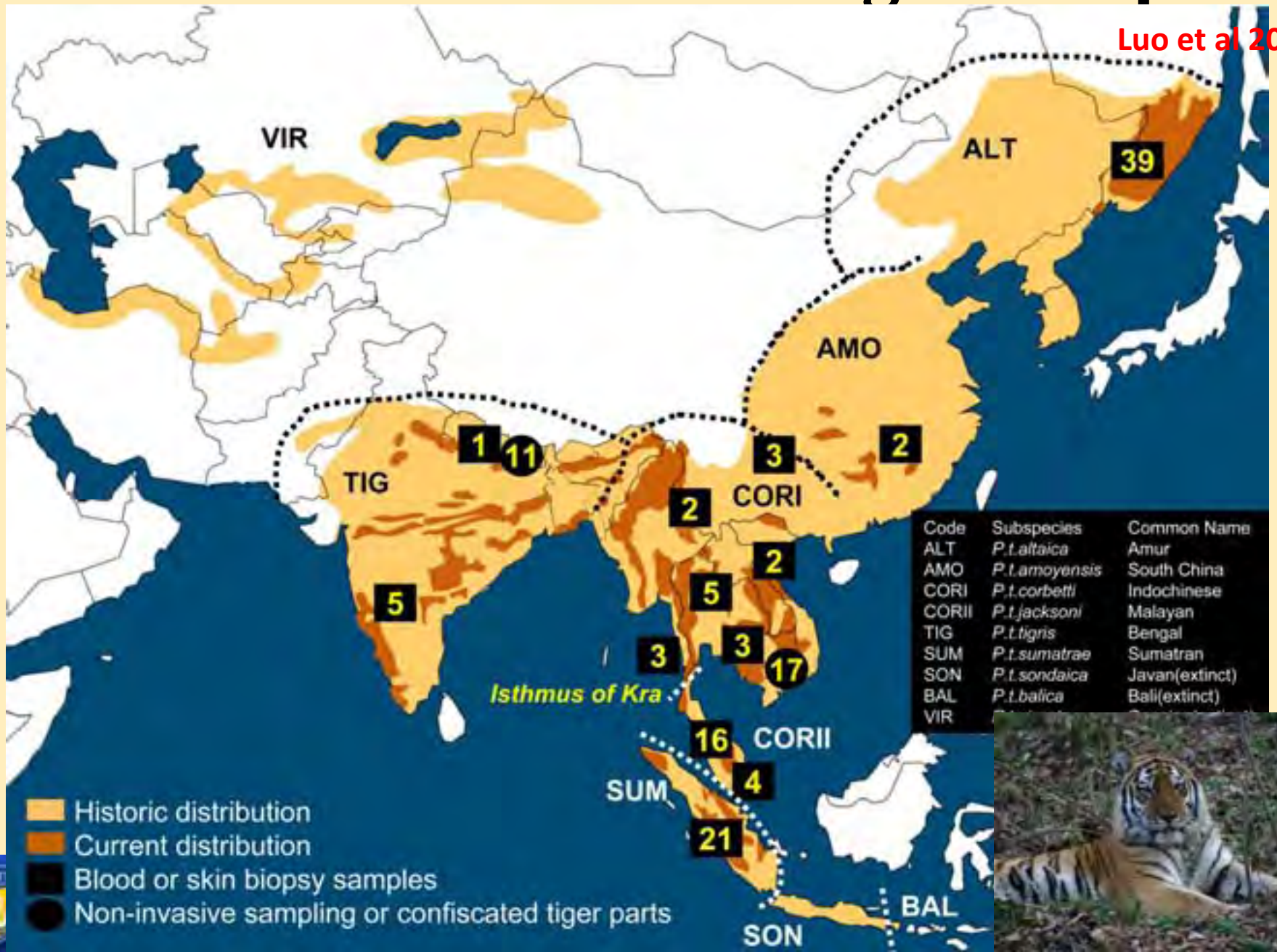
Benefits of the genetic tools for population monitoring

