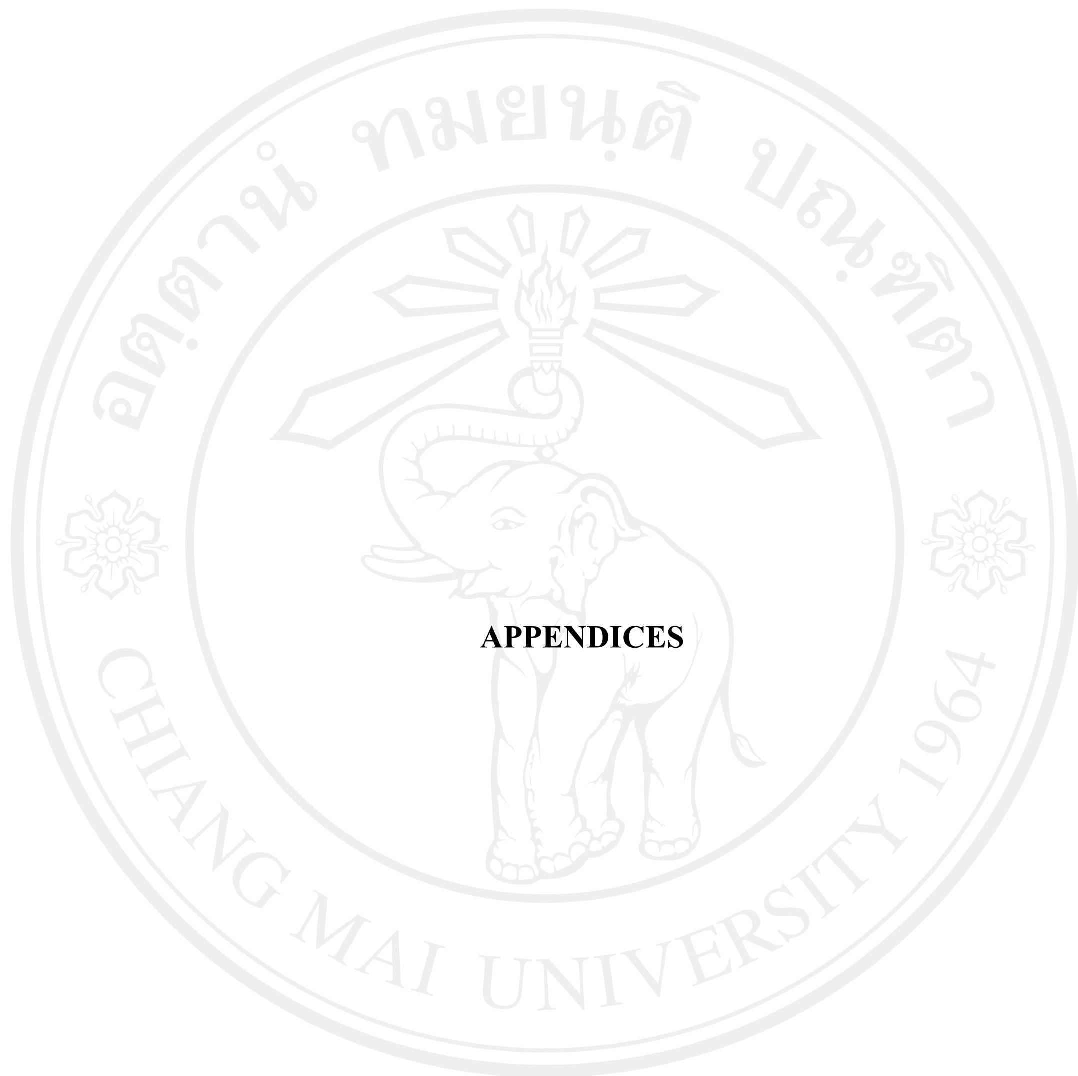

APPENDICES

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APPENDIX A

The Existed Elephants

The ancestor of the elephants, *Moeritherium*, first presented in 55-60 million years ago in *Eocine* era and its fossil was found in Africa. The most of elephant species were extinct. Only two existing elephants are left in nowadays, African elephants (*Loxodonta africana)* and Asian elephants (*Elephas maximus*). These elephants were classified their taxonomy as the following details.

