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OPEN READING FRAME DETECTION AND PRIMER DESIGNING FOR COP1 GENE OF ARABIDOPSIS THALIANA

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Abstract: In Arabidopsis, light changes plant improvement and movement all through the entire life cycle, which is most profoundly spread out by seedling morphogenesis. Arabidopsis thaliana is a little blooming plant which has a spot with the mustard family. Some uncommon traits of Arabidopsis thaliana, like little genome, short life period, rapid turn of events, etc. makes it a fair inherited model living thing. Since Arabidopsis thaliana is a model living thing, most of the investigations are driven using this plant. The Arabidopsis Information Resource (TAIR) is a data set giving a wide scope of data about Arabidopsis thaliana. Arabidopsis thaliana had been basically utilized and need as a model organic entity for research. TAIR stores the data about explanations on quality design, quality/protein practical and metabolic pathway. Primary and useful comment incorporates the acknowledgment of quality areas, coding districts and expectation of its capacity. Primary and useful comment incorporates the acknowledgment of quality areas, coding districts and expectation of its capacity.

COP1 gene is one of the gene of Arabidopsis thaliana. CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), an expert controller of light flagging. Found over twenty years prior in the model plant Arabidopsis thaliana (Arabidopsis). COP1 gene, a Really Interesting New Gene (RING)- finger E3 ubiquitin ligase, exists broadly in eukaryotes including warm blooded creatures.

ORF finder searches for open getting housings (ORFs) in the DNA sequence plan you enter.

Keywords- Arabidopsis thaliana, TAIR, COP1 gene, ORF, Primer.

INTRODUCTION

Light is quite possibly the main natural variables for photosynthetic plants, as a fuel source as well as an instructive sign to balance different formative cycles. Outstanding amongst other described light-controlled formative cycles is morphogenesis. Plants display different morphogenesis designs, incorporating photomorphogenesis in the light. Photomorphogenesis is portrayed by the restraint of hypocotyl and stem stretching, open cotyledons, chloroplast separation and the aggregation of chlorophyll, and leaf development. In Arabidopsis, light tweaks plant development and advancement all through the entire life cycle, which is most drastically outlined by seedling morphogenesis.

Arabidopsis thaliana is a little blossoming plant which has a place with the mustard family. Some few exceptional attributes of Arabidopsis thaliana, similar to the little genome, short and small life period, speedy development and so on makes it a decent hereditary model life form. Since *Arabidopsis thaliana* is basically a model life form, the greater part of the explores are led utilizing this plant. The Arabidopsis Information Resource i.e. (TAIR) is a data set which is a simple method to get a wide scope of data about Arabidopsis thaliana. It gives the information including qualities, quality item, quality articulation, the total genome successions, hereditary markers, quality construction, actual markers, clones, freaks, digestion, seed stocks, genome maps, grouping polymorphism, research distributions and about the exploration local area (Nachimuthu, 2011). My project manages open reading frame detection and primer designing for COP1 gene of *Arabidopsis thaliana*.

Arabidopsis thaliana have number of genomes. I have basically selected a gene of interest (COP1) for analyzing. Protein COP1 (Constitutive Photomorphogenesis 1) is a critical controller of plant photomorphogenesis. In dark, COP1 influences articulation of over 20% of Arabidopsis thaliana qualities; principally, this is related with the association of COP1 in proteolysis of light-controlled record factors; COP1

smothers articulation of photomorphogenesis qualities. Some particular highlights of guideline grant COP1 to control various phases of plant advancement. COP1 shows movement of E3 ubiquitin ligase, and at the same time it is associated with the guideline of UV-B-responsive quality record.

Photoreceptors achieve a significant piece of their flagging capacities by means of controlling the exercises of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), an expert controller of light flagging. Found over twenty years prior in the model plant *Arabidopsis thaliana* (Arabidopsis). COP1, an Interesting Gene (RING)-finger E3 ubiquitin ligase, exists broadly in eukaryotes including warm blooded creatures. E3 ubiquitin ligases recognize and partner with protein substrates, enroll the ubiquitin forming chemical E2, and help or straightforwardly include in the exchange of ubiquitin atoms from E2 to the substrates (Han, Huang, WangDeng,2020).

COP1 Plays Evolutionarily Conserved and Divergent Roles in Plants. (Kim, Song and Seo 2017). COP1 also have a vital role in temperature responses which is described in detailed. COP1, an E3 ubiquitin ligase, has been shown to assume an indispensable part in the guideline of cell expansion, apoptosis and DNA fix. So, it has an important role in cancer development and therapy (Song,2020).

The area of a nucleotide that beginnings from an inception codon and closures with a stop codon is called an Open Reading Frame (ORF). Proteins are framed from ORF. By investigating the ORF we can foresee the conceivable amino acids that may be delivered during interpretation. It perceives all ORF or possible protein-coding districts from six different agreement edges.

Through ORF (Open Reading Frame) finder, detected the ORF of a particular locus for COP1 gene of *Arabidopsis thaliana*. ORF finder searches for open getting housings (ORFs) in the DNA sequence plan you enter. The program returns the extent of each ORF, close by its protein understanding.

Designing the primers for COP1 gene of *Arabidopsis thaliana* through ApE software which has many much features for designing primer according to our desire. ApE is an instrument that can be utilized to discover groundworks from a given grouping.

1.REVIEW OF LITERATURE

1.1 Mouse-ear cress: *Arabidopsis thaliana*

A. thaliana is a little blossoming weed plant which having a place with the mustard family (Meinke; Cherry;1998). It is similar to *D. melanogaster* and *C. elegans* in the animals of the world collectively. It is utilized as a model life form to contemplate plant science. Studies on Arabidopsis began in the mid-1900s, however just in 1998 was it's anything but a model creature. (Nachimuthu, 2011) It has no immediate significance to horticulture as it's anything but a weed. It is available across the world and there are numerous ecotypes or increases accessible. For sub-atomic science and genome considers, cultivars, for example, Columbia and Landsberg are utilized. It has five sets of chromosomes ($2n = 10$) and genome size is 157 million base sets. It has the littlest genome size among blossoming plants. The total genome succession was distributed in 2000 and the quantity of anticipated qualities is 25,498, which are spread all through the genome. The Arabidopsis thaliana Information Resource (TAIR) keeps up the data relating to *A. thaliana*. One of the significant parts of transgenic improvement in *A. thaliana* is the botanical plunge technique which stays away from tissue culture. Upwards of 30,000 quality knockout assortments are accessible for study (Nachimuthu, 2011).

Since Arabidopsis thaliana has a little genome with five chromosomes, hereditary controls are simpler making the plant valuable for sequencing and hereditary planning. As one may know, sequencing is the way toward tracking down the amino corrosive grouping of protein though hereditary planning is the way toward making genome maps. Genome maps are the graphical portrayal of a quality on a chromosome which gives data about its area. The plant has a short life period. The fast development and little size of Arabidopsis thaliana makes

it appropriate for development in relatively more limited time for an enormous scope. *Arabidopsis thaliana* has an indispensable part in the exchange of DNA in plant biotechnology. These highlights of *A. thaliana* make it a decent hereditary model creature. The data about *Arabidopsis thaliana* would be extremely useful for analysts particularly for investigates in other blossoming plants of same species (Goodman; Ecker; and Dean 1995).



