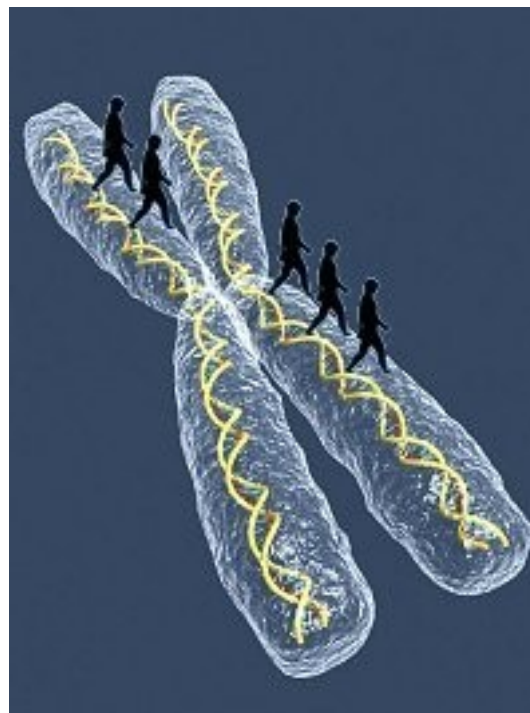


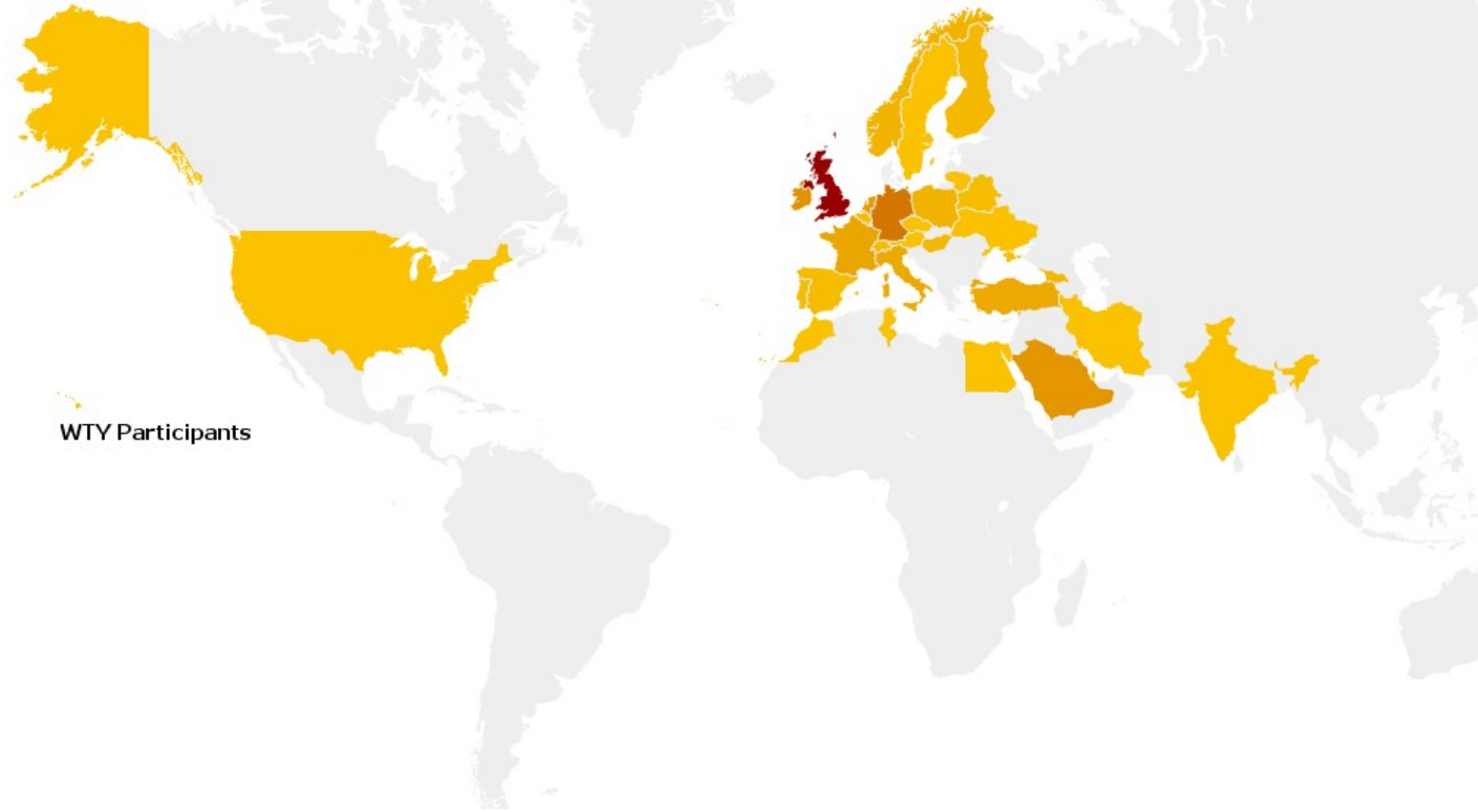
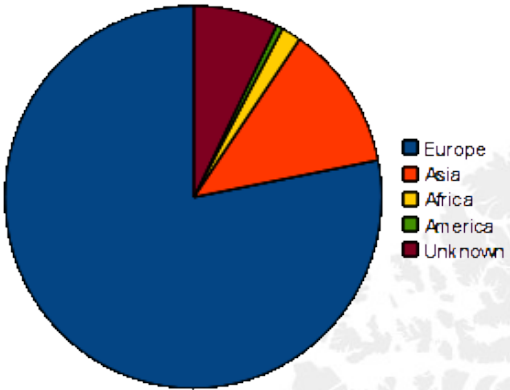
Walk Through The Y Project



*FTDNA Conference 2010
Houston TX*

Dipl.- Ing. Thomas Krahn

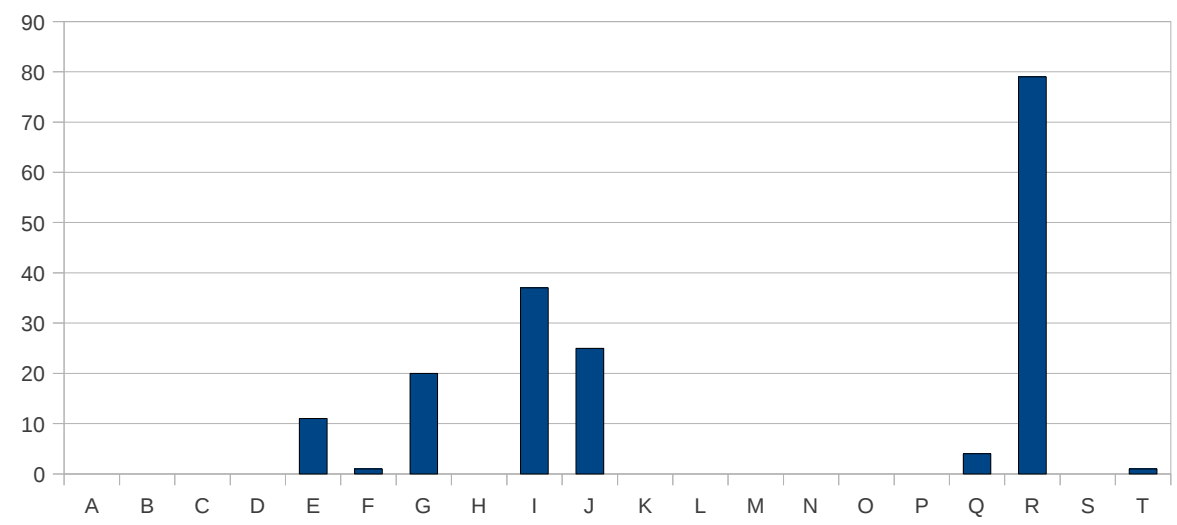
Participants - Origin



WTY Participants

| | |
|-------------------------|----|
| Austria | 1 |
| Basque | 2 |
| Belarus | 2 |
| Belgium | 2 |
| Czech Republic | 1 |
| Egypt | 1 |
| Finland | 3 |
| France | 7 |
| Georgia | 3 |
| Germany | 17 |
| Hungary | 3 |
| India | 1 |
| Iran | 1 |
| Ireland | 11 |
| Italy | 6 |
| Kuwait | 1 |
| Lithuania | 1 |
| Mahican/Wappanoe Nation | 1 |
| Morocco | 1 |
| Netherlands | 9 |
| Northern Ireland | 2 |
| Norway | 4 |
| Poland | 4 |
| Portugal | 2 |
| Qatar | 1 |
| Saudi Arabia | 10 |
| Sweden | 1 |
| Switzerland | 2 |
| Tunisia | 1 |
| Turkey | 6 |
| Ukraine | 1 |
| United Kingdom | 42 |
| Unknown | 28 |

Participants - Haplogroups



Would like to see some Central African and East Asian HGs
What about N?

