BOOALI PATHOBIOLOGY LAB

آزمایشگاه بوعلی

Introduction to Common Medical Genetics Tests Performed in the Laboratory

Dr.Sadeghi
Ph.D in Medical Genetics

- Medical Genetic Tests
  - Molecular Genetics
    - Mutation Detection by Sager Seq
      - Every Genes
    - SNPs analysis By ARMS and …
      - JAK2 *and …*
    - *MLPA and …*
  - Cytogenetics
    - Karyotyping and FISH

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Mutatation Detection by Sager Sequencing

- *1- Physician Test Request*
- *2- Primer Design*
- *3-BLAST*
- *4- SNP Check*
- *5- Primer Ordering*
- *6- Sanger Sequencing*
- *7- Analysis*

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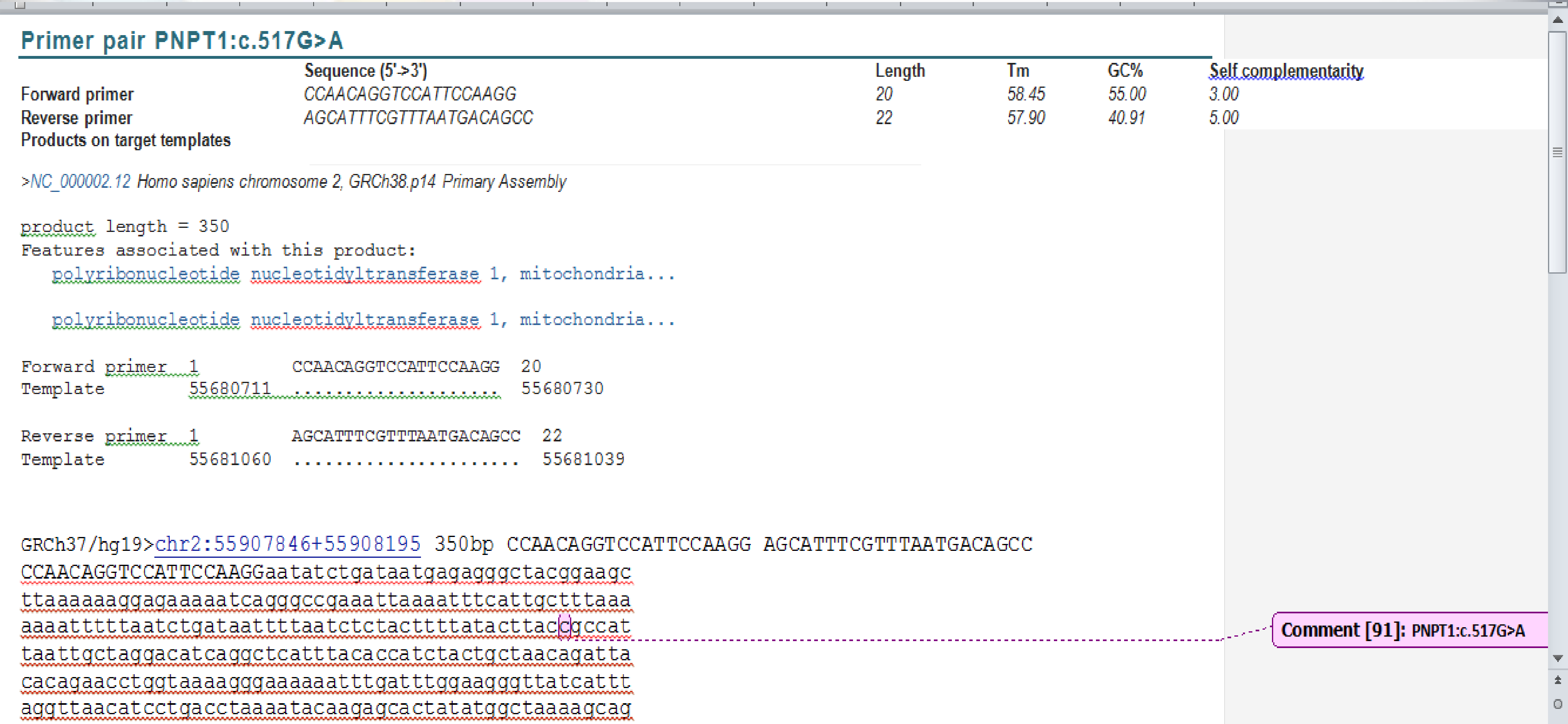
Kwon Familial Mutation



Primer pair PNPT1:c.517G>A

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity
Forward primer	*CCAACAGGTCCATTCCAAGG*	*20*	*58.45*	*55.00*	*3.00*
Reverse primer	*AGCATTTCGTTTAATGACAGCC*	*22*	*57.90*	*40.91*	*5.00*
Products on target templates					

>NC_000002.12 Homo sapiens chromosome 2, GRCh38.p14 Primary Assembly

```
product length = 350
Features associated with this product:
   polyribonucleotide nucleotidyltransferase 1, mitochondria...

   polyribonucleotide nucleotidyltransferase 1, mitochondria...

Forward primer  1         CCAACAGGTCCATTCCAAGG  20
Template        55680711  ....................  55680730

Reverse primer  1         AGCATTTCGTTTAATGACAGCC  22
Template        55681060  ......................  55681039
```

```
GRCh37/hg19>chr2:55907846+55908195 350bp CCAACAGGTCCATTCCAAGG AGCATTTCGTTTAATGACAGCC
CCAACAGGTCCATTCCAAGGaatatctgataatgagagggctacggaagc
ttaaaaaaggagaaaaatcagggccgaaattaaaatttcattgctttaaa
aaaattttaatctgataattttaatctctacttttatacttaccgccat
taattgctaggacatcaggctcatttacaccatctactgctaacagatta
cacagaacctggtaaaagggaaaaaatttgatttggaagggttatcattt
aggttaacatcctgacctaaaatacaagagcactatatggctaaaagcag
```

Comment [91]: PNPT1:c.517G>A

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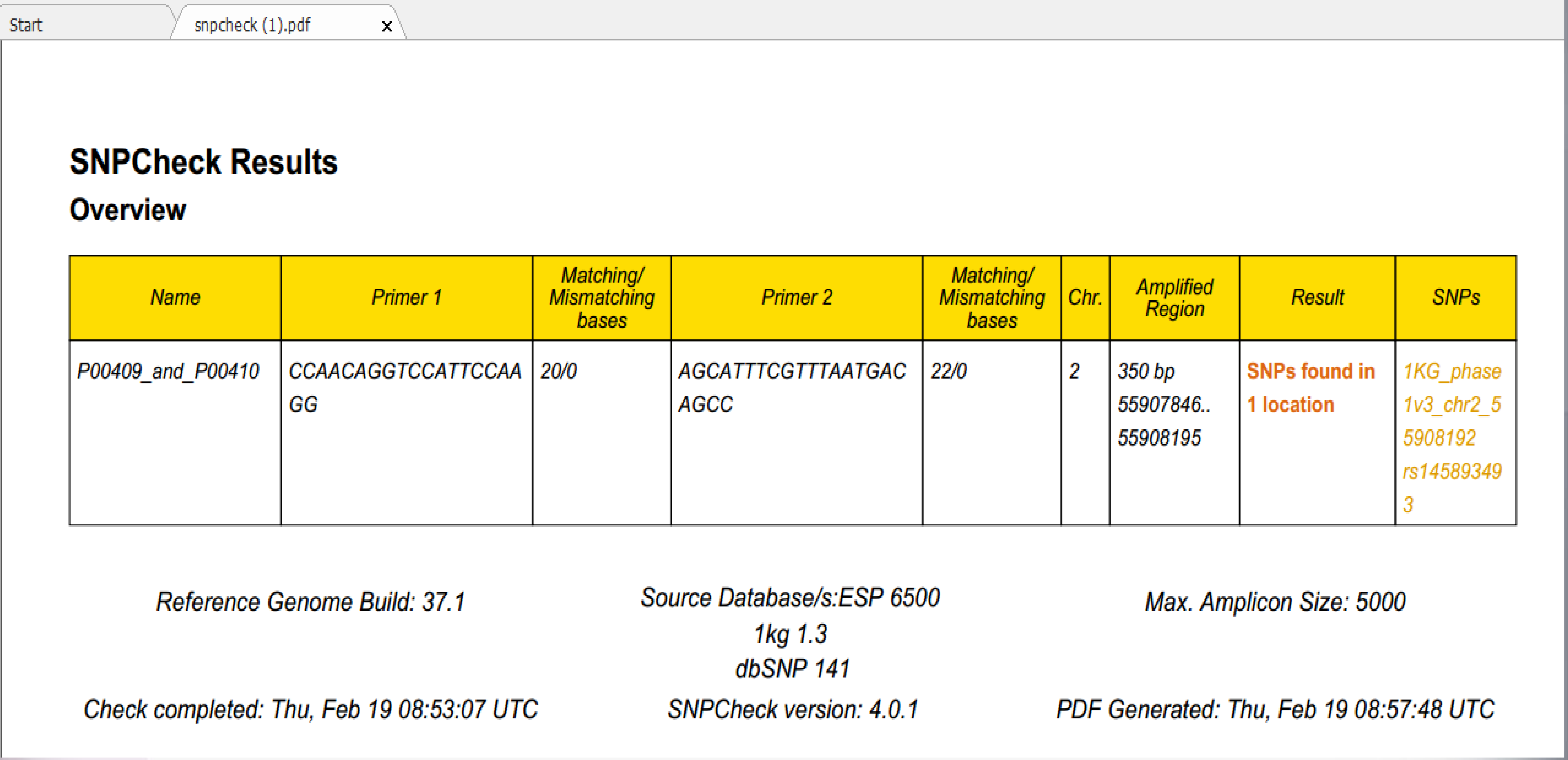
SNP Check