(Figure-1) Representing *Arabidopsis thaliana*

1.2 Striking highlights of *Arabidopsis thaliana*

1. Little size plant (6–12-inch stature).
 2. Can be filled in Petri dish.
 3. Life cycle (5 a month and a half).
 4. Little genome contrasted with other plant genomes.
 5. Enormous number of seeds per plant (10,000 for each plant).
 6. Simple to produce transgenic plants.
 7. Genome contains substantially less dull DNA.
 8. Very much read for light detecting and bloom improvement.
 9. Biggest assortments of freaks are accessible.
 10. Clear nature of the plant parts can be utilized to take fluorescent pictures to act in situ investigation.
- (Nachimuthu, 2011).

1.3 Benefits of utilizing *A. Thaliana* as Model Organism

A. thaliana is not difficult to care for contrasted and creature model organic entities. It develops rapidly, produces numerous exceptionally little seeds, has a little genome ~114.5 Mb and is hereditarily very much described because of the volume of work being centered around this plant. As an individual from the Brassicaceae family it is connected to more significant developed species like cabbage, mustard and radish. Uncovering *A. thaliana* to *Agrobacterium tumefaciens* gives a method for proficient change vector making *A. thaliana* a flexible model organic entity for use in the science research center. *A. thaliana* is broadly utilized in the fields of plant science, hereditary qualities and development and has encouraged our comprehension of germination and parts of plant development that are significant in business crops. 1,2 as of late *A. thaliana* has even become a model life form for the investigation of the biochemical and sub-atomic cycles associated with human sicknesses (Mullan; Marsh 2019).

2. GENE OF INTEREST (COP1)

E3 ubiquitin ligases containing the RING area address the biggest E3 family and basically collaborate with both the protein like substrates and E2 ubiquitin forming compounds. (Han, Huang, WangDeng,2020). COP1 protein contains the 3 utilitarian areas needed for its anything but: an N-terminal RING finger space, a center curled loop space, and a C-terminal WD40 area.(Holm, S. Hardtke, Gaudet 2001) COP1 was first recognized as an E3 ligase of the downstream photomorphogenesis-advancing record factor HY5; COP1 was found to straightforwardly communicate with HY5 by means of its WD40 space and debase HY5 in haziness through the ubiquitin–proteasome framework (Han,Huang,WangDeng,2020).The ubiquitination movement of COP1 can be recognized toward the two its objective proteins and itself in vitro. Light conditions and COP1-connecting proteins influence its E3 ligase action in vivo (Yi and Deng, 2005).

COP1 is likewise a focal point of examination in vertebrates on account of its diverse parts in mammalian turn of events, digestion, and tumorigenesis (Sanchez-Barcelo et al., 2016). In spite of the fact that it is universally communicated and harbors three monitored spaces like Arabidopsis COP1 gene, mammalian COP1 neglected to basically save the imperfections of Arabidopsis (cop1) freaks. Be that as it may, also with Arabidopsis COP1, the limitation of mammalian COP1 could be directed by light when communicated in plant cells (Han, Huang, WangDeng,2020). What's more, human COP1 has inborn E3 ligase action in its RING area along with the snaked loop space in vitro (Dornan et al., 2004), and targets tumorigenic factors for debasement in vivo. Human COP1 fundamentally confines in the atomic envelope, and extracellular prompts quickly incite its nucleoplasm restriction (Ouyang et al., 2020).

COP1 is a basic controller that influences a scope of the plant development and formative cycles like the guideline of phytochrome bounty by COP1. Phytochromes are photoreceptors with a direct tetrapyrrole chromophore that see red and far-red light (Kim; Song 2017).

Guideline of UV-B photoreceptor by COP1. Bright B (UV-B) light, which goes from 280 nm to 315 nm, is significant for managing plant development and advancement by controlling the statement of various qualities engaged with different plant measures, including digestion, morphogenesis, photosynthesis, UV-security, and protection against irritations and microbes.

Regulation of the wealth of different light flagging and photomorphogenesis-related factors by COP1, and ‘regulation of phytochrome and cryptochrome flagging joining by COP1 (Kim, Song and Seo 2017).

Dull developed seedlings go through the skotomorphogenesis and basically show the etiolation portrayed by the stretched hypocotyl, shut and yellow cotyledons, and a firmly collapsed apical snare. The extended hypocotyl improves the probability that a dirt covered seedling arrives at the light. Thus, energy is redirected from cotyledon improvement to prolongation development. In spa fourfold and cop1 freaks, these record factors additionally amass in obscurity, causing constitutive photomorphogenesis in complete dimness. (Ponnu, Hoecker, 2021)

COP1 protein would be actuated at 28°C. It was discovered that COP1 record and protein strength of COP1 are not perceptibly affected by warm temperatures). It is realized that COP1 is moved from the cytoplasm to the core in obscurity. Remarkably, subcellular fractionation measures uncovered that COP1 is moved to the core at 28°C under both consistent light and dim conditions (Park,2017).

During their life cycle, plants are tested with variances in temperature. Outrageous temperatures (ice, inordinate warmth) lead to pressure reactions, while more modest changes lead to versatile development. Such versatile development reactions to high surrounding temperature (27–29°C for Arabidopsis) emulate development reactions in shelter conceal: hypocotyls and internodes extend and leaves show up (hyponastic) development, maybe to cool the plant surface and to shield the meristem from soil heat (Ponnu, Hoecker, 2021). Light and temperature flagging are unequivocally interconnected. Test results affirm this thought: both COP1 and SPA are needed for the reaction to high surrounding temperature (Gangappa and Kumar, 2017).

COP1, an E3 ubiquitin ligase, had been shown to assume a indispensable part in the guideline of cell expansion, apoptosis and DNA fix. Gathered proof has uncovered that COP1 is engaged with carcinogenesis through focusing on its substrates, including p53, c-Jun, ETS, β -catenin, STAT3, MTA1, etc. for ubiquitination and debasement. COP1 can play tumor suppressive and oncogenic jobs in human malignancies. (Song,2020)

3. TAIR DATABASE (For retrieving gene information)

The Arabidopsis Information Resource i.e. (TAIR's) objective is to give a planned, best in class point of view on Arabidopsis science from genome to phenome from various wellsprings of information going from

singular correspondence with experts to disseminated composing (Rhee, 2003). It hopes to work with correspondence inside an investigation neighborhood through and through produces and refines a common assortment of data. I have performed retrieving gene information for COP1 gene through TAIR database.

The Arabidopsis Information Resource i.e. (TAIR) is an data set giving a wide scope of data about *Arabidopsis thaliana*. TAIR information base gives information including qualities, quality item, quality articulation, the total genome successions, hereditary markers, quality construction, actual markers, clones, freaks, digestion, seed stocks, genome maps, arrangement polymorphism, research distributions and about the exploration local area.

It stores the data about explanations on quality design, quality/protein practical and metabolic pathway. Primary and useful comment incorporates the acknowledgment of quality areas, coding districts and expectation of its capacity.