1. Reliability and reproducibility
2. Complement traditional methods (camera trapping etc.)
3. Provides additional information for conservation (Genetic diversity, population structure, gene flow, reintroduction & geographic assignment of confiscated articles, management of captive populations)

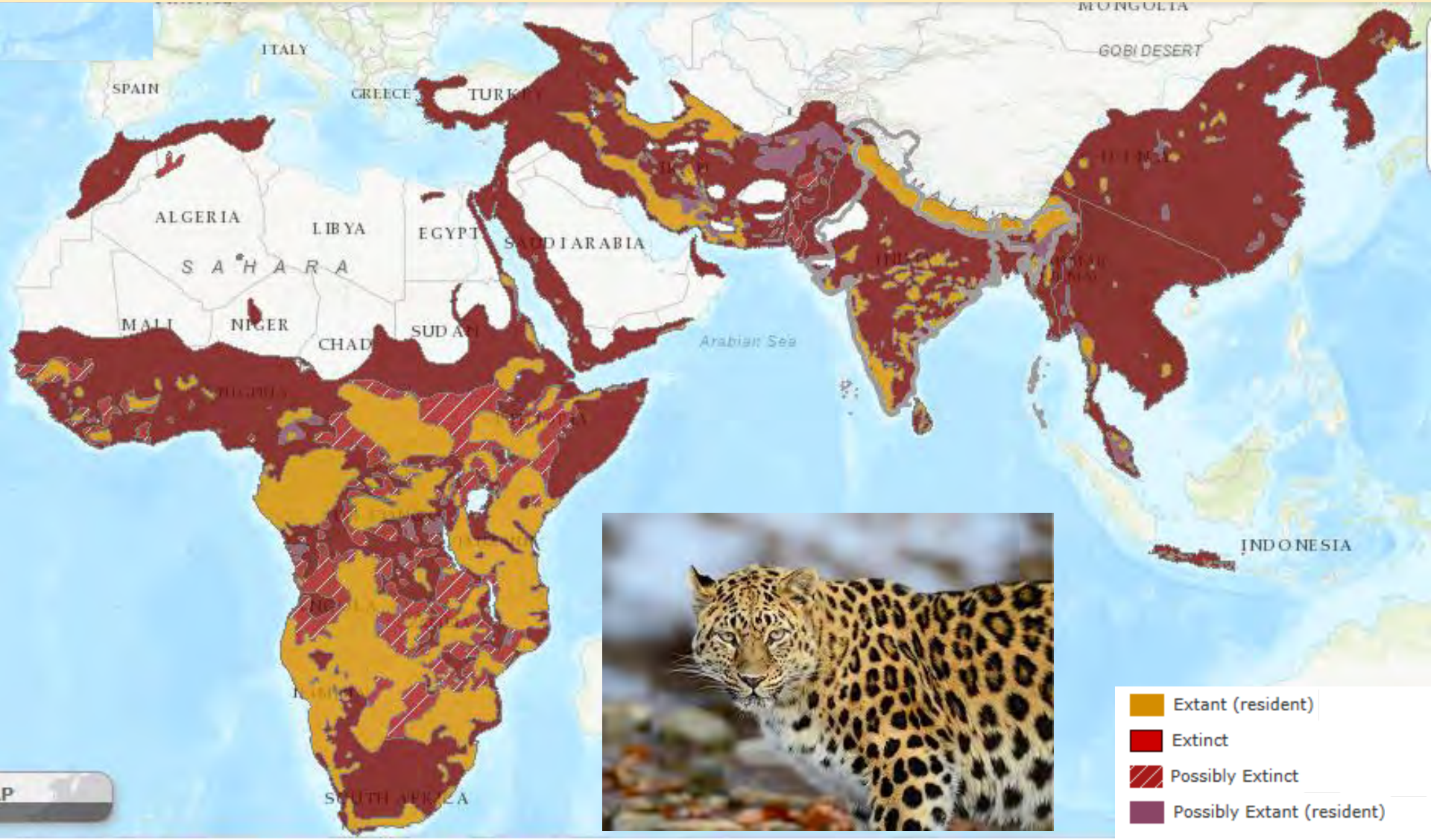
Asian Big Cats

1. Tiger (*Panthera tigris*) – Endangered 호랑이
2. Leopard (*Panthera* piardus) – Vulnerable (P. p. orientalis – Critically endangerd) 표범
3. Lion (*Panthera leo*) – Endangered (only l population in wild) 사자
4. Snow leopard (*Panthera uncia*) – Vulnerable 눈표범
5. Clouded leopard (*Neofelis nebulosa*) – Vulnerable 구름표범