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vetebrata
Class	Mammalia
Subclass	Eutheria
Order	Probosidae
Suborder	Paenangulata
Family	Elephantidae
Species	*Elephas maximus* (Asian elephant)
	Loxodanta africana (African bush elephant)
	Loxodomta cyclotis (African forest elephant)

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The Asian elephants are subdivided into three subspecies, according to their morphological characteristic and distribution in different ranges. Sri Lankan subspecies, *Elephas maximus maximus*, the biggest Asian elephants are living in Sri Lunka which have dark colored body. Mainland or Indian subspecies, *Elephas maximus indicus*, the elephants are found in Indian subcontinent, Southeast Asia and Peninsular Malaysia. The last subspecies is Sumatran, *Elephas maximus sumatranus*, the smallest Asian elephants are found in Sumatra in Indonesia and which have light colored body.

Fernando *el at* (2003b) reported the unique subspecies of Asian elephant living in Borneo. They found that the Borneo elephants were different to the other subspecies by genetic analyses. There indicated the highly significant divergence in Borneo elephant which compared to other subspecies, that mean the Borneo's elephants are indigenous to Borneo, *Elephas maximus borneensis*. The new information is rejects the hypothesis, the Borneo elephants were introduced from the continent range. The Borneo elephants is similar to Indian or Sumatran subspecies because of there ever been presented under those subspecies with used morphological base before, belief that the origins of the Borneo elephant were mainland and peninsular Malaysia or Sumatra. The morphological data is the inadequacy of description, they suggest that a formal reinstatement of the *E. m. borneensis* taxa await a detailed morphological analysis of Borneo elephants and their comparison with other subspecies.

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APPENDIX B

Blood and Body Fluid Spin Protocol

QIAamp® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook

Page 27-29

Blood and Body Fluid Spin Protocol

- Equilibrate samples to room temperature (15–25°C).
- Heat a water bath or heating block to 56°C for use in step 4.
- Equilibrate Buffer AE or distilled water to room temperature for elution in step 10.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared
according to the instructions on page 24.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.
- All centrifugation steps should be carried out at room temperature.
- Use carrier DNA if the sample contains <10,000 genome equivalents
- 200 µl of whole blood yields 3–12 µg of DNA. Preparation of buffy coat is recommended if a higher yield is required.

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1. Pipet 20 µl QIAGEN Protease (or Proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.

2. Add 200 µl sample to the microcentrifuge tube. Use up to 200 µl whole blood, plasma,serum, buffy coat, or body fluids, or up to 5 x 106 lymphocytes in 200 µl PBS.

If the sample volume is less than 200 µl, add the appropriate volume of PBS. QIAamp Spin Columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

Note: It is possible to add QIAGEN Protease (or Proteinase K) to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.

In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. If the sample volume is larger than 200 µl, increase the amount of QIAGEN Protease (or Proteinase K) and Buffer AL proportionally; e.g., a 400 µl sample will require 40 µl QIAGEN Protease (or Proteinase K) and 400 µl Buffer AL. If sample volumes larger than 400 µl are required, use of QIAamp DNA Blood Midi or Maxi Columns is recommended; these can process up to 2 ml or up to 10 ml of sample, respectively.

Note: Do not add QIAGEN Protease or Proteinase K directly to Buffer AL.

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4. Incubate at 56°C for 10 min.

DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation

times have no effect on yield or quality of the purified DNA.

5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

6. Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

If the sample volume is greater than 200 µl, increase the amount of alcohol proportionally; e.g., a 400 µl sample will require 400 µl of alcohol.

7. Carefully apply the mixture from step 6 to the QIAamp Spin Column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.

Close each spin column in order to avoid aerosol formation during centrifugation. Centrifugation is performed at 6000 x *g* (8000 rpm) in order to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Spin Column is empty.

Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

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8. Carefully open the QIAamp Spin Column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.

It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200 µl.

9. Carefully open the QIAamp Spin Column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min. Continue directly with step 10, or to eliminate any chance of possible Buffer AW2 carryover, perform step 9a, and then continue with step 10.

Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Buffer AW2, coming into contact with the QIAamp Spin Column. Removing the QIAamp Spin Column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp Spin Column. In these cases, the optional step 9a should be performed.

9a. (Optional): Place the QIAamp Spin Column in a new 2 ml collection tube (not provided) and discard the collection tube with the filtrate. Centrifuge at 20,000 x g (14,000 rpm) for 1 min.

10. Place the QIAamp Spin Column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Spin Column and add 200 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.

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Incubating the QIAamp Spin Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield. A second elution step with a further 200 µl Buffer AE will increase yields by up to 15%. Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. For samples containing less than 1 µg of DNA, elution in 50 µl Buffer AE or water is recommended. Eluting with 2 x 100 µl instead of 1 x 200 µl does not increase elution efficiency. For long-term storage of DNA, eluting in Buffer AE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis. A 200 µl sample of whole human blood (~5 x 106 leukocytes/ml) typically yields 6 µg of DNA in 200 µl water (30 ng/µl) with an *A*260/*A*280 ratio of 1.7–1.9. For more information about elution and how to determine DNA yield, purity, and length.

Preparation of Buffy Coat

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500 x *g* for 10 min at room temperature. After centrifugation, three different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

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APPENDIX C

Protocol of Gene Scan

Mixture per one sample for PCR

Milli Q water	3.24	μl
M13 forward primer (1 pmol/μl)	0.2	μl
Reverse primer (10 pmol/ μl)	0.25	μl
M13 FAM (10 pmol/ μl)	0.25	μl
PCR buffer, without $MgCl_2$ (gold)	1.5	μl
$MgCl_2$ (gold) 25 mM	1.5	μl
DNTPs (1 mM)	3	μl
Amplified Taq (gold)	0.06	μl
DNA (5 ng/ μl)	5	μl
Total	**15**	**μl**

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PCR program

Initial Denature	95°C for 5 min
Denaturation	95°C for 30 seconds
Primer Annealing	FH94 and FH102 at 60°C for 40 seconds
	LafMS03 at 50°C for 40 seconds
Primer extension	72°C for 60 seconds
	X 35 cycles
Final extension step	72°C for 5 min

Gene Scan preparation

Mixture for GeneScan (in Gene Scan plate) for one sample

Hi-di formamide	9	µl
PCR product	2	µl
Size standard (Liz 500,ABI)	0.2	µl

Denature program

95.0 c for 5 minutes

Put in the ice for 5 minutes

If there is a bubble in the sample, spin down briefly by centrifuge.

Put in the Gene Scan machine.

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APPENDIX D

Big Dye Sequencing

I: Title

Big Dye Terminator Cycle Sequencing

II: Aim of the protocol

Sequencing

III: Reagents

-ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit; Applied Biosystems ; Part. no. 4390242 = 100 Rxn, Part. no. 4390244 = 1000 Rxn.

-3 M Sodium Acetate, pH 5.2

-96 % cold Ethanol

-70 % Ethanol

-5x Sequence Buffer: solve 4,84 g Tris (400 mM) and 0,203 g $MgCl_2$ (10 mM) in 80 ml Aqua Dest. Adjust pH to 9.0 with 2N HCL. Fill up to 100 ml with Aqua Dest. Store at –20°C.

-Sephadex G-50 Superfine (Amersham: cat.no.: 17-0041-01.)

IV: Equipment

-MicroAmp® Optical 96-Well Reaction Plate ; Applied Biosystems: Part. no. N 801-056.

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-3100 Genetic Analyzer Plate Retainer 96-Well; 4/Pk; Applied Biosystems: Part. no. 4317241.

-3100 Genetic Analyzer Plate Base 96-Well; 4/Pk; Applied Biosystems: Part. no. 4317237..

-3100 Genetic Analyzer Plate Septa 96-Well; 20/Pk; Applied Biosystems: Part. no. 4315933.

-MultiScreen plates: MAHV N45.

-Multiscreen Column Loader MACL 096 45.

-MJ PTC-100 Thermal Cycler

-Eppendorf Centrifuge 5415 C or 5415 R.

-Hermle Centrifuge ZK 510.