TAIR continues to progress and develop to fuse new data types and assessment mechanical assemblies. It is centered around improving data content by industrious curation and data assessment worked with by the composed endeavors with and basically contributions from the investigation neighborhood. Ordered information about the database TAIR project, including the full suggestion, data sources, projects being chipped away at and documentation of the item and curation techniques. (Rhee, 2003)

For COP1 gene, it shows 37 loci as shown in figure (figure 2).

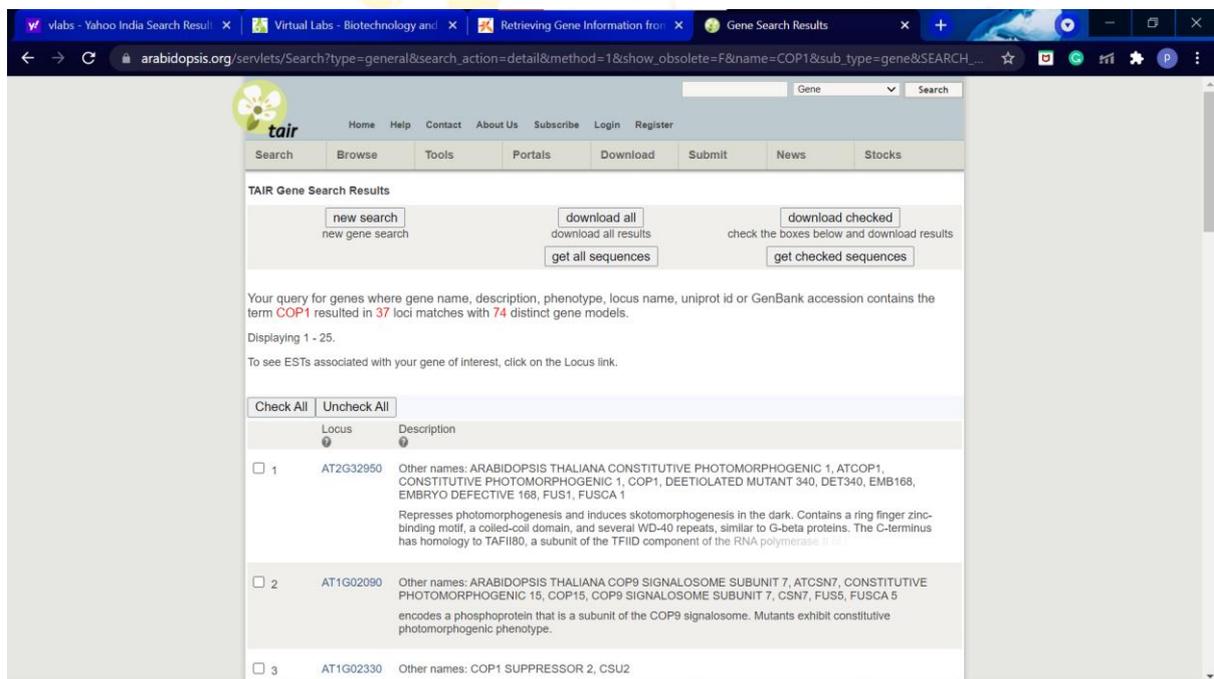
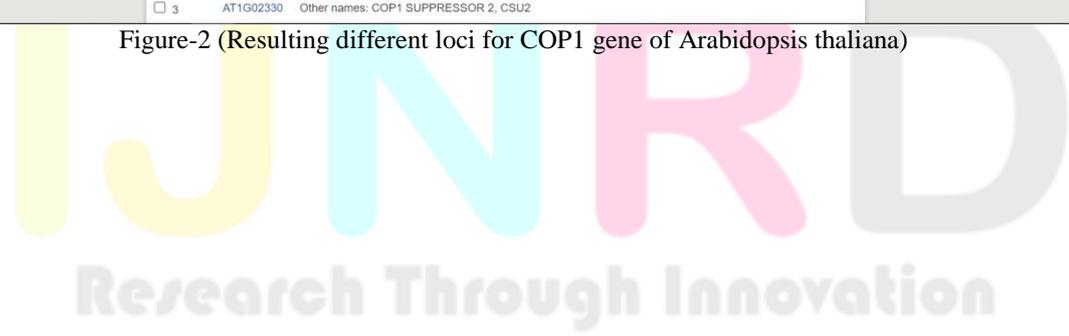


Figure-2 (Resulting different loci for COP1 gene of Arabidopsis thaliana)



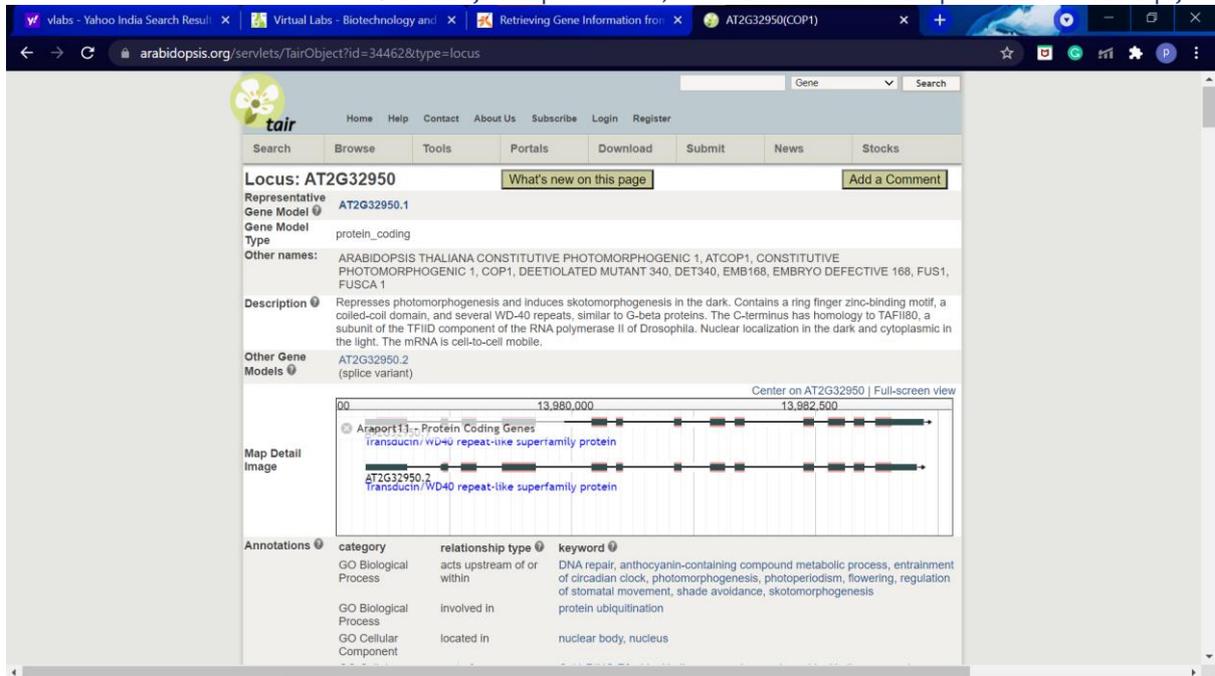


Figure-3 (Representing specific loci [AT2G32950] for COP1 gene of *Arabidopsis thaliana*)