DYS464X Quality Control

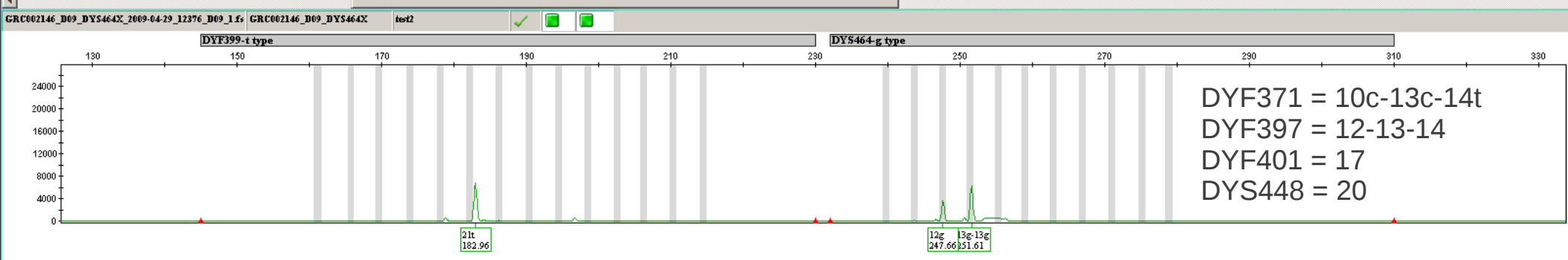
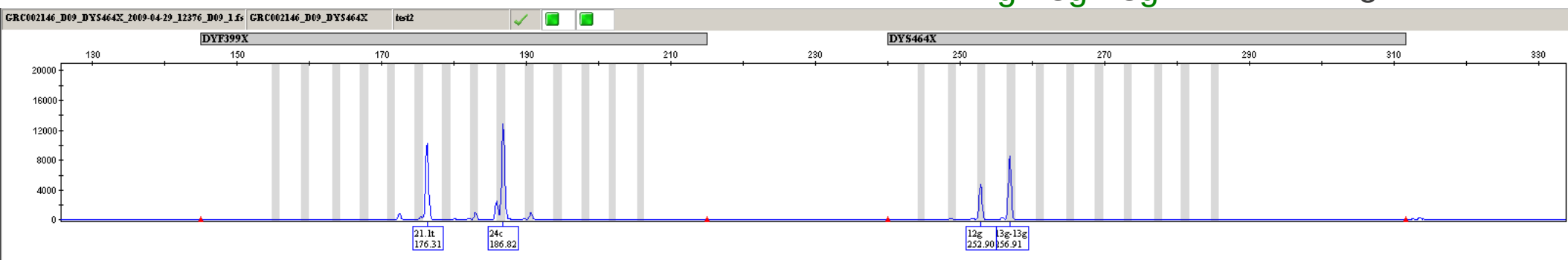
- All fresh samples passed the amplifiable DNA requirement
- Most DYS464X alleles matched previous DYS464 results (one was different: 15-15-16-16 vs. 15c-15c-15c-16g)
- 5 participants have micro-alleles at DYS464. One of them turned out to be a good indicator for a family study.
- 6 participants have duplications
- 3 participants have deletions in the P1/P2 region
- One 5 allelic and one 3 allelic pattern were found

GRC002146 DYF399X / DYS464X

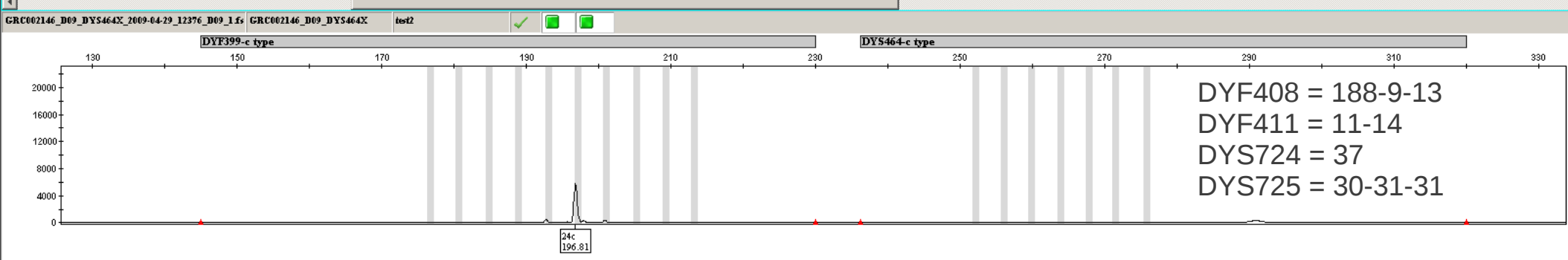
21t-24c

12g-13g-13g

Hg = G-P303*



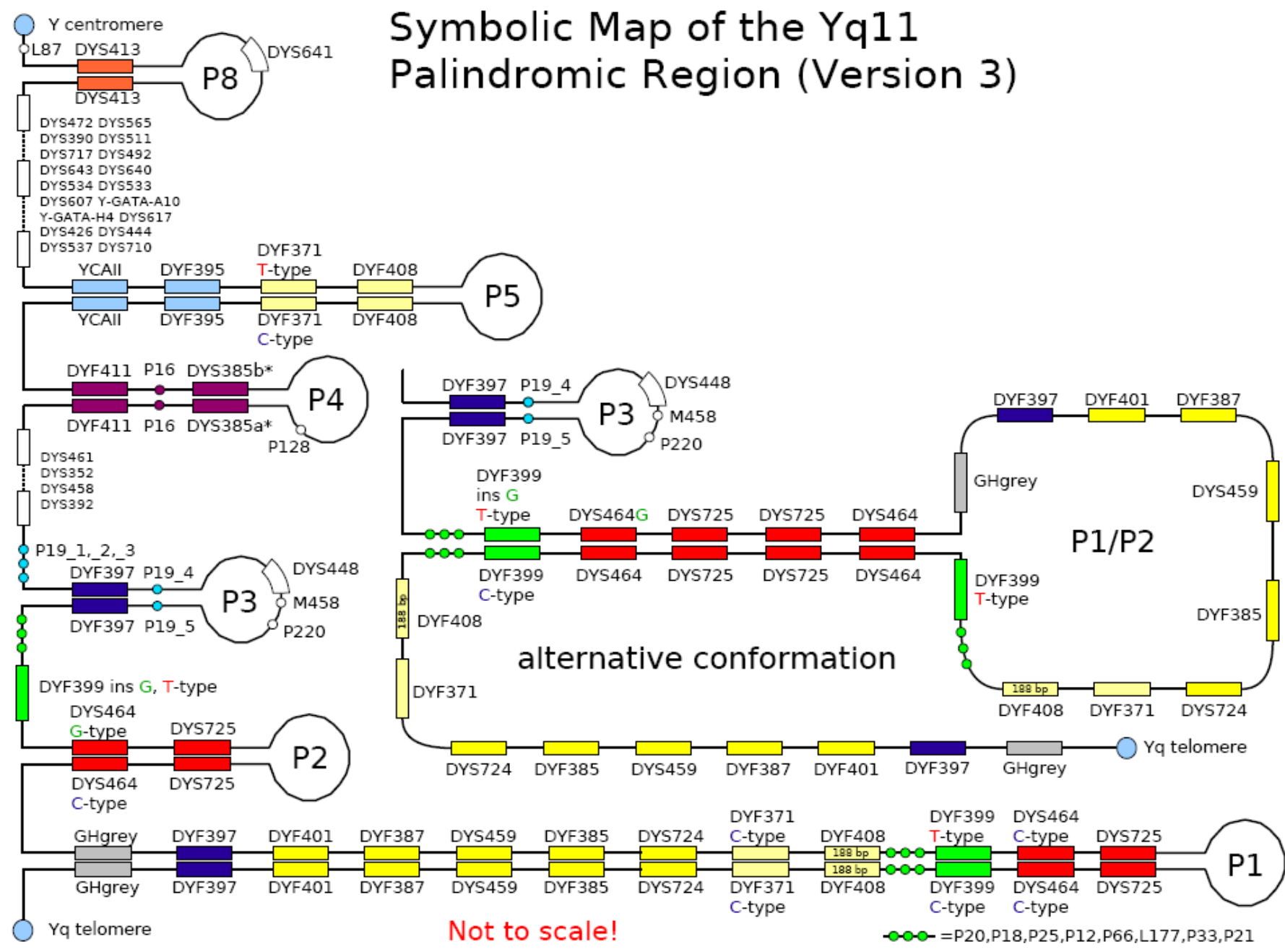
DYF371 = 10c-13c-14t
 DYF397 = 12-13-14
 DYF401 = 17
 DYS448 = 20



DYF408 = 188-9-13
 DYF411 = 11-14
 DYS724 = 37
 DYS725 = 30-31-31

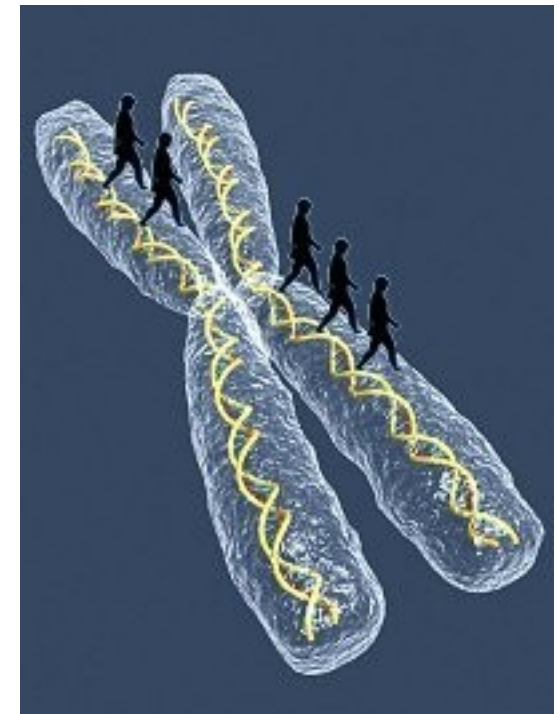
Unusual P1 deletion. Distal arm of the P1 is missing?

Symbolic Map of the Yq11 Palindromic Region (Version 3)