SNPCheck Results

Overview

Name	*Primer 1*	*Matching/ Mismatching bases*	*Primer 2*	*Matching/ Mismatching bases*	*Chr.*	*Amplified Region*	*Result*	*SNPs*
P00409_and_P00410	*CCAACAGGTCCATTCCAAGG*	*20/0*	*AGCATTTCGTTTAATGACAGCC*	*22/0*	*2*	*350 bp* *55907846..55908195*	**SNPs found in 1 location**	*1KG_phase1v3_chr2_55908192* *rs145893493*

Reference Genome Build: 37.1

Source Database/s:ESP 6500
1kg 1.3
dbSNP 141

Max. Amplicon Size: 5000

Check completed: Thu, Feb 19 08:53:07 UTC

SNPCheck version: 4.0.1

PDF Generated: Thu, Feb 19 08:57:48 UTC

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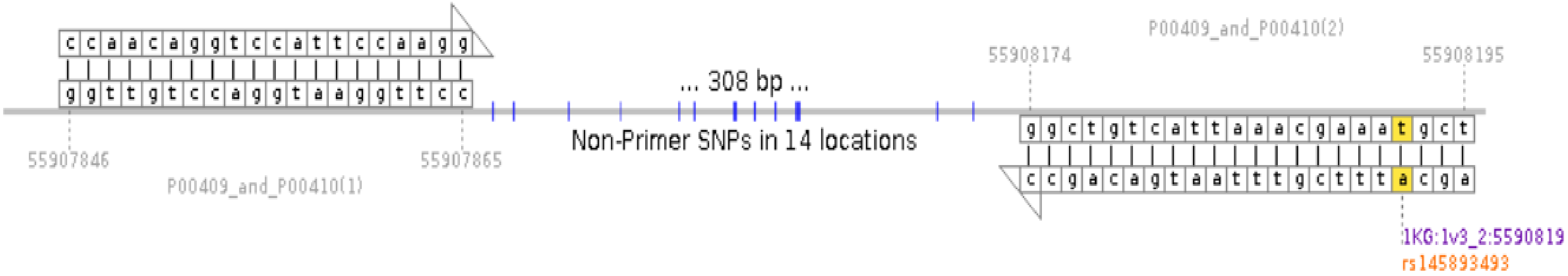
SNP Check

P00409_and_P00410



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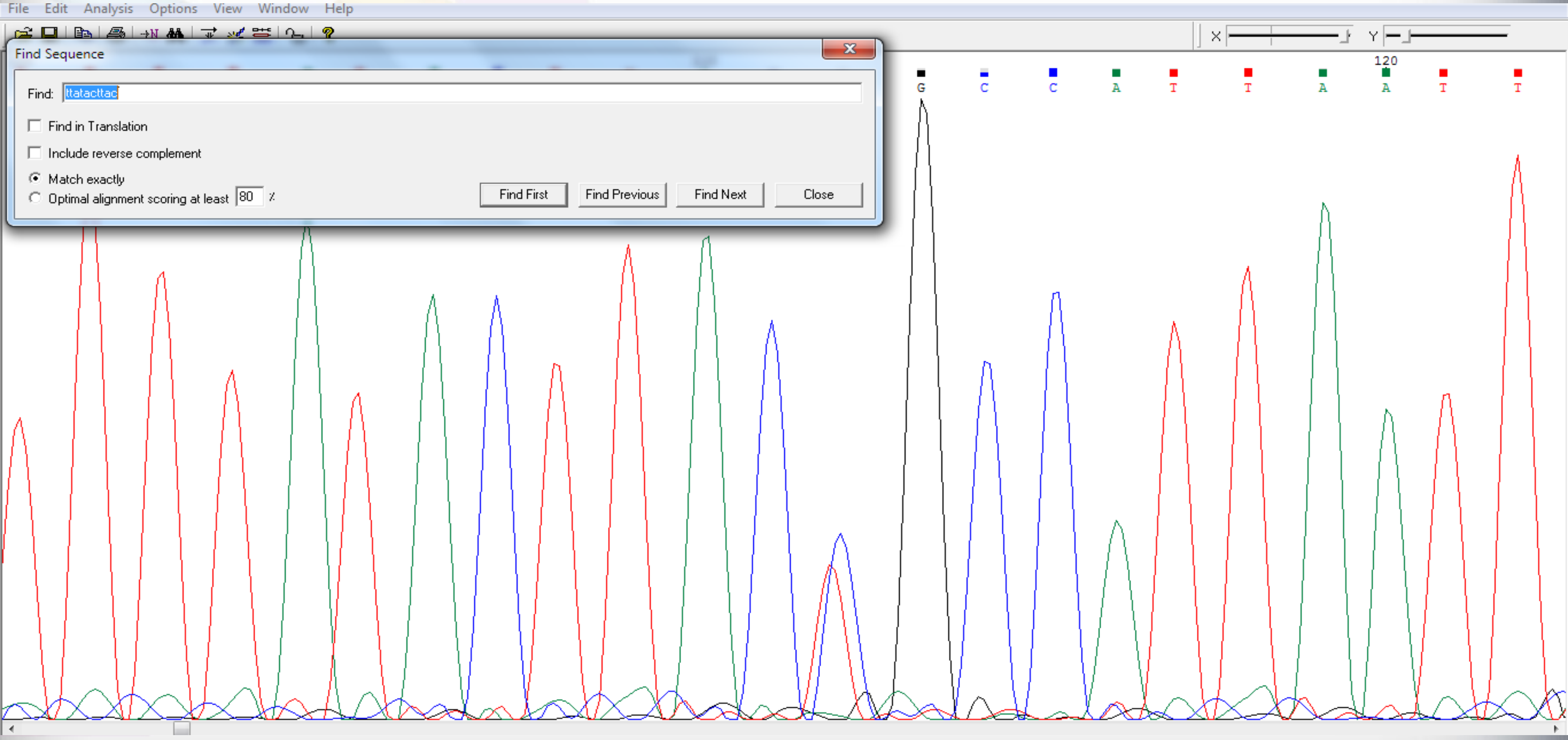
Sequencing Result



Find Sequence

Find: ttatacttac

☐ Find in Translation

☐ Include reverse complement

● Match exactly

○ Optimal alignment scoring at least 80 %

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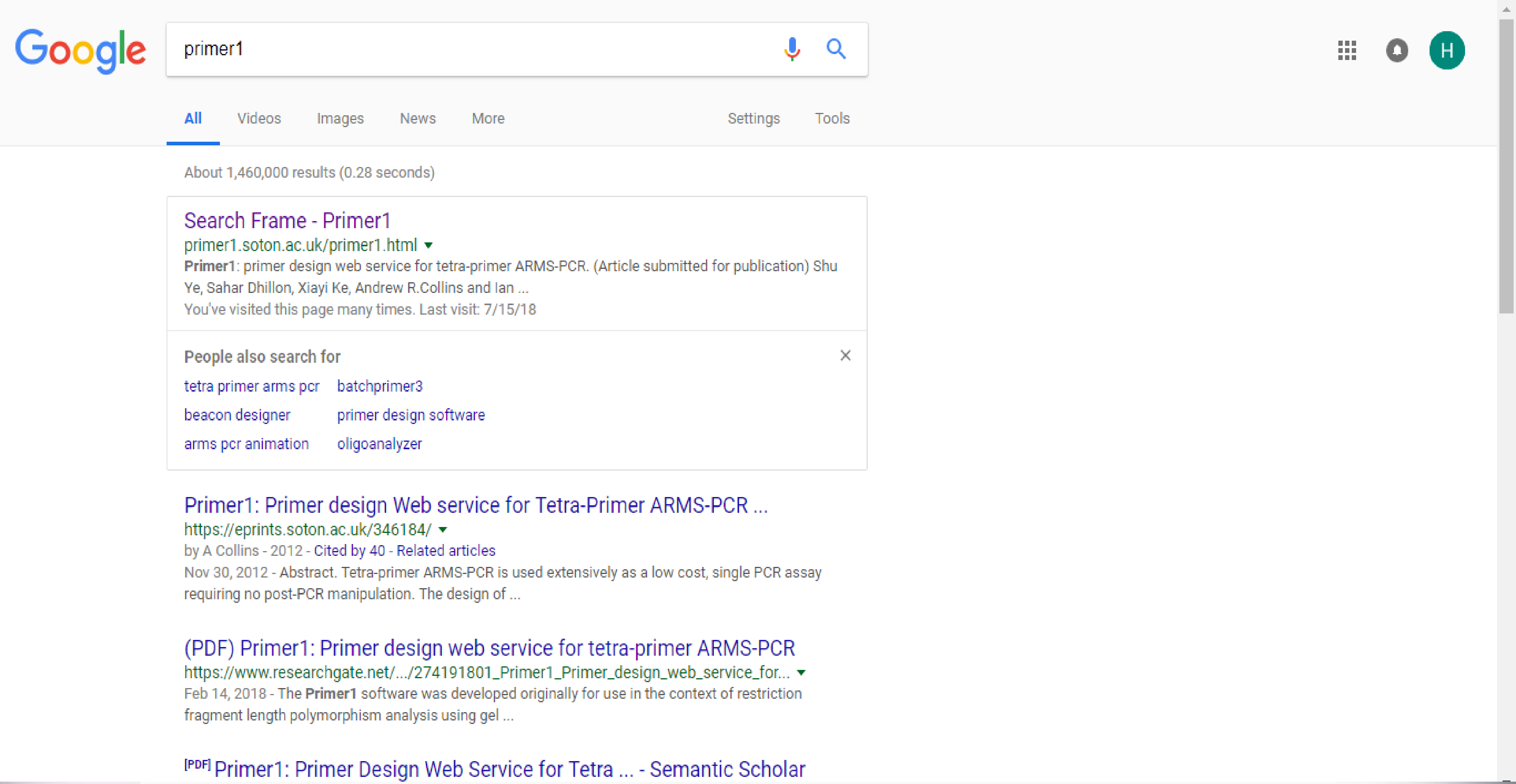

