Class Demo 1: NCBI

PIPRA

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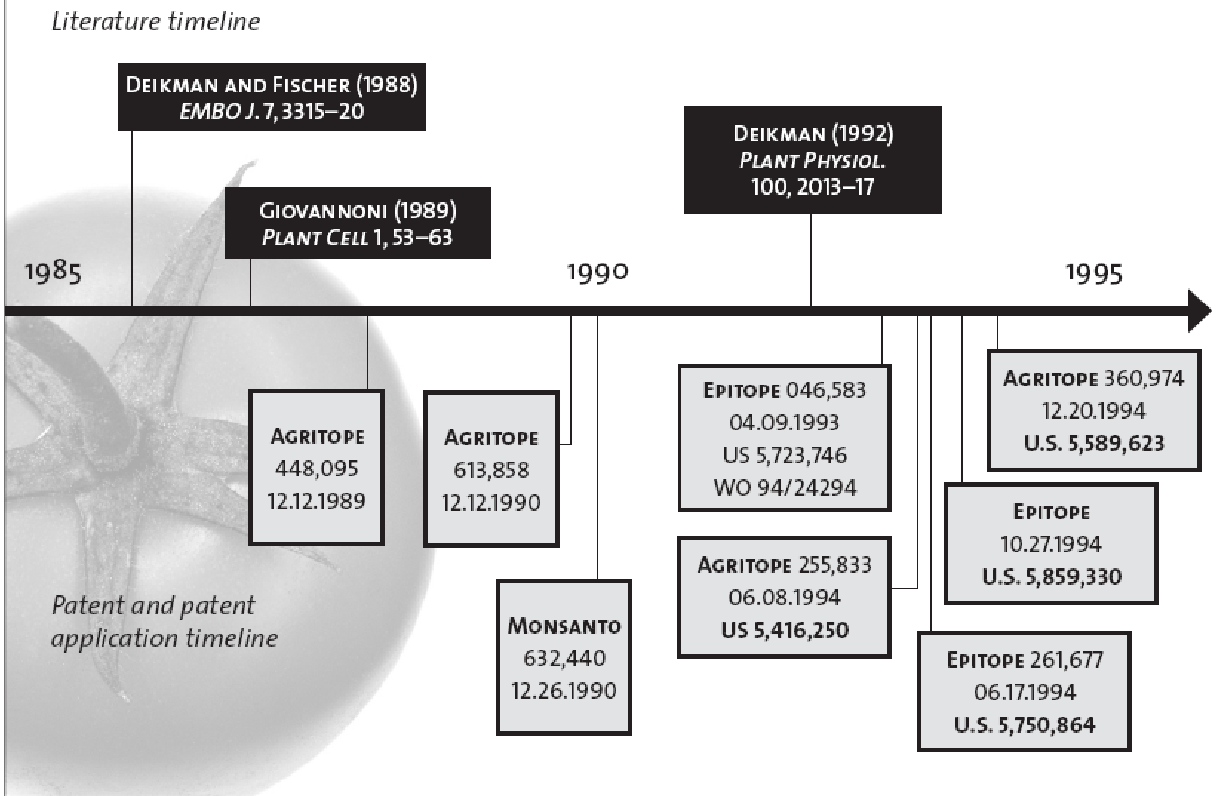
<u>Demonstration Task:</u> Identify patents related to Tomato E8 promoter to drive expression of a target gene (i.e. oral vaccine) in ripening fruit tissue

1. Identify DNA sequence for FTO Target Tomato Fruit E8 Promoter
2. Conduct preliminary DNA search using NCBI's Patent Database

