Population Structure and Conservation Genetics of Juvenile Loggerhead (*Caretta caretta*) and Green Sea Turtles (*Chelonia mydas*) in North Carolina

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Prepared for

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Introduction

Advances in the application of genetic techniques to the study of marine turtles have resulted in the acquisition of more information pertaining to aspects of their natural history (see Bowen & Avise, 1996), evolution (Encalada et al., 1996), and behavior (Fitzsimmons, 1996; Peare & Parker, 1996). In addition genetic markers provide a means of assessing the population structure and composition of foraging grounds. The relevance of this data to management plans has been demonstrated (Bowen et al., 1995; 1996).

The population of turtles in the Pamlico-Albemarle Estuarine complex has been recognized as an indicator of the overall health of loggerhead and green turtle foraging populations in the US (Epperly et al., 1995). Not only does the cooperation of pound net fisherman in the area provide data on movement, density and demography of these populations, but it also allows for the collection of other information, such as blood samples for genetic analysis. Genetic markers provide a baseline to evaluate changes in composition of foraging cohorts. These data can demonstrate which nesting populations may be impacted by human induced and natural mortality. Genetic studies of foraging ground individuals can also provide information on migratory patterns of marine turtles inhabiting US waters.

The goals of this project are two-fold:

1. To provide baseline data on the genetic identity of juvenile loggerhead and green turtles foraging in the Pamlico-Albemarle Estuarine Complex.
2. To estimate the contribution of regional management units to this foraging ground area.

Materials and Methods

Blood samples were collected from 97 juvenile loggerhead and 29 juvenile green turtles, placed in 9 ml of lysis buffer (100 mM Tris-HCl, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; pH 8.0), and stored at room temperature. Whole DNA isolations from blood samples were conducted with the phenol/chloroform method described by Hillis et al. (1996).

For the loggerheads, a 380 base-pair fragment located in the control region of the mitochondrial genome (mtDNA) was amplified with PCR methodology (Mullis & Faloona, 1987), using the primers TCR-5 and TCR-6 of Norman et al. (1994) and standard reaction conditions (Saiki et al., 1988). For the greens, a 510 base-pair fragment located in the control region of the mtDNA genome was amplified using the primers LTCM1 and HDCM1 of Allard et al. (1994). Cycle sequencing was conducted with an ABI Prism kit and florescently labeled dideoxynucleotides at the University of Florida DNA Sequencing Core and the labeled extension products were analyzed with an automated DNA sequencer (Applied Biosystems model 373A). Sequences were compared to known loggerhead haplotypes (Encalada et al., submitted) and known green turtle haplotypes (Encalada et al., 1996) and assigned a haplotype designation.

To test for differences in haplotype frequencies among management units and the foraging ground, chi square tests of independence (Sokal & Rohlf, 1981) with the Monte Carlo randomization method in the program CHIRXC were conducted (Zaykin & Pudovkin, 1993). Maximum likelihood (ML) estimates of the percent contribution of regional rookeries were obtained using the program UCON (Masuda et al., 1991). Standard deviations of the percent contribution were generated using the infinitesimal jackknife method in UCON.

Results

Caretta caretta

Blood samples from 97 juvenile loggerhead turtles captured in the Pamlico-Albemarle Estuarine complex were analyzed to determine the mtDNA haplotype of the individual. Six haplotypes were identified. The majority of individuals carried haplotype A (57%) or B (38%). Rare haplotypes among the NC foraging ground animals include C, D, G, and J (Table 1).

Table 1. Haplotypes and haplotype frequencies of loggerheads sampled from the Pamlico-Albemarle Estuarine complex. Surveyed Atlantic loggerhead rookeries in which these haplotypes are present (Encalada et al, submitted) are indicated to the right.

Haplotype	Frequency	Relative Frequency	Management Unit
A	55	0.57	NWFL[†], SFL[£], NC-NEFL[§]
B	37	0.38	NWFL, SFL, Mexico, Greece
C	2	0.02	NWFL, SFL, Mexico
D	1	0.01	Brazil
G	1	0.01	NWFL & SFL
J	1	0.01	Mexico

[†] NWFL = Management unit including nesting locations in north-west Florida (See Encalada et al. for locations and sample sizes).
[£] SFL = Management unit including nesting locations in south-east and south-west Florida.
[§] NC-NEFL = Management unit including nesting locations from Northeast Florida to North Carolina.

To test for differences in haplotype frequencies between the nesting locations and NC foraging ground, chi-square tests were conducted. These tests indicated that the haplotype frequencies found at the juvenile foraging ground were significantly different from all management units in the Atlantic and Mediterranean at the $p = 0.05$ level (Table 2) with the exception of the large source population located in south Florida (SFL).

Table 2. Chi-square tests of haplotype frequency between the North Carolina foraging ground and loggerhead nesting locations in the Atlantic and Mediterranean.

NWFL	SFL	NC-NEFL	MEXICO	BRAZIL	GREECE
14.33	5.44	53.97	44.23	97.97	32.18
$p<0.05$	NS	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

Maximum likelihood analyses were conducted assuming that their was an equal probability of contributions from all management units. Essentially the null hypothesis was that all management units are potential contributors and will contribute at equivalent levels. Analyses were conducted both including and excluding the Mediterranean nesting colony as a possible contributor. Regardless of whether Greece was included in the analysis, the estimated contribution varied little (Tables 3 & 4). Both analyses indicated that the SFL management unit was the major contributor at an estimated 63%. The next largest contributor was the NC-NEFL management unit at an estimated 32%. Contributions from the NWFL, Mexican and Brazilian management units were estimated at less than 5%.

Table 3. Results of ML analysis for North Carolina juvenile loggerheads including Kiparissia Bay, Greece as a potential source population.

Population	% Contribution	Standard Deviation
North-West Florida	0.0018	0.0107
South Florida	0.6364	0.1009
NC-NEFL	0.3195	0.0923
Mexico	0.0314	0.0308
Brazil	0.0108	0.0108
Greece	0.0000	0.0000

Table 4. Results of ML analysis for juvenile loggerheads from North Carolina excluding the management unit at Kiparissia Bay, Greece.

Population	% Contribution	Standard Deviation
North-West Florida	0.0014	0.0085
South Florida	0.6364	0.1009
NC-NEFL	0.3197	0.0921
Mexico	0.0314	0.0308
Brazil	0.0108	0.0108

Chelonia mydas

Blood samples from 29 juvenile green turtles were analyzed using the same methodology as described above for loggerhead samples. Two samples were problematic and their haplotype has not been identified, as of yet. It is notable that of the 27 individuals typed, 5 haplotypes were found in the NC foraging ground (Table 5). Haplotype CMI was found at a frequency of 37%, CMIII, at a frequency of 33%, and haplotypes CMV, CMXVIII, and CMVIII accounted for the remaining 30% of the individuals sampled.

Maximum likelihood analyses were not conducted due to the small sample size (see Broderick & Moritz, 1996). Additional samples will allow robust conclusions about the origins of juvenile green turtles in North Carolina waters.

Table 5. Haplotype frequencies of green turtles sampled from the Pamlico-Albemarle Estuarine complex. Surveyed Atlantic green turtle rookeries in which these haplotypes are present (Encalada, et al. 1996) are indicated on the right.