primer1

About 1,460,000 results (0.28 seconds)

Search Frame - Primer1
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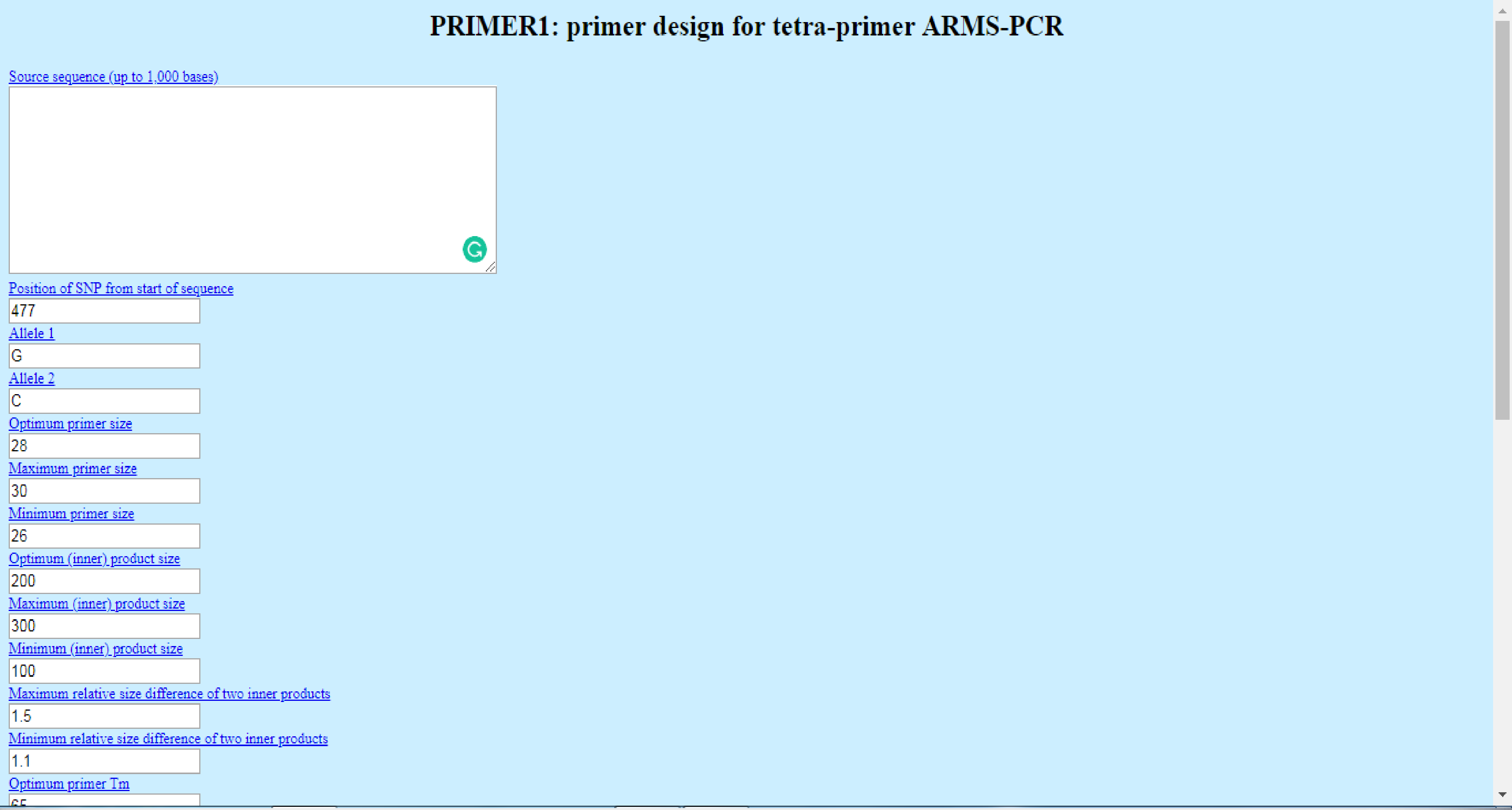

PRIMER1: primer design for tetra-primer ARMS-PCR

Source sequence (up to 1,000 bases)

Position of SNP from start of sequence
477

Allele 1
G

Allele 2
C

Optimum primer size
28

Maximum primer size
30

Minimum primer size
26

Optimum (inner) product size
200

Maximum (inner) product size
300

Minimum (inner) product size
100

Maximum relative size difference of two inner products
1.5

Minimum relative size difference of two inner products
1.1

Optimum primer Tm
65

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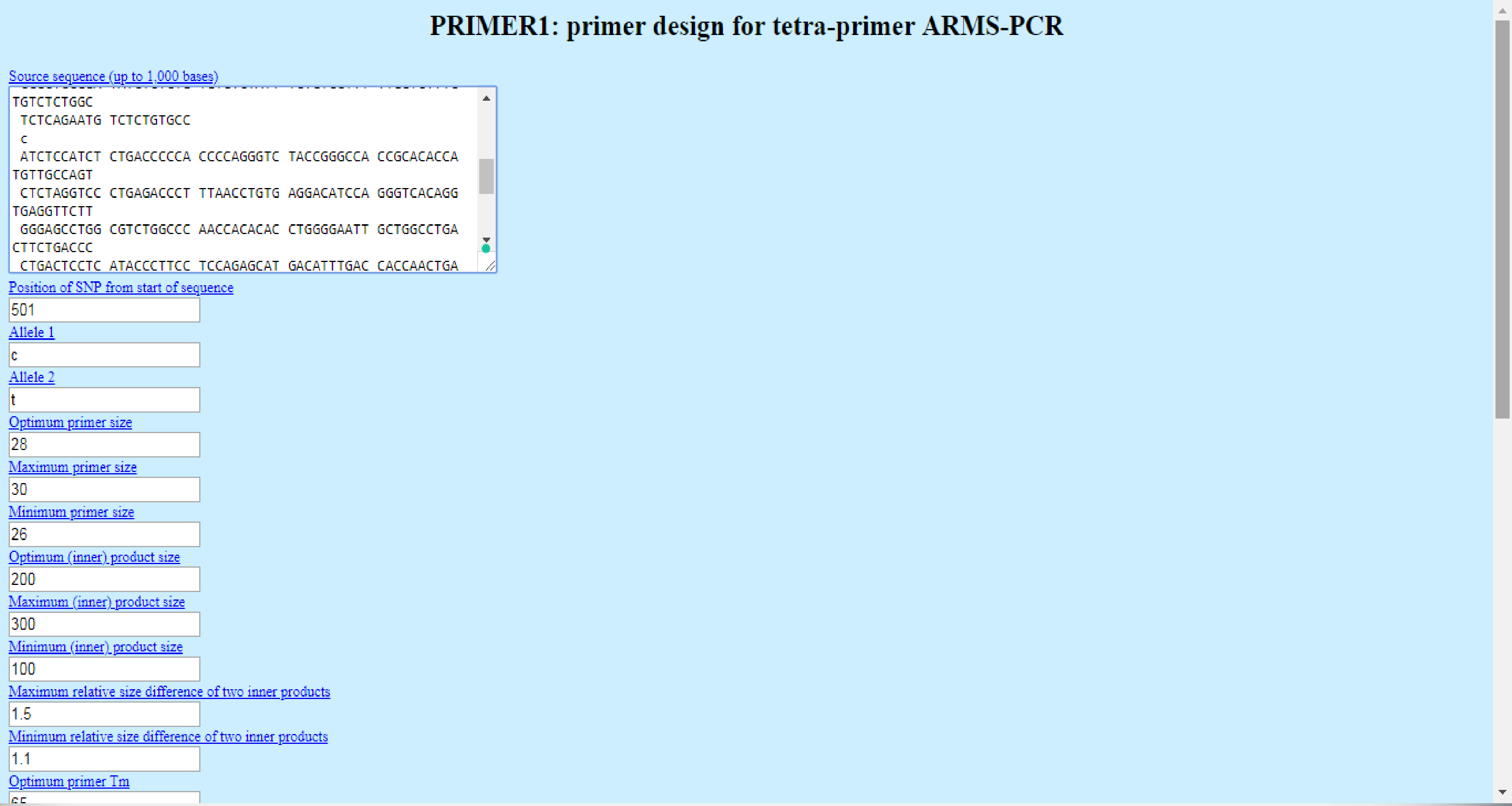