Class Demo: Searching for Patents using NCBI Patent Database

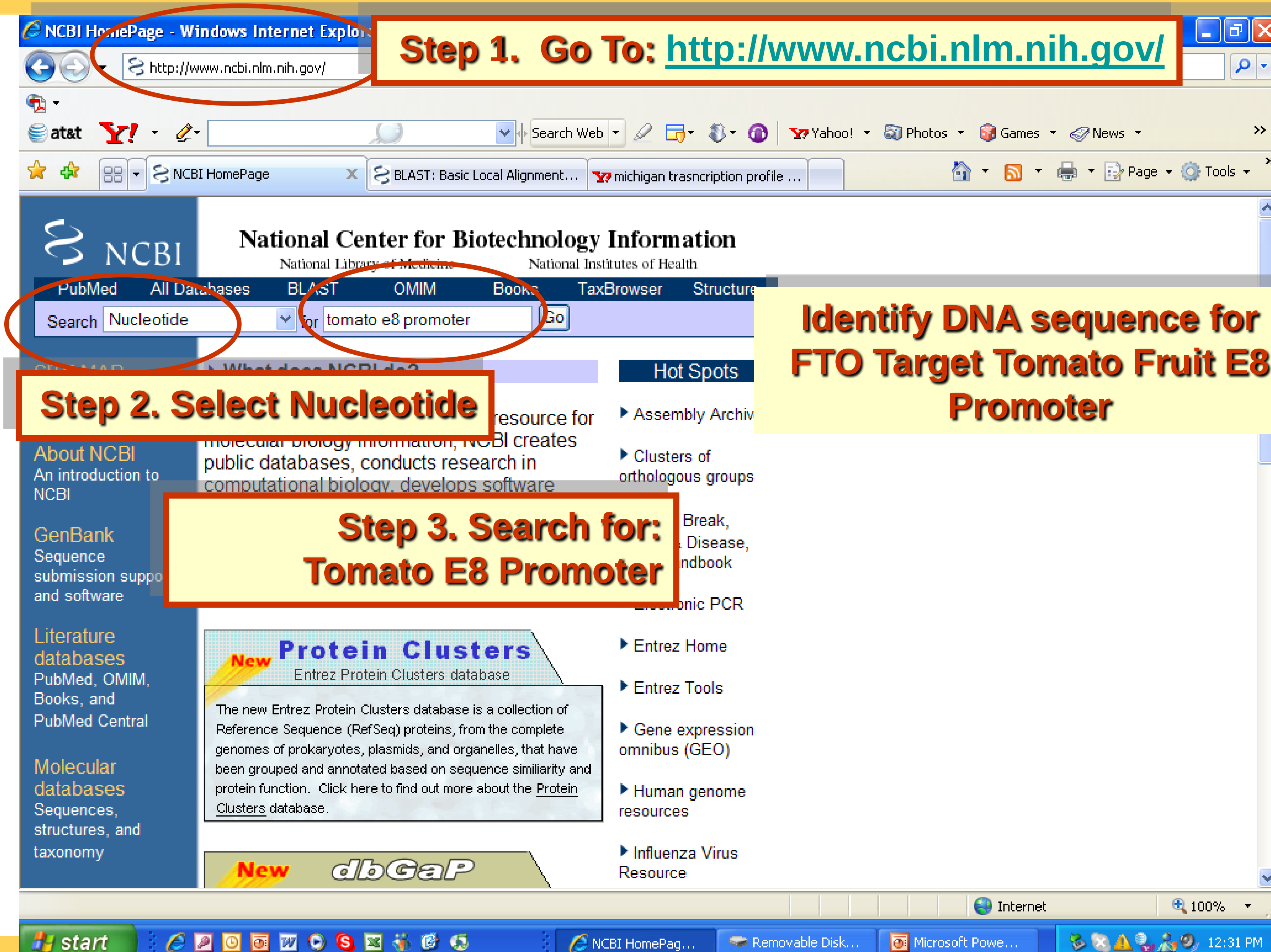
Tomato E8 Tissue Specific Promoter

FIGURE 1: TIMELINE OF TOMATO E8 SCIENTIFIC PUBLICATIONS AND PATENTS



Scientific and patent literature, including patents and patent applications, illustrate the existing prior art at the time that related patent applications were filed. The priority dates of each patent and patent application relative to the publication dates of the main scientific literature are shown.