Historical distribution of tiger subspecies



Panthera pardus leopard



Panthera leo lion

SPAIN
GREECE
TURKEY
IRAN
CHINA
HIMALAYA
ALGERIA
LIBYA
EGYPT
SAUDI ARABIA
SAHARA
INDIA
MYANMAR
BURMA
MALI
NIGER
CHAD
SUDAN
Arabian Sea
NIGERIA
DR CONGO
NAMIBIA
SOUTH AFRICA
Ocean

Panthera leo persica
(Gir National Park,
India)

Extant (resident)
Possibly Extinct

Panthera uncia snow leopard

Neofelis nebulosi clouded leopard

Microsatellite markers: Ultimate solution to Wildlife Managers

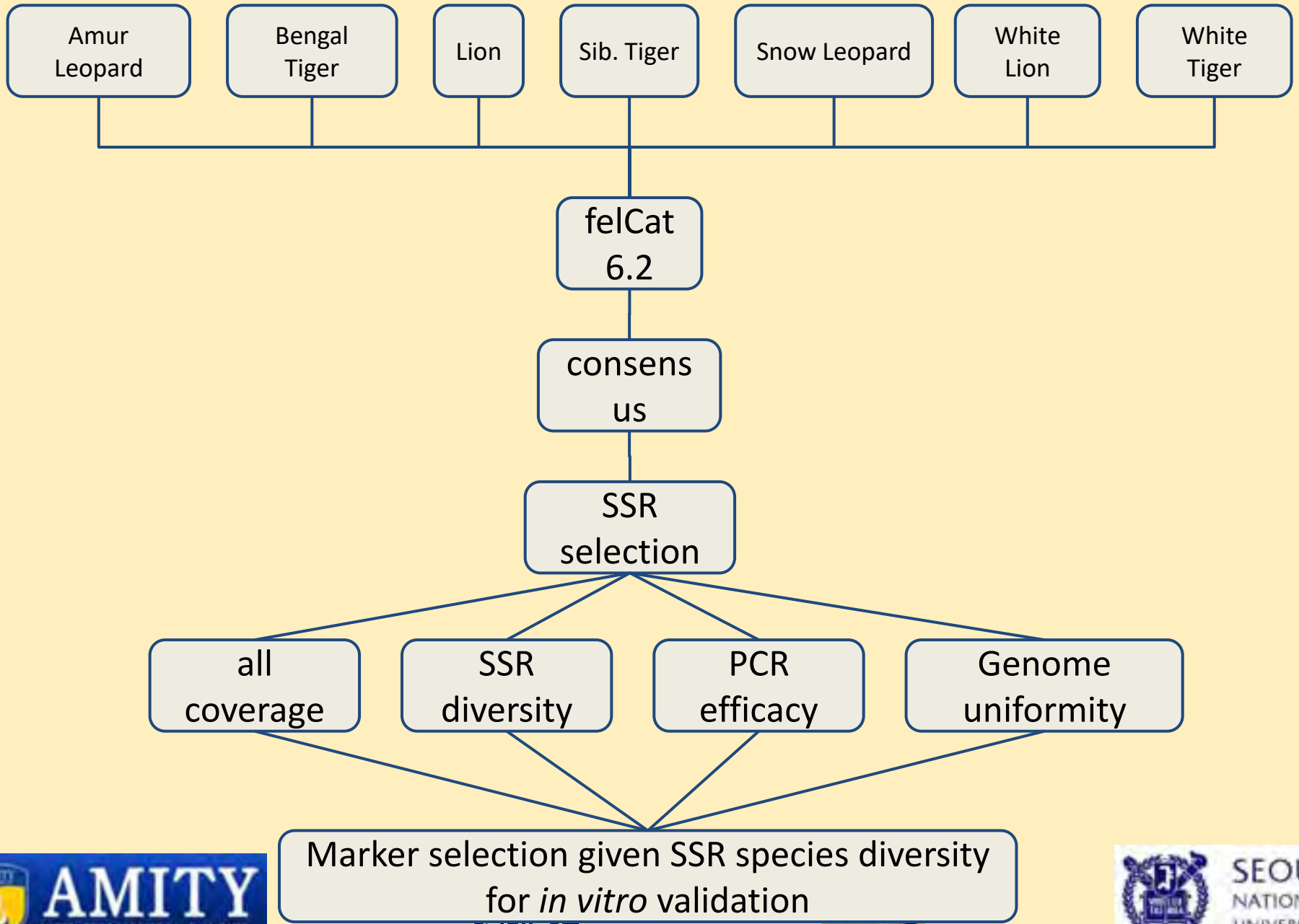
1. **Individual identification** and **population monitoring**
2. **Genetic diversity** Assessment
3. Examination of extent of **gene flow** between populations
4. Selection of potential candidates for **restoring** wild populations in lost ranges
5. Inferring source of origin of wildlife seizures to strengthen **law enforcement**