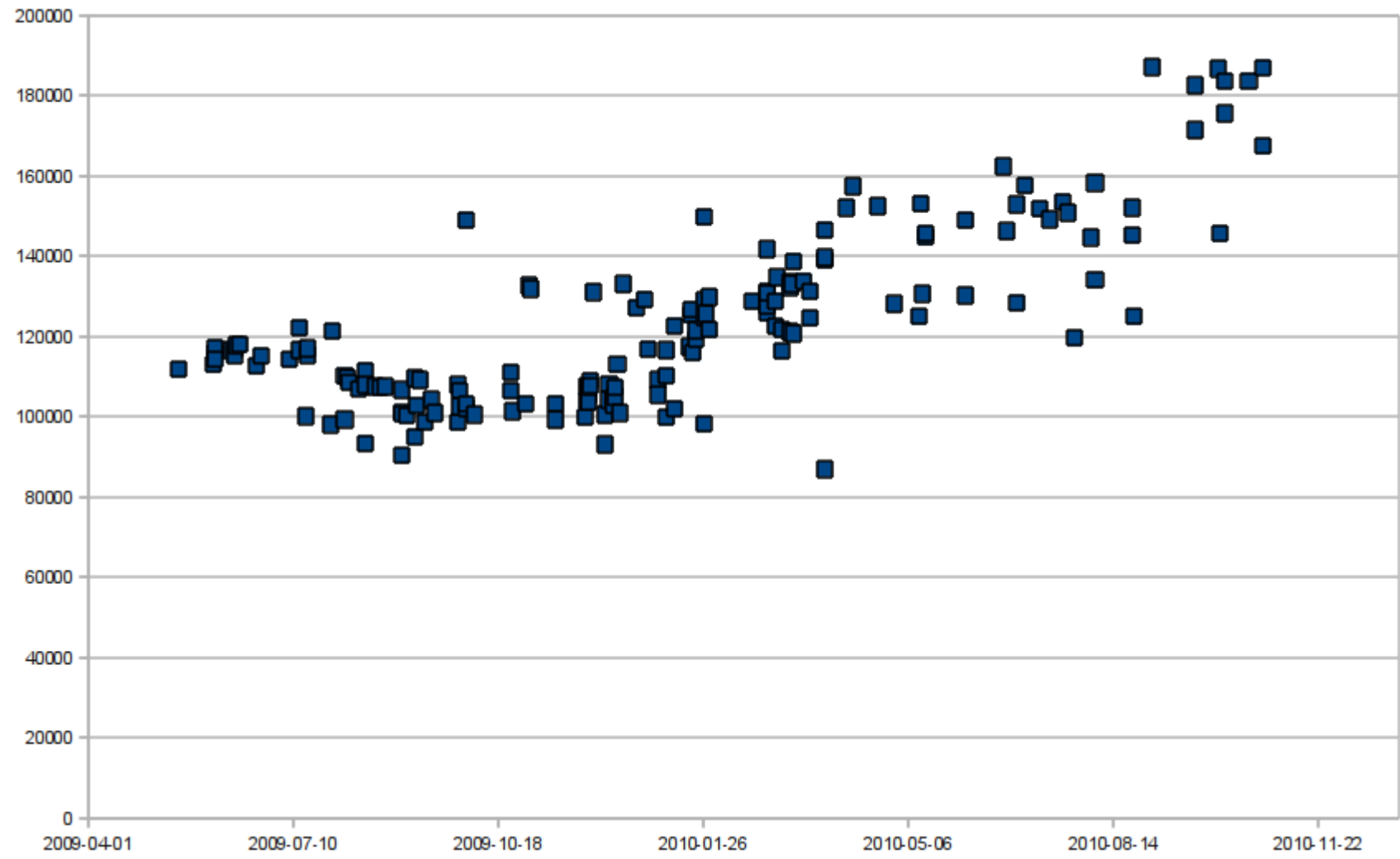


WTY Project in Numbers

- **178** Participants
- **130,182** Sequencing traces
- **53.7 Million** basepairs sequenced
- **20.5 Million** bp of Y chromosome DNA was covered
- **123,000 bp** average coverage per participant
- **137** previously undocumented Y-SNP markers were found
- **96** Participants didn't find a new SNP in their DNA



WTY Coverage Has Increased



New segments were added because they became available from research activities. Chance of finding a new SNP stays approximately constant because old segments are extensively explored and chance of finding a new SNP there is decreasing.

Private Vs. Public Participation

80% of the participants have chosen "public"

finch2 Enter GRC: Links: User: [64047](#) Oct 28, 2010 11:11

Home > BLAST Requests > Result Details > Query Details > hspDetails

System
[Admin](#)
Results
[Folders](#)
BLAST
[Requests](#)
[New Request](#)
[Databanks](#)
Alleles
[Alleles](#)
[Array](#)
[Compare ht](#)
[Aldi](#)
[Add Alleles](#)
[Samples](#)
[Markers](#)
FTDNA
[Chromats](#)
[FGS](#)
[WTY](#)
[SNP-Info](#)

BLAST Alignment

| | | | |
|-------------------|---|------------------|---------|
| Query | GRC013186_WTY_E17_sL16v2_R_WTY2 943323 Re- GRC013186_WTY_E17_sL16v2_R_WTY22053_R_E17.ab1 | | |
| Subject | Ic ChrY No definition line found | | |
| E Value | 0 | Score | 546 |
| Align Len | 546 | Bit Score | 1090.46 |
| Subj Len | 546 | Query Len | 546 |
| Identities | 546 | Positives | 546 |

Q: 553
 S: 7232910

ChrY
 7232910 7232920 7232930 7232940 7232950 7232960 7232970 7232980 7232990

STR
 dbSNP
 M series SNPs
 P series SNPs
 Other Variations

47
 72

Public participants can review their traces and exchange data on the Finch2 platform

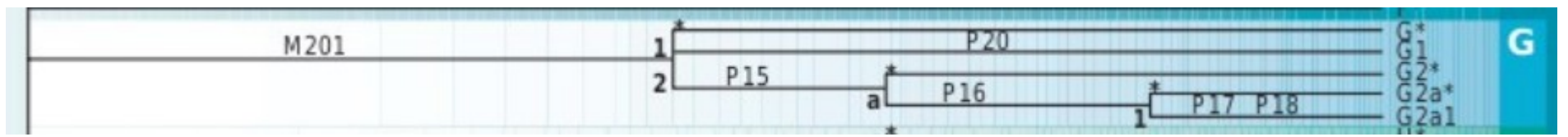
WTY Success Stories

- Irish Type III found a SNP that covers the whole group and is now hg R-**L226**
- **L140** and **L141** define major branches in the G2 haplogroup tree
- **L222**(.2) has become the most discussed marker among Arab J1
- **L245** defines a major branch downstream of Q-M378
- **L161** is a significant branch downstream I-M423
- **L257** is the fourth large branch below R-U106 next to R-L48, R-L1 and R-U198
- A lot of “private” SNPs with importance on the family / genealogy level.