Haplotype	Frequency	Relative Frequency	Rookery
CMI	10	0.37	FL, Yucatan
CMIII	9	0.33	FL, Yucatan, Costa Rica, Venezuela
CMV	3	0.11	Yucatan, Venezuela, Surinam
CMXVIII	3	0.11	Yucatan
CMVIII	2	0.08	Brazil, Ascension, Guinea Bissau (West Africa)

Discussion

The sample sizes are insufficient to make robust conclusions about green turtles, but it is possible to make several conclusions about the loggerhead population. Qualitatively, the occurrence and origin of multiple loggerhead haplotypes among the individuals sampled from the NC foraging ground indicate that more than one management unit is contributing to this area. The presence of haplotypes A and B at high frequencies is not surprising due to the predominance of these haplotypes in loggerhead rookeries analyzed to date (Encalada et al., submitted). Chi-square tests of the haplotype frequencies indicate that the haplotype frequencies found on the NC foraging ground are significantly different from all loggerhead management units except for the SFL management unit. Due to differences in haplotype frequencies, the data are sufficient to estimate the contribution of management units to this foraging ground (Pella & Milner, 1987; Chapman, 1996).

Although the majority of the animals sampled carry haplotype A, 31% of the juveniles on the foraging ground originate from the NC-NEFL management unit. The SFL management unit which encompasses nesting on both the eastern and western sides of the Florida peninsula was estimated to be the largest contributor, 63%. In terms of the ML analysis, this is most likely due to the high frequency of haplotype B among the NC foraging assemblage, and the occurrence of the rarer haplotypes, C & G, in the SFL management unit.

Murphy & Hopkins (1984) reported that approximately 90% of the nesting effort in the southeast US is in southern Florida, and most of the remaining nesting effort is in the unit we define as NC-NEFL. Hence the smaller northern population seems to contribute disproportionately to the North Carolina feeding ground. These findings indicate that mortality in North Carolina waters will have greater consequences for the nesting populations in northern Florida, Georgia, South Carolina, and North Carolina.

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Appendix 1

List of loggerhead turtles analyzed with haplotype designations.

REL_LRTA	REL_RRTA	BEECS NUMBER	HAPLOTYPE
INJURE	QQS299	CC691	B
PPX080	PPX079	CC662	A
PPX084	PPX083	CC695	A
PPX085	PPX086	CC680	A
PPX087	PPX088	CC681	A
PPX091	PPX092	CC674	A
PPX093	PPX095	CC727	A
PPX098	PPX099	CC665	B
PPZ803	QQT727	CC658	A
PPZ806	PPZ807	CC689	A
PPZ808	PPZ809	CC728	B
PPZ810	PPZ811	CC700	B
PPZ812	PPZ813	CC710	A
PPZ816	PPZ815	CC714	A
PPZ820	PPZ819	CC729	A
PPZ821	PPZ822	CC654	A
PPZ824	PPZ823	CC720	A
QQS000	PPZ825	CC687	B
QQS248	QQS247	CC643	A
QQS280	QQS281	CC644	B
QQS282	QQS283	CC690	B
QQS284	QQS285	CC726	B
QQS286	QQS287	CC705	J
QQS300	PPX076	CC724	B
QQS311	QQS363	CC721	A
QQS313	QQS312	CC732	A
QQS316	QQS315	CC723	A
QQS322	QQS321	CC731	B
QQS329	QQS330	CC706	B
QQS335	QQS334	CC715	A
QQS337	QQS336	CC702	B
QQS339	QQS338	CC712	B
QQS340	QQS341	CC668	A
QQS342	QQS343	CC663	B
QQS345	QQS344	CC664	A
QQS347	QQS346	CC661	B
QQS349	QQS348	CC713	B
QQS350	QQV275	CC699	B
QQS354	QQS353	CC646	A
QQS357	QQS356	CC671	A
QQS359	QQS358	CC649	A
QQS360	QQS361	CC648	A
QQS705	QQS704	CC645	A

QQS712	QQS711	CC725	B
QQS714	QQS713	CC642	A
QQS716	QQS715	CC641	A
QQS720	QQS719	CC736	D
QQS852	QQS853	CC679	B
QQS854	QQS855	CC682	B
QQS858	QQS859	CC697	B
QQS926	QQS927	CC650	B
QQS928	QQS929	CC647	A
QQS930	QQS931	CC651	B
QQS932	QQS933	CC652	A
QQS938	QQS939	CC694	A
QQS942	QQS943	CC656	A
QQS945	QQS946	CC683	C
QQS949	QQS950	CC703	A
QQS976	QQS977	CC685	A
QQS986	QQS987	CC677	A
QQS995	QQS994	CC667	B
QQS996	QQS997	CC659	A
QQS998	QQS999	CC676	A
QQT726	QQS728	CC733	A
QQT731	QQT730	CC734	B
QQT733	QQT732	CC735	B
QQT739	QQT740	CC707	B
QQT744	QQT743	CC722	A
QQT747	QQT748	CC730	A
QQT826	QQT827	CC653	A
QQT828	QQT829	CC657	A
QQT830	QQT831	CC698	B
QQT832	QQT833	CC693	A
QQT836	QQT837	CC719	A
QQT838	QQT839	CC711	G
QQT840	QQT841	CC709	C
QQT842	QQT843	CC716	A
QQT844	QQT845	CC717	B
QQT846	QQT847	CC686	B
QQT848	QQT849	CC678	A
QQT850	QQS851	CC701	A
QQV005	QQV006	CC684	A
QQV009	QQV010	CC696	A
QQV011	QQV012	CC670	A
QQV014	QQV013	CC672	B
QQV019	QQV017	CC666	A
QQV020	QQV021	CC673	B