Challenges in Adopting Microsatellites based techniques for the management of Asian Big Cats

1. Rather complicated technology and high cost
2. Lack of sufficient genetic labs and expertise
3. Lack of consensus on the adoption of standardized markers

Work accomplished

- AIWS, SNU, WII, & UNIST have worked in collaboration to develop novel microsatellite markers by **screening the whole genome sequences** of tiger, leopard, lion, and snow leopard.
- **Developed microsatellites** (n = 32) are uniformly distributed in genome and have showed uniformity in **variability** across the Asian Big Cats species (n = 90).
- Developed microsatellites are optimized in **multiplex PCRs** to achieve simplification of technique and cost effectiveness



Fluorescently labeled microsatellite primers

Primer name	Primer type	Orientation	Seq	Dye Label	Prod size	Motif	Motif Len
Pan1_C2_F	SSR	FORWARD	CCTCAAGGTAACAGCAACA	6FAM	173	CA	2
Pan1_D1_F	SSR	FORWARD	CCTACATCAACATAAACACACC	NED	200	TG	2
Pan1_D2_F	SSR	FORWARD	AAAGGCATGGATACAGTCAG	NED	218	AG	2
Pan10_C2_F	SSR	FORWARD	ACTCCACTTGTCATCATTTGC	NED	160	TG	2
Pan14_C2_F	SSR	FORWARD	GCAAGAACTAAGACTCCAACC	VIC	206	CA	2
Pan15_C2_F	SSR	FORWARD	TTCTGTAGGGTGTGGGTTC	PET	198	CAA	3
Pan16_C2_F	SSR	FORWARD	AAGTCAGGAGAAGATGGATG	PET	204	TTG	3
Pan1A1_F	SSR	FORWARD	CTCCTTATTGTGACCCTGATT	PET	243	TC	2
Pan1A2_F	SSR	FORWARD	GCAGAGGAGGAGAGTATAGATTAG	PET	176	AC	2
Pan1C1_F	SSR	FORWARD	CTTTCTCTCCCTCTTTCTCTCTCT	NED	169	ATC	3
Pan2_D1_F	SSR	FORWARD	TCTTGGTTCCTTCCTCTGT	VIC	165	GAAT	4
Pan2_D2_F	SSR	FORWARD	ACCCACAGACAACCACAC	6FAM	193	TG	2
Pan2A1_F	SSR	FORWARD	AACCCAGAGCCCAACACA	NED	239	TAT	3
Pan2C1_F	SSR	FORWARD	CTCCCATACCCTCACACA	VIC	99	CT	2