NCBI: National Center for Biotechnology Information
http://www.ncbi.nlm.nih.gov/



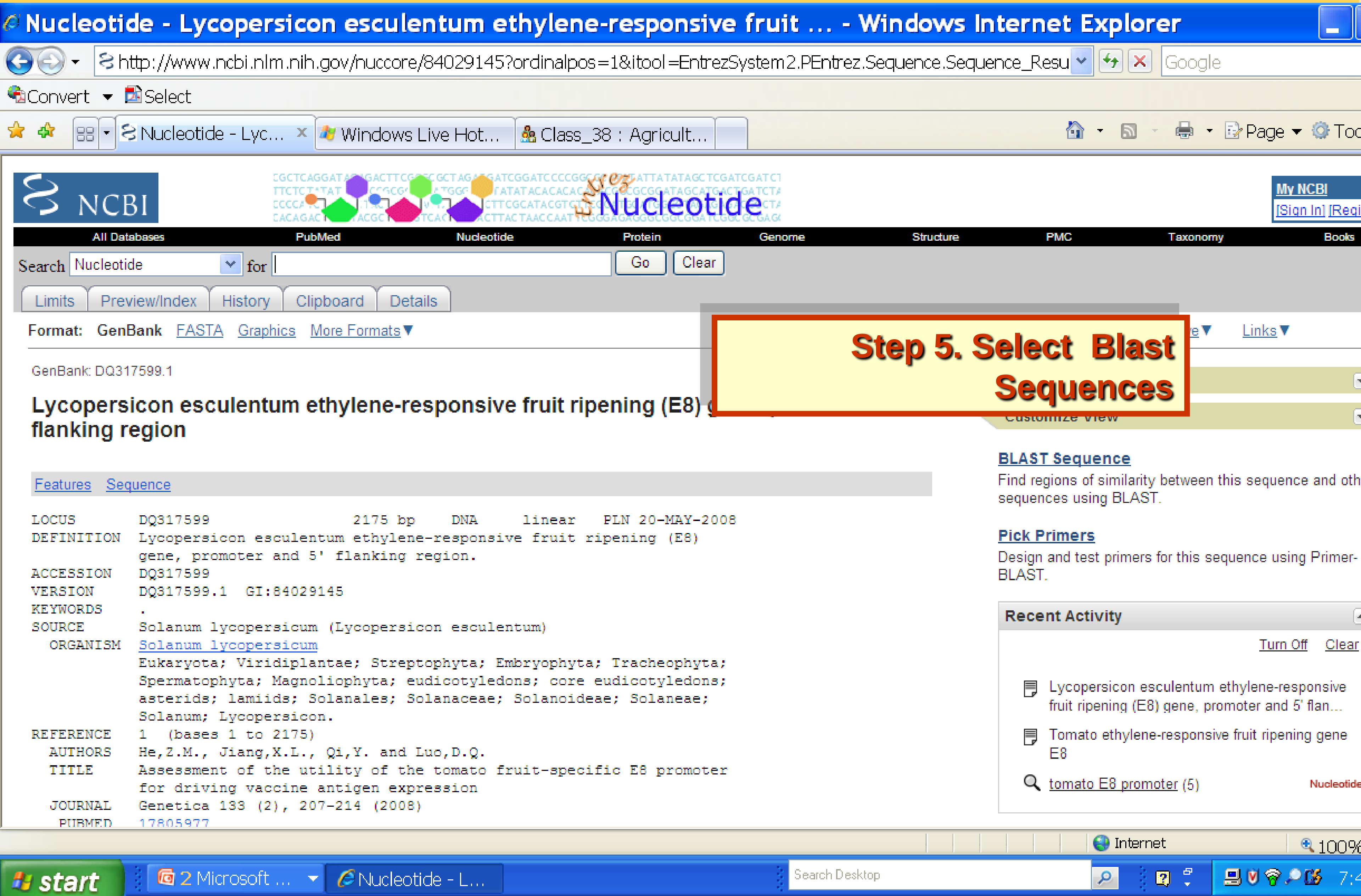
Step 1. Go To: http://www.ncbi.nlm.nih.gov/