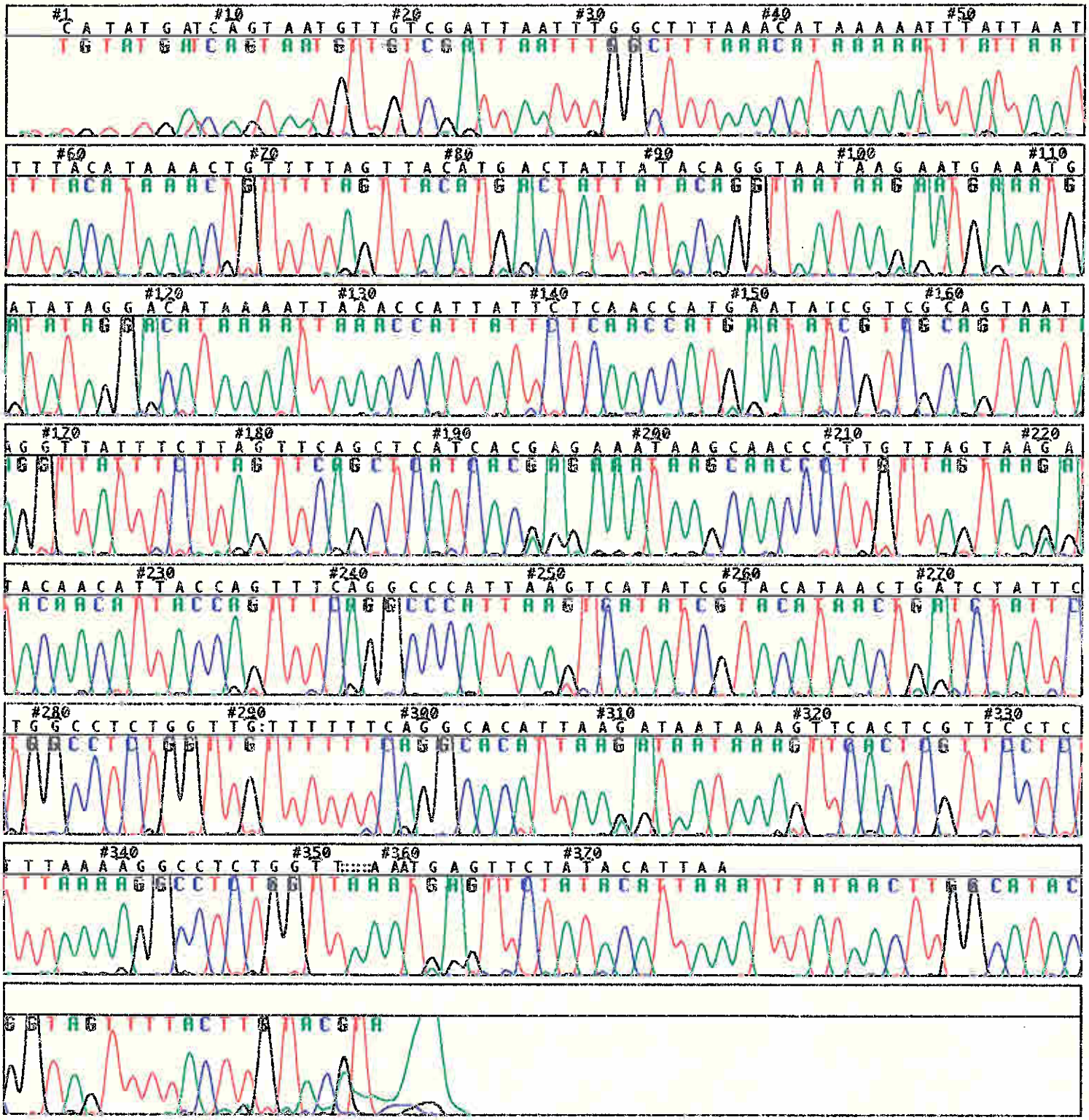
QQV022	QQV023	CC669	B
QQV024	QQV025	CC692	A
QQV259	QQV258	CC704	A
QQV260	no tag	CC675	A
QQV264	QQV263	CC718	A
QQV265	QQV266	CC655	B
QQV267	QQV268	CC737	A
QQV270	QQV269	CC688	B
QQV272	QQV271	CC708	B
QQV273	QQV274	CC660	B

Appendix 2

Chromatograms of loggerhead haplotypes found in the North Carolina foraging ground. Haplotype designation is listed on the upper left hand corner of the chromatogram.

Haplotype A

{Experimental Data} CC692/CR1 11.26.96 334 04.1
Sequencer NC-CC [User Name]



Haplotype B

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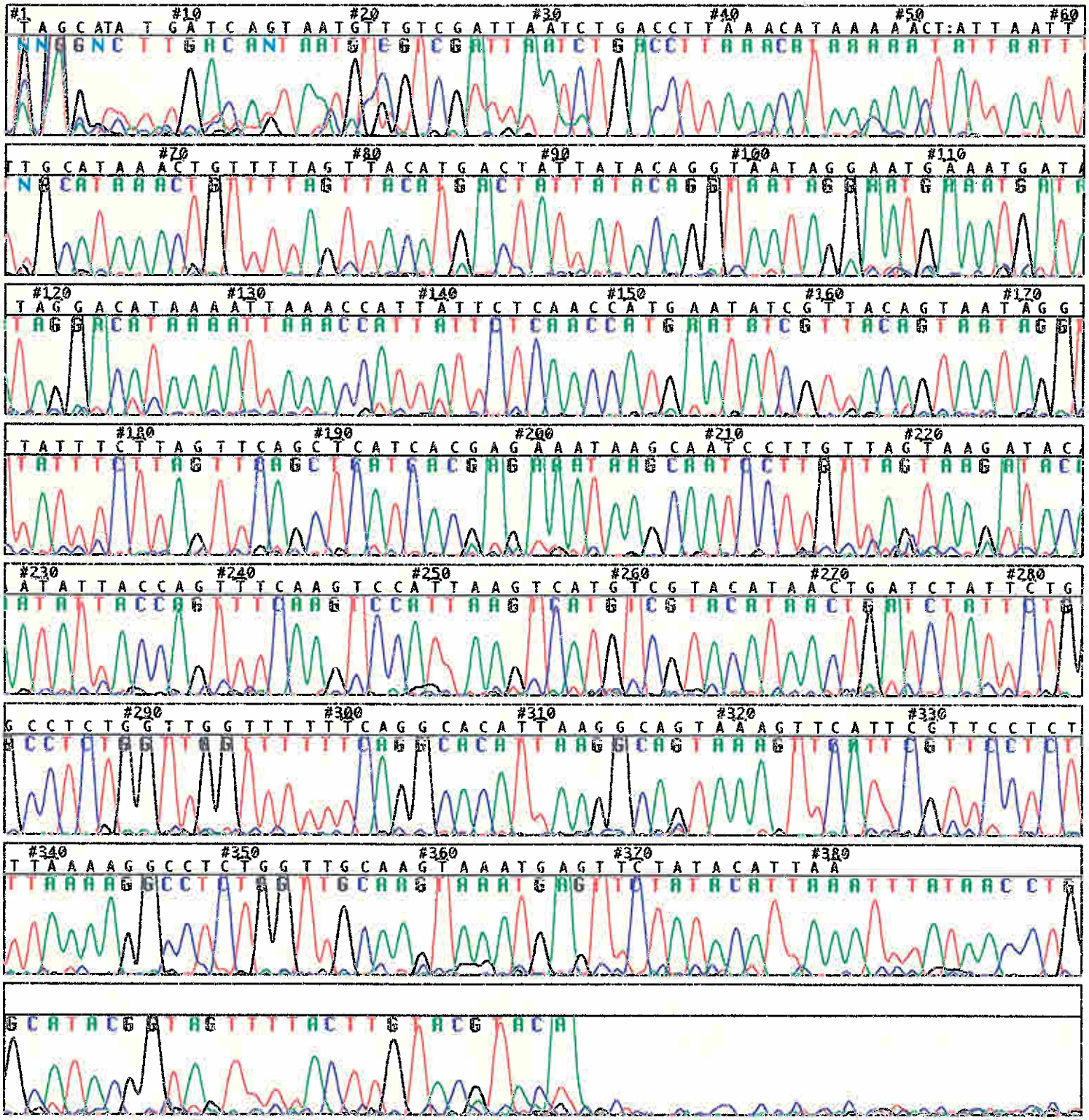
Sequencer NC-CC [User Name]