Pan3_C2_F	SSR	FORWARD	ATCTGACCCTTATGAGTATGTGAG	6FAM	116	CT	2
Pan3_D1_F	SSR	FORWARD	TCTTGTGGTTCGTGATTTG	VIC	230	CT	2
Pan3_D2_F	SSR	FORWARD	GTGCGTGTGTGTATCTGTG	VIC	171	TG	2
Pan3A1_F	SSR	FORWARD	CTTGCTAATCCTGTGTTTGTC	NED	205	AC	2
Pan3A2_F	SSR	FORWARD	TTTCTGATTCGGCCCTTT	PET	225	AGAC	4
Pan4_D1_F	SSR	FORWARD	CTGTGTCTCCCTGTCTTTGT	PET	167	TC	2
Pan4A1_F	SSR	FORWARD	TTTGGATTTCGTGTAGTGTG	VIC	185	TG	2
Pan4A2_F	SSR	FORWARD	GAGAAGCATTACAAGAAGCA	VIC	151	AACA	4
Pan5_D1_F	SSR	FORWARD	CTTTGTCTCTCAGCTCTTTGT	6FAM	158	AG	2
Pan5A1_F	SSR	FORWARD	CTTCCTCATTCTCTTTGCTCTT	6FAM	204	ATG	3
Pan6_C2_F	SSR	FORWARD	AGAGAAGCCAACCACAAA	6FAM	213	GA	2
Pan6A1_F	SSR	FORWARD	CCAAGTGTCCATCCAAAG	6FAM	166	CA	2
Pan6A2_F	SSR	FORWARD	ATTCTGTCTCTCTGCTCCTC	NED	143	TC	2
Pan7_C2_F	SSR	FORWARD	GGCTCTATTCTATCCCTACACA	VIC	202	TGA	3
Pan7A1_F	SSR	FORWARD	TACATCCCTCCTTCCATCT	NED	177	ATCT	4
Pan8_C2_F	SSR	FORWARD	GATTGTCTCTTTCTCTCCCTCT	PET	134	AAT	3
Pan8A1_F	SSR	FORWARD	GGGTGAAGATGGTGTTGATAG	PET	159	AG	2
Pan9_C2_F	SSR	FORWARD	GGTAGGAGGTGGGAACAT	6FAM	233	AAC	3

Genotyping Results (Initial): Species wise (Na and Ne)

Locus/Species	Number of Alleles				Effective number of Alleles			
	Leopard	Lion	Snow Leopard	Tiger	Leopard	Lion	Snow Leopard	Tiger
Pan6A1	9	5	5	8	5.8	1.5	3.1	5.2
Pan2D2	11	7	4	10	4.8	4.8	1.7	3.5
Pan5A1	6	3	5	4	2.5	2.1	1.7	1.4
Pan9C2	4	3	3	4	1.3	2.4	1.9	1.5
Pan6C2	5	3	3	5	1.5	1.1	1.3	2.4
Pan3C2	5	2	4	4	2.3	1.2	1.5	2.2
Pan1C2	8	4	4	9	6.8	2.3	1.7	2.3
Pan5D1	6	4	6	7	1.9	2.3	3.3	2.6
Pan14C2	7	1	4	5	3.0	1.0	2.8	2.5
Pan7C2	4	2	3	6	1.5	1.5	1.4	2.6
Pan4A1	7	6	7	10	2.6	3.5	5.8	4.3
Pan4A2	4	1	3	3	2.1	1.0	1.5	1.1
Pan2D1	4	2	2	3	1.6	1.5	1.3	1.2
Pan3D2	6	1	1	11	1.9	1.0	1.0	5.5
Pan2C1	3	3	1	4	1.9	1.4	1.0	1.3
Pan3D1	4	2	4	9	1.3	1.1	2.0	4.1