PRIMER1: primer design for tetra-primer ARMS-PCR

Source sequence (up to 1,000 bases)

```
TGTCTCTGGC
 TCTCAGAATG TCTCTGTGCC
 c
 ATCTCCATCT CTGACCCCCA CCCCAGGGTC TACCGGGCCA CCGCACACCA
TGTTGCCAGT
 CTCTAGGTCC CTGAGACCCT TTAACCTGTG AGGACATCCA GGGTCACAGG
TGAGGTTCTT
 GGGAGCCTGG CGTCTGGCCC AACCACACAC CTGGGGAATT GCTGGCCTGA
CTTCTGACCC
 CTGACTCCTC ATACCCTTCC TCCAGAGCAT GACATTTGAC CACCAACTGA
```

Position of SNP from start of sequence: 501

Allele 1: c

Allele 2: t

Optimum primer size: 28

Maximum primer size: 30

Minimum primer size: 26

Optimum (inner) product size: 200

Maximum (inner) product size: 300

Minimum (inner) product size: 100

Maximum relative size difference of two inner products: 1.5

Minimum relative size difference of two inner products: 1.1

Optimum primer Tm

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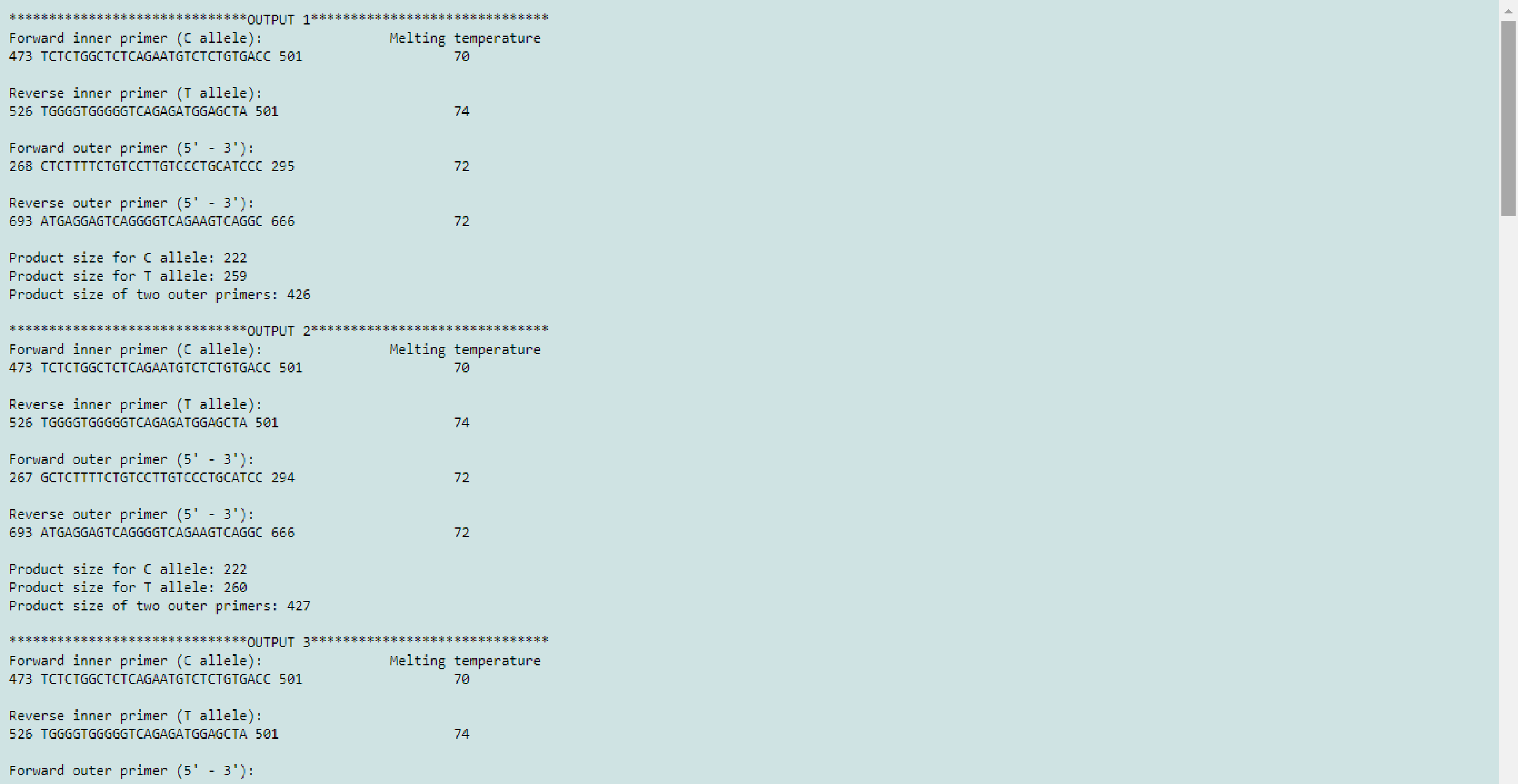
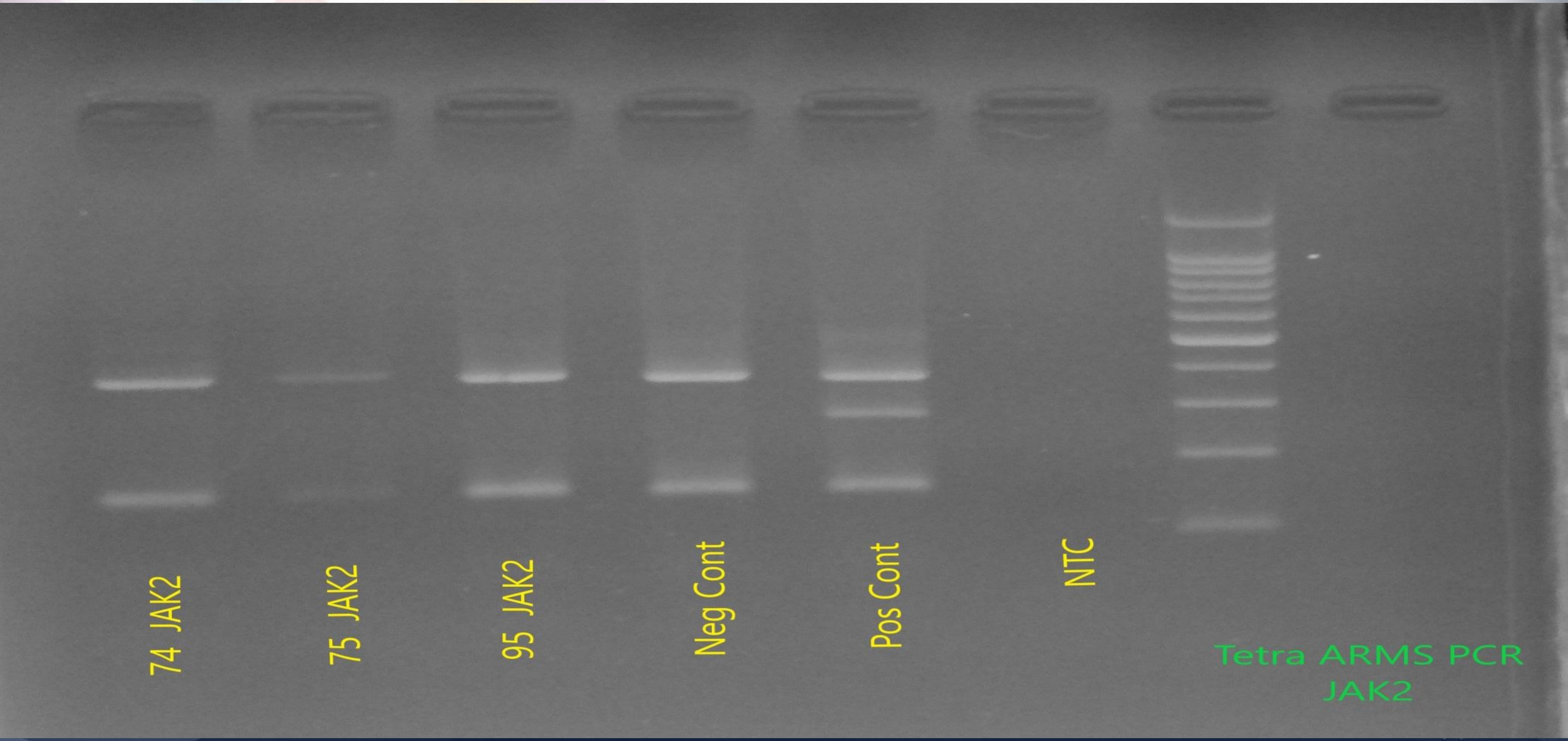


