Title: Genetic Variation of Crabapples (*Malus spp.)* found on Governors Island and NYC Area

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School: Manhattan Comprehensive Night & Day High School

Crab

Apple

Crabapple variation?

MCN + DHS

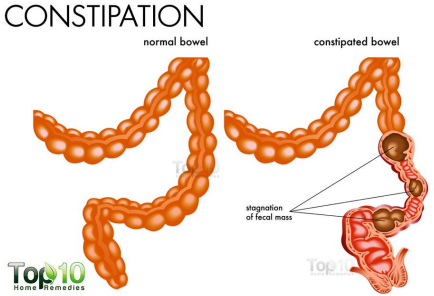
YEAH IT'S CRABAPPLE!!!!!

Medicinal uses

"Accelerate cerebral circulation and oxygenation and regulates the secretion of neuromodulators to help facilitate intellectual work and mental fatigue"
http://www.forresthealth.com/Crab-Apple-Bud.html

"Crabapple buds are rich in beneficial phyto-chemicals such growth factors and plant hormones, enzymes, nucleic acids, oligoelements, and phytonutrients such as polyphenols and flavonoids."

http://www.goldenneedleonline.com/Crab-Apple-Bud.html



To treat constipation and worms

Crabapple for jelly and cider

Crabapple fruits have sour and bitter taste if you eat them raw. But the fruits can be made jelly and cider and crabapple wood can also be used to grill and smoke food to add aroma

Abstracts

Crabapple trees are one of the most common flowering fruit trees found along streets and parks and around landmark buildings in NYC. Their colorful blossom and different size fruits make them one of the most popular ornament trees for landscape. In fact, apples and crabapples belong to the rose family, Rosacea and genus *Malus*. All over the world there are approximately 35 different species of crabapple and 700 cultivars and they can be hybrids that result from more than one species. Our observation of the crab apple samples showed some variations in physical characteristic and therefore we hypothesized that those crabapple trees found on Governors Island, NY and in other NYC area may have come from those 35 species or hybrids of 700 cultivars and there may be genetic variation among them. Our research objective is to determine if there is genetic variation among crabapple trees found on Governors Island of New York and in New York City area. Total of 35 samples of crab apple trees (13 samples from the Governors Island, NY, 8 samples from Gramercy Park, NY, and 3 from Central Park, NY) were collected, and their DNA was isolated and identified using DNA barcode. The analysis of the DNA barcode result suggested that all the samples from the Governors Island and NYC areas belong to different species of Malus. *Malus baccata, Malus micromalus, Malus domestica, Malus pumila.*

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Introduction

According to research, there is genetic diversity among crabapple trees because those trees vary in size, tree characteristic, types of blooms and fruits and there are over 35 species and 700 cultivated varieties of crab apples. At New York Botanical Garden, there are 80 varieties in the crabapple collection.

Research Questions:

Do all the crabapple trees on the Governors Island and NY belong to one type of ***Malus species*** or a varieties of species?

Do all the crabapple trees found in the NYC areas belong to different ***Malus species***?

Hypothesis:

We hypothesized that those crabapple trees found on Governors Island, NY and other NYC area may have come from those 35 species or hybrids of 700 cultivars and there may be genetic variation among them. Our research objective is to find out if there is genetic variation among crabapple trees found on Governors Island of New York and New York City area. We hope to contribute to DNA Database of Biodiversity of NYC through our DNA barcode research.

Materials and Methods

Majority of our crab apple samples were collected from Governors Island .Collection of leaf and fruit samples will be difficult in the winter due to cold weather. Therefore, before we started writing our proposal, we had collected fruit and leaf samples of crabapple trees on Governors Island, NY, Central Park, Gramercy Park. We took photos of those samples with a digital camera, put them in the zip lock bags and properly labeled and stored them in the freezer. We extracted DNA of the samples following the protocol provided by the DNA Learning Center. We took a small amount of leaf tissues from each samples from-25 samples and fruit tissues from 6 samples. In order to identify genetic diversities of the crabapple samples, we used DNA sequences of chloroplast rbcL genes following the Barcoding protocol from NYC Urban Barcode Project. Then the samples were amplified using the rbcl primers through PCR. We also used ITS primers for a few of the samples that rbcl primers did not work well with. When we received the PCR products, we analyzed PCR products by running Gel electrophoresis. Finally we requested the DNA Learning Center to send our PCR products to DNA sequencing services. When we received the sequence result, we uploaded the sequences to DNA Subway and used BLAST to find DNA sequences in a Database to see if our sample DNA sequences matched with those from the Database. Based on the result, we concluded if our hypothesis is supported by the result.

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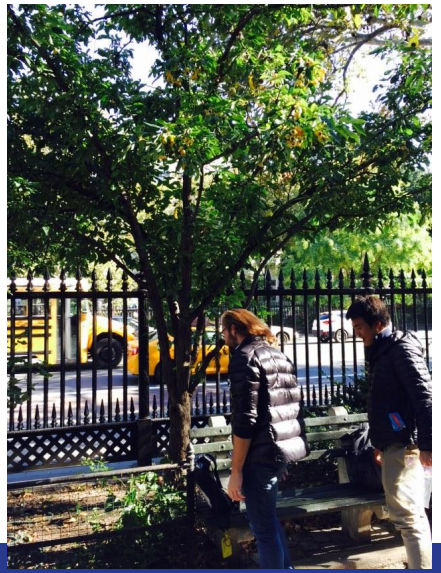
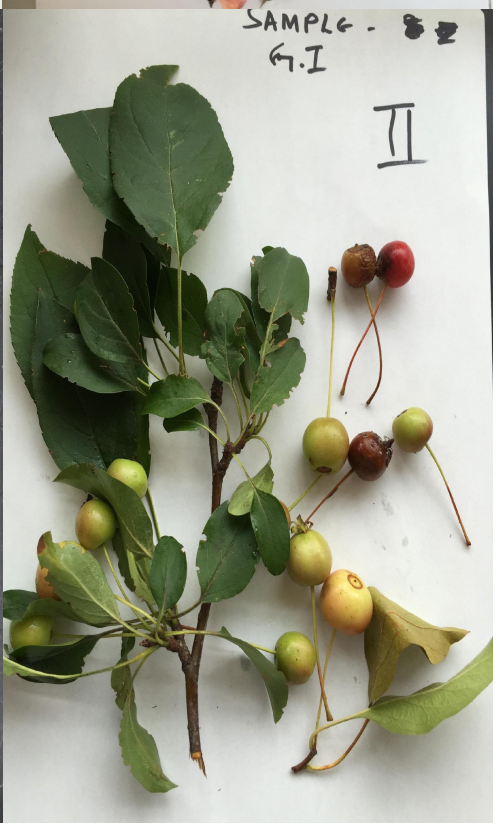
Sample collection

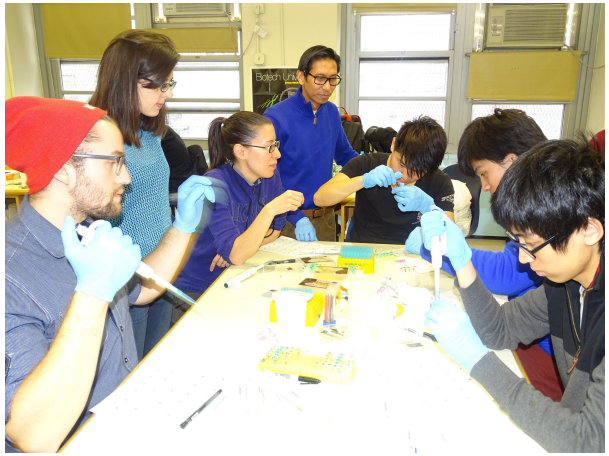


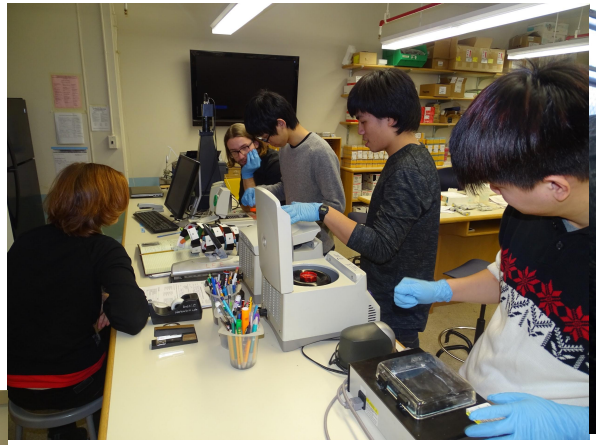

SAMPLE -
G.I
III

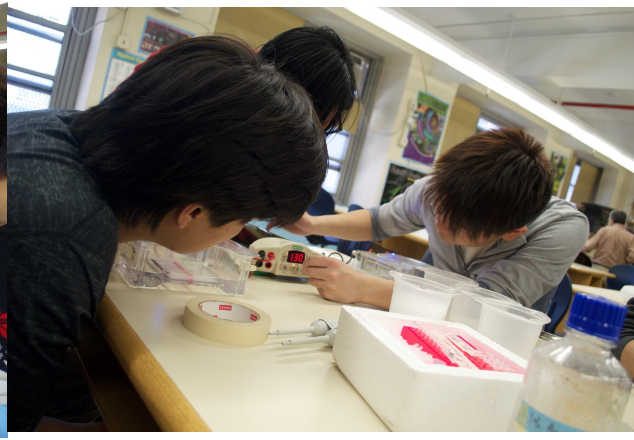

S-6


SAMPLE -
G.I
II

DNA extraction with qiagen kits

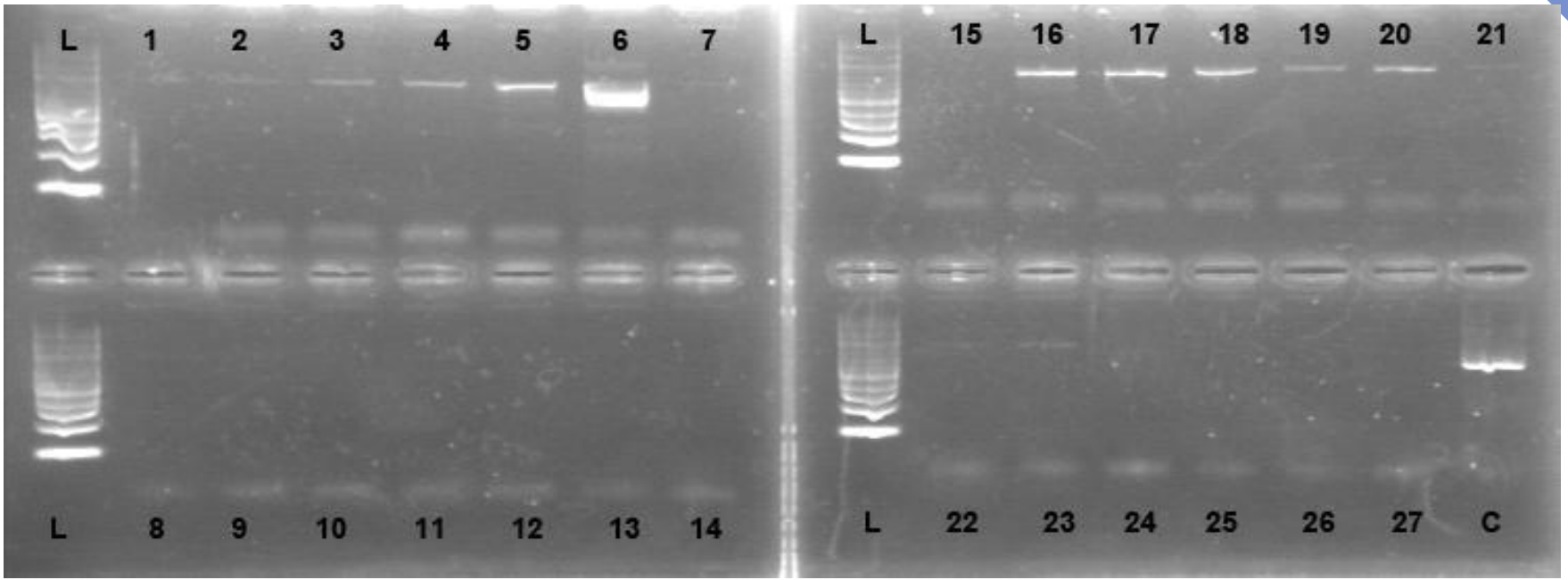