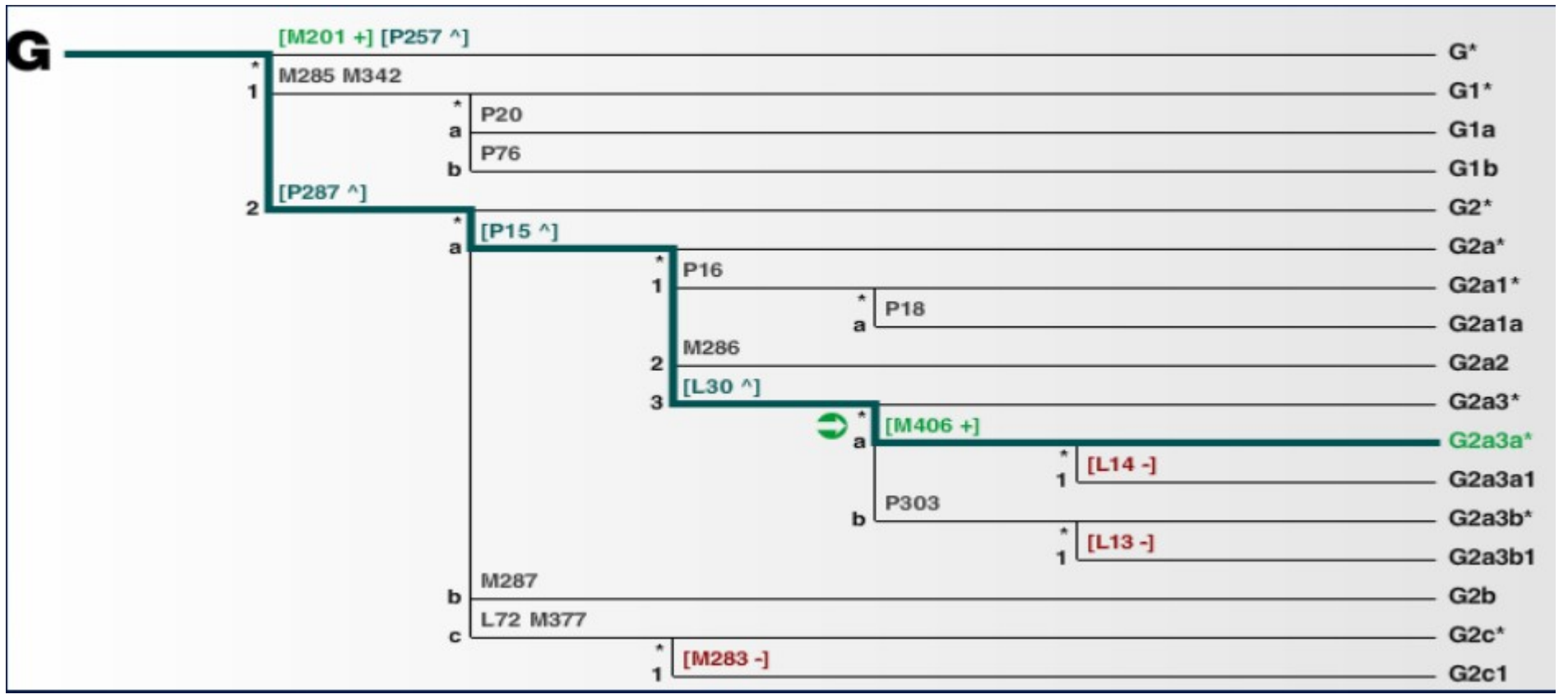


The Y Tree Has Grown

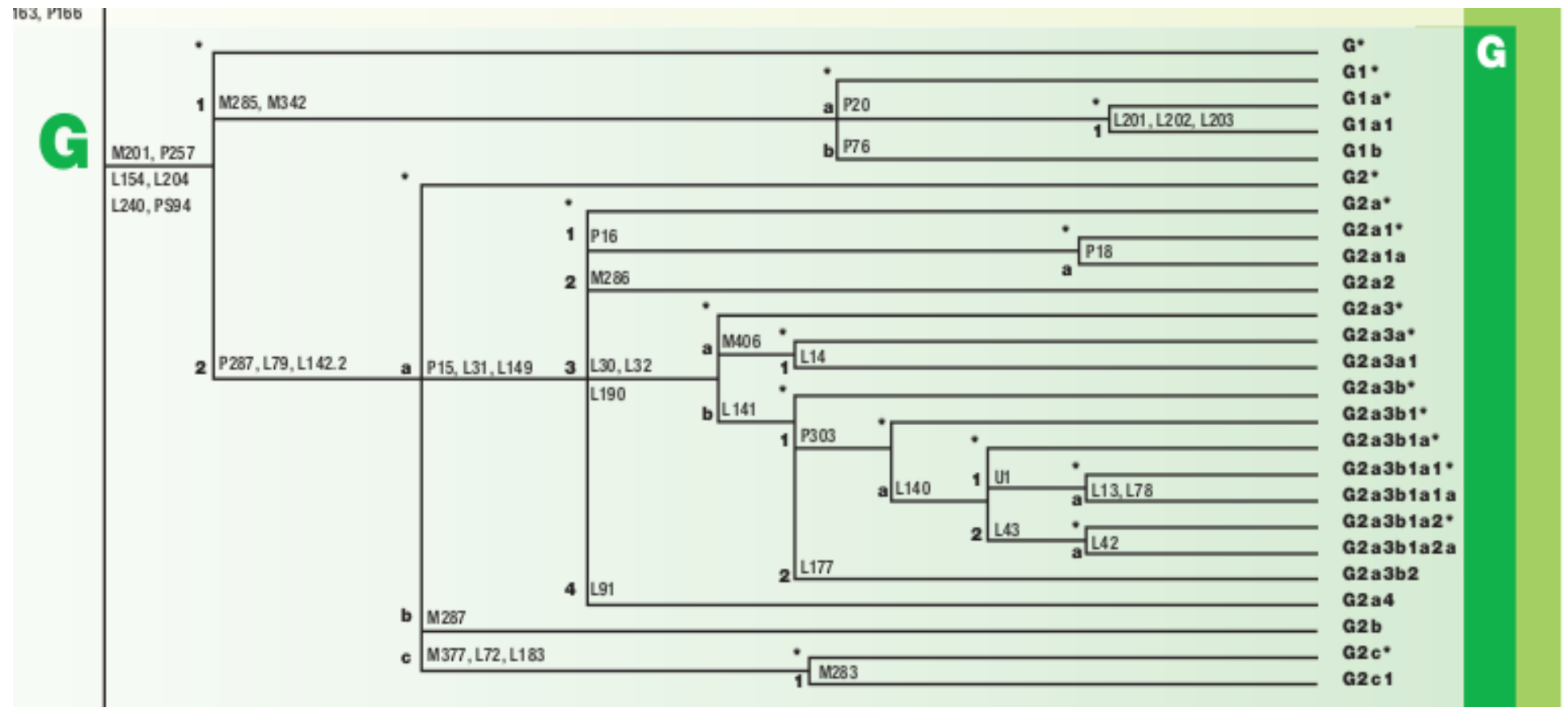
Nature 2003



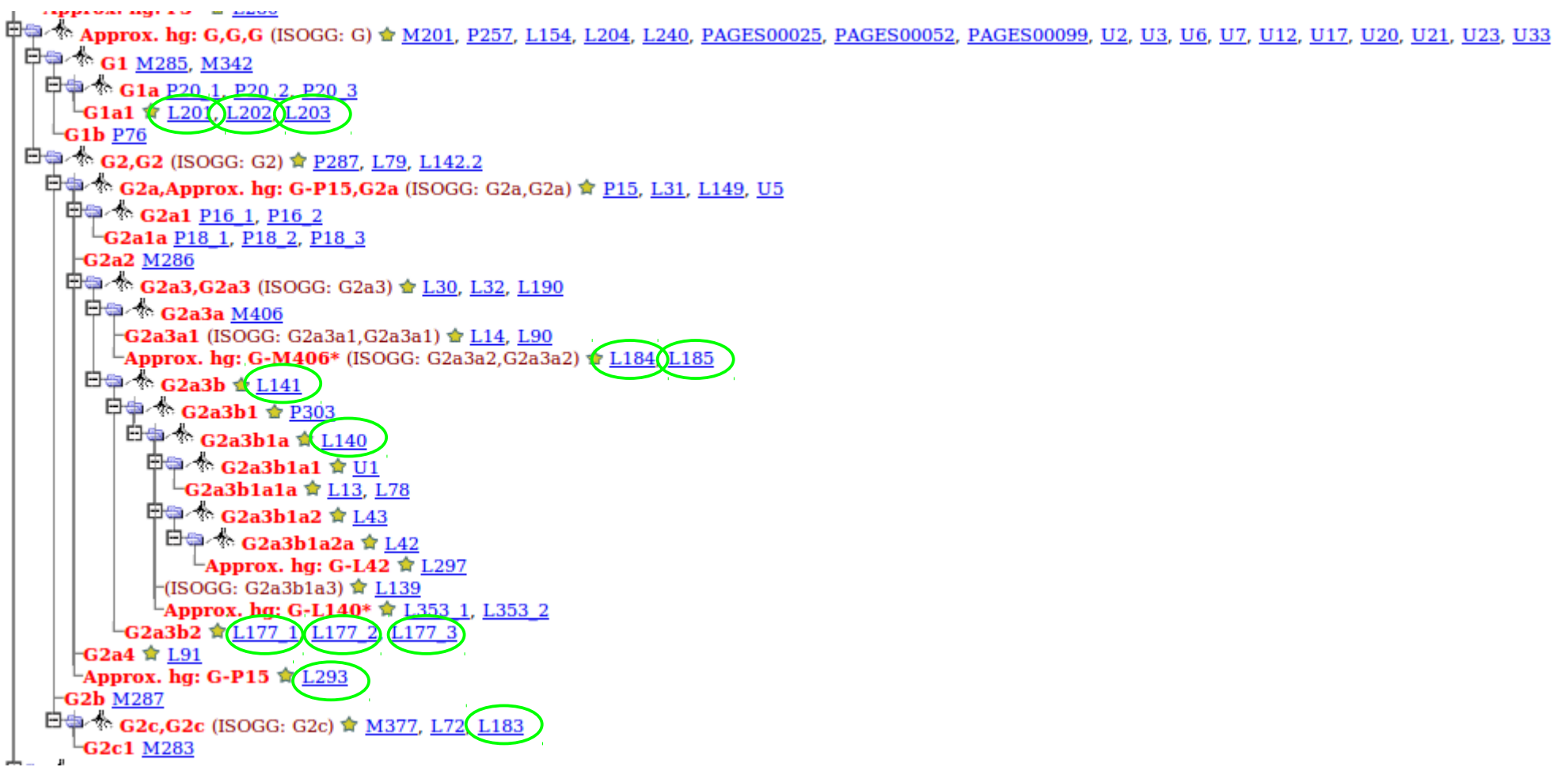
YCC2008



FTDNA Poster 2010



Draft Tree 2010

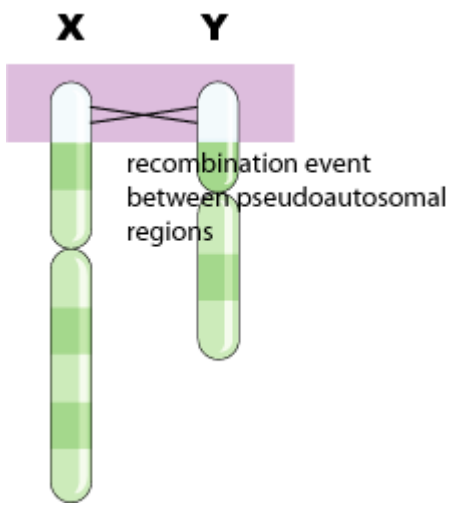


The circled SNPs have been found in the WTY Project

Recombination

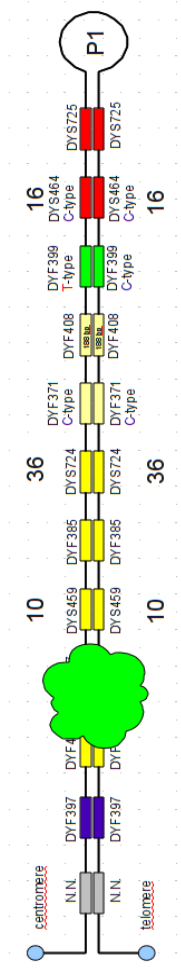
Recombination is a very important factor for the generation of SNP mutations on the ChrY

PAR



Known since 2000 [1]

recLOH



New Findings:

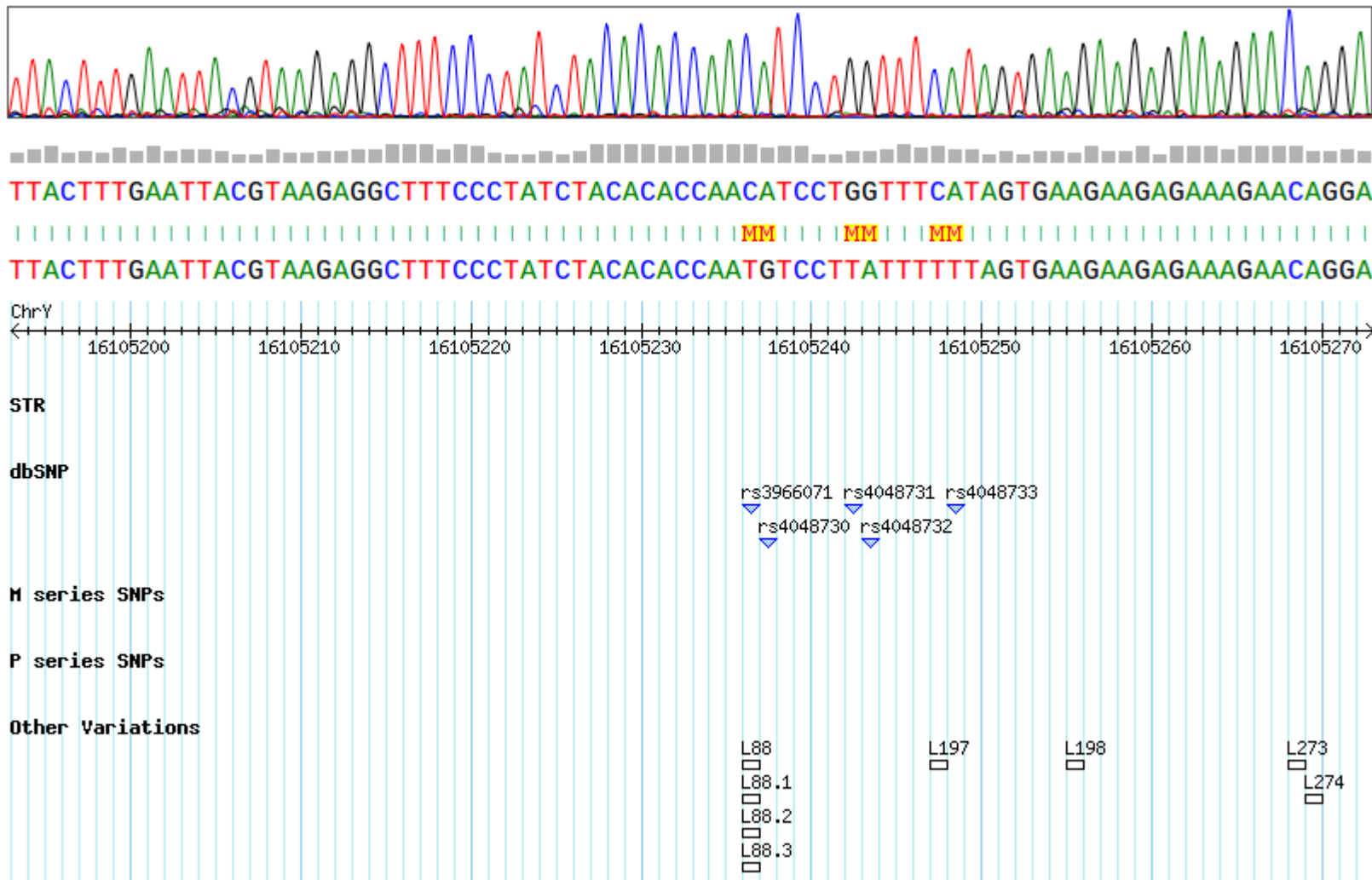
- Recombination takes place everywhere on the ChrY.
- Many of the derived SNPs on the existing Y tree have their derived allele from the ChrX or other autosomes.
- Often independent recombination events happen in different Hgs. This forces us to assign .1 and .2 variants to the same marker on the Y tree.
- The term NRY (non recombining part of the Y chromosome) is completely obsolete!