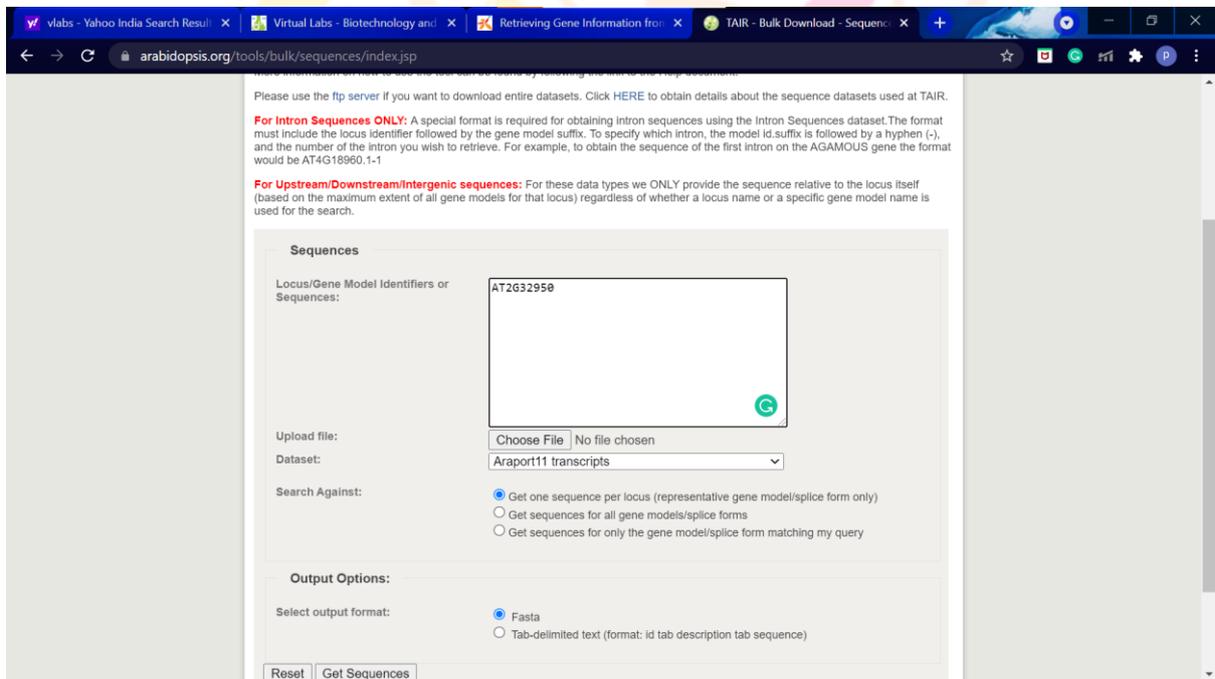


Figure-4 (Through particular loci, getting FASTA sequence for analysis)

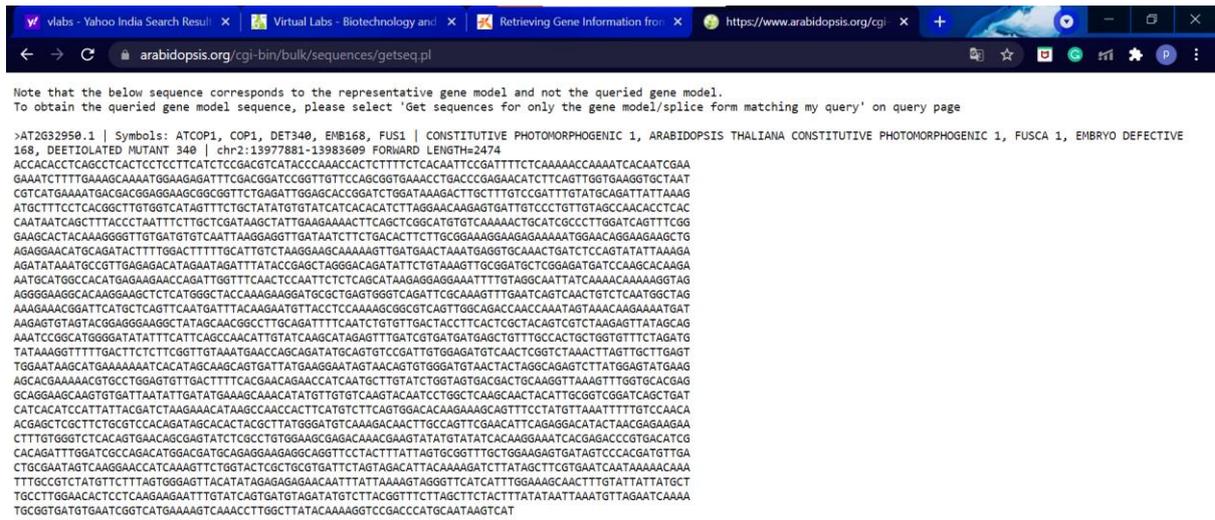


Figure-5 (Representing FASTA format sequence)

4. ORF [Open Reading Frame] FINDER (For ORF detection)

Secondly, I have successfully performed ORF detection for a particular locus of COP1 gene through ORF finder. ORF detector searches for the open getting housings (ORFs) basically in the DNA sequence you enter. The program basically returns the extent of each and every ORF, close by its protein-understanding. Use ORF finder to basically filter as of late sequenced DNA for basically the potential-protein encoding parcels, affirm expected protein basically using as of late made BLAST or the standard BLASTP.

The space of a nucleotide that basically beginnings from the beginning codon and the terminations with stop codons are basically known as an Open Reading Frame i.e. (ORF). Proteins are basically framed from the ORF. By seeing and investigating the ORF we can foresee the conceivable amino acids that may be delivered during interpretation. It's basically recognizes all ORF or conceivable protein-coding locales from the six diverse understanding edges. The ORF result coming for COP1 gene of Arabidopsis thaliana is shown in the figure (Figure 7).