V: Procedure

A: General precautions and safety aspects

B: Pretreatment of samples

Samples do not have to be purified.

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C: Analysis

Preparing Sequencing Reactions

Step	**Action**
1	For each reaction, add the following reagents to a separate tube:
2	Mix well and spin briefly.

Terminator Ready Reaction Mix	1 µl
Template:	1 µl:
PCR product DNA :100-200 bp	1-3 ng
200-500 bp	3 -10 ng
500-1000 bp	5 - 20 ng
1000-2000 bp	10-40 ng
Primer, 3.2 pmol	1 µl
5x Sequence buffer	2 µl
MilliQ	5 µl
Total volume	10 µl

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Cycle sequencing on the MJ PTC-100

Step	**Action**
1	Place the tubes in a thermal cycler: with heated lid.
2	Repeat the following for 25 cycles: ♦ rapid thermal ramp to 96 °C ♦ 96 °C for 30 sec. ♦ rapid thermal ramp to 50 °C ♦ 50 °C for 15 sec. ♦ rapid thermal ramp to 60 °C ♦ 60 °C for 2 min.
3	Rapid thermal ramp to 4 °C and hold until ready to purify.
4	Spin down the contents of the tubes in a microcentrifuge.
5	Proceed to "*Sephadex purification*" or "*Ethanol/Sodium Acetaat Precipitation*".

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***Sephadex purification*:**

Dye Terminator Removal Using MultiScreen 96-Well Filtration Plates

Step	**Action**
1	Load dry Sephadex into all 96-wells of a Multiscreen MAHV plate using the column loader as follows: - Add Sephadex G-50 to the Column Loader. - Remove excess resin off the top of the column loader with the scraper. - Place Multiscreen MAHV plate upside-down on the top of the Column Loader. - Invert both Multiscreen MAHV plate and the Column Loader. - Tap om top or side of the Column Loader to release the resin.
2	Using a multi-channel pipettor, add 300 µl milli-Q water to each well to swell resin. Incubate at room temperature for 3 hr. -Once the mini-columns are swollen in Multiscreen plates, they can be stored in the refrigerator at 4^0C for up to two weeks, by tightly sealing the plates with parafilm.
3	Place a Centrifuge Alignment Frame on top of a standard 96-well microplate, then place the MAHV plate on the assembly, **without lid**. Centrifuge at 1900 rpm for 5 min. to pack the mini-columns.

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4	Carefully add 20 µl milliQ water to the sequencing reactions (10 µl) and pipet the 30 µl to the center of the columns.
5	Tape off the unused mini-columns.
6	Place the MAHV plate (**without lid**) on top of a sequencing plate (an MicroAmp® Optical 96-Well Reaction Plate) and centrifuge at 1900 rpm for 5 min. (The position of the samples must correspond with empty wells in the sequencing plate !!)
7	Proceed to *Electrophoresis on the ABI Prism 3100*

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Ethanol/Sodium Acetate Precipitation

Step	Action
1	For each sequencing reaction, prepare a 1.5 ml microcentrifuge tube containing the following: ♦ 2.0 µl of 3M Sodium Acetate (NaOAC), pH 5.2 ♦ 50 µl of 96% cold ethanol (EtOH)
2	Pipet the entire contents of each extinction reaction into a tube of sodium acetate/ethanol mixture. Mix thoroughly. **To remove reactions run on the MJ PTC-100:** Place the pipette tip into the bottom of the reaction and carefully remove the reaction from the oil. **IMPORTANT** Transfer as little oil as possible.
3	Vortex the tubes and place at –20 °C for 20 minutes to precipitate the extension products.
4	Spin the tubes in a microcentrifuge for 20 minutes at 14,000 rpm
5	Carefully aspirate the supernatant with a pipette and discard.
6	Rince the pellet with 100 µl of 70% EtOH.
7	Spin for 2 minutes in a microcentrifuge at 14,000 rpm. Again, carefully aspirate the supernatant and discard.
8	Dry the pellet and resuspend in 15 µl milliQ water.