[1] Ciccodicola et al.: Differentially regulated and evolved genes in the fully sequenced Xq/Yq pseudoautosomal region Hum. Mol. Genet. (2000) 9 (3): 395-401.

L88 Region in HG J-L26/L27



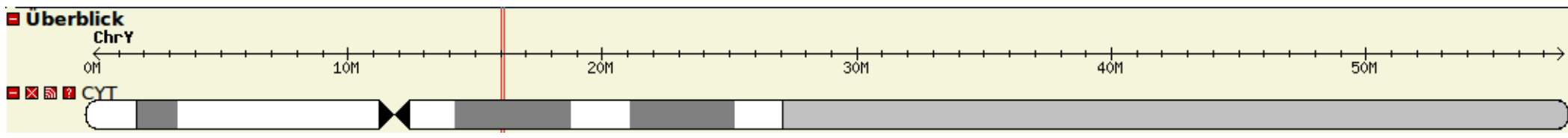
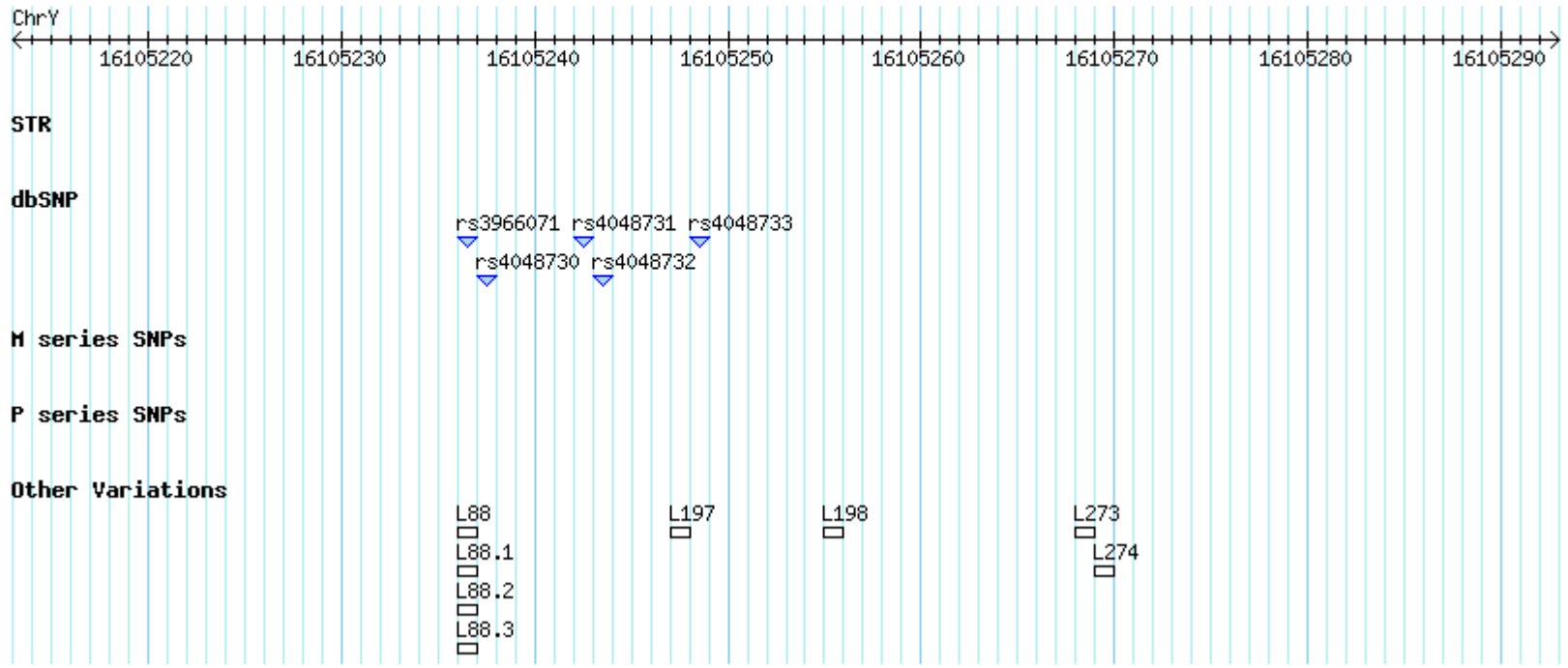
L88 Region in HG E-M2



L88 Region of Highly Similar ChrX Sequence

Q:312 GGCTTTCCCTGCCTACACACCAACATCCTGGTTTCATAGTGAGGAAGAGAAAGAATGGGAATGTAATTATTGCAATTATT ChrX

S:16105213 GGCTTTCCCTATCTACACACCAATGTCCTTATTTTTTAGTGAAGAAGAGAAAGAACAGGAATGTAATTATTGCAATTATT ChrY

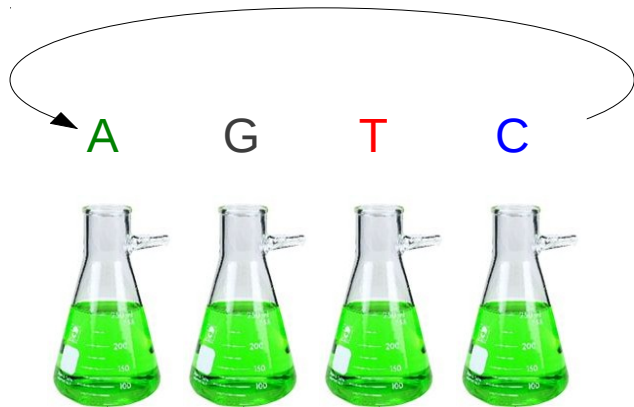


L88

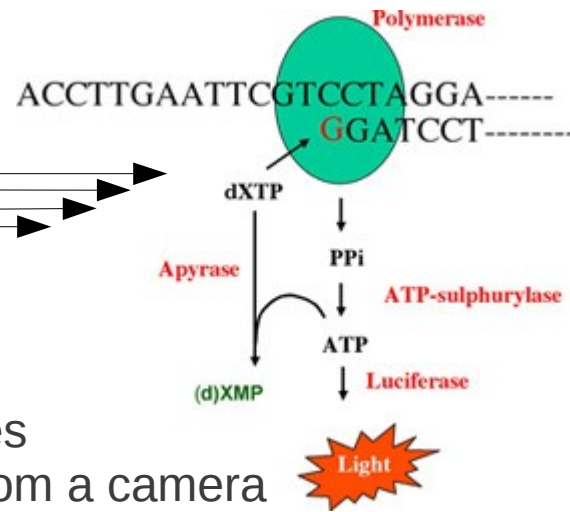
New Perspective for ChrY Sequencing?



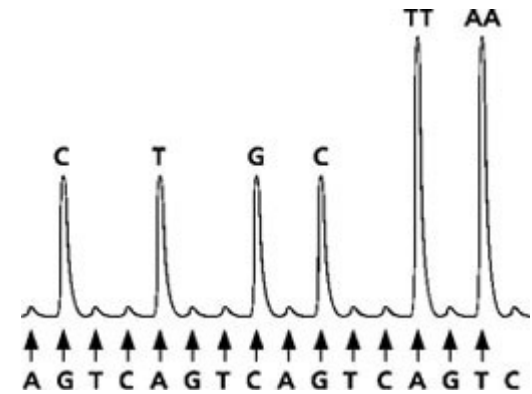
Pyrosequencing



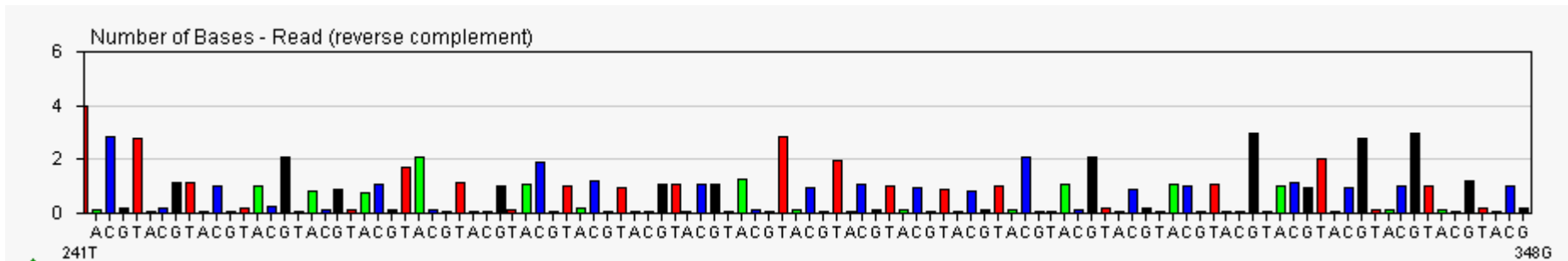
Nucleotides are added one after the other



