Mining microsatellites markers for big cats (Genus: *Panthera*; Oken 1816) using next generation sequencing data

Why?

- Except for Tigers and Lions, species specific microsatellite markers were not developed for big cats
- Most of the genetic studies have used heterologous markers (mainly *Felis catus* microsatellite markers)
- Heterologous markers show less polymorphism with greater chances of null alleles (Lopes et al 2010)

A need for microsatellite marker development

TIGER

LEOPARD

Microsatellite development using **whole genome sequencing data** is not only **efficient** but also **cost effective** as compared to traditional approaches (Vartia *et al.*, 2014).

LION

SNOW LEOPARD

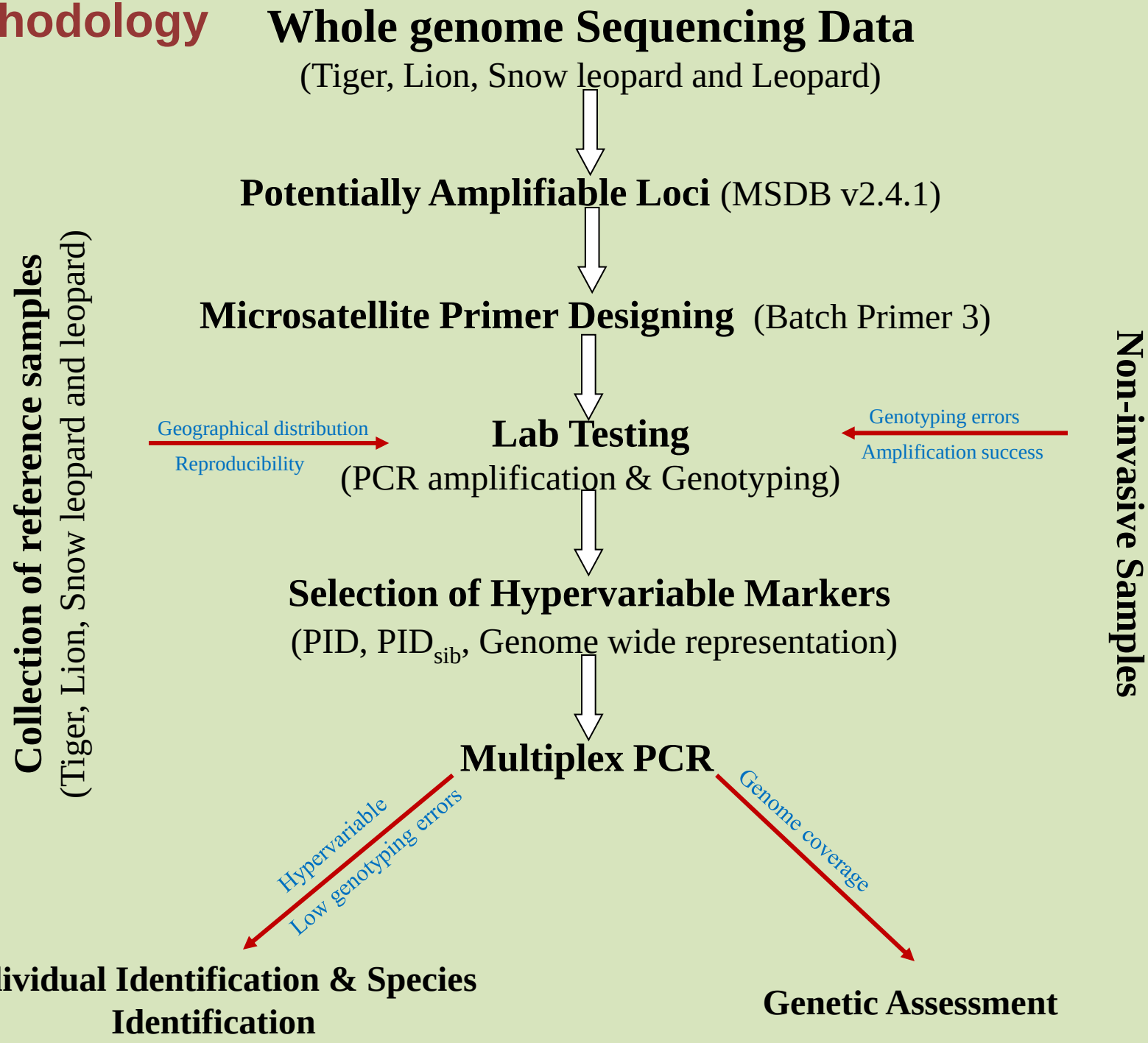
Objectives

- Screening and selection of **polymorphic microsatellite markers** with conserved flanking regions across big cats
- Optimization of polymorphic markers to develop **multiplex PCR system** for species, sex, individual identification; and for genetic assessment (with wider genome coverage) of all big cat species and subspecies