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Electrophoresis on the ABI Prism 3100

Step	Action
1	Do NOT denaturate the samples,
2	Refer to the *ABI Prism 3100 Genetic Analyzer protocol.*

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APPENDIX E

Spectophotometry and DNA concentration

The $OD_{260/280}$ and DNA concentration (ng/µl) of the samples in this study.

Family	Code	Sample	OD 260/280	DNA conc. (ng/µl)	Remark
TECC-01	LP1-B-TD	blood	1.81	20.5	
	LP1-C-SK	blood	1.61	30.6	
	LP1-Cc-NU	hair follicle	2.18	42.4	
TECC-02	LP1-B-PM	blood	1.76	10.7	
	LP1-C-PP	blood	1.47	15.9	
	LP1-Cc-AN	hair follicle	2.11	95.1	
MSEC-01	CM1-B-BP	blood	1.49	93.7	
	CM1-C-ND	hair follicle	2.11	70.9	
	CM1-Cc-DP	blood	1.48	19.5	
MSEC-02	CM1-B-KH	blood	1.86	20.9	
	CM1-C-NO2*	blood	1.85	34.7	
	CM1-Cc-CT	blood	1.66	61	
MSEC-03	CM1-B-YA	blood	1.83	83.1	
	CM1-C-NO2*	blood	1.85	34.7	mother 1
	CM1-Cc-WP2	hair follicle	1.75	21.7	
	CM1-C-MH	hair follicle	2.1	107.3	mother 2
	CM1-Cb-LC	blood	1.98	57.3	
	CM1-Cb-TP	blood	1.47	42.4	

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The $OD_{260/280}$ and DNA concentration (ng/µl) of the samples in this study (cont.).

Family	Code	Sample	OD 260/280	DNA conc. (ng/µl)	Remark
	CM1-C-SY	hair follicle	2.05	101.2	mother 3
	CM1-Cb-SP	blood	1.6	33.4	
	CM1-Cb-WP	blood	2.11	126	
	CM1-C-SN	hair follicle	2.08	104.3	mother 4
	CM1-Cb-TT	blood	1.52	206.3	

* = same animal

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APPENDIX F

Mitochondrial DNA sequence

MTCB marker

(cytochrome *b*)

All mother and calf in all families

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            ************************************************************* **************************************
  LP1-C-PP TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 LP1-Co-AN TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
  LP1-C-SK TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 LP1-Co-NU TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
  CM1-C-ND TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCCATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Co-DP TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCCATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-C-NO2 TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Co-CT TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
CM1-Co-WP2 TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
  CM1-C-MH TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Cb-LC TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Cb-TP TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
  CM1-C-SY TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Cb-SP TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Cb-WP TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
  CM1-C-SN TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
 CM1-Cb-TT TCCTAACAGGATTATTCCTAGCCATACATTACACACCTGACACAATAACTGCATTTTCATCTATATCCCATATCTGCCGAGACGTCAACTACGGCTGAAT  100
     ruler 1.......10........20........30........40........50........60........70........80........90.......100

            ************ ******************** ** *************************************************** ***********
  LP1-C-PP TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
 LP1-Co-AN TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
  LP1-C-SK TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
 LP1-Co-NU TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
  CM1-C-ND TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
 CM1-Co-DP TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
 CM1-C-NO2 TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
 CM1-Co-CT TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
CM1-Co-WP2 TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
  CM1-C-MH TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
 CM1-Cb-LC TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
 CM1-Cb-TP TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
  CM1-C-SY TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
 CM1-Cb-SP TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
 CM1-Cb-WP TATTCGACAACTGCACTCAAACGGAGCATCTATCTTTTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTATCTATACTCAGAA  200
  CM1-C-SN TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
 CM1-Cb-TT TATTCGACAACTACACTCAAACGGAGCATCTATTTTCTTCCTCTGCCTATACACACACATTGGACGAAACATCTACTATGGATCCTACCTATACTCAGAA  200
     ruler .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200