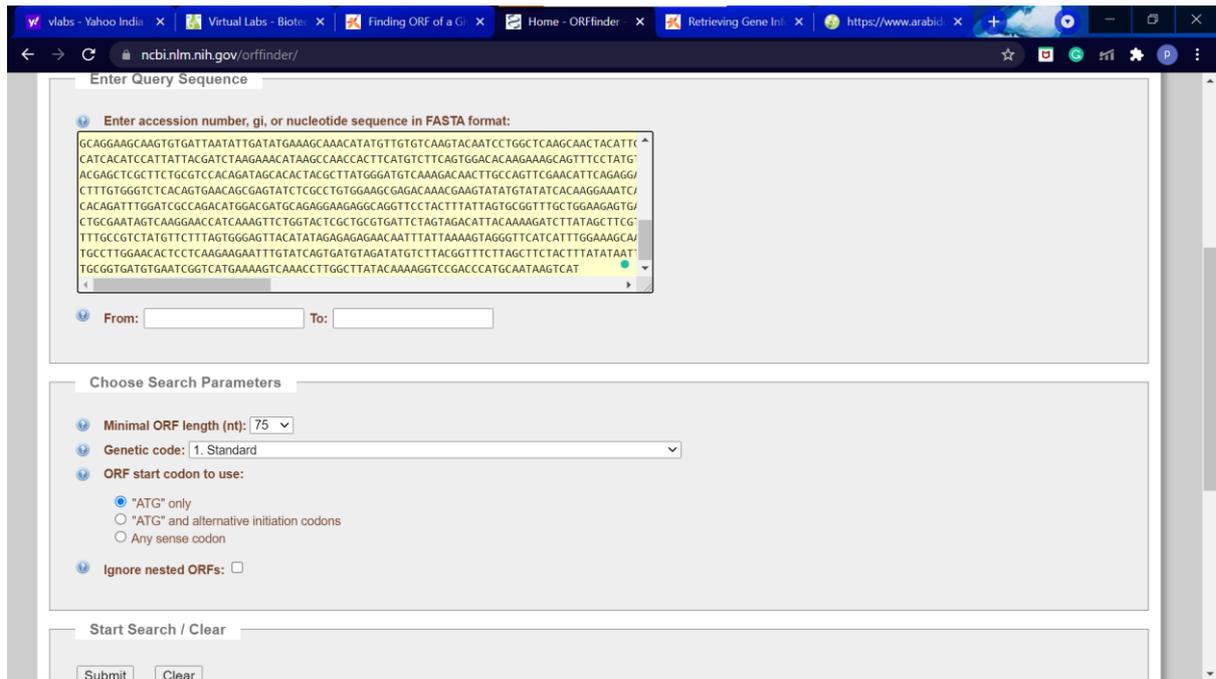


Figure-6 (Shows finding of ORF detection through ORF Finder)

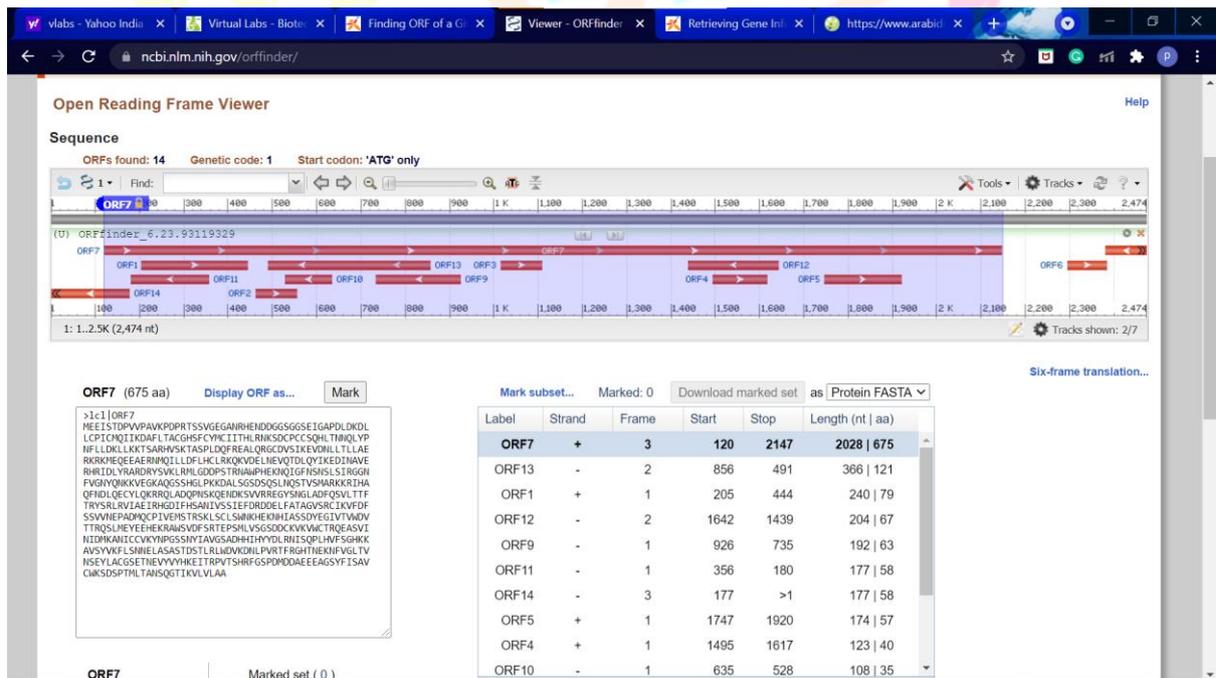


Figure-7 (Result of ORF detection for particular locus and selecting longest orf [orf7] for analysis)

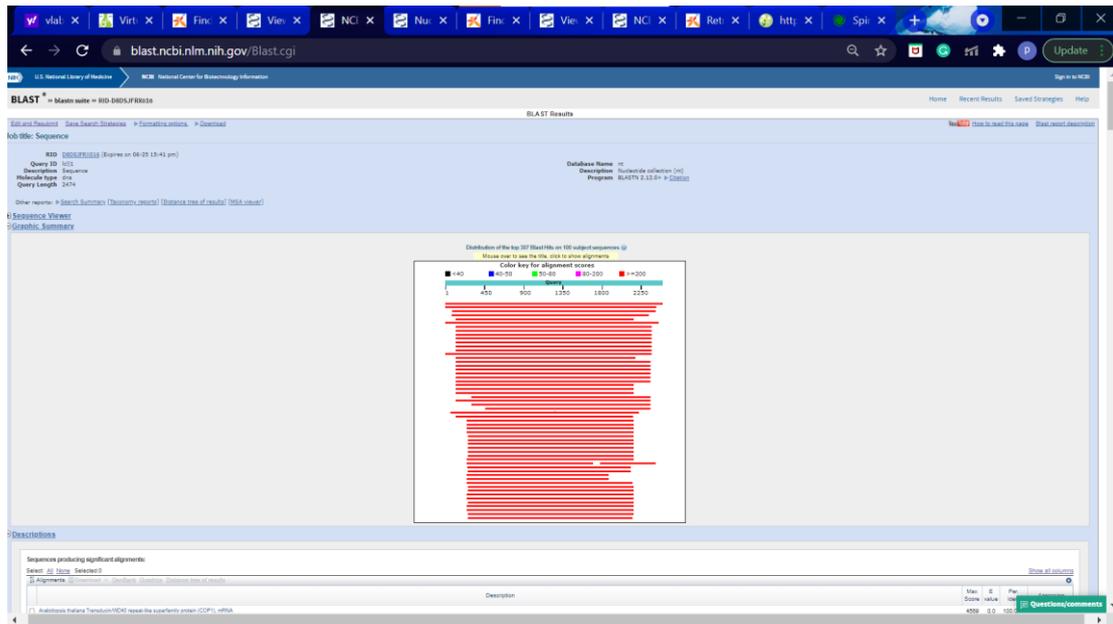


Figure-8 (For graphical representation, representing result after performing [orf7] BLAST)

5. ApE SOFTWARE (For primer designing)

Lastly, performed primer designing for COP1 gene of *Arabidopsis thaliana* through ApE software which has many features to design primer according to your desire. Groundwork can be characterized as short nucleic corrosive successions. It can go about as a beginning stage for DNA blend. The polymerase protein begins adding nucleotides in the 3'- finish of the preliminary. Interaction like DNA sequencing (to decide the specific request of nucleotides in a DNA) and polymerase chain reaction (or PCR used to intensify DNA successions) require DNA groundworks while for normal DNA replication short groupings of RNA are utilized as preliminary (Ye; Coulouris; Zaretskaya; Rozen; and; Madden 2012).

Generally, the length of the groundwork is 18 to 24 nucleotides. ApE is an instrument that can be utilized to discover groundworks from a given grouping.

Primers designed for COP1 gene of *Arabidopsis thaliana* are shown in the figure (Figure 11).

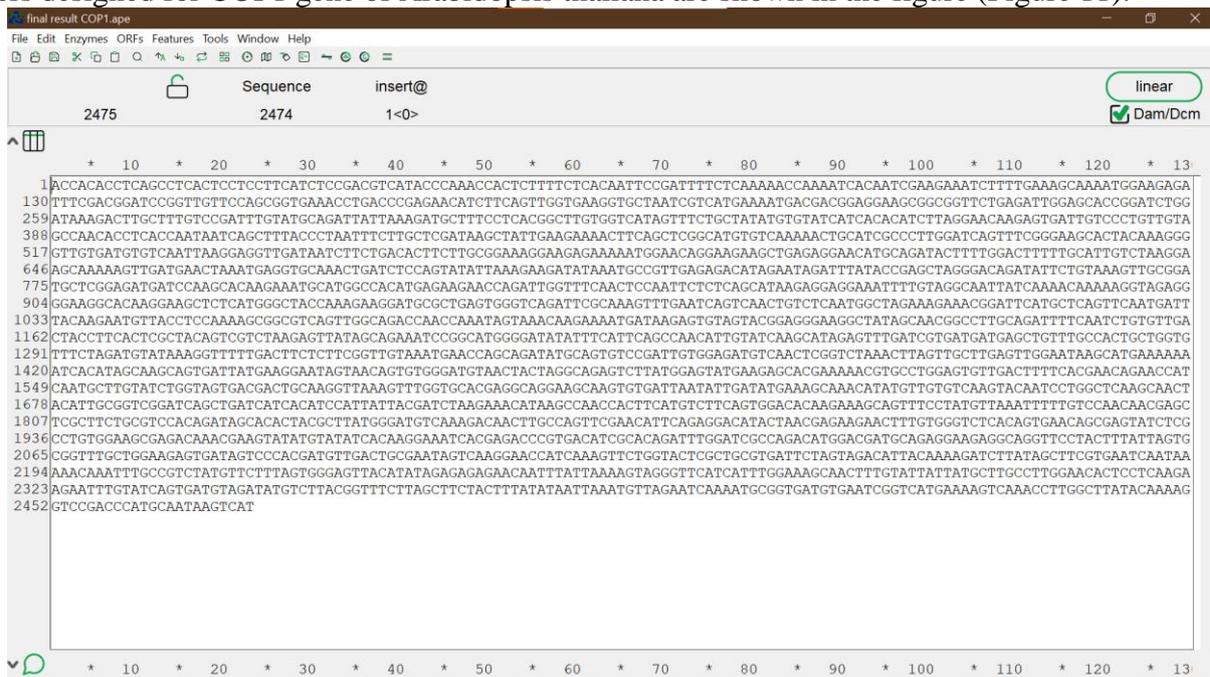


Figure-9 (Representing primer designing in ApE tool for COP1 gene)

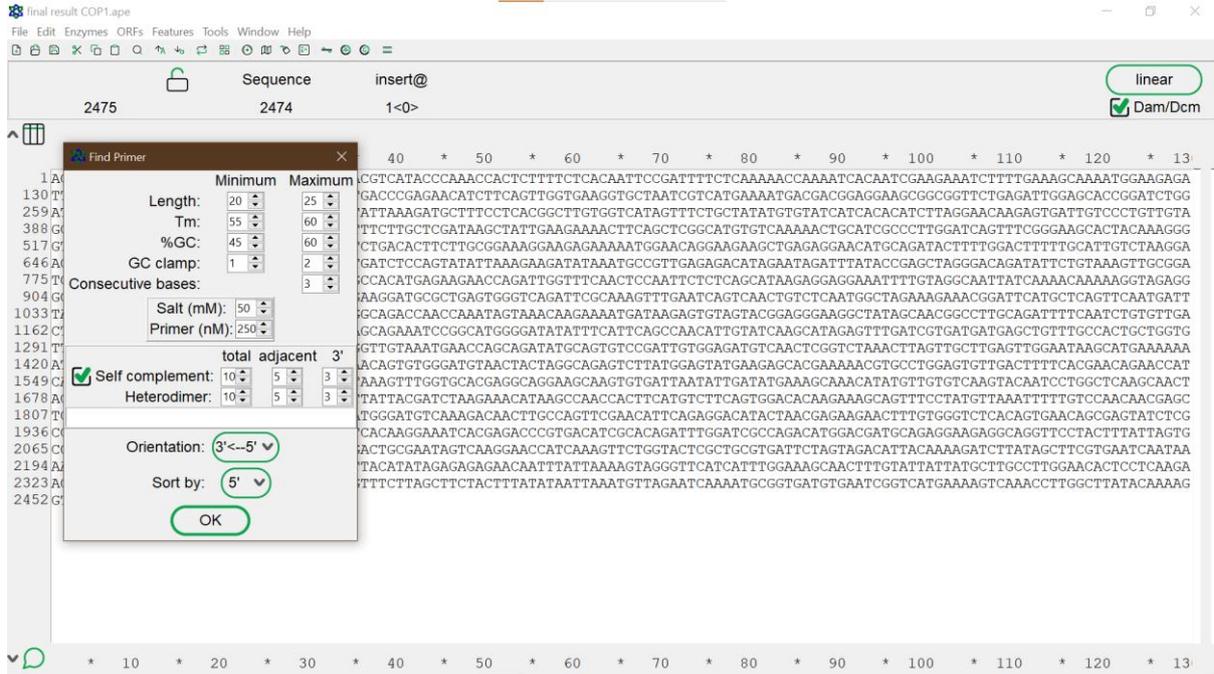


Figure-10 (Finding of primers by clicking on OK button to get primers)

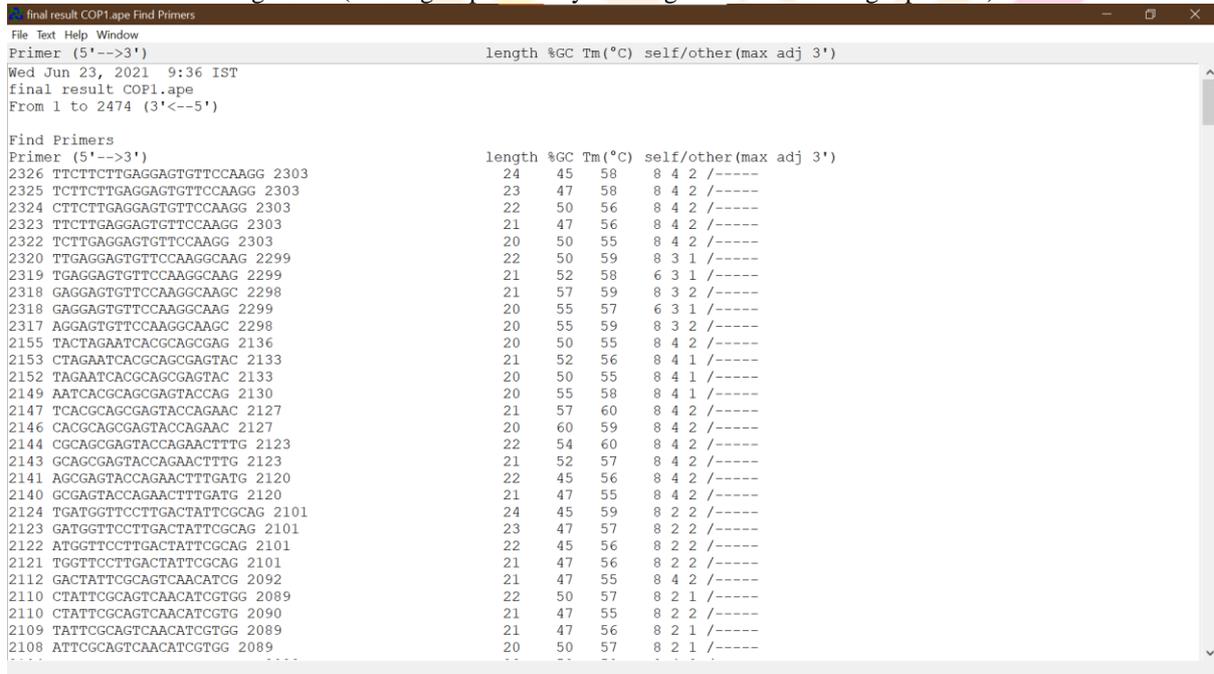
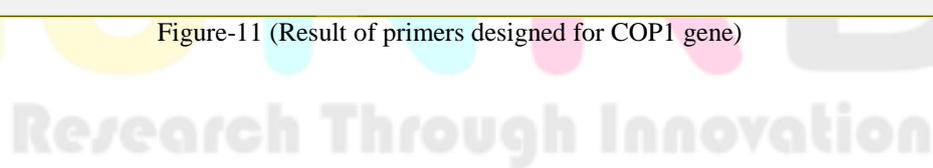


Figure-11 (Result of primers designed for COP1 gene)



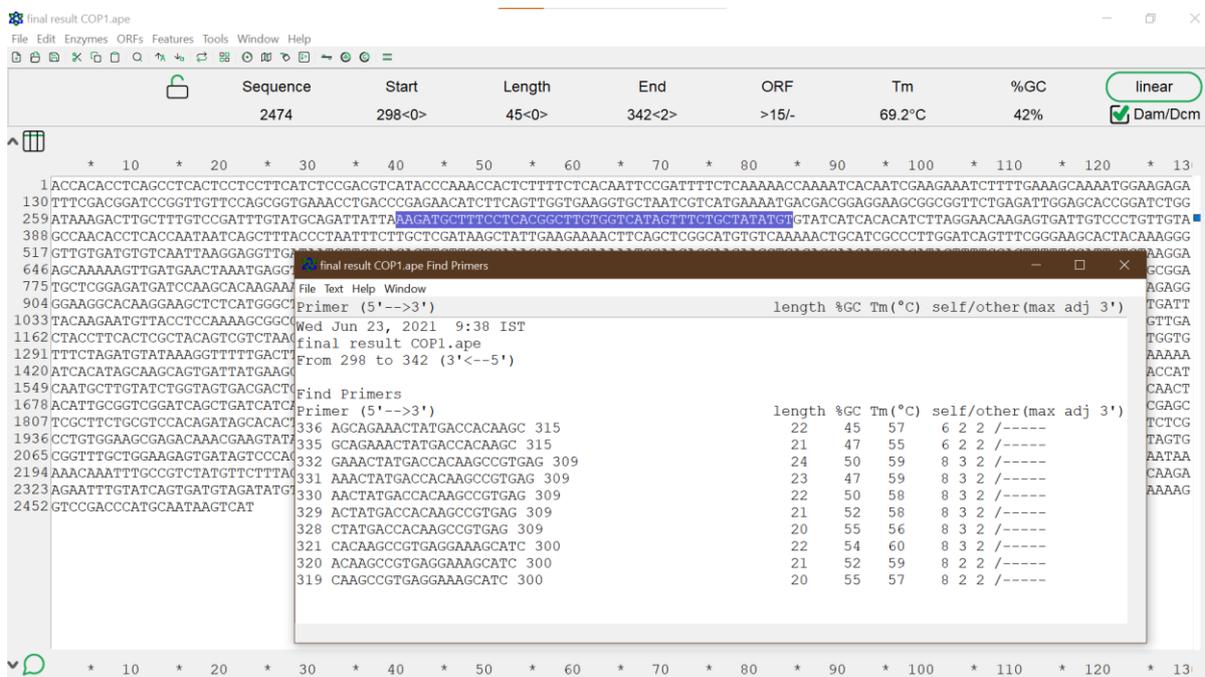
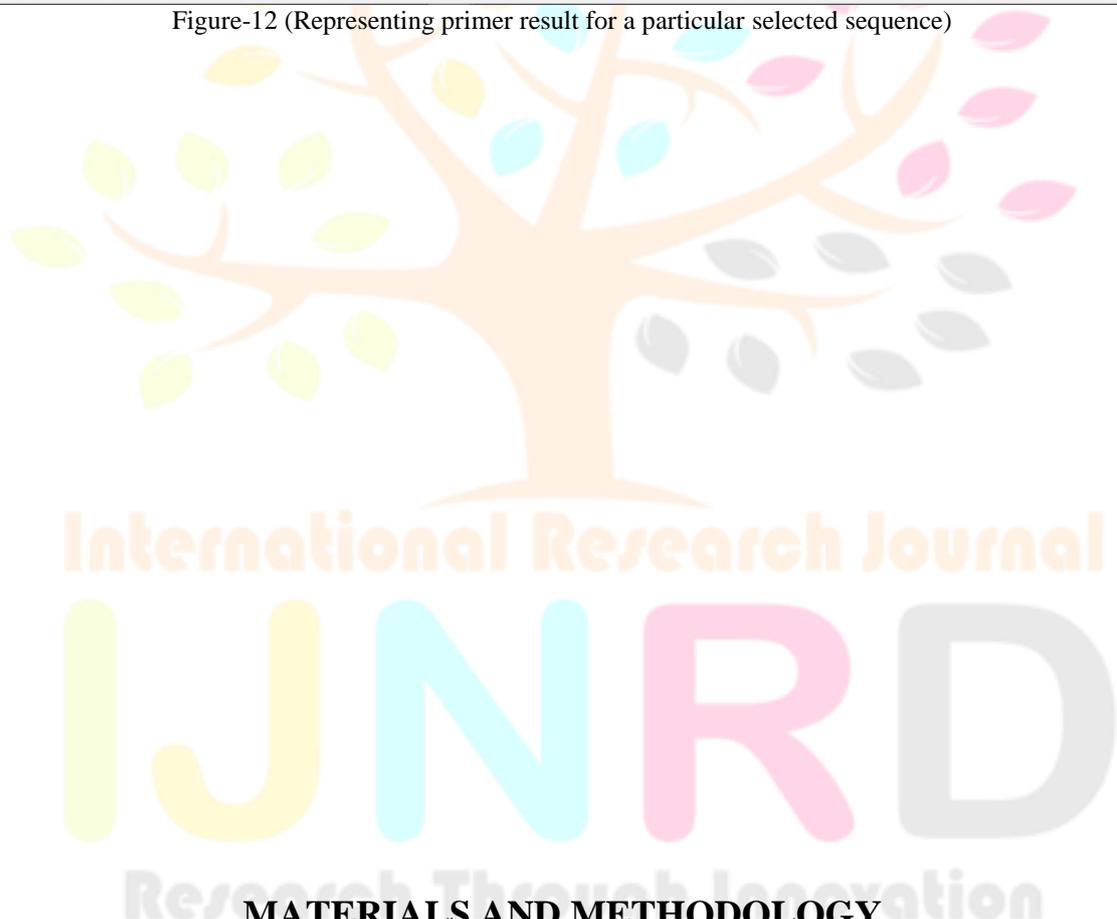


Figure-12 (Representing primer result for a particular selected sequence)



MATERIALS AND METHODOLOGY

MATERIALS: -

Gene model organism- *Arabidopsis thaliana*

Gene- COP1

Locus- Out of 37 loci, selected one locus [AT2G32950].

Database tools- TAIR database for retrieving gene information.

ORF (Open Reading Frame) Finder for ORF detection

ApE software for primer designing.

METHODOLOGY: -

<p>Step-1 RETRIVING GENE INFORMATION THROUGH TAIR DATABASE</p>	<ol style="list-style-type: none"> 1. Selected a gene of interest. 2. Search for locus of gene. 3. Selected a particular locus. 4. By that locus, generate the FASTA sequence. 5. Getting sequence in FASTA format.
<p>Step-2 ORF (OPEN READING FRAME) DETECTION THROUGH ORF FINDER</p>	<ol style="list-style-type: none"> 1. Taking the sequence that retrieve in previous step and pasting in ORF finder. 2. On submitting the sequence, the ORF result found shows all the possible six reading frames. 3. For graphical representation, performing BLAST for a particular ORF sequence.
<p>Step-3 PRIMER DESIGNING FROM ApE SOFTWARE</p>	<ol style="list-style-type: none"> 1. Pasting the FASTA sequence of a particular locus for COP1 gene in ApE software. 2. By clicking on a particular primer, it will also show the position of the particular primer in the input. 3. Click “OK” button to get primer. 4. After performing all the procedure, sorting the primer according to the Tm, 5', 3', GC%, and length. 5. Primer found in the input.

Finally, selected the most suitable primer out of it for COP1 gene of *Arabidopsis thaliana*.

RESULTS AND DISCUSSION

Hence ORF detection and primer designing for COP1 gene of *Arabidopsis thaliana* is successfully done and satisfying conditions for a suitable primer.

The first 5 primers out of all as shown in figure (Figure- 13) are most suitable primers for COP1 gene of *Arabidopsis thaliana*.

Primer (5'-->3')	length	%GC	Tm(°C)	self/other(max adj 3')
2326 TCTCTTTGAGGAGTGTTCGAAGG 2303	24	45	58	8 4 2 /-----
2325 TCTCTTTGAGGAGTGTTCGAAGG 2303	23	47	58	8 4 2 /-----
2324 CTCTCTTGGAGAGTGTTCGAAGG 2303	22	50	56	8 4 2 /-----
2323 TTTCTTGGAGAGTGTTCGAAGG 2303	21	47	56	8 4 2 /-----
2322 TCTCTTGGAGAGTGTTCGAAGG 2303	20	50	55	8 4 2 /-----
2320 TTGAGGAGTGTTCGAAGGCAAG 2299	22	50	59	8 3 1 /-----
2319 TGAGGAGTGTTCGAAGGCAAG 2299	21	52	58	6 3 1 /-----
2318 GAGGAGTGTTCGAAGGCAAG 2298	21	57	59	8 3 2 /-----
2318 GAGGAGTGTTCGAAGGCAAG 2299	20	55	57	6 3 1 /-----
2317 AGGAGTGTTCGAAGGCAAG 2298	20	55	59	8 3 2 /-----
2155 TACTAGATTCACGCGAGCGAG 2136	20	50	55	8 4 2 /-----
2153 CTAGATTCACGCGAGCGAGTAC 2133	21	52	56	8 4 1 /-----
2152 TAGATTCACGCGAGCGAGTAC 2133	20	50	55	8 4 1 /-----
2149 AATCAGCGAGCGAGTACCGAG 2130	20	55	58	8 4 1 /-----
2147 TCAGCGAGCGAGTACCGAAG 2127	21	57	60	8 4 2 /-----
2146 CACGCGAGCGAGTACCGAAG 2127	20	60	59	8 4 2 /-----
2144 CGCAGCGAGTACCGAAGCTTTG 2123	22	54	60	8 4 2 /-----
2143 GCAGCGAGTACCGAAGCTTTG 2123	21	52	57	8 4 2 /-----
2141 AGCGAGTACCGAAGCTTTGATG 2120	22	45	56	8 4 2 /-----
2140 GCGAGTACCGAAGCTTTGATG 2120	21	47	55	8 4 2 /-----
2124 TGATGGTTCCTTGACTATTCCGAG 2101	24	45	59	8 2 2 /-----
2123 GATGGTTCCTTGACTATTCCGAG 2101	23	47	57	8 2 2 /-----
2122 ATGGTTCCTTGACTATTCCGAG 2101	22	45	56	8 2 2 /-----
2121 TGGTTCCTTGACTATTCCGAG 2101	21	47	56	8 2 2 /-----
2112 GACTATTCCGAGTCAACATCG 2092	21	47	55	8 4 2 /-----
2110 CTATTCCGAGTCAACATCGTGG 2089	22	50	57	8 2 1 /-----
2110 CTATTCCGAGTCAACATCGTG 2090	21	47	55	8 2 2 /-----
2109 TATTCCGAGTCAACATCGTGG 2089	21	47	56	8 2 1 /-----

Figure- 13 (Presenting top 5 most suitable primers for COP1)

CONCLUSION AND FUTURE PROSPECT

Primers are short, specially made stretches of oligonucleotides that are combined in different lengths. Great groundwork finds some kind of harmony among particularity and enhancement efficiency. The more limited the primer, the more proficient they can strengthen to target DNA.

Great PCR can be utilized in significant regions like a finding of hereditary infection, biomedical examination, legal application, transformative investigations, and some more.

Primer designed for COP1 gene of *Arabidopsis thaliana* can be used in further research. The data about *Arabidopsis thaliana* would be useful for analysts and can be utilized further in wet labs particularly for explores in other blossoming plants of similar species.

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