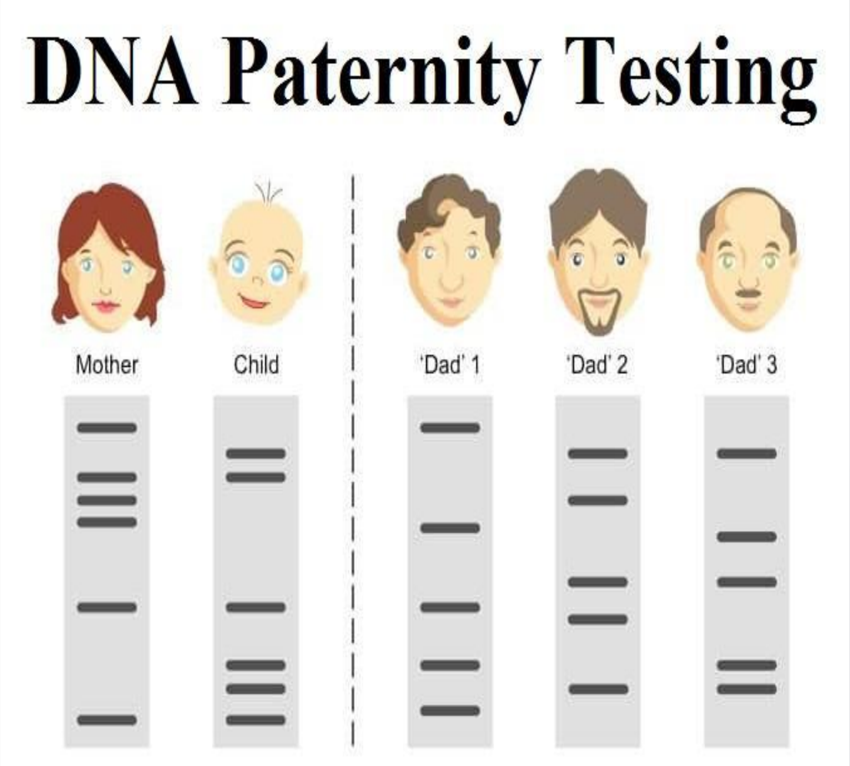
```
******************************OUTPUT 1******************************
Forward inner primer (C allele):                Melting temperature
473 TCTCTGGCTCTCAGAATGTCTCTGTGACC 501                   70

Reverse inner primer (T allele):
526 TGGGTGGGGGTCAGAGATGGAGCTA 501                       74

Forward outer primer (5' - 3'):
268 CTCTTTTCTGTCCTTGTCCCTGCATCCC 295                    72

Reverse outer primer (5' - 3'):
693 ATGAGGAGTCAGGGGTCAGAAGTCAGGC 666                    72

Product size for C allele: 222
Product size for T allele: 259
Product size of two outer primers: 426

******************************OUTPUT 2******************************
Forward inner primer (C allele):                Melting temperature
473 TCTCTGGCTCTCAGAATGTCTCTGTGACC 501                   70

Reverse inner primer (T allele):
526 TGGGTGGGGGTCAGAGATGGAGCTA 501                       74

Forward outer primer (5' - 3'):
267 GCTCTTTTCTGTCCTTGTCCCTGCATCC 294                    72

Reverse outer primer (5' - 3'):
693 ATGAGGAGTCAGGGGTCAGAAGTCAGGC 666                    72

Product size for C allele: 222
Product size for T allele: 260
Product size of two outer primers: 427

******************************OUTPUT 3******************************
Forward inner primer (C allele):                Melting temperature
473 TCTCTGGCTCTCAGAATGTCTCTGTGACC 501                   70

Reverse inner primer (T allele):
526 TGGGTGGGGGTCAGAGATGGAGCTA 501                       74

Forward outer primer (5' - 3'):
```

JAK2 RESULT


74 JAK2
75 JAK2
95 JAK2
Neg Cont
Pos Cont
NTC
Tetra ARMS PCR
JAK2

Paternity Test

- 1- DNA extraction
- 2- PCR
- 3- Fragment Analysis
- 4- Reports



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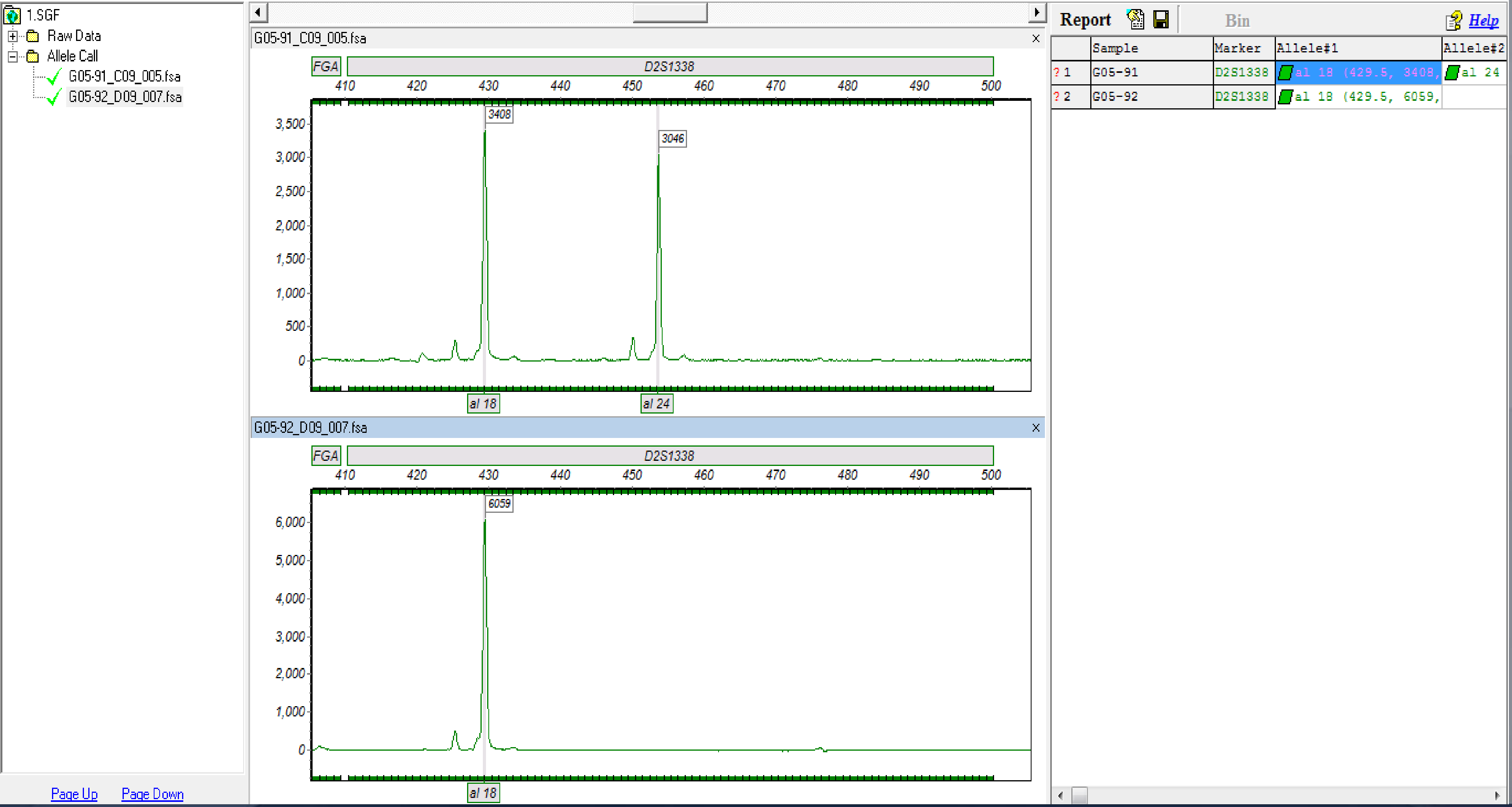
Paternity Result



	Sample	Marker	Allele#1	Allele#2
? 1	G05-91	D2S1338	al 18 (429.5, 3408,	al 24
? 2	G05-92	D2S1338	al 18 (429.5, 6059,	