If the nucleotide matches
Light will be captured from a camera



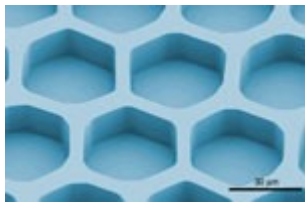
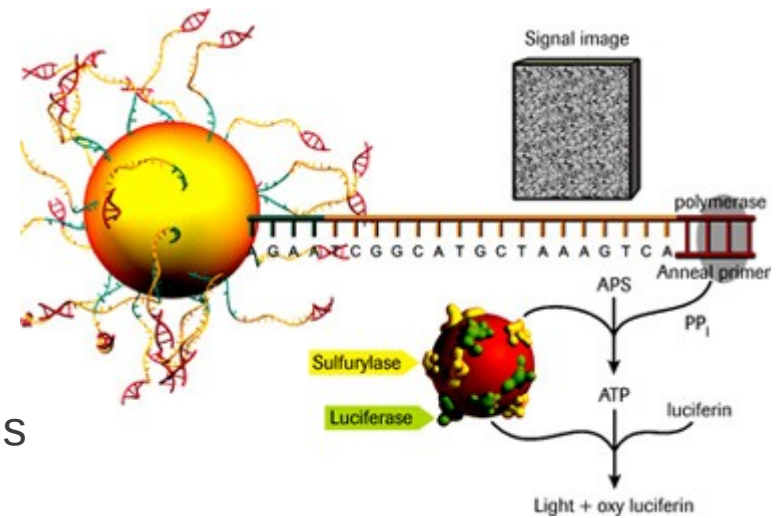
Flowgram



Roche 454 Titanium Sequencer

Principle of 454 Sequencing

- Single DNA molecules are captured on a bead
- PCR around bead in an oil emulsion
- Beads with PCR products are distributed on a PTP
- Instrument performs millions of pyrosequencing reactions in parallel

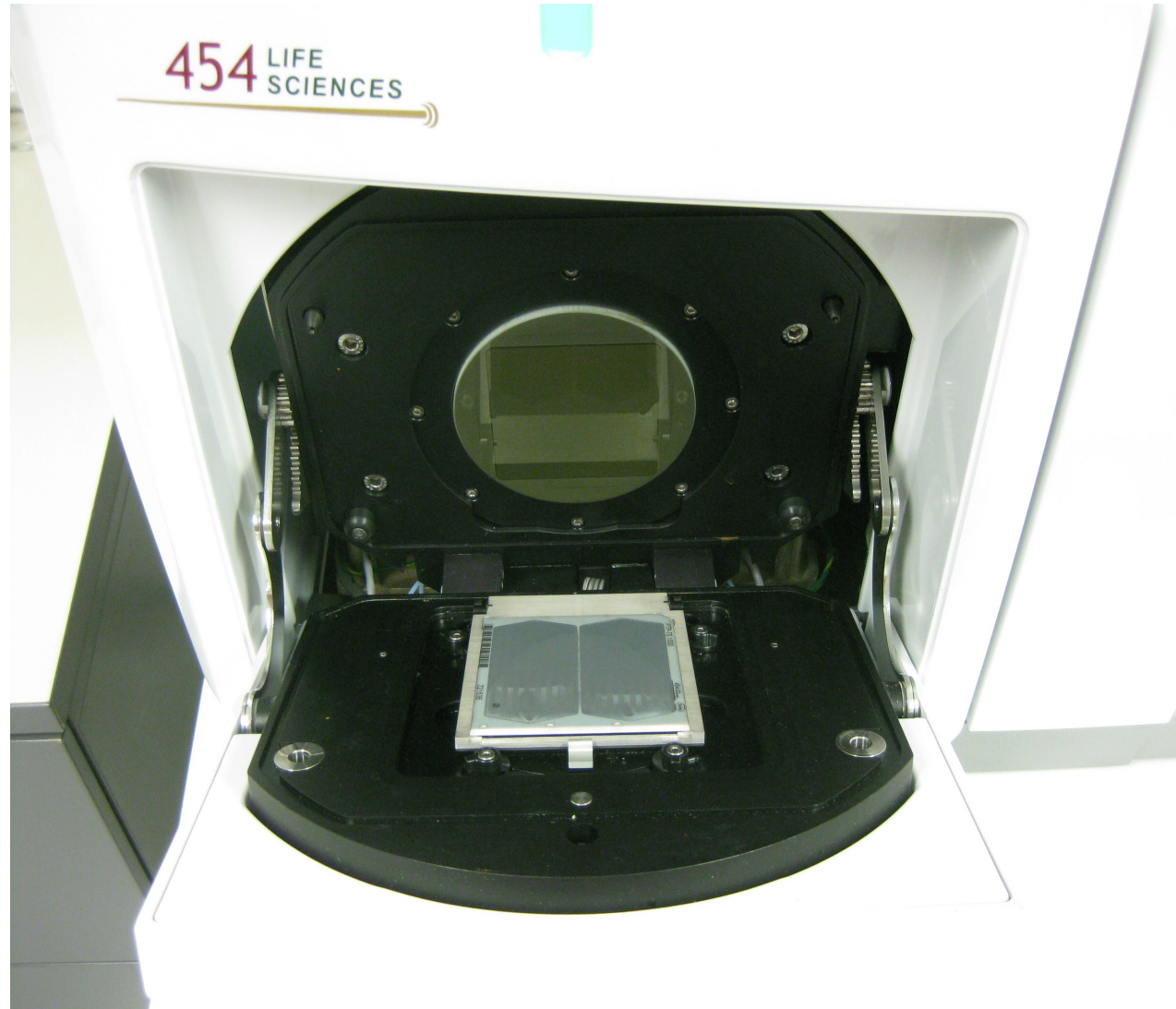


Picotiter Plate (PTP)



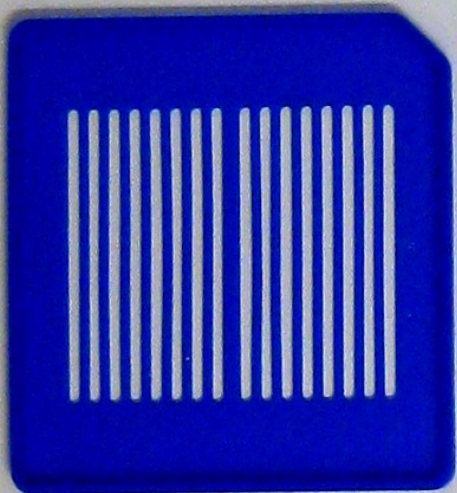
Roche 454 Titanium Sequencing Instrument

PTP and Camera



Roche 454 Titanium Sequencing Gaskets

Loading gaskets



Up to 20 Mbp/region



55 Mbp/region



110 Mbp/region

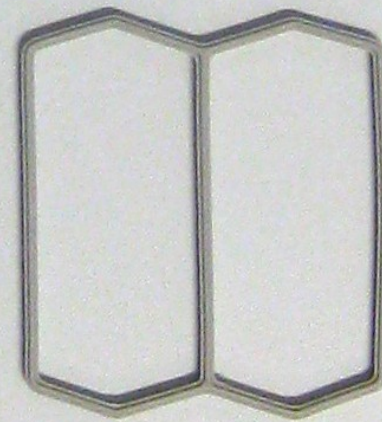


280 Mbp/region

Picotiter plate



ChrY 10x coverage is approx. 600 Mbp

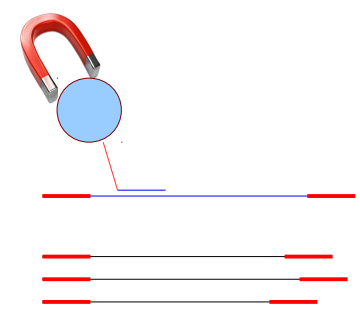
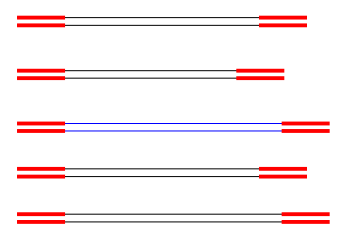
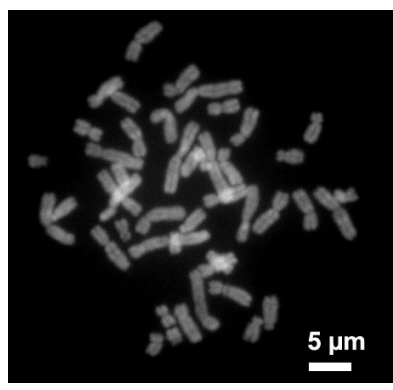


Run gasket

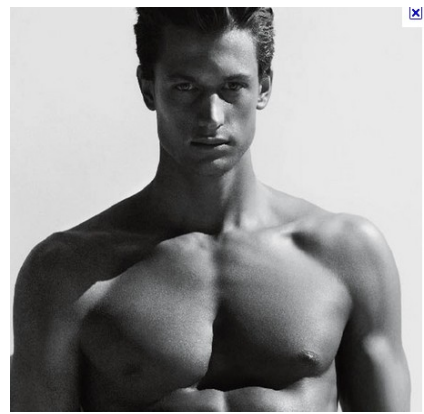
Loosing real estate when using multiple regions

Sample Processing on 454

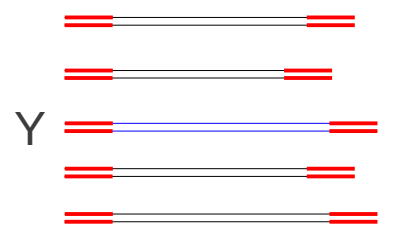
Genomic DNA → Library → Enrichment → Pyrosequencing