Haplotype C

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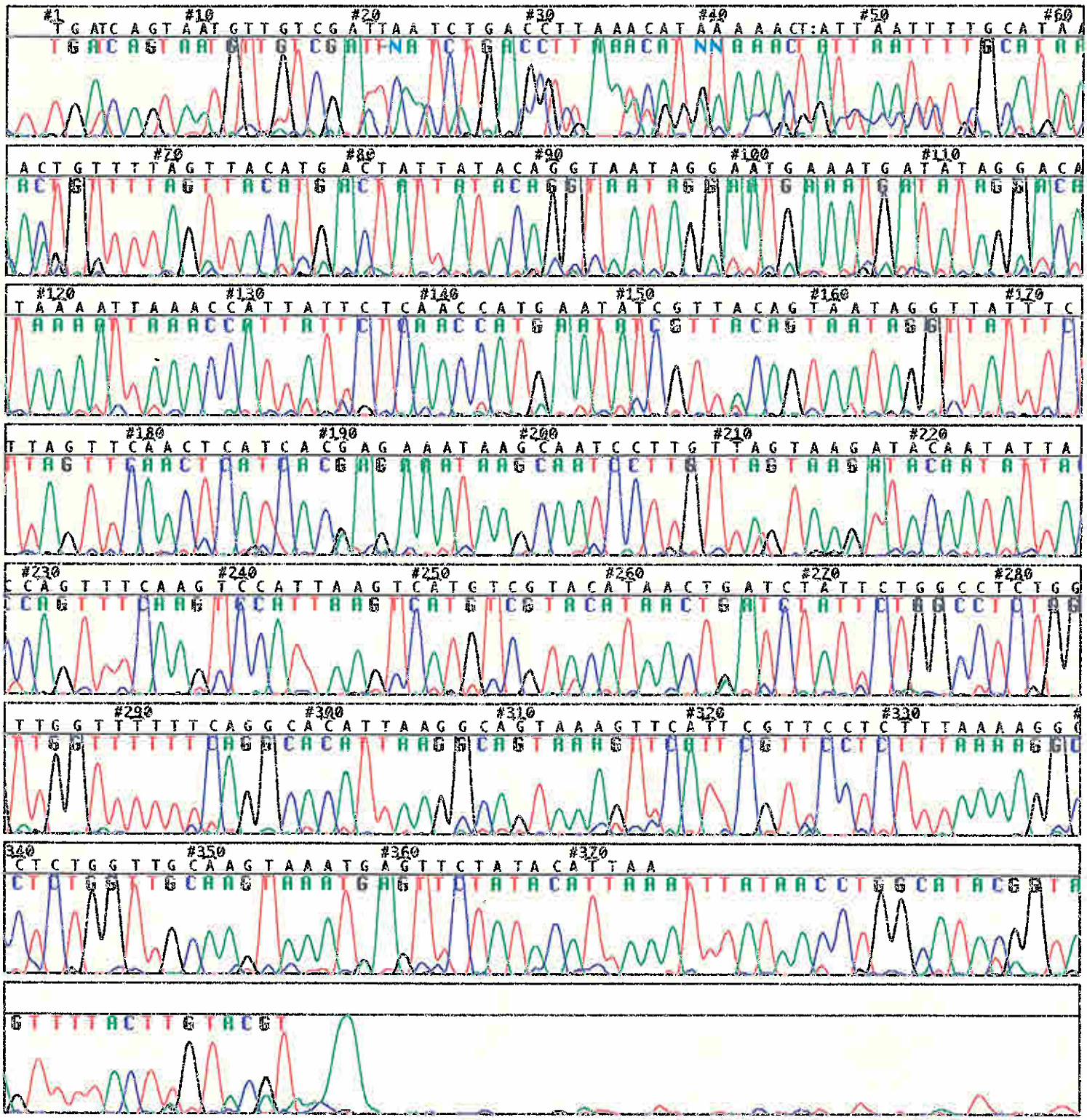
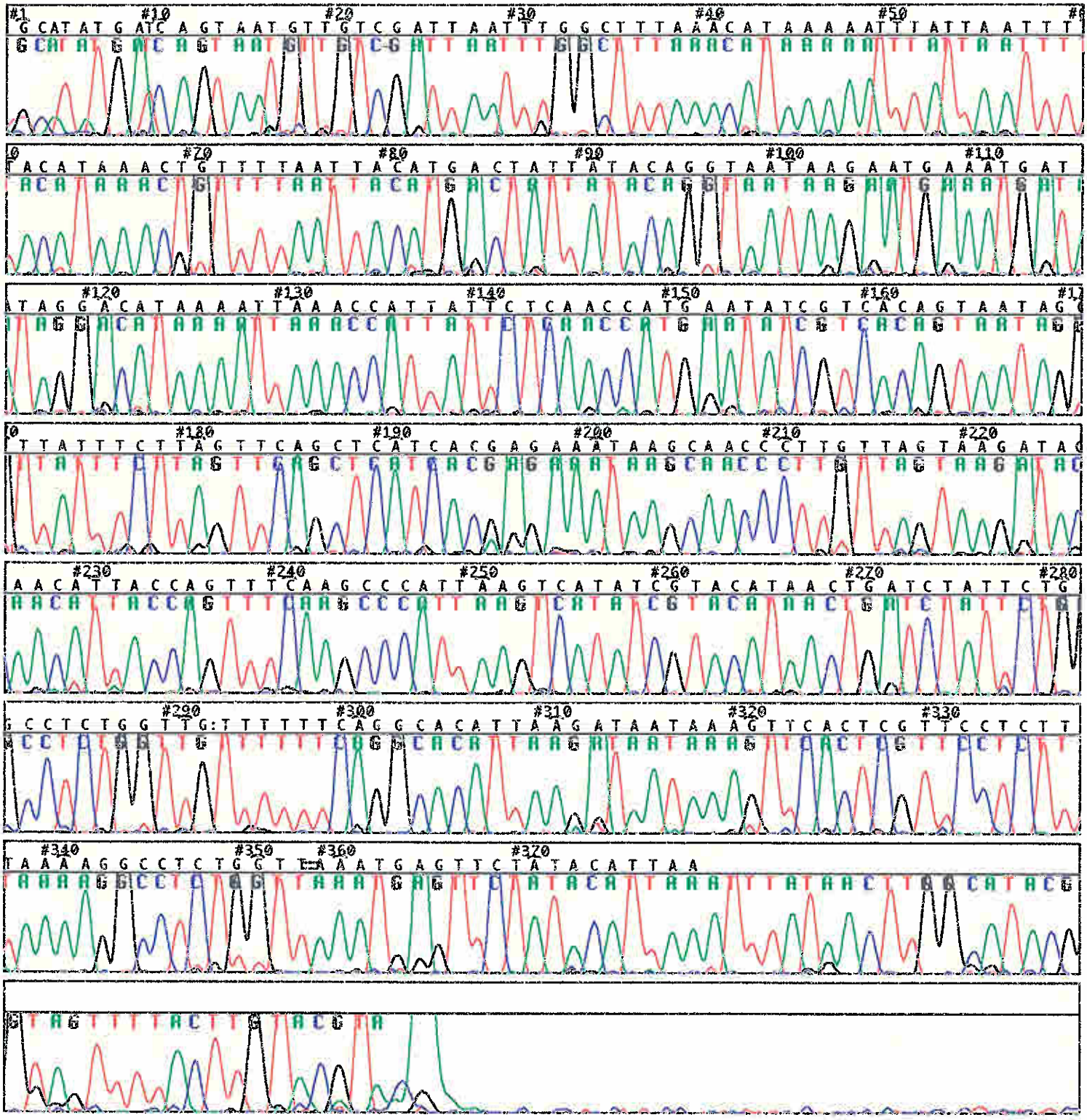


Haplotype D

{Experimental Data} CC736/CR1 12.16.96 334 07.1
Sequencer NC-CC [User Name]