Identify DNA sequence for FTO Target Tomato Fruit E8 Promoter

Step 2. Select Nucleotide

Step 3. Search for: Tomato E8 Promoter

Step 4. Select DQ317599

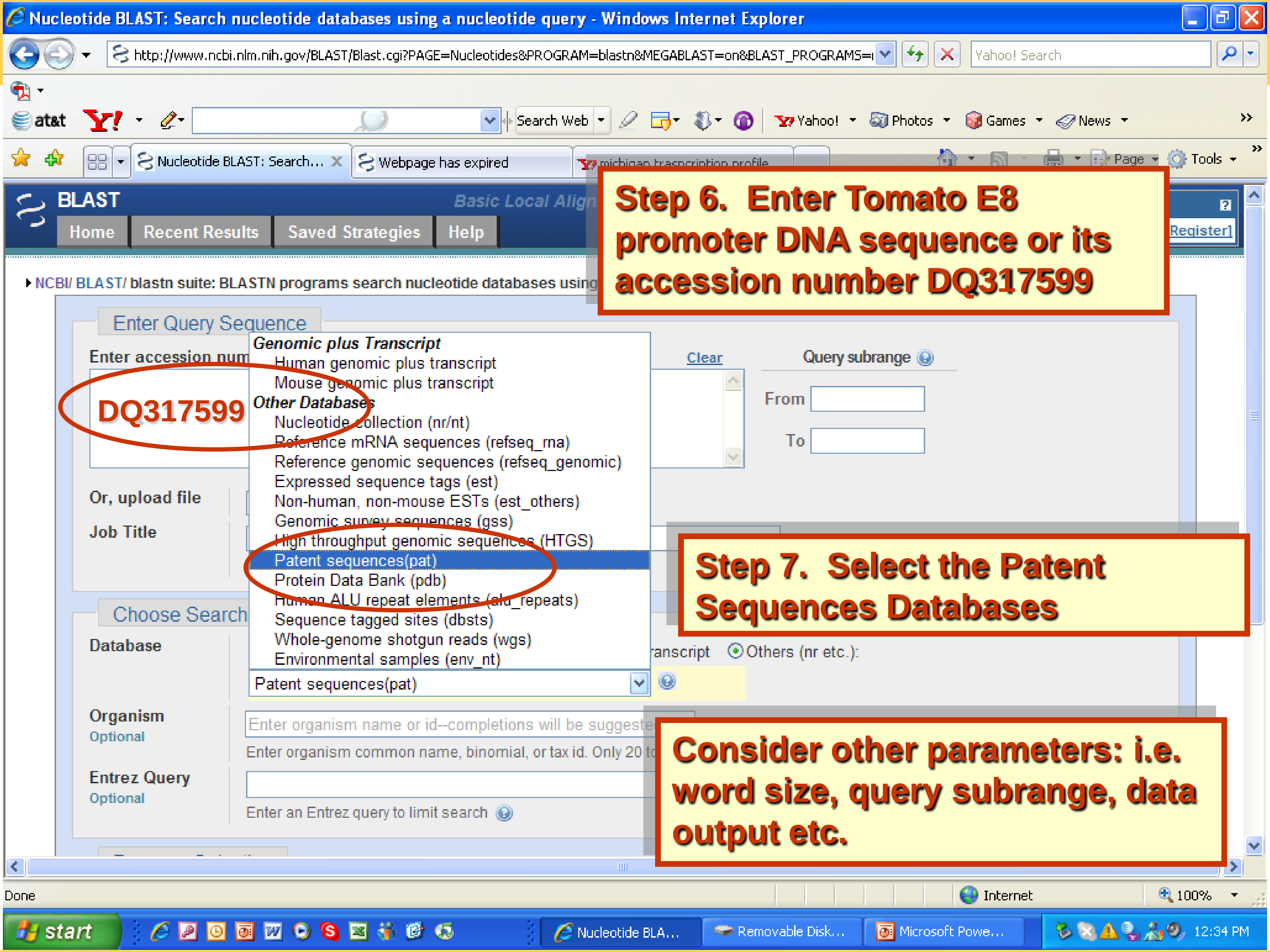


Step 5. Select Blast Sequences

Step 6. Enter Tomato E8 promoter DNA sequence or its accession number DQ317599

Step 7. Select the Patent Sequences Databases

Consider other parameters: i.e. word size, query subrange, data output etc.



NCBI Blast:Nucleotide sequence (2191 letters) - Windows Internet Explorer

http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi

Distance tree of results NEW

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
BD077997.1	Synthetic hybrid plant promoter	[illegible]	[illegible]	[illegible]	0.0	99%
AR029480.1	Sequence 24 from patent US 5859330	[illegible]	[illegible]	[illegible]	0.0	99%
AR007550.1	Sequence 3 from patent US 5750870	[illegible]	[illegible]	[illegible]	0.0	99%
I90253.1	Sequence 12 from patent US 5723746	3967	3967	100%	0.0	99%
AR029482.1	Sequence 27 from patent US 5859330	2037	2037	51%	0.0	99%
I86984.1	Sequence 10 from patent US 5702933	2028	2028	51%	0.0	99%
I19978.1	Sequence 10 from patent US 5512466	2028	2028	51%	0.0	99%
BD077991.1	Synthetic hybrid plant promoter	1901	1901	48%	0.0	99%
I24835.1	Sequence 1 from patent US 5545815	1332	1896	47%	0.0	99%
BD077996.1	Synthetic hybrid plant promoter	1240	1240	31%	0.0	99%

