

**Supplementary material for 'Genetic diversity and origin of captive lion (*Panthera leo*) in South Africa: an assessment and comparison to wild populations'**

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**File S1. Questions from National Captive Lion Survey of privately-owned captive lions in South Africa (Williams & 't Sas-Rolfes, 2019) that were relevant to this study and an explanation of the additional information that was extracted for this study.**

Q5. What year did the facility open?

*Linked to Q30 – details below*

29. From where has the current lion stock in the facility been sourced: (select all that apply to your facility)

- Lions bred in this facility
- Lions from breeders in South Africa
- Lions from breeders elsewhere in Africa
- Lions from breeders elsewhere
- Wild-sourced lions from South Africa
- Wild-sourced lions from elsewhere in Africa
- Do not know
- Other\*
- Other (please specify)

*Summarised in Figure 1b*

30. From where was the original lion stock for the facility sourced: (select all that apply to your facility)

- Lions bred in this facility
- Lions from breeders in South Africa
- Lions from breeders elsewhere in Africa
- Lion breeders elsewhere
- Circuses
- Wild-sourced lions from South Africa
- Wild-sourced lions from elsewhere in Africa
- Do not know
- Other\*
- Other (please specify)

*Summarised in four-year increments based on the answers to Q5 – Figure 1a*

36. Has the facility ever introduced wild lions into its breeding stock?

If yes, indicate when, where and/or which populations(s) the stock was from

*Used to supplement answers from Q29 and Q30 to capture any wild lion introductions that were not part of the original stock and are no longer present in the facility.*

Williams, V. L., & 't Sas-Rolfes, M. J. (2019). Born captive: A survey of the lion breeding, keeping and hunting industries in South Africa. *PLOS ONE*, 14(5), e0217409.  
<https://doi.org/10.1371/journal.pone.0217409>

**File S2.** Breakdown of data available, data manipulations and dataset used for each analysis based on Table/Figure numbers

**Data**

| <b>Source</b>                          | <b>Genotypes original</b> | <b>Genotypes 13</b> | <b>Subset unrelated at 0.25 level</b> |
|--|---------------------------|---------------------|---------------------------------------|
| Standardisation exercise               | 39                        | 39                  |                                       |
| VGL – captive                          | 38                        | 38                  |                                       |
| Unistel – captive                      | 729                       | 704                 |                                       |
| SANBI – captive                        | 120                       | 0*                  |                                       |
| <b>Total captive</b>                   | <b>926</b>                | <b>781</b>          | <b>128</b>                            |
| VGL – Kruger NP                        | 54                        | 51                  |                                       |
| VGL – Kgalagadi TP                     | 11                        | 10                  |                                       |
| VGL – Pilansberg NP (Etosha NP origin) | 26                        | 25                  |                                       |
| VGL – Mapungubwe TFCA                  | 10                        | 9                   |                                       |
| <b>Total SA open systems</b>           | <b>101</b>                | <b>95</b>           |                                       |
| <b>VGL - metapopulation</b>            | <b>242</b>                | <b>170</b>          |                                       |

\*not enough overlapping markers to include existing SANBI genotypes in most analyses

**Analyses**

Table 1 – Summary statistics: Genotypes 13 – Kruger NP, Total captive and subset captive, also included SANBI captive for markers where data was available

Figure 2 – STRUCTURE analysis: Genotypes 13– all open systems; Subset – Total captive

Figure 4 – Individual heterozygosity: Genotypes 13 – Total captive, Kruger NP, metapopulation

Figure 5 – Relatedness connectivity: Genotypes 13 – metapopulation; Subset – Total captive

Figure 6 – Relatedness box plot: Genotypes 13 – Kruger NP, metapopulation; Subset – Total captive