Na= observed number of alleles, Ne= expected number of alleles

Locus/Species	Number of Alleles				Effective number of Alleles			
	Leopard	Lion	Snow Leopard	Tiger	Leopard	Lion	Snow Leopard	Tiger
Pan1C1	7	2	3	7	2.7	1.6	1.7	2.7
Pan2A1	5	4	4	5	4.3	2.9	1.9	2.4
Pan1D2	3	3	3	5	2.1	2.9	2.0	1.2
Pan3A1	5	2	5	4	1.9	1.6	3.8	2.2
Pan6A2	5	4	3	6	2.2	2.3	1.3	2.7
Pan7A1	6	2	3	10	2.9	1.1	1.7	6.0
Pan10C2	4	2	6	4	1.5	1.9	3.8	1.3
Pan1D1	2	2	3	3	1.3	1.9	1.7	1.3
Pan1A1	4	2	7	4	2.0	1.9	2.8	2.6
Pan4D1	8	4	2	7	4.8	1.9	1.2	3.1
Pan15C2	7	5	3	6	3.7	3.7	1.3	1.6
Pan16C2	4	4	5	7	2.3	3.0	3.1	3.1
Pan8C2	3	3	4	7	1.1	2.2	1.8	3.2
Pan8A1	3	1	2	3	1.2	1.0	2.0	1.2
Pan3A2	3	3	1	4	1.5	1.3	1.0	1.7
Pan1A2	8	1	4	3	7.0	1.0	1.7	1.5
Mean	5.31	2.91	3.66	5.84	2.66	1.93	2.06	2.55
S.E.	0.366	0.263	0.375	0.426	0.282	0.162	0.182	0.231

Genotyping Results (Initial): Species wise (Ho and He)

Locus	Leopard		Lion		Snow leopard		Tiger	
	Ho	He	Ho	He	Ho	He	Ho	He
6A1	0.5	0.8	0.4	0.3	0.3	0.7	0.6	0.8
2D2	0.6	0.8	0.7	0.8	0.1	0.4	0.4	0.7
5A1	0.4	0.6	0.2	0.5	0.3	0.4	0.1	0.3
9C2	0.0	0.2	0.3	0.6	0.4	0.5	0.1	0.3
6C2	0.0	0.3	0.1	0.1	0.1	0.2	0.3	0.6
3C2	0.3	0.6	0.0	0.1	0.3	0.3	0.3	0.5
1C2	0.5	0.9	0.4	0.6	0.1	0.4	0.2	0.6
5D1	0.4	0.5	0.3	0.6	0.3	0.7	0.4	0.6
14C2	0.4	0.7	0.0	0.0	0.4	0.6	0.4	0.6
7C2	0.3	0.3	0.0	0.3	0.3	0.3	0.5	0.6
4A1	0.5	0.6	0.8	0.7	0.7	0.8	0.6	0.8
4A2	0.5	0.5	0.0	0.0	0.1	0.3	0.1	0.1
2D1	0.0	0.4	0.4	0.3	0.0	0.2	0.1	0.2
3D2	0.3	0.5	0.0	0.0	0.0	0.0	0.8	0.8
2C1	0.3	0.5	0.1	0.3	0.0	0.0	0.0	0.2
3D1	0.0	0.2	0.1	0.1	0.1	0.5	0.7	0.8

Ho= observed heterozygosity, He= expected heterozygosity

Locus	Leopard		Lion		Snow leopard		Tiger	
	Ho	He	Ho	He	Ho	He	Ho	He
6A1	0.5	0.8	0.4	0.3	0.3	0.7	0.6	0.8
1C1	0.3	0.6	0.5	0.4	0.0	0.4	0.1	0.6
2A1	0.4	0.8	0.6	0.7	0.3	0.5	0.3	0.6
1D2	0.2	0.5	0.6	0.7	0.0	0.5	0.1	0.2
3A1	0.1	0.5	0.0	0.4	0.9	0.7	0.1	0.6
6A2	0.3	0.5	0.3	0.6	0.1	0.2	0.4	0.6
7A1	0.6	0.7	0.1	0.1	0.1	0.4	0.7	0.8
10C2	0.0	0.3	0.4	0.5	0.3	0.7	0.0	0.2
1D1	0.1	0.3	0.6	0.5	0.0	0.4	0.1	0.2
1A1	0.2	0.5	0.4	0.5	0.5	0.6	0.5	0.6
4D1	0.8	0.8	0.5	0.5	0.2	0.2	0.6	0.7
15C2	0.7	0.7	0.6	0.7	0.1	0.3	0.3	0.4
16C2	0.6	0.6	0.7	0.7	0.4	0.7	0.5	0.7
8C2	0.0	0.1	0.5	0.5	0.1	0.5	0.4	0.7
8A1	0.0	0.2	0.0	0.0	0.0	0.5	0.0	0.2
3A2	0.2	0.3	0.1	0.2	0.0	0.0	0.2	0.4
1A2	0.6	0.9	0.0	0.0	0.1	0.4	0.0	0.4
Mean	0.32	0.52	0.30	0.38	0.21	0.42	0.32	0.51
S.E.	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04