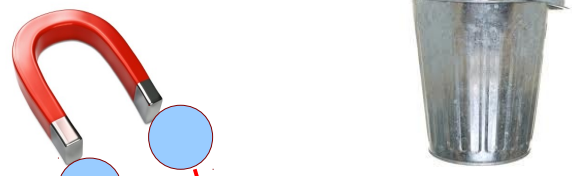
Possible Enrichment Strategy



Male DNA library

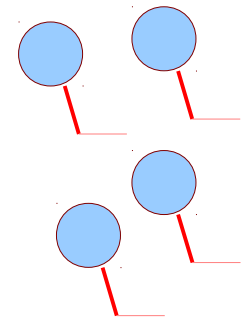


Pull out and discard all autosomal DNA except ChrY

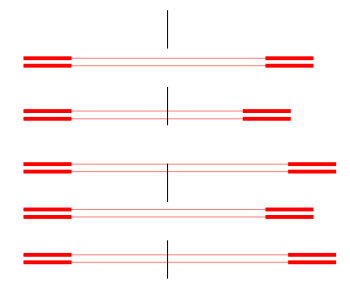


ChrY DNA remains in solution

Attach to mag. beads



Female DNA library






cut






Results from Female Subtraction “Enrichment”

| Reference | Unique matching reads | % of unique matches | % of all reads | % coverage of reference |
|-----------|-----------------------|---------------------|----------------|-------------------------|
| Chr1 | 336 | 8.40 | 6.30 | 12.07 |
| Chr2 | 336 | 8.40 | 6.30 | 11.39 |
| Chr3 | 255 | 6.40 | 4.70 | 11.28 |
| Chr4 | 251 | 6.30 | 4.70 | 10.90 |
| Chr5 | 205 | 5.10 | 3.80 | 12.02 |
| Chr6 | 218 | 5.40 | 4.10 | 11.50 |
| Chr7 | 192 | 4.80 | 3.60 | 11.73 |
| Chr8 | 187 | 4.70 | 3.50 | 11.50 |
| Chr9 | 182 | 4.50 | 3.40 | 11.55 |
| Chr10 | 258 | 6.40 | 4.80 | 12.18 |
| Chr11 | 195 | 4.90 | 3.60 | 13.03 |
| Chr12 | 180 | 4.50 | 3.40 | 11.28 |
| Chr13 | 94 | 2.30 | 1.80 | 11.10 |
| Chr14 | 114 | 2.80 | 2.10 | 10.73 |
| Chr15 | 114 | 2.80 | 2.10 | 12.54 |
| Chr16 | 176 | 4.40 | 3.30 | 11.79 |
| Chr17 | 135 | 3.40 | 2.50 | 10.52 |
| Chr18 | 119 | 3.00 | 2.20 | 10.77 |
| Chr19 | 132 | 3.30 | 2.50 | 10.77 |
| Chr20 | 89 | 2.20 | 1.70 | 10.72 |
| Chr21 | 36 | 0.90 | 0.70 | 9.21 |
| Chr22 | 69 | 1.70 | 1.30 | 9.96 |
| ChrX | 78 | 1.90 | 1.50 | 11.82 |
| ChrY | 38 | 0.90 | 0.70 | 9.80 |
| mtDNA | 16 | 0.40 | 0.30 | 25.49 |
| | sum | sum | sum | average |
| | 4005 | 99.8 | 74.9 | 11.83 |
| | Approx. basepairs: | | | |
| | 2002500 | | | |

New SNP found in 454 Alignment

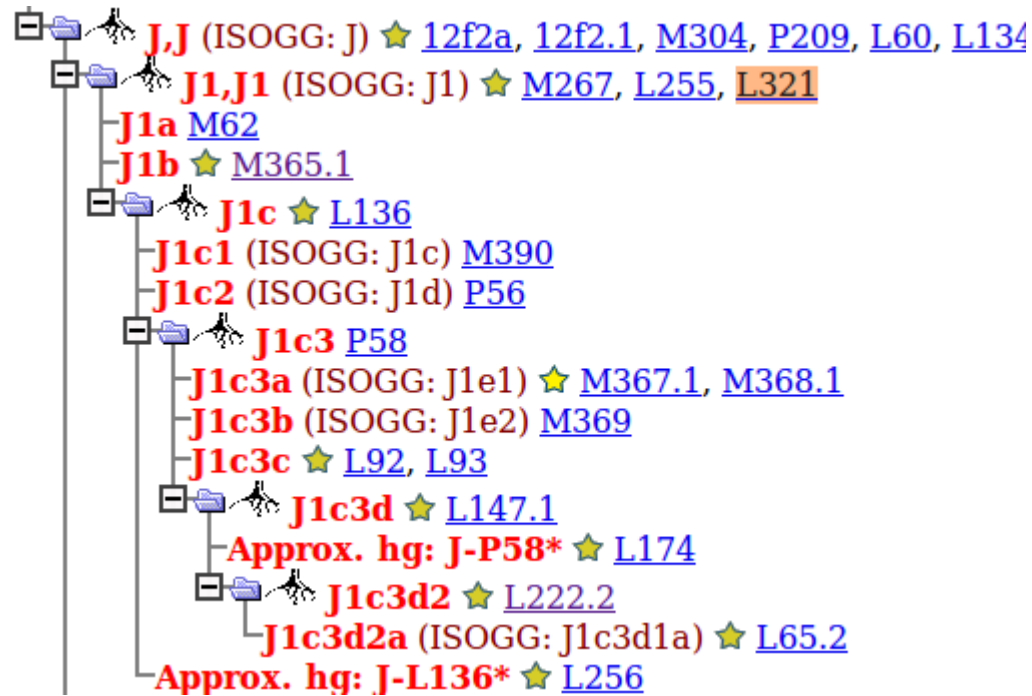
Ref: ChrY - 57,772,954 bp.  Positions of Interest  

Base left selected right   

ChrY
contig91341
GKDCRZV01B10AE
GKDCRZV01B22X0


AAAATTCAGGTGATCGCCTCTCACTAAATGCCATCAGCCCACGT -GCACCATGACACAAAGTCTCTGAAAGTTTCACTTGGGGTCTGTGTTTG
AAAATTCAGGTGATCGCCTCTCACTAAATGCCATCAGCCCACGT T-CACCATGACACAAAGTCTCTGAAAGTTTCACTTGGGGTCTGTGTTTG
AAAATTCAGGTGATCGCCTCTCACTAAATGCCATCAGCCCACGT T-CACCATGACACAAAGTCTCTGAAAGTTTCACTTGGGGTCTGTGTTTG
AAAATTCAGGTGATCGCCTCTCACTAAATGCCATCAGCCCACGT T-CACCATGACACAAAGTCTCTGAAAGTTTCACTTGGGGTCTGTGTTTG




Add New SNP to The J1 Tree



So far so good...

Another "SNP" from the 454 traces

Ref: ChrY - 57,772,954 bp.  Positions of Interest

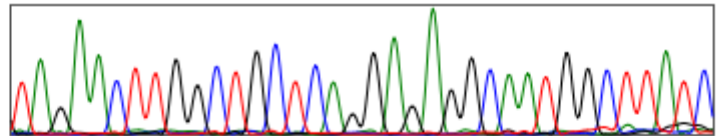
Base left selected right   

| Base | left | selected | right |
|------|------------|------------|------------|
| ChrY | 12,874,347 | 12,874,392 | 12,874,439 |

```
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTG-TAGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
GCAGTGCTGCTCCGAGGTTGTTGATGACTACTTCTCTTTGCTTTGC-AGAACTTGGCTGCTCAGGAGAGGCAATGGCTTATCAGCTGTTGCTGA
```


No Confirmation With Sanger Method

Q:481

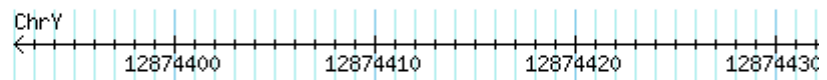


TAGAACTTGGCTGCTCAGGAGAGGCCAATGGCTTATC

S:12874392

|||||

TAGAACTTGGCTGCTCAGGAGAGGCCAATGGCTTATC



- STR
- dbSNP
- M series SNPs
- P series SNPs
- Other Variations
- L323
-

DEPRECIATED

Only 1 in 5 Mismatches are Real

| SNP | Chr | Start | End | Mutation | hg | Forward Primer | Reverse Primer |
|------|------|----------|----------|----------|------------|-----------------------------|---------------------------|
| L321 | ChrY | 7231834 | 7231834 | G to T | J1 | ccccaattgaagaagcagat | gagagtgcgtcgggagaggcc |
| L322 | ChrY | 4978881 | 4978881 | A to G | deprecated | aaaatattaattggtctttg | acattctgtaaattttctagata |
| L323 | ChrY | 12874392 | 12874392 | T to C | deprecated | gttctaaacctggtaccaaataa | gataagccattgcctctctcg |
| L324 | ChrY | 7232074 | 7232074 | G to A | deprecated | L321-f | L321-r |
| L328 | ChrY | 20541419 | 20541419 | A to G | deprecated | gtgagcaagttcacataagaac (v2) | gctgtgtaggccattcttgc (v2) |