Figure 7 – PCA: Genotypes 13 – Total captive, Total SA open systems

Figure 8 – Assignment test: Genotypes 13 – Total captive, Total SA open systems

**Table S1** Microsatellite markers used for lion genotypes at all three laboratories and correction factors developed for the three laboratories.  
 \* indicates used in retrospective analysis of captive genotypes

| Microsat | VGL | Unistel | SANBI | Correction Factors |        | Notes   |
|----------|-----|---------|-------|--------------------|--------|---|
|          |     |         |       | Unistel            | SANBI  |   |
| FCA057*  | Y   | Y       | Y     | +10                | +7     |   |
| FCA275*  | Y   | Y       | Y     | +9                 | +6     |   |
| FCA097*  | Y   | Y       |       | +10                | +8     |   |
| FCA224*  | Y   | Y       | Y     | +10                | +4     |   |
| FCA391*  | Y   | Y       | Y     | +9                 | +6     |   |
| FCA453*  | Y   | Y       | Y     | +6                 | +7     |   |
| FCA026*  | Y   | Y       | Y     | +10/+12            | +8/+10 | A shift from odd to even; likely caused by a 1bp insertion  |
| FCA240*  | Y   | Y       |       | +10                |        | X-linked; useful for parentage  |
| FCA272*  | Y   | Y       |       | +9                 |        |   |
| FCA506*  | Y   | Y       |       | +11                |        |   |
| FCA628*  | Y   | Y       |       | +11                |        |   |
| F42*     | Y   | Y       |       | +5                 |        |   |
| FCA031*  | Y   | Y       |       | +10                |        |   |
| FCA075   | Y   | Y       |       | +11/+9             |        | One small allele that was rounded up at VGL and down at Unistel; useful going forward                                 |
| FCA105   | Y   | Y       |       |                    |        | No longer used at VGL as linked to FCA113, but with FCA113 removed, we could reinstate it; historically used at SANBI |
| FCA096   | Y   |         | Y     |                    | +6     | Historic challenges at Unistel  |
| FCA113   | Y   | Y       |       |                    |        | Some single bp repeats, hard to standardize   |
| FCA126   | Y   | Y       | Y     |                    |        | Rounding issues at larger alleles   |
| FCA001   | Y   | Y       |       |                    |        | Rounding issues at lower end; also a “messy” marker, not great for standardization                                    |
| FCA230   | Y   | Y       |       |                    |        | Does not amplify consistently at VGL or Unistel   |
| FCA310   | Y   | Y       | Y     |                    |        | No longer used at VGL due to linkage with other, more informative markers   |
| FCA069   | Y   | Y       | Y     |                    |        | No longer used at VGL due to linkage with other, more informative markers   |
| FCA085   | Y   |         | Y     |                    |        | No longer used at VGL due to linkage with other, more informative markers   |
| FCA441   | Y   |         |       |                    |        | Historically used at SANBI  |
| FCA193   | Y   |         | Y     |                    |        |   |
| FCA008   | Y   |         | Y     |                    |        |   |

| Microsat | VGL | Unistel | SANBI | Correction Factors |       | Notes |
|----------|-----|---------|-------|--------------------|-------|-------|
|          |     |         |       | Unistel            | SANBI |       |
| F37      | Y   |         |       |                    |       |       |
| FCA719   |     |         | Y     |                    |       |       |
| FCA247   |     |         | Y     |                    |       |       |
| FCA171   |     |         | Y     |                    |       |       |
| FCA211   |     |         | Y     |                    |       |       |
| FCA212   |     |         | Y     |                    |       |       |
| FCA139   |     |         | Y     |                    |       |       |
| FCA077   |     |         | Y     |                    |       |       |
| FCA032   |     |         | Y     |                    |       |       |
| FCA043   |     |         | Y     |                    |       |       |
| FCA014   |     |         | Y     |                    |       |       |
| FCA005   |     |         | Y     |                    |       |       |