Haplotype G

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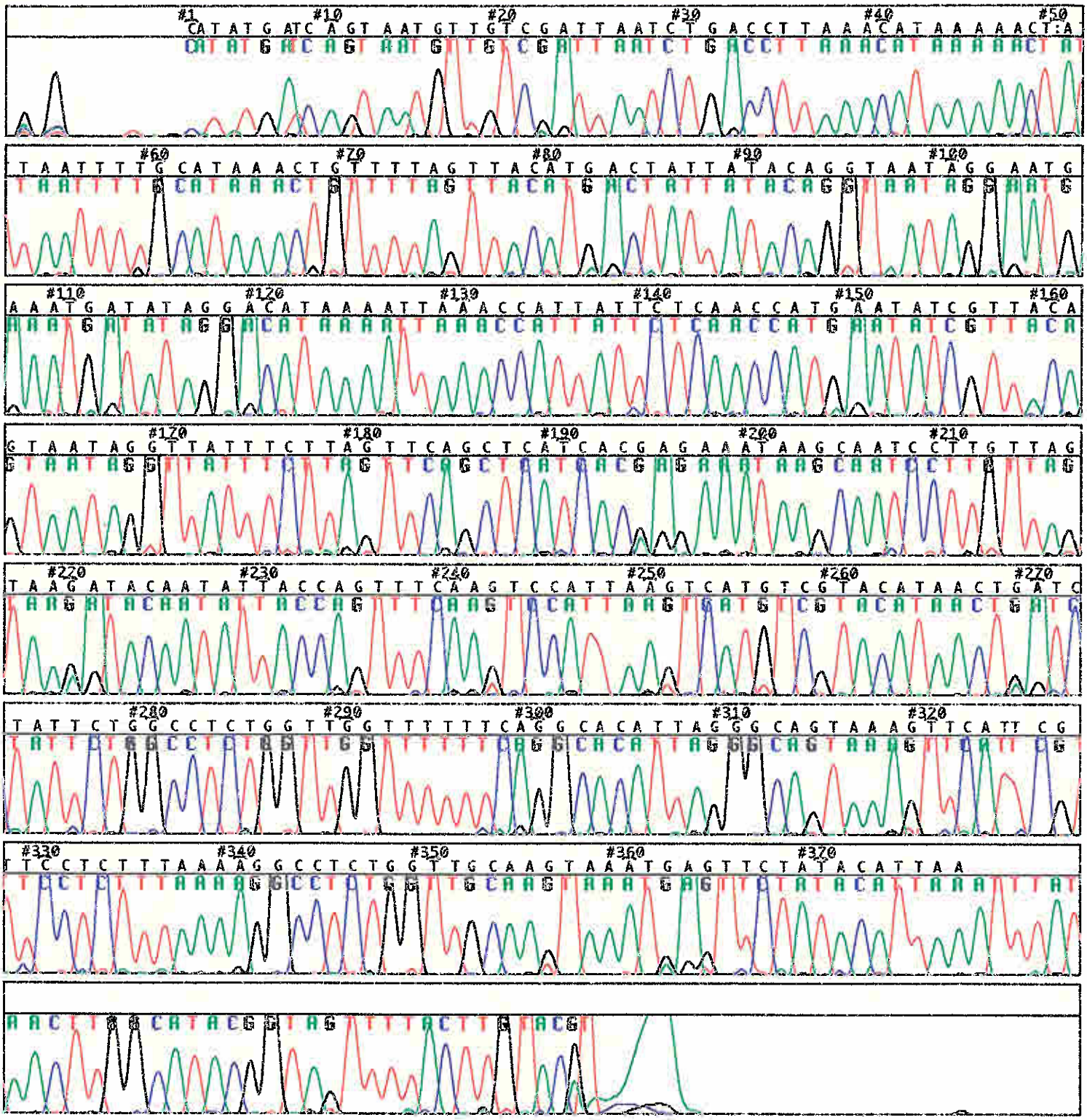
Sequencer NC-CC [User Name]



Haplotype J

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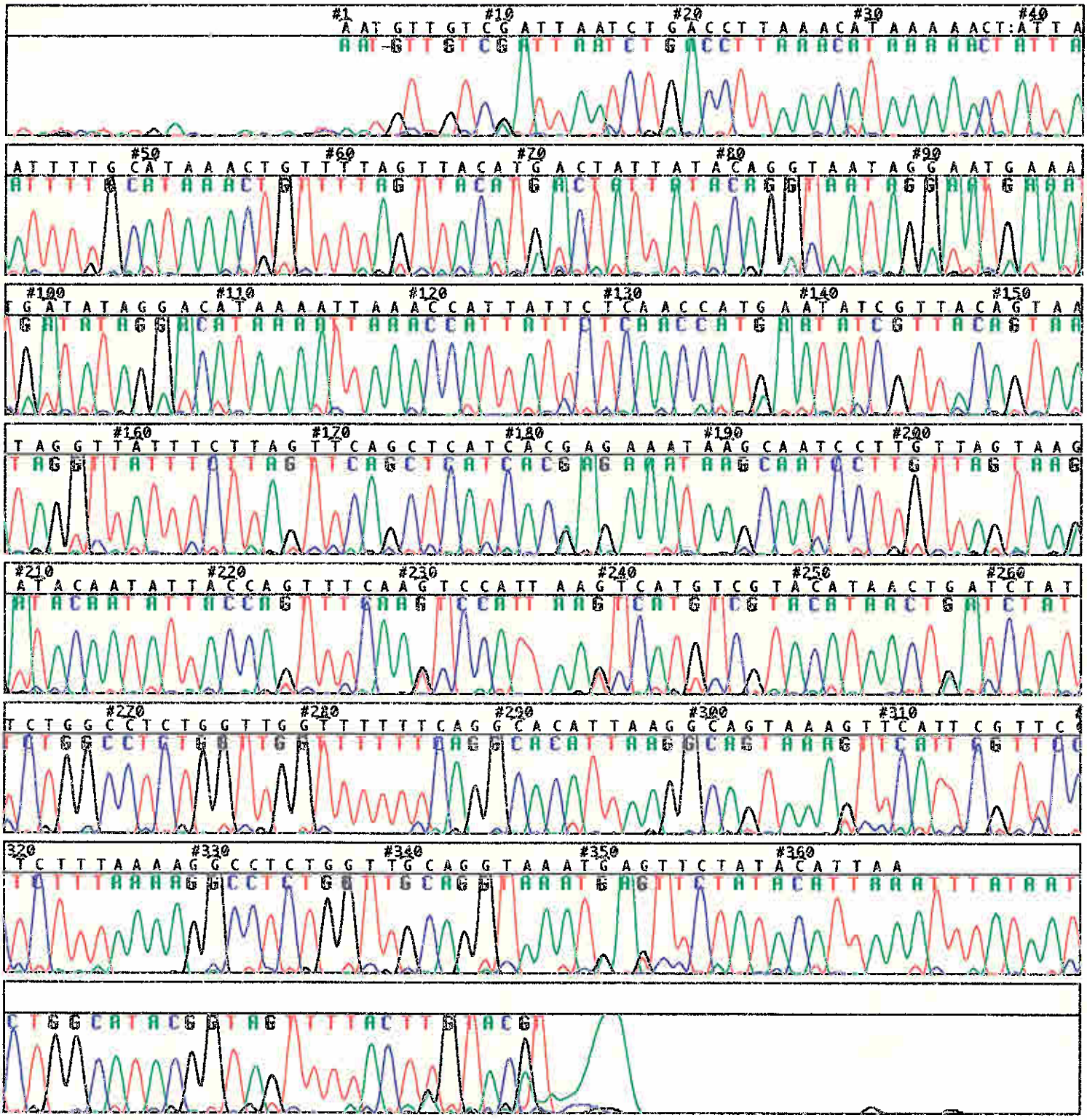
Sequencer NC-CC [User Name]



Appendix 3

List of green turtles analyzed with haplotype designations.