            ******** ***** ****** *********** ***********************************
  LP1-C-PP ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 LP1-Co-AN ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
  LP1-C-SK ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 LP1-Co-NU ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
  CM1-C-ND ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Co-DP ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-C-NO2 ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Co-CT ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
CM1-Co-WP2 ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
  CM1-C-MH ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Cb-LC ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Cb-TP ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
  CM1-C-SY ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Cb-SP ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Cb-WP ACCTGAAACACAGGTATTATACTACTACTAATCACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
  CM1-C-SN ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
 CM1-Cb-TT ACCTGAAATACAGGCATTATATTACTACTAATTACCATAGCCACCGCCTTCATAGGATATGTCCTTCCA  269
     ruler .......210.......220.......230.......240.......250.......260.........
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(D-loop)

Mother and calf in Family MSEC-03 (excluded CM1-C-NO2 and CM1-Cc-WP2)

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           ****************************************************************************************************
  CM1-C-MH TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
 CM1-Cb-TP TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
  CM1-C-SY TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
 CM1-Cb-SP TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
 CM1-Cb-WP TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
  CM1-C-SN TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
 CM1-Cb-TT TCTATGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCA   100
     ruler 1.......10........20........30........40........50........60........70........80........90.......100
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           ****************************************************************************************************
  CM1-C-MH TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
 CM1-Cb-TP TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
  CM1-C-SY TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
 CM1-Cb-SP TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
 CM1-Cb-WP TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
  CM1-C-SN TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
 CM1-Cb-TT TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   200
     ruler .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200
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           ****************************************************************************************************
  CM1-C-MH AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
 CM1-Cb-TP AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
  CM1-C-SY AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
 CM1-Cb-SP AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
 CM1-Cb-WP AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
  CM1-C-SN AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
 CM1-Cb-TT AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   300
     ruler .......210.......220.......230.......240.......250.......260.......270.......280.......290.......300
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           *****************************************************************************
  CM1-C-MH CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
 CM1-Cb-TP CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
  CM1-C-SY CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
 CM1-Cb-SP CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
 CM1-Cb-WP CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
  CM1-C-SN CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
 CM1-Cb-TT CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   377
     ruler .......310.......320.......330.......340.......350.......360.......370.......
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Mother and calf in Family MSEC-02 and MSEC-03 (CM1-C-NO2 is mother)

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           ****************************************************************************************************
 CM1-C-NO2 TGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCATGTA   100
 CM1-Co-CT TGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCATGTA   100
CM1-Co-WP2 TGTTTAATCTTACATACACCATACTATGTATAATCGTATATCACATTATTTACCCCATGCTTATAAGCAAGTATATTCGGTCAATGTAGACAGTCATGTA   100
     ruler 1.......10........20........30........40........50........60........70........80........90.......100

           ****************************************************************************************************
 CM1-C-NO2 ATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACATAGCT   200
 CM1-Co-CT ATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACATAGCT   200
CM1-Co-WP2 ATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACATAGCT   200
     ruler .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200

           ****************************************************************************************************
 CM1-C-NO2 CATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTATCCCT   300
 CM1-Co-CT CATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTATCCCT   300
CM1-Co-WP2 CATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTATCCCT   300
     ruler .......210.......220.......230.......240.......250.......260.......270.......280.......290.......300

           *************************************************************************
 CM1-C-NO2 CTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   373
 CM1-Co-CT CTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   373
CM1-Co-WP2 CTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   373
     ruler .......310.......320.......330.......340.......350.......360.......370...
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Mother and calf in Family MSEC-01

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           ****************************************************************************************************
  CM1-C-ND TACTTAATCTTACATAGACCTTACTATGTATAATCGTGCATCACATTATTTACCCCATGCTTATAAGCAAGCACTGTTTAATCAATGTGTTGAGTCATAT   100
 CM1-Co-DP TACTTAATCTTACATAGACCTTACTATGTATAATCGTGCATCACATTATTTACCCCATGCTTATAAGCAAGCACTGTTTAATCAATGTGTTGAGTCATAT   100
     ruler 1.......10........20........30........40........50........60........70........80........90.......100

           ****************************************************************************************************
  CM1-C-ND TCTTGTAGATTCACAGGTTATGTTTTAGCTCATGGATATTATTCACCTACGATAAACCATAGTCTTACATAGCACATTAAAGCTCTTGATCGTGCATAGC   200
 CM1-Co-DP TCTTGTAGATTCACAGGTTATGTTTTAGCTCATGGATATTATTCACCTACGATAAACCATAGTCTTACATAGCACATTAAAGCTCTTGATCGTGCATAGC   200
     ruler .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200