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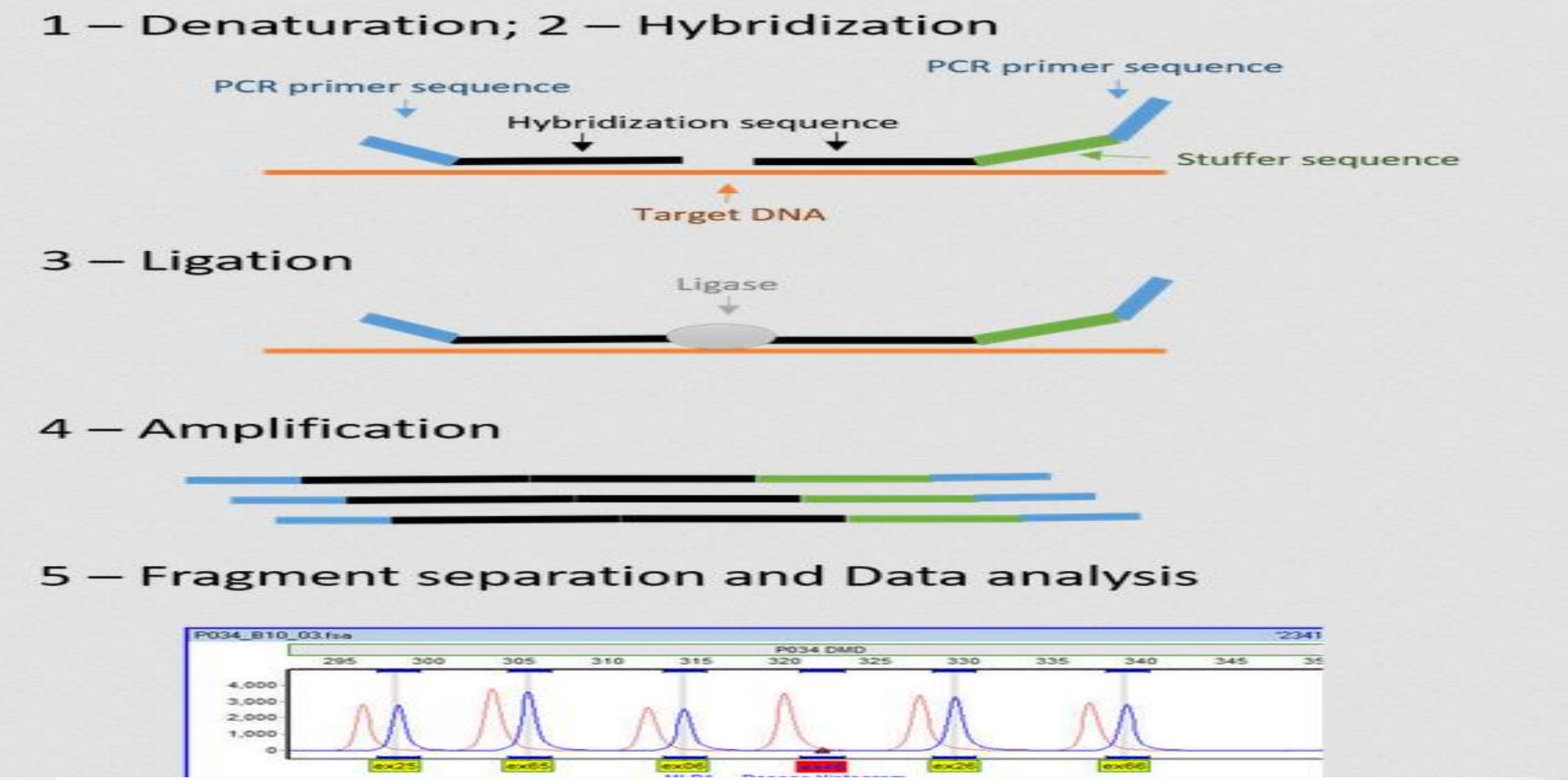
MLPA



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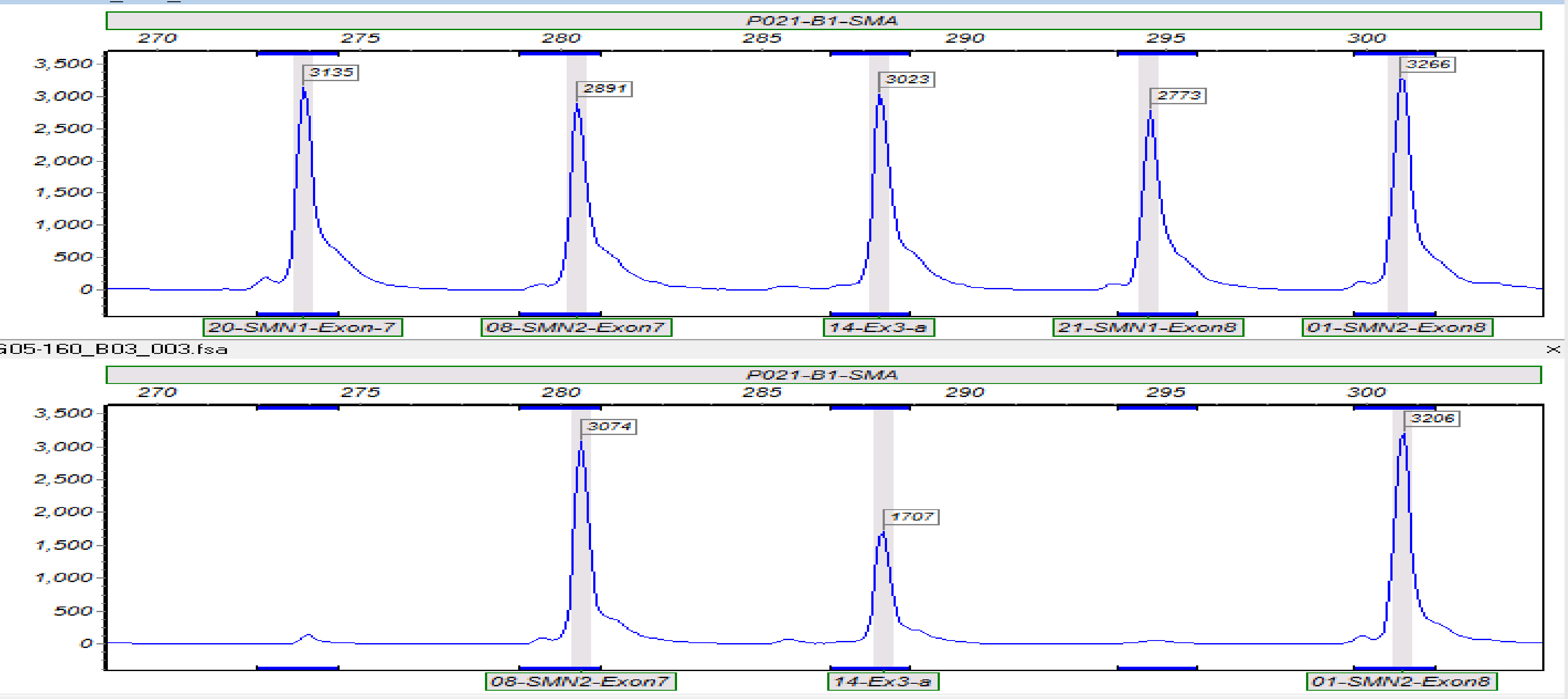
MLPA for SMA