| SNP | Chr | Start | End | Mutation | Comment | Forward Primer | Reverse Primer |
|------------|------|----------|----------|----------|--------------------------------------|---------------------------|----------------------------|
| YSC0000001 | ChrY | 3224594 | 3224594 | T to C | Found in a hg J-P58 person | CCTTTGAAGAGTCACATAGTATGAG | GATAATTC AAGTAAATATCCATACG |
| YSC0000002 | ChrY | 8282500 | 8282500 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000003 | ChrY | 8543648 | 8543648 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000004 | ChrY | 9211276 | 9211276 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000005 | ChrY | 9999916 | 9999916 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000006 | ChrY | 11911004 | 11911004 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000007 | ChrY | 11950155 | 11950155 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000008 | ChrY | 11984871 | 11984871 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000009 | ChrY | 13359588 | 13359588 | A to G | deprecated | M180P88-f | M180P88-r |
| YSC0000010 | ChrY | 13892301 | 13892301 | T to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000011 | ChrY | 13955573 | 13955573 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000012 | ChrY | 14166184 | 14166184 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000013 | ChrY | 14348553 | 14348553 | C to T | Found in a hg J-P58 person | TBD | TBD |
| YSC0000014 | ChrY | 14584021 | 14584021 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000015 | ChrY | 15396010 | 15396010 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000016 | ChrY | 15461747 | 15461747 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000017 | ChrY | 15695702 | 15695702 | G to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000018 | ChrY | 17374692 | 17374692 | C to T | Found ancestral in a hg J-P58 person | TBD | TBD |
| YSC0000019 | ChrY | 19817701 | 19817701 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000020 | ChrY | 19824299 | 19824299 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000021 | ChrY | 20356650 | 20356650 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000022 | ChrY | 21298406 | 21298406 | G to A | Found ancestral in a hg J-P58 person | TBD | TBD |
| YSC0000023 | ChrY | 21526511 | 21526511 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000024 | ChrY | 21526652 | 21526652 | G to A | Found in a hg J-P58 person | TBD | TBD |
| YSC0000025 | ChrY | 21549214 | 21549214 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000026 | ChrY | 21718473 | 21718473 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000027 | ChrY | 21718479 | 21718479 | T to A | Found in a hg J-P58 person | TBD | TBD |
| YSC0000028 | ChrY | 22222237 | 22222237 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000029 | ChrY | 27030547 | 27030547 | T to C | Found in a hg J-P58 person | TBD | TBD |
| YSC0000030 | ChrY | 27132639 | 27132639 | A to G | Found in a hg J-P58 person | TBD | TBD |
| YSC0000031 | ChrY | 57237843 | 57237843 | C to A | Found in a hg J-P58 person | TBD | TBD |
| YSC0000032 | ChrY | 21658766 | 21658766 | C to T | Found in a hg R-M198 person | TBD | TBD |
| YSC0000033 | ChrY | 15862538 | 15862538 | C to T | Found in a Illumina SNP assay | TBD | TBD |
| YSC0000034 | ChrY | 10342008 | 10342008 | G to A | Found in a Illumina SNP assay | TBD | TBD |
| YSC0000035 | ChrY | 17269381 | 17269381 | G to T | Found in a 454 trace of GRC000001 | TBD | TBD |
| YSC0000036 | ChrY | 17550160 | 17550160 | A to G | Found in a 454 trace of GRC000001 | TBD | TBD |
| YSC0000037 | ChrY | 19782983 | 19782983 | A to G | Found in a 454 trace of GRC000001 | TBD | TBD |
| YSC0000038 | ChrY | 20688016 | 20688016 | T to A | Found in a 454 trace of GRC000001 | TBD | TBD |
| YSC0000039 | ChrY | 21376059 | 21376059 | G to A | Found in a 454 trace of GRC000001 | TBD | TBD |
| YSC0000040 | ChrY | 8673154 | 8673154 | T to C | Found in a 454 trace of GRC000001 | TBD | TBD |
| YSC0000041 | ChrY | 12178088 | 12178088 | T to C | Found in a 454 trace of GRC000001 | TBD | TBD |



Long list of Y chromosome SNP Candidates are pending confirmation with Sanger

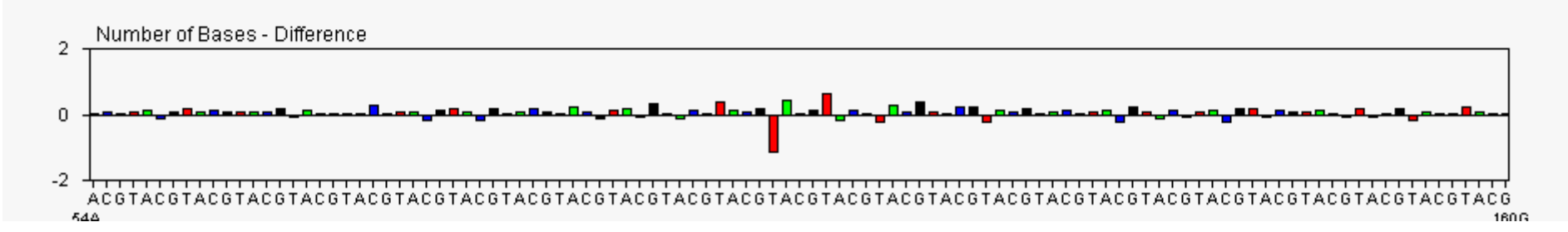
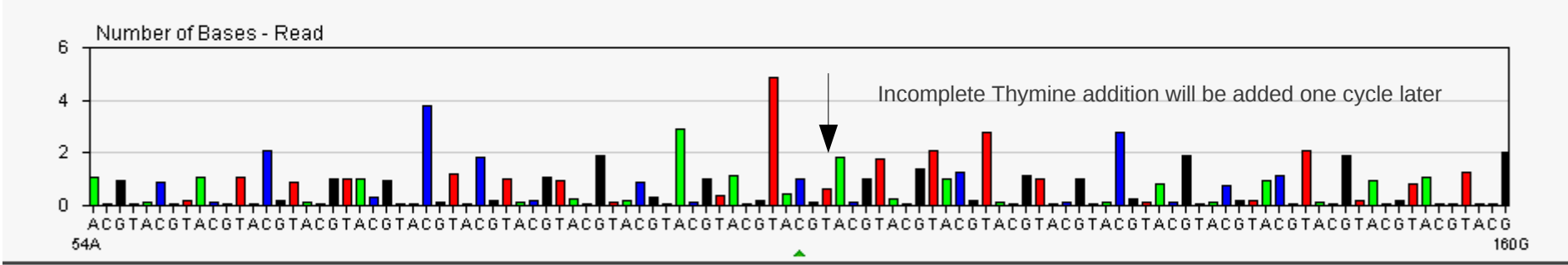
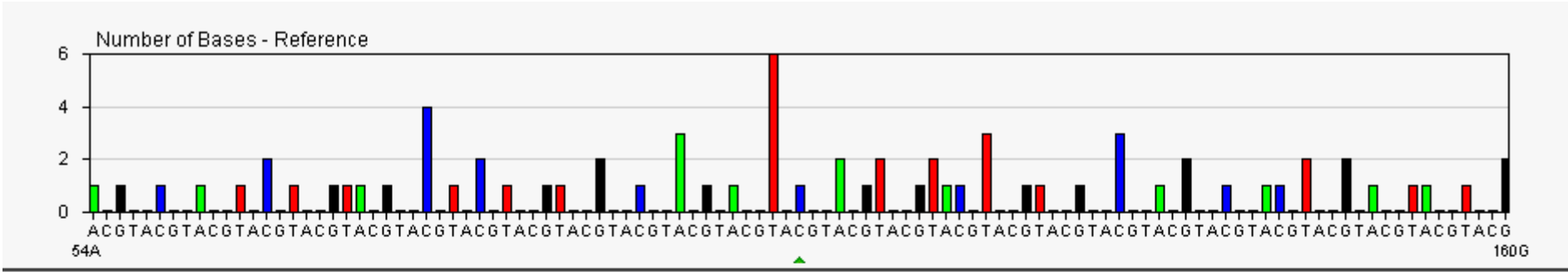
"Carry Forward" Effect

Ref: ChrY - 57,772,954 bp. Positions of Interest:

Base left selected right What looks like two independent mutations

```

ChrY
contig91335
GKDCRZV01BJXFX
GNLDEY102BR5YK
CAGATCAGCATCCTGTAGCCCCCTCTGTGGCAAAGATTTTTTC - AAGTTGTTACTTTGTGCCCAGGCACCTGGATATGGTGGTAACTAAGACAG
CAGATCAGCATCCTGTAGCCCCCTCTGTGGCAAAGATTTTTTC - AAGTTGTTACTTTGTGCCCAGGCACCTGGATATGGTGGTAACTAAGACAG
CAGATCAGCATCCTGTAGCCCCCTCTGTGGCAAAGATTTTTTC - AAGTTGTTACTTTGTGCCCAGGCACCTGGATATGGTGGTAACTAAGACAG
CAGATCAGCATCCTGTAGCCCCCTCTGTGGCAAAGATTTTTTC - AAGTTGTTACTTTGTGCCCAGGCACCTGGATATGGTGGTAACTAAGACAG
    
```



How Does The WTY Continue?

- We're working on next generation sequencing but we're not ready
- FTDNA is still accepting WTY applications
- Coverage will be further extended above 200 kB
- Evaluating a concept for WTY upgrade package
- Reduce time from SNP discovery to availability on advanced orders page

How to participate?

<http://www.familytreedna.com/walk-y-application.aspx>

\$ 750 per participant



A BIG thank you to

All WTY participants

The active WTY group
administrators

The GRC laboratory staff

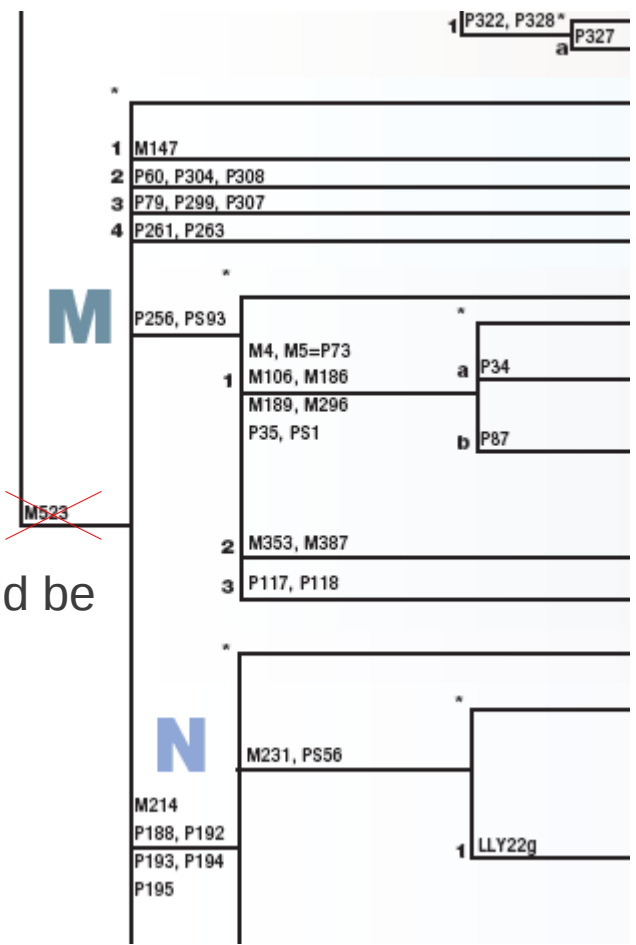
FTDNA customer support

Elliott (for all my extras I
require on the FTDNA page)

Bennett and Max (who let us
play with a non high income
project)

Y'all for patiently listening

Errors on the Printed Y-Tree



| | | |
|------------------------|--------------------------|---|
| F | P14, M89, M213, P133 * | |
| | P134, P135, P136, P138 1 | P91, P104 |
| | P139, P140, P141, P142 2 | M427, M428 |
| | P145, P146, P148, P149 3 | P96, M282, L279, L281, L284, L285, L286 |
| | P151, P157, P158, P159 4 | P254 |
| P160, P161, P163, P166 | | |
| P197 P216 | | |

P254 is really hg H3