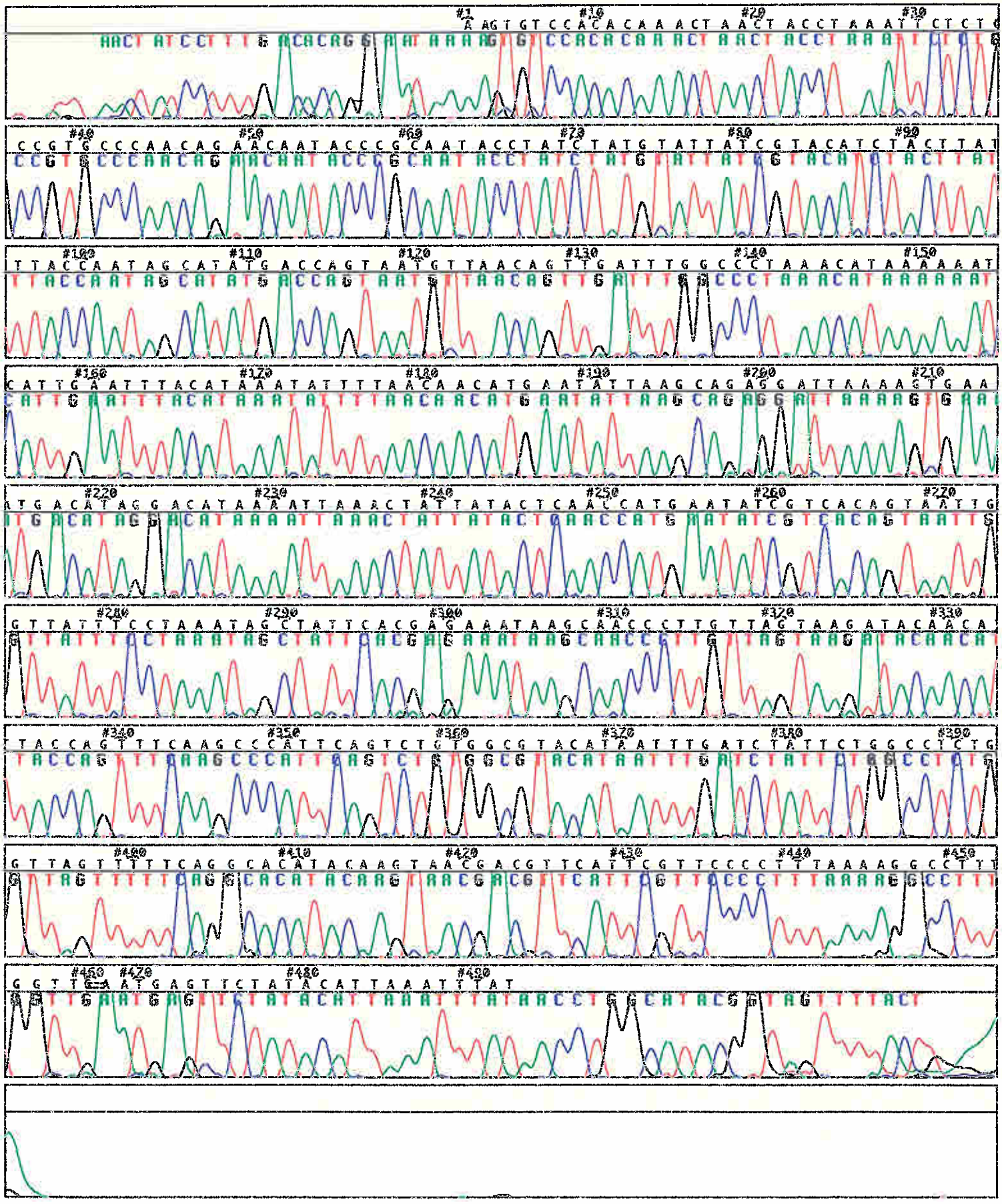
REL_LRTA	REL_RRTA	BEECS NUMBER	HAPLOTYPE
PPX089	PPX090	CM655	CM III
PPZ805	PPZ804	CM643	CM VIII
PPZ814	QQS326	CM663	CM I
PPZ817	PPZ818	CM662	CM III
QQS276	QQS279	CM659	CM V
QQS288	QQS289	CM661	CM III
QQS292	QQS293	CM652	CM III
QQS294	QQS295	CM665	CM III
QQS319	QQS318	CM645	CM I
QQS325	QQS324	CM648	CM III
QQS327	QQS328	CM668	CM I
QQS702	QQS703	CM644	CM I
QQS706	QQS707	CM670	CM V
QQS718	QQS717	CM669	CM III
QQS934	QQS935	CM671	?
QQS936	QQS937	CM646	CM I
QQS940	QQS941	CM656	CM VIII
QQS947	QQS948	CM660	CM XVIII
QQS978	QQS979	CM647	CM I
QQS990	QQS989	CM650	CM III
QQS991	QQS993	CM649	CM I
QQT729	QQT728	CM651	CM I
QQT736	NO TAG	CM653	CM I
QQT738	QQT737	CM664	CM XVIII
QQT742	QQT741	CM666	CM I
QQT834	QQT835	CM657	CM XVIII
QQV003	QQV004	CM654	CM V
QQV015	QQV016	CM658	CM III
QQV262	QQV261	CM667	?

Appendix 4

Chromatograms of green haplotypes found in the North Carolina foraging ground. Haplotype designation is listed on the upper left hand corner of the chromatogram.

Haplotype CM I

{Experimental Data} CM644/CR7 12.12.96 449 17.1
Sequencer On from NC [User Name]