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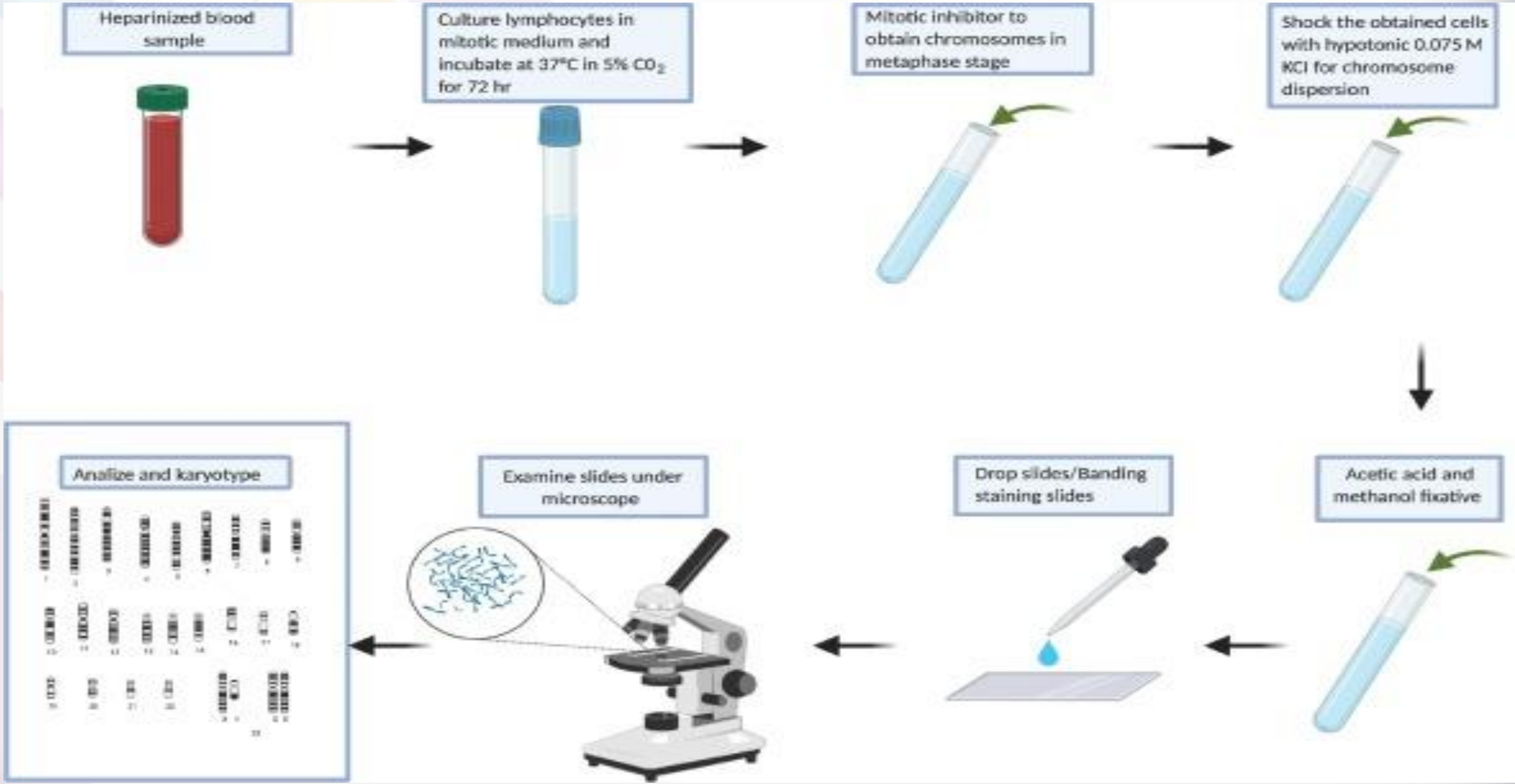
Karyotype procedure



Heparinized blood sample

Culture lymphocytes in mitotic medium and incubate at 37°C in 5% CO_2 for 72 hr

Mitotic inhibitor to obtain chromosomes in metaphase stage

Shock the obtained cells with hypotonic 0.075 M KCl for chromosome dispersion

Acetic acid and methanol fixative

Drop slides/Banding staining slides

Examine slides under microscope

Analize and karyotype

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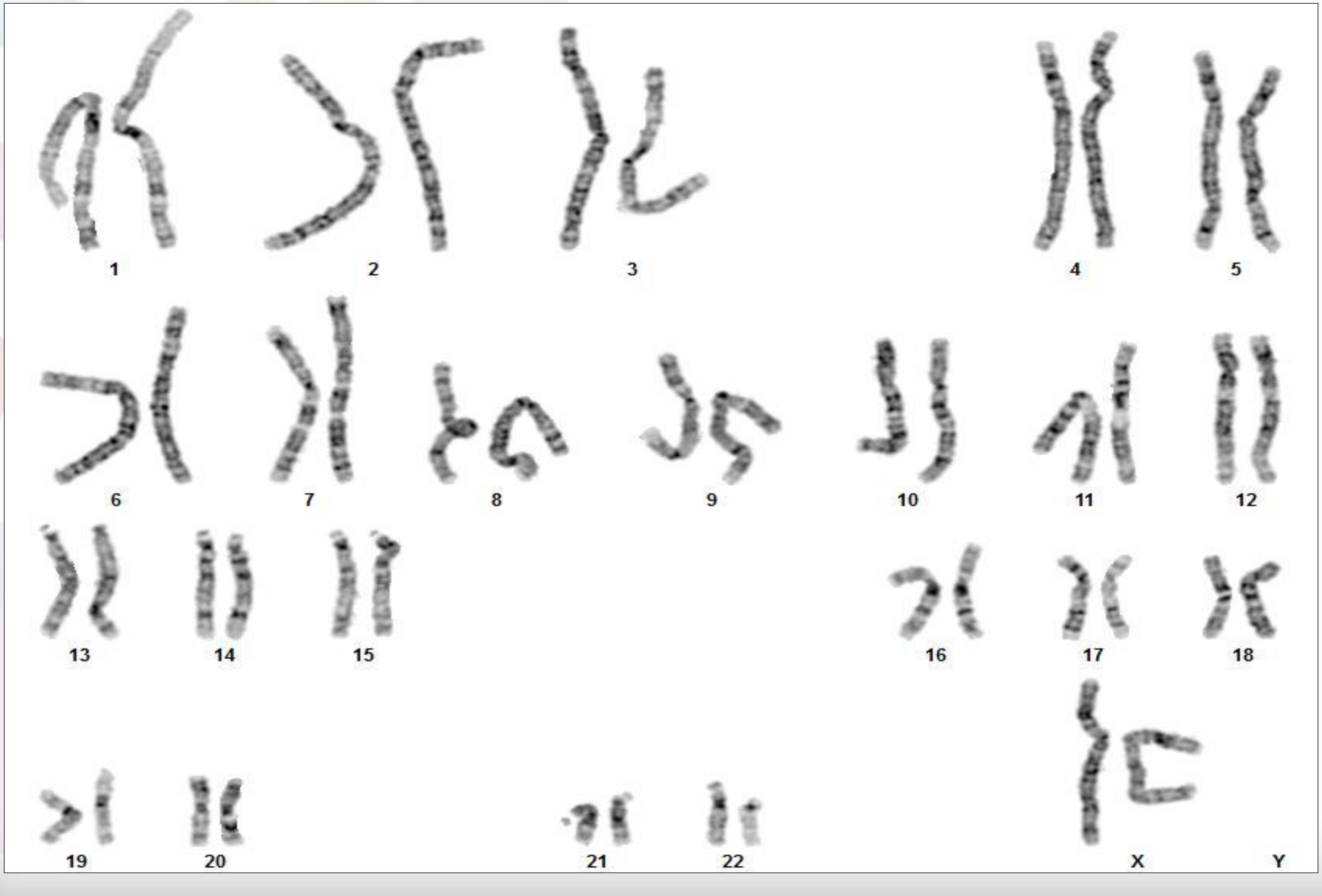
Karyotype Result



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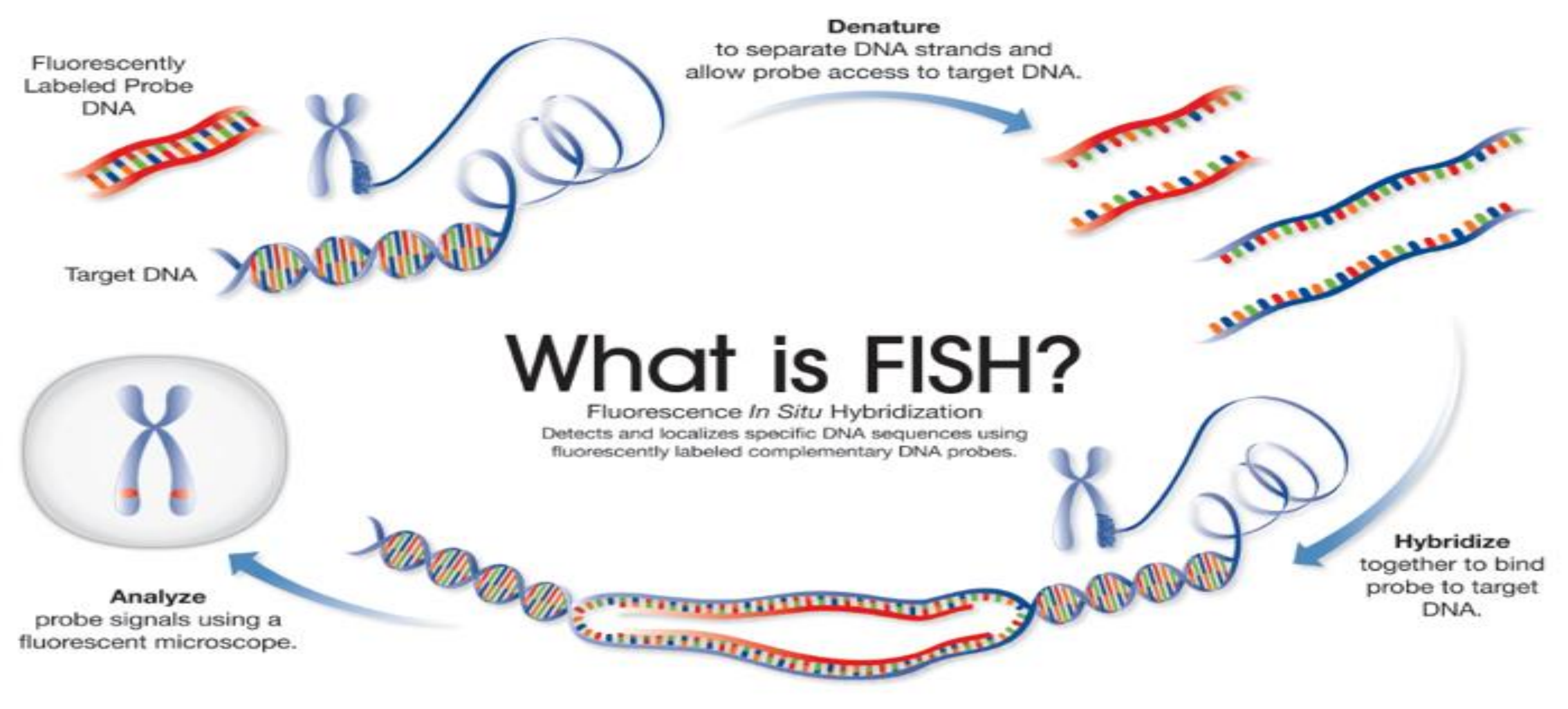
Fluorescence in Situ Hybridization



What is FISH?