           ****************************************************************************************************
  CM1-C-ND GCATTACTGAGAAATCTCTAGTCATCATGCATATCACCTCCAACGGTTGTACCTTAACTACCTACCTCCGAGAAACCATCAACCCGCCCATCTTCGTGTC   300
 CM1-Co-DP GCATTACTGAGAAATCTCTAGTCATCATGCATATCACCTCCAACGGTTGTACCTTAACTACCTACCTCCGAGAAACCATCAACCCGCCCATCTTCGTGTC   300
     ruler .......210.......220.......230.......240.......250.......260.......270.......280.......290.......300

           *****************************************************************************
  CM1-C-ND CCTCTTCTCGCTCCGGGCCCATCAATTGTGGGGGTTTCTATACTGGATCTATACCTGGCATCTGCTTCCTACTTCAA   377
 CM1-Co-DP CCTCTTCTCGCTCCGGGCCCATCAATTGTGGGGGTTTCTATACTGGATCTATACCTGGCATCTGCTTCCTACTTCAA   377
     ruler .......310.......320.......330.......340.......350.......360.......370.......
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Mother and calf in Family TECC-01

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           ****************************************************************************************************
LP1-C-PP   GATCCACAGGTTATGTTCTAGTTCATGGATATTGTTCACCTACGATAAACCATAGTCTTACATAGCACATTAAAGCTCTTGATCGTGCATAGCGCATTAC   100
LP1-Cc-AN  GATCCACAGGTTATGTTCTAGTTCATGGATATTGTTCACCTACGATAAACCATAGTCTTACATAGCACATTAAAGCTCTTGATCGTGCATAGCGCATTAC   100
ruler      1.......10........20........30........40........50........60........70........80........90.......100

           ****************************************************************************************************
LP1-C-PP   TGAGAAATTTCTAGTCATCATGCATATCACCTCCAACGGTTGTATCTTAACTACCTACCTCCGAGAAACCATCAACCCGCCCATCTTCGTGTCCCTCTTC   200
LP1-Cc-AN  TGAGAAATTTCTAGTCATCATGCATATCACCTCCAACGGTTGTATCTTAACTACCTACCTCCGAGAAACCATCAACCCGCCCATCTTCGTGTCCCTCTTC   200
ruler      .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200

           **********************************************************************
LP1-C-PP   TCGCTCCGGGCCCATCAATTGTGGGGGTTTCTATACTGGATCTATACCTGGCATCTGCTTCCTACTTCAA   270
LP1-Cc-AN  TCGCTCCGGGCCCATCAATTGTGGGGGTTTCTATACTGGATCTATACCTGGCATCTGCTTCCTACTTCAA   270
ruler      .......210.......220.......230.......240.......250.......260.......270
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Mother and calf in Family TECC-02

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           ****************************************************************************************************
LP1-C-SK   TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   100
LP1-Cc-NU  TGTAATTCACGAATCCCCTAACACTTATTGAACCCATGGATATTATTTCCCTAGGTAAATCCTAGTTCCACACAGTACATTCAAATCCTTGATCGTACAT   100
ruler      1.......10........20........30........40........50........60........70........80........90.......100

           ****************************************************************************************************
LP1-C-SK   AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   200
LP1-Cc-NU  AGCTCATTACTGAGAAATCTCTAGGCACCATGCATATCACCTCCAATAGATTTTCTTTACTGCCTACCTCTGAGAAACCAGCAACCCGCCCAATTTGTAT   200
ruler      .......110.......120.......130.......140.......150.......160.......170.......180.......190.......200

           *****************************************************************************
LP1-C-SK   CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   277
LP1-Cc-NU  CCCTCTTCTCGCTCCGGCCCATCAATTGTGGGGGTTTCTATTCTGAATCTATACCTGGCATCTGCTTCCTACTTCAA   277
ruler      .......210.......220.......230.......240.......250.......260.......270.......
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