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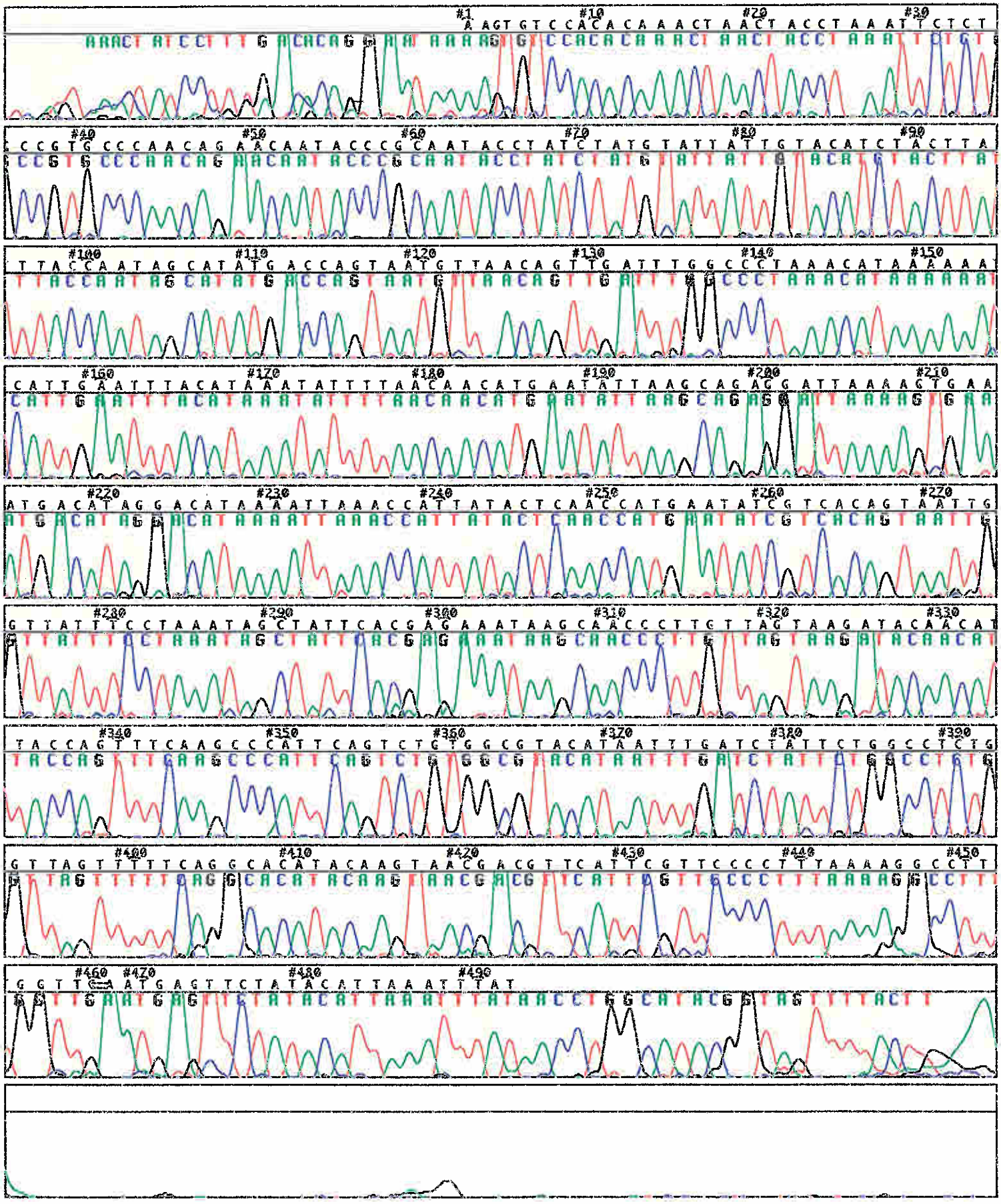
Haplotype CM III

{Experimental Data} CM650/CR7 12.12.96 449 29.1
Sequencer Cm from NC [User Name]



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Haplotype CM V

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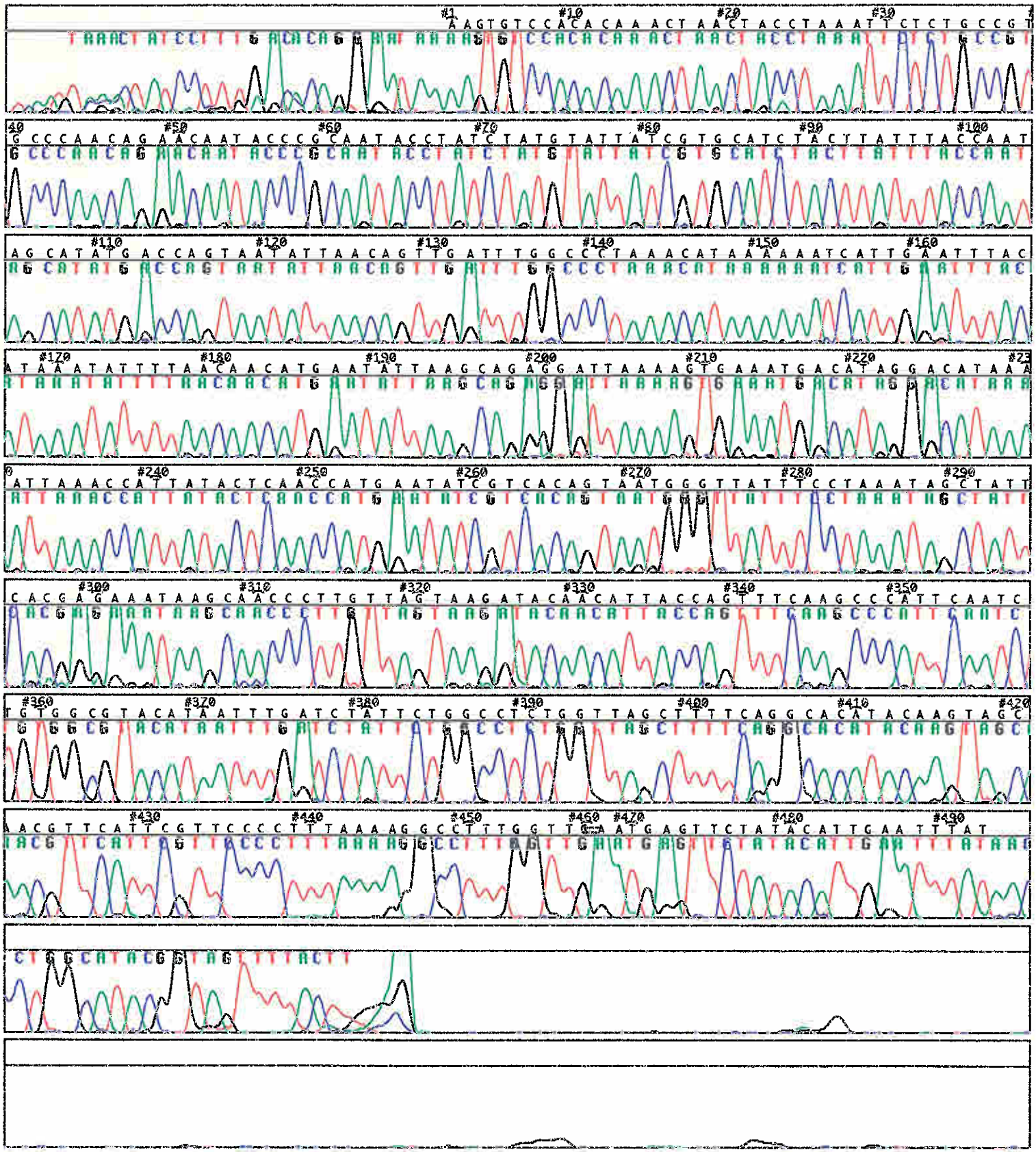
Sequencer Cm from NC [User Name]