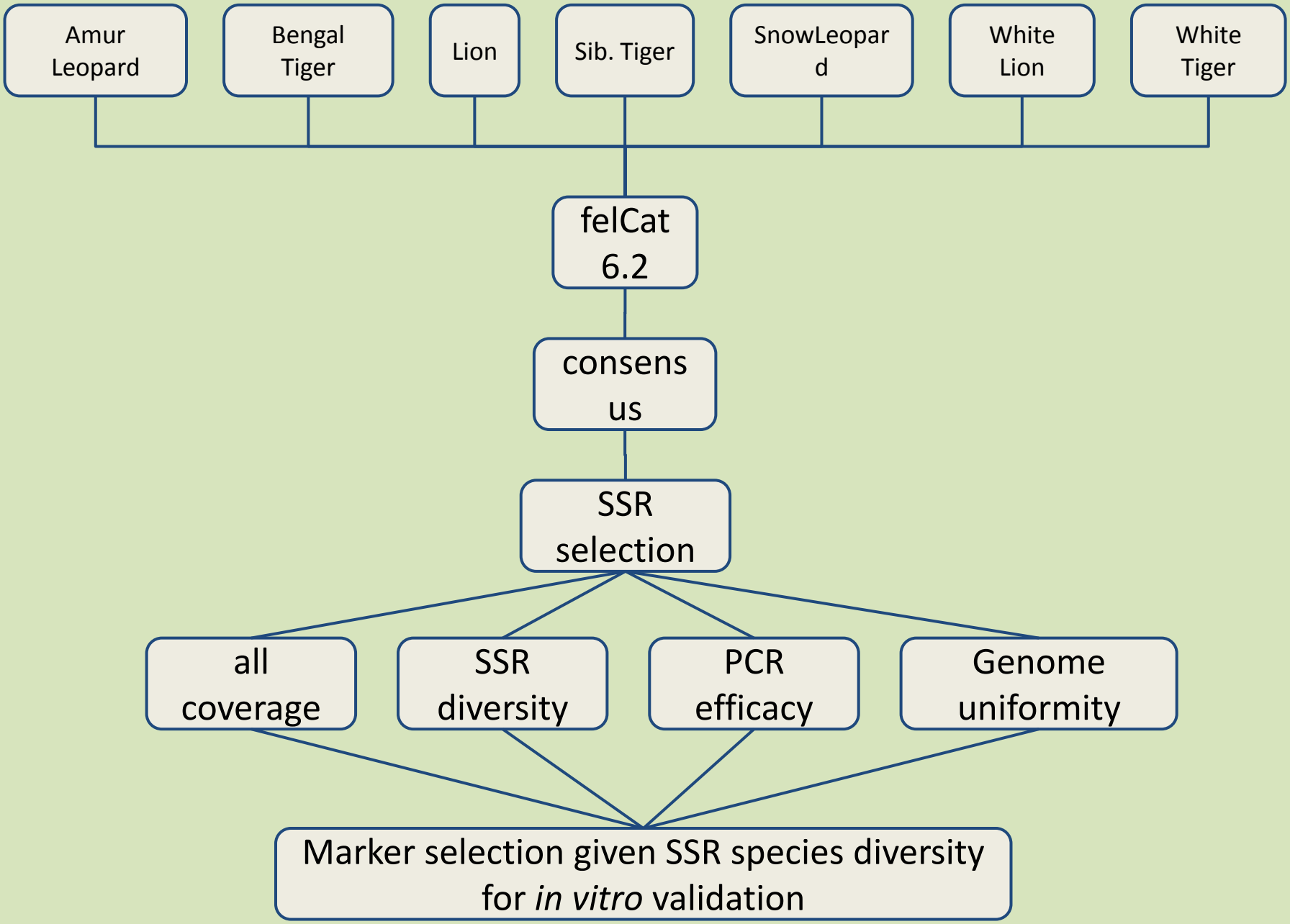
Benefits

- A multi-utility **simplified multiplex PCR kit** (like human identification kit, Canine or Bovine genotyping kit) approach that may be used by veterinarians or biologist in zoological parks, natural reserves etc.
- Selection and optimization of hypervariable markers in multiplex PCR will **reduce cost of sample processing**.
- Use of uniform microsatellite markers will lead to development of **consensus database** and such data may be used for future conservation studies.

Methodology



Initial Findings



Initial Findings

	3uniq/7	4uniq/7	5uniq/7	6uniq/7
Possible Variants (Union 5 samples)	80474871	80474871	80474871	80474871
Filtered (no het, DP>4, #uniqAlleles, INDEL)	282202	49107	8947	1261
Unique Variant Positions	234310	37659	6283	822
All sample cov (DP>4 for 300bp region: +-150 bp flanking)	75398	14807	3026	396
Total Variants Within Regions	825946	174127	31849	4529
Consensus Generation	62526	12483	2614	351

Initial Findings

- Microsatellite primer selection and designing

Microsatellite Repeat	Batch Primer 3 (default settings)	Primers screened (no secondary structure and high GC content)
Di-nucleotides	704	45
Tri-nucleotides	155	16
Tetra-nucleotides	179	20
Penta-nucleotides	43	2
Hexa-nucleotides	12	0

Way Ahead

- **Reference sample collection** (different species, subspecies) across the distribution range
- **PCR validation of primers** and **optimization of multiplex PCR system**
- Collection of **fecal samples** (wild) to applicability of developed marker system for species identification and genetic assessment

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