Genotyping Results (Initial): Polymorphic Information Content

Locus	Leopard	Lion	Snow leopard	Tiger	Locus	Leopard	Lion	Snow leopard	Tiger
6A1	0.80	0.30	0.63	0.78	**1C1**	0.60	0.31	0.37	0.59
2D2	0.77	0.76	0.39	0.69	**2A1**	0.73	0.60	0.44	0.51
5A1	0.54	0.44	0.40	0.25	**1D2**	0.43	0.58	0.45	0.19
9C2	0.21	0.49	0.43	0.32	**3A1**	0.42	0.29	0.69	0.47
6C2	0.31	0.12	0.22	0.53	**6A2**	0.50	0.51	0.22	0.57
3C2	0.52	0.13	0.31	0.49	**7A1**	0.62	0.07	0.35	0.81
1C2	0.84	0.50	0.39	0.54	**10C2**	0.31	0.36	0.70	0.21
5D1	0.43	0.52	0.66	0.59	**1D1**	0.22	0.37	0.37	0.23
14C2	0.61	0.00	0.59	0.51	**1A1**	0.45	0.36	0.63	0.56
7C2	0.30	0.27	0.27	0.54	**4D1**	0.77	0.45	0.16	0.64
4A1	0.58	0.68	0.80	0.73	**15C2**	0.70	0.68	0.24	0.37
4A2	0.42	0.00	0.29	0.05	**16C2**	0.54	0.60	0.64	0.66
2D1	0.34	0.27	0.20	0.15	**8C2**	0.09	0.44	0.43	0.65
3D2	0.44	0.00	0.00	0.79	**8A1**	0.17	0.00	0.38	0.16
2C1	0.42	0.27	0.00	0.20	**3A2**	0.29	0.21	0.00	0.38
3D1	0.20	0.07	0.46	0.72	**1A2**	0.84	0.00	0.39	0.39

Thank you for listening!

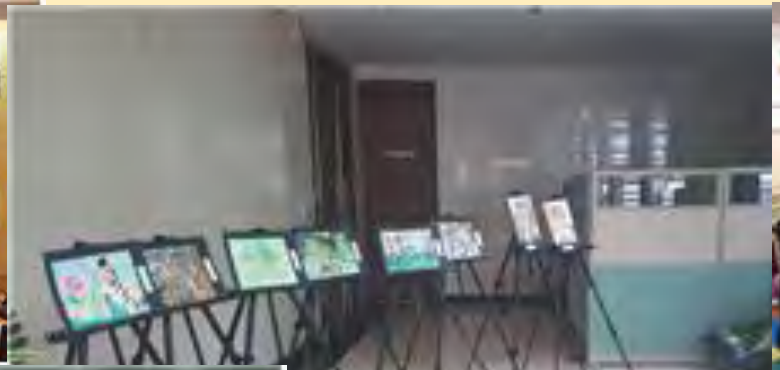
Questions?

Tiger and Leopard Conservation Campaign: Children's Tiger Drawing Contest


제 5회 한국-러시아
어린이 호랑이그리기 대회 시상식
일시 2017년 5월 13일 (토)
주최 (사)한국범보전기금, 피닉스기금
협력
FANTASTIC

SEOUL NATIONAL UNIVERSITY


"응답하라 호랑이!"




어린이 호랑이 표범 그림 전시회