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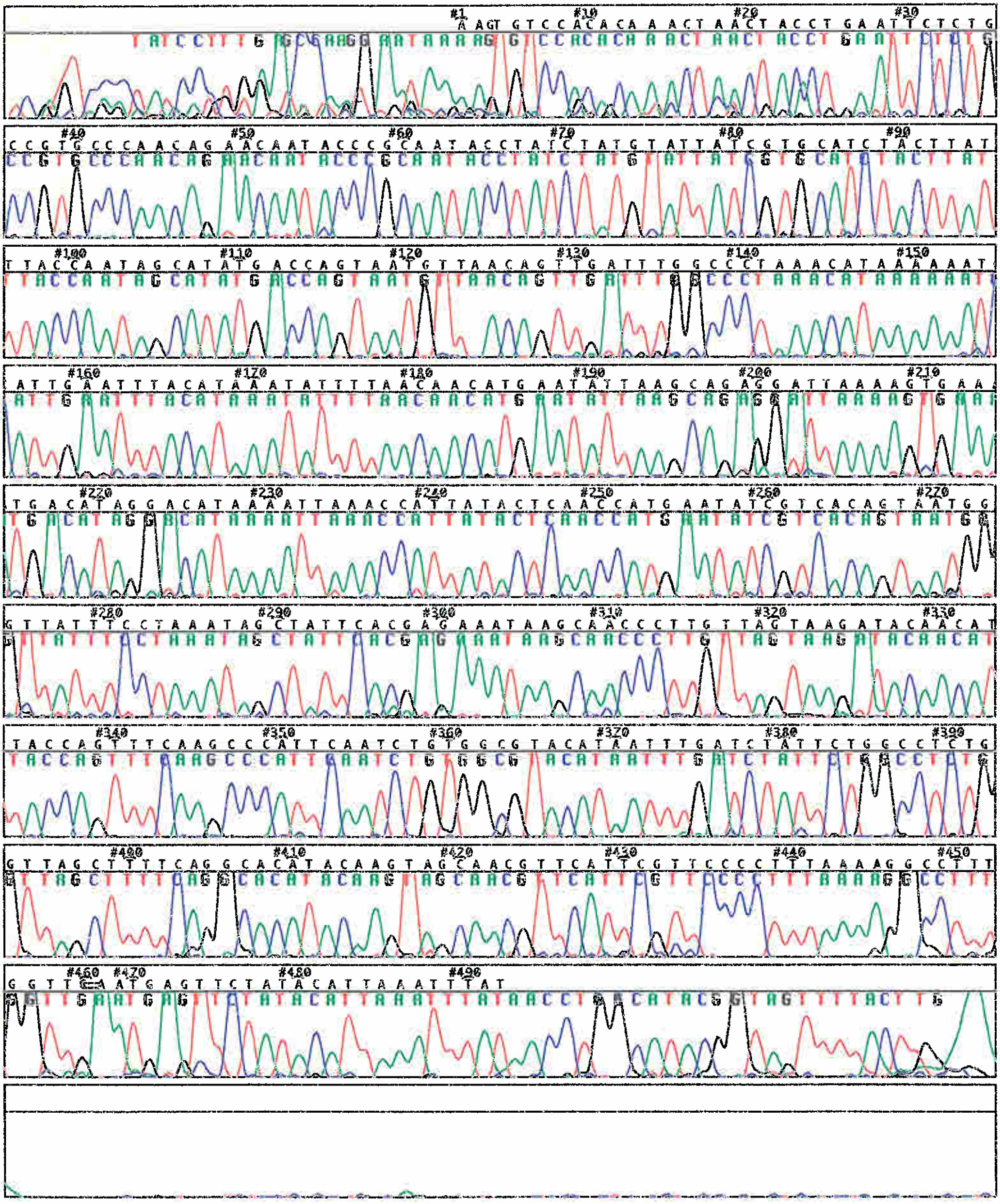
Sequencer On from NC [User Name]



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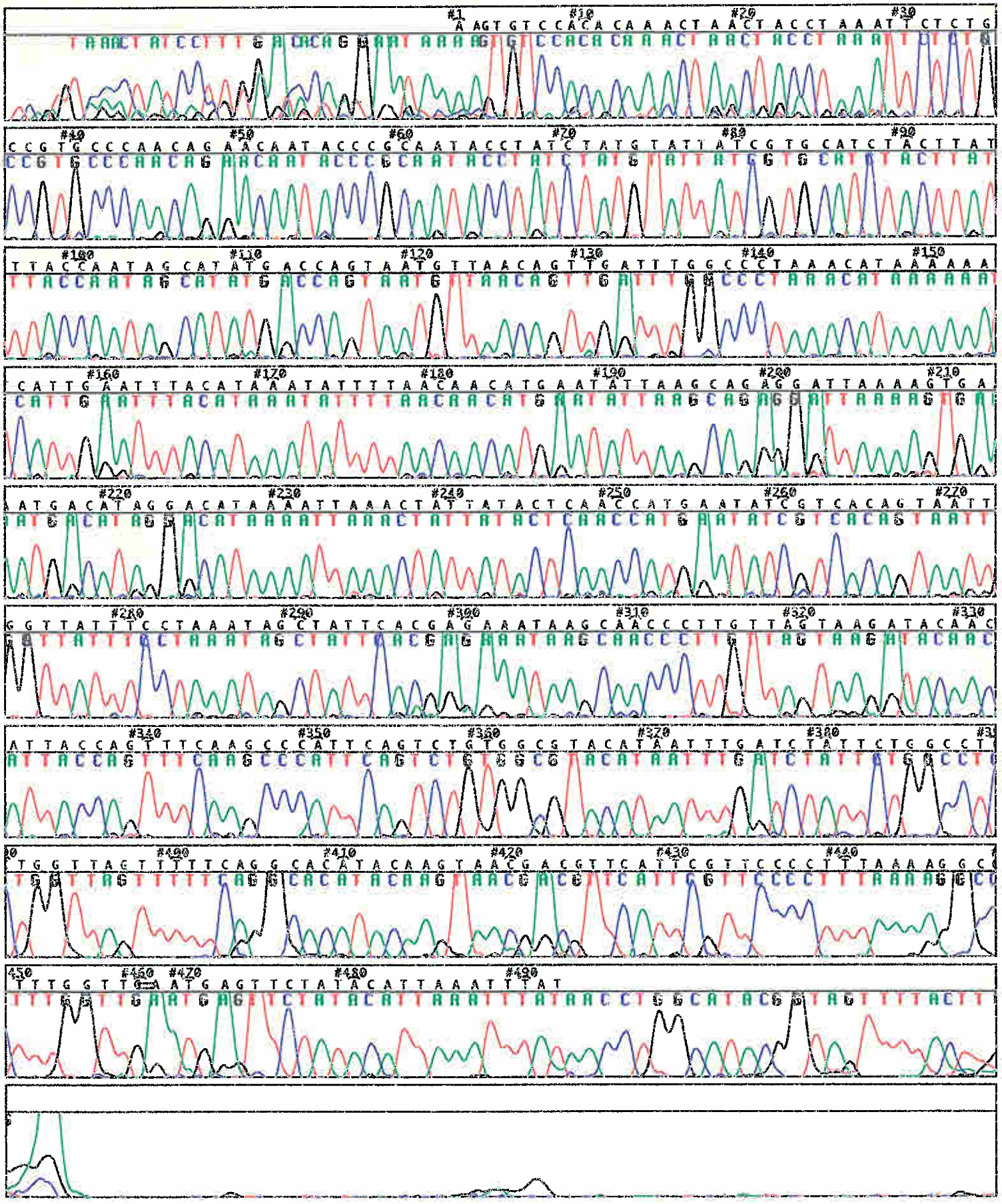
Haplotype CM XVIII {Experimental Data} CM657/CR7 12.16.96 334 27.1
Sequencer Cm from NC [User Name]



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