Fluorescence *In Situ* Hybridization

Detects and localizes specific DNA sequences using fluorescently labeled complementary DNA probes.

Denature to separate DNA strands and allow probe access to target DNA.

Hybridize together to bind probe to target DNA.

Analyze probe signals using a fluorescent microscope.

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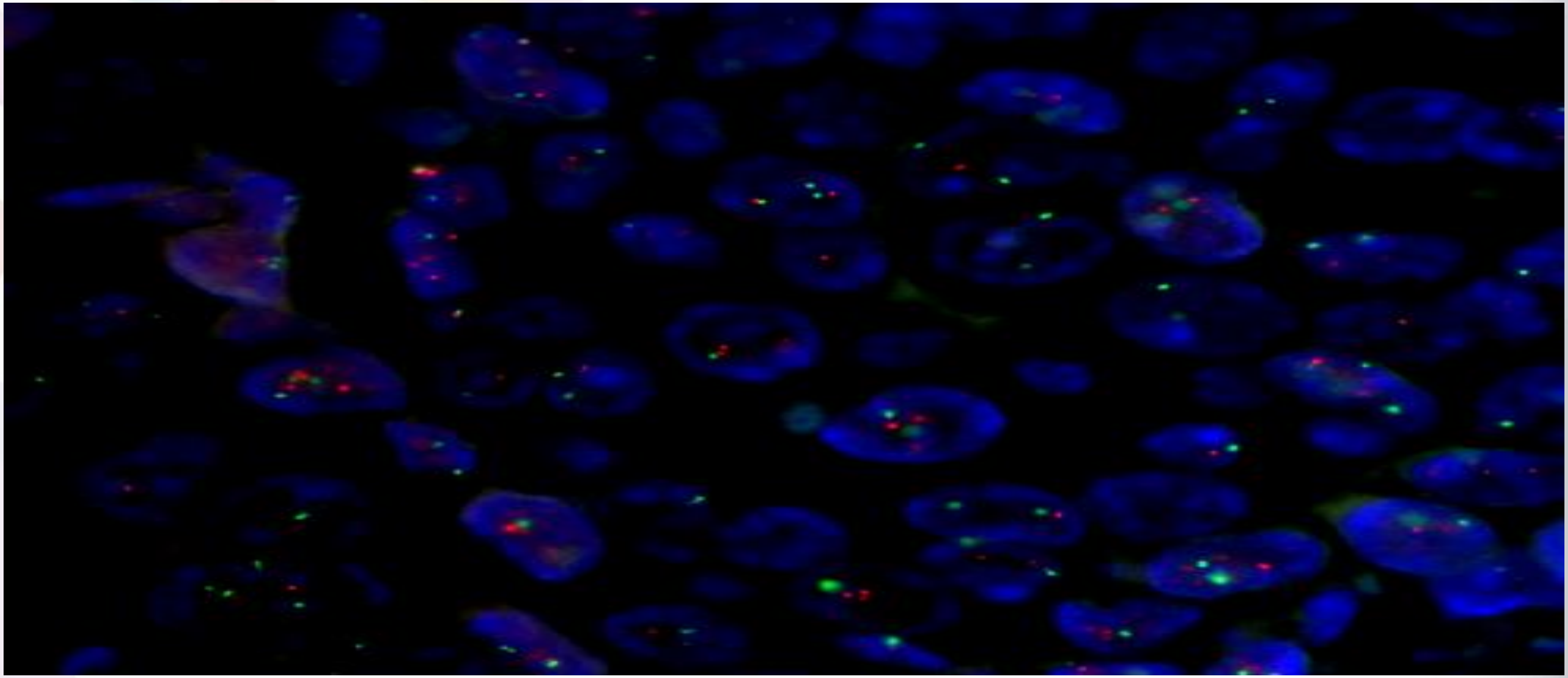
Fluorescence in Situ Hybridization



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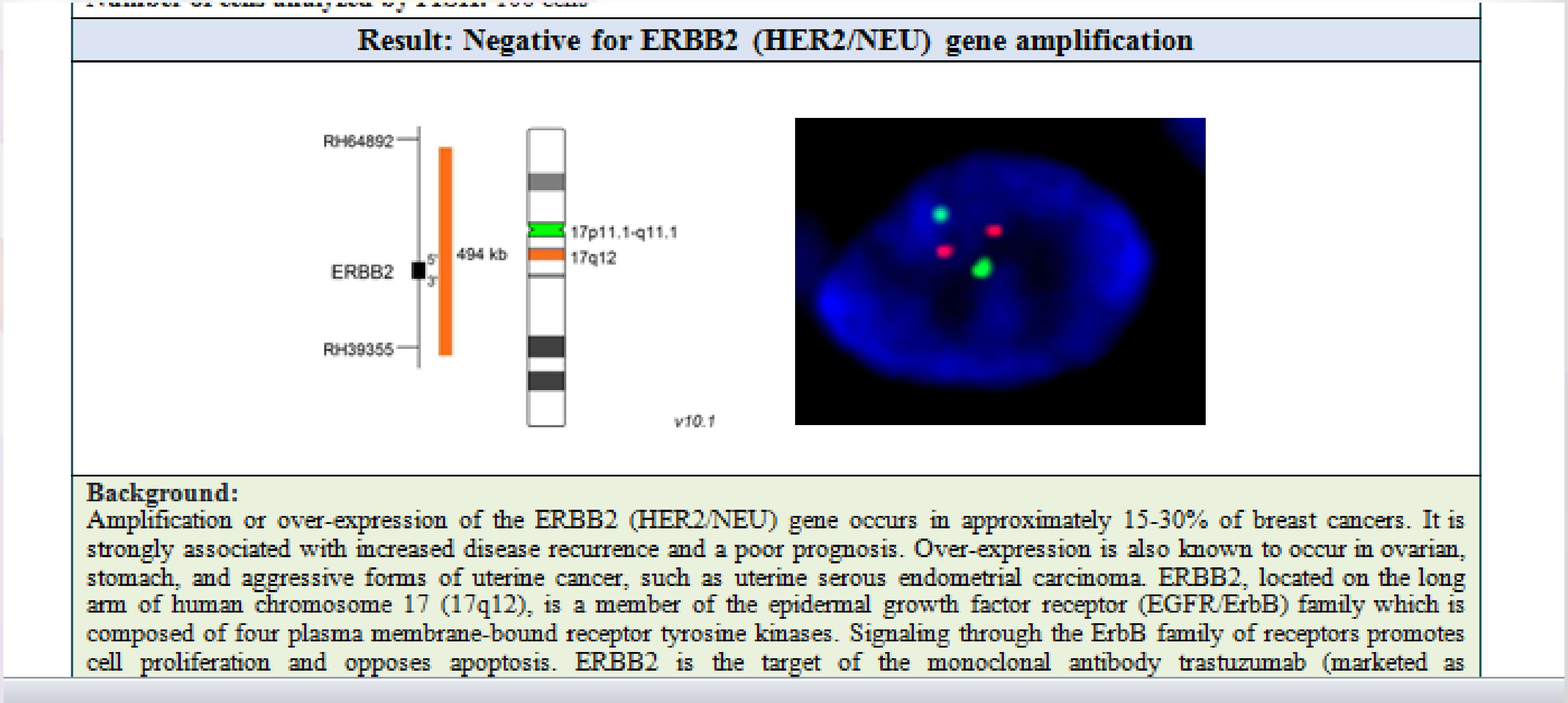
FISH Result



Result: Negative for ERBB2 (HER2/NEU) gene amplification

Background:

Amplification or over-expression of the ERBB2 (HER2/NEU) gene occurs in approximately 15-30% of breast cancers. It is strongly associated with increased disease recurrence and a poor prognosis. Over-expression is also known to occur in ovarian, stomach, and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma. ERBB2, located on the long arm of human chromosome 17 (17q12), is a member of the epidermal growth factor receptor (EGFR/ErbB) family which is composed of four plasma membrane-bound receptor tyrosine kinases. Signaling through the ErbB family of receptors promotes cell proliferation and opposes apoptosis. ERBB2 is the target of the monoclonal antibody trastuzumab (marketed as

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Thank you