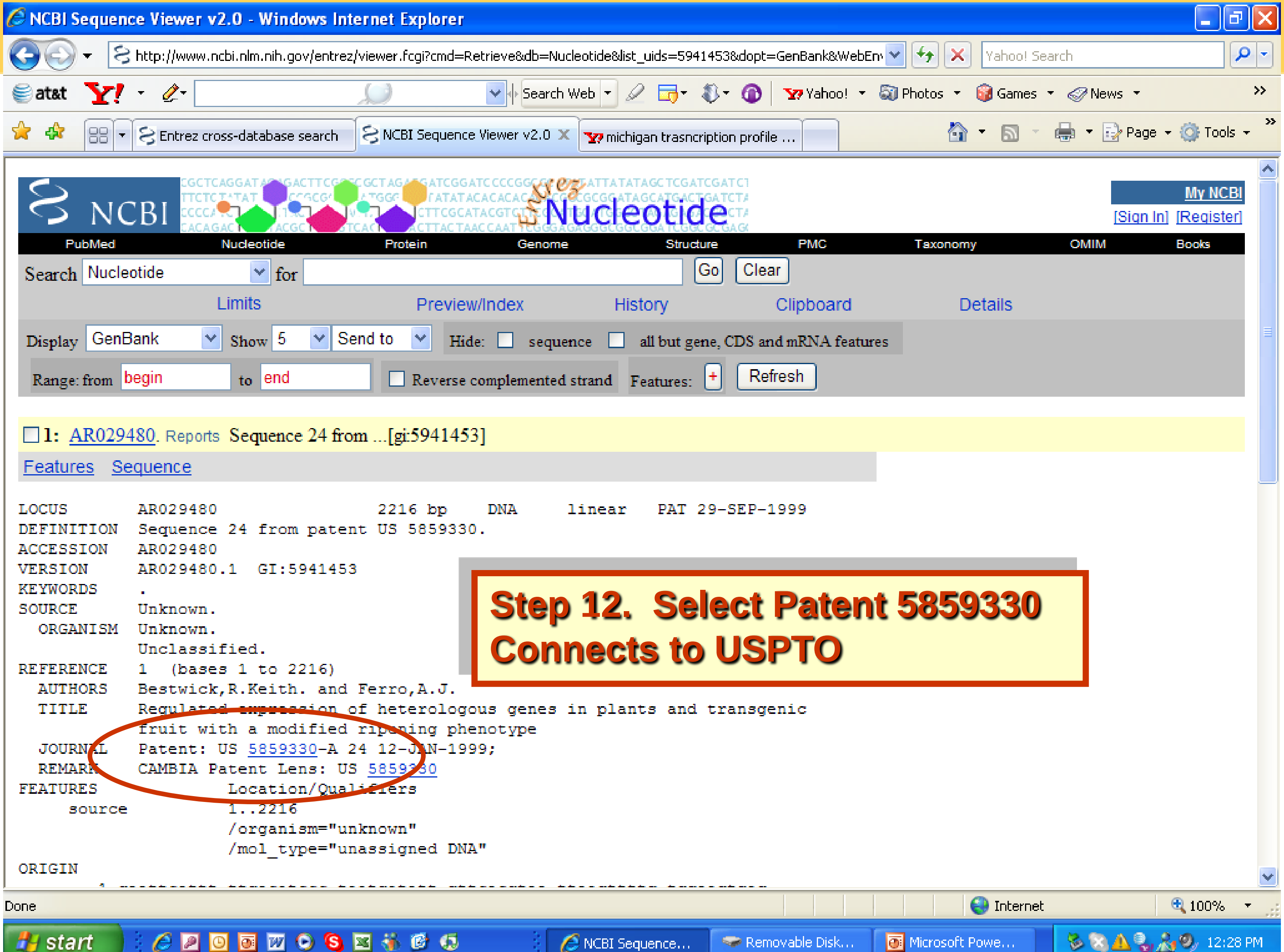
Step 11. Select Patent 5859330

Alignments

Get selected sequences | Select all | Deselect all | Distance tree of results

> dbj|BD077997.1| Synthetic hybrid plant promoter
Length=2298

Score = 3967 bits (2148), Expect = 0.0


NCBI Sequence Viewer v2.0 - Windows Internet Explorer
http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?cmd=Retrieve&db=Nucleotide&list_uids=5941453&dopt=GenBank&WebEn
Entrez cross-database search
NCBI Sequence Viewer v2.0
michigan trasncription profile ...
NCBI
Entrez Nucleotide
My NCBI
[Sign In] [Register]
PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM Books
Search Nucleotide for Go Clear
Limits Preview/Index History Clipboard Details
Display GenBank Show 5 Send to Hide: sequence all but gene, CDS and mRNA features
Range: from begin to end Reverse complemented strand Features: + Refresh
1: AR029480. Reports Sequence 24 from ...[gi:5941453]
Features Sequence
LOCUS AR029480 2216 bp DNA linear PAT 29-SEP-1999
DEFINITION Sequence 24 from patent US 5859330.
ACCESSION AR029480
VERSION AR029480.1 GI:5941453
KEYWORDS .
SOURCE Unknown.
ORGANISM Unknown.
Unclassified.
REFERENCE 1 (bases 1 to 2216)
AUTHORS Bestwick,R.Keith. and Ferro,A.J.
TITLE Regulated expression of heterologous genes in plants and transgenic fruit with a modified ripening phenotype
JOURNAL Patent: US 5859330-A 24 12-JAN-1999;
REMARK CAMBIA Patent Lens: US 5859330
FEATURES Location/Qualifiers
source 1..2216
/organism="unknown"
/mol_type="unassigned DNA"
ORIGIN
Step 12. Select Patent 5859330
Connects to USPTO
Done
Internet
100%
start
NCBI Sequence...
Removable Disk...
Microsoft Powe...
12:28 PM

United States Patent: 5859330 - Windows Internet Explorer

http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchn

USPTO Patent Full-Text and Image Database

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United States Patent **5,859,330**

Bestwick , et al. **January 12, 1999**

Regulated expression of heterologous genes in plants and transgenic fruit with a modified ripening phenotype

Abstract

The present invention relates to chimeric genes having (i) a DNA sequence encoding a product that is effective to reduce ethylene biosynthesis in fruit from the plant, and (ii) a promoter whose expression is induced during fruit ripening or by ethylene synthesis by the fruit, where the DNA sequence is heterologous to the promoter and the DNA sequence is operably linked to the promoter to enable expression of the product. The invention describes vectors and transgenic plants, vegetables, flowers and fruits carrying the chimeric gene, as well as methods related thereto.