**Table S2** PCR protocol for microsatellite marker amplification of samples in the standardization exercise.

| Panel 1 | Dye label | Concentration | Size Range |
|---------|-----------|---------------|------------|
| FCA230  | FAM       | 0.08          | 98-116     |
| FCA026  | FAM       | 0.07          | 135-152    |
| FCA096  | FAM       | 0.06          | 200-218    |
| FCA193  | VIC       | 0.07          | 111-119    |
| FCA057  | VIC       | 0.08          | 164-180    |
| FCA275  | NED       | 0.07          | 128-136    |
| FCA506  | NED       | 0.07          | 186-233    |
| ZnF     | PET       | 0.07          |            |
| Panel 2 |           |               |            |
| FCA272  | FAM       | 0.08          | 105-111    |
| FCA224  | FAM       | 0.08          | 158-177    |
| FCA097  | VIC       | 0.08          | 144-164    |
| FCA453  | VIC       | 0.06          | 192-204    |
| FCA001  | NED       | 0.08          | 133-169    |
| F41     | PET       | 0.07          | 101-110    |
| F42cht  | PET       | 0.07          | 234-249    |
| Panel 3 |           |               |            |
| FCA240  | FAM       | 0.08          | 189-211    |
| FCA031  | FAM       | 0.06          | 239-251    |
| FCA628  | VIC       | 0.08          | 108-119    |
| FCA113  | VIC       | 0.06          | 149-159    |
| FCA075  | NED       | 0.06          | 105-137    |
| FCA391  | NED       | 0.07          | 201-233    |
| FCA126  | PET       | 0.07          | 186-222    |

PCR conditions: 10µl reaction with 2x Kapa Master Mix, 1µl DNA

Thermocycler set to:

95°C for 3 minutes

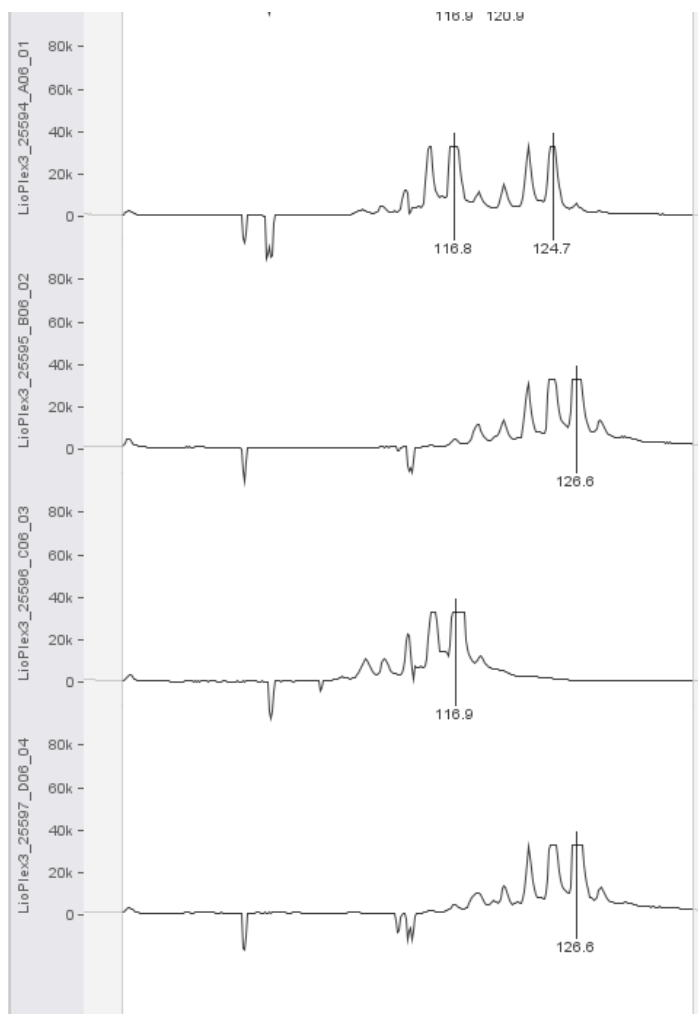
30 cycles of: 95°C for 15 seconds

59°C for 30 seconds

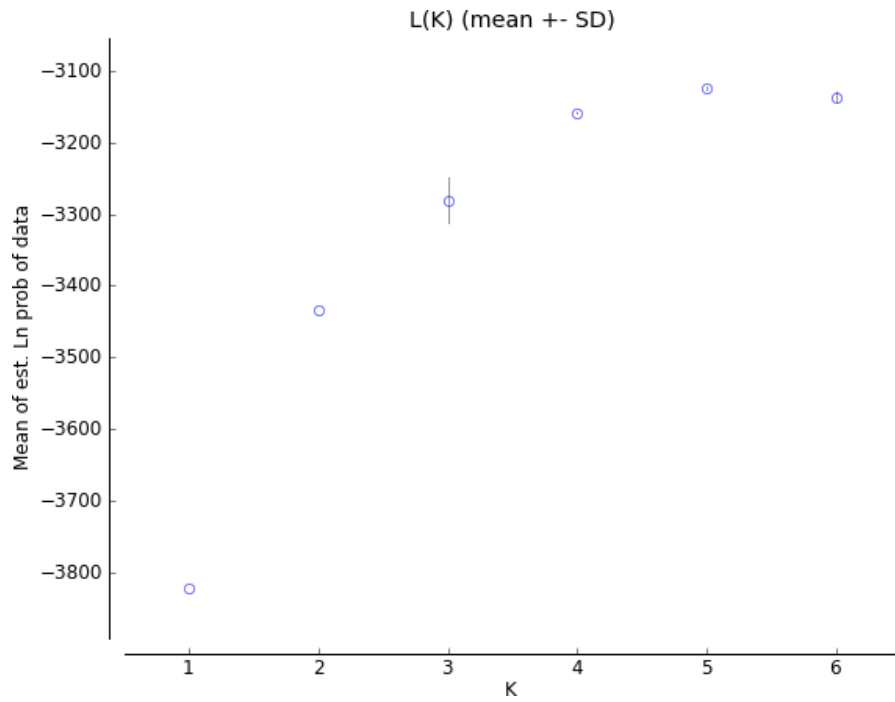
72°C for 30 seconds

72°C for 10 minutes

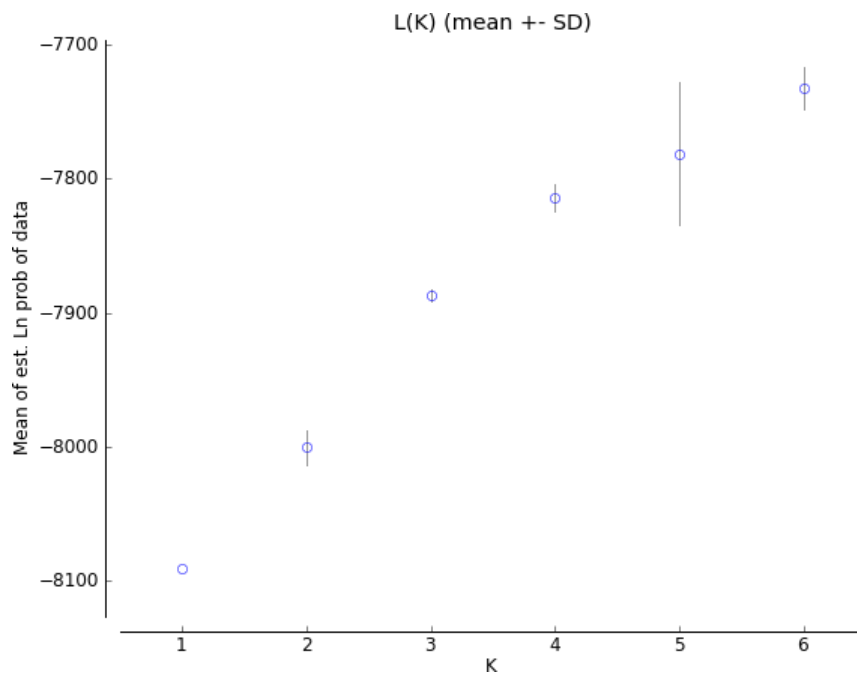
Hold at 4°C



**Fig S1.** Example of traces of sheared off microsatellite allele peaks for FCA075. The largest peaks are sheared off and thus look similar in size/height to the stutter peaks immediately to the left. It is therefore impossible to reliably tell individuals that are homozygous apart from those that are heterozygous for two alleles that are only two base pairs apart.



**Fig. S2a** Plot of the mean of the estimated Ln of the probability for the Open System STRUCTURE run (Figure 1) as created by Structure Harvester.



**Fig. S2b** Plot of the mean of the estimated Ln of the probability for the Captive STRUCTURE run (Figure 5) as created by Structure Harvester