Inventors:	**Bestwick; Richard Keith** (Portland, OR), **Ferro; Adolph J.** (Lake Oswego, OR)
Assignee:	**Epitope, Inc.** (Beaverton, OR)
Appl. No.:	**08/331,355**
Filed:	**October 27, 1994**

Done | Internet | 100%

start | United States P... | Removable Disk... | Microsoft Powe... | 12:29 PM and a continuation-in-part of co-owned, U.S. patent application Ser. No. 08/255,833, filed 8 Jun. 1994, herein incorporated by reference, now U.S. Pat. No. 5,416,250, which is a file-wrapper continuation of co-owned, U.S. patent application Ser. No. 07/613,858, filed 12 Dec. 1990, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 448,095 filed Dec. 12, 1989, now abandoned.

Claims

It is claimed:

1. A transgenic fruit-bearing plant, comprising:

(i) a DNA sequence encoding S-adenosylmethionine hydrolase, and operably linked to said DNA sequence (ii) a promoter selected from the group consisting of: a promoter of a tomato E4 gene, wherein said E4 gene is composed of a series of nucleotides that is at least 60% identical to that presented in SEQ ID NO: 8, a promoter of a tomato E8 gene, wherein said gene is composed of a series of nucleotides that is at least 60% identical to that presented in SEQ ID NO: 27, and an avocado cellulase gene promoter,

where expression regulated by the promoter is induced during fruit ripening or by ethylene synthesis by fruit produced by said plant.

2. A transgenic plant of claim 1, wherein the promoter comprises nucleotides 1-1173 of SEQ ID NO: 10.

3. A transgenic plant of claim 1, wherein the promoter is from a tomato E4 or E8 gene.

4. A transgenic plant of claim 1, wherein the promoter is an avocado cellulase gene promoter.

5. A method for modifying ripening fruit of a fruit bearing plant, comprising:

growing the plant of claim 1, to produce a transgenic plant bearing fruit, wherein fruit produced by said plant has an initial burst of ethylene production, followed by a reduction in the level of ethylene synthesis by said fruit, resulting in a fruit having a modified ripening phenotype in which the time course of ripening is delayed relative to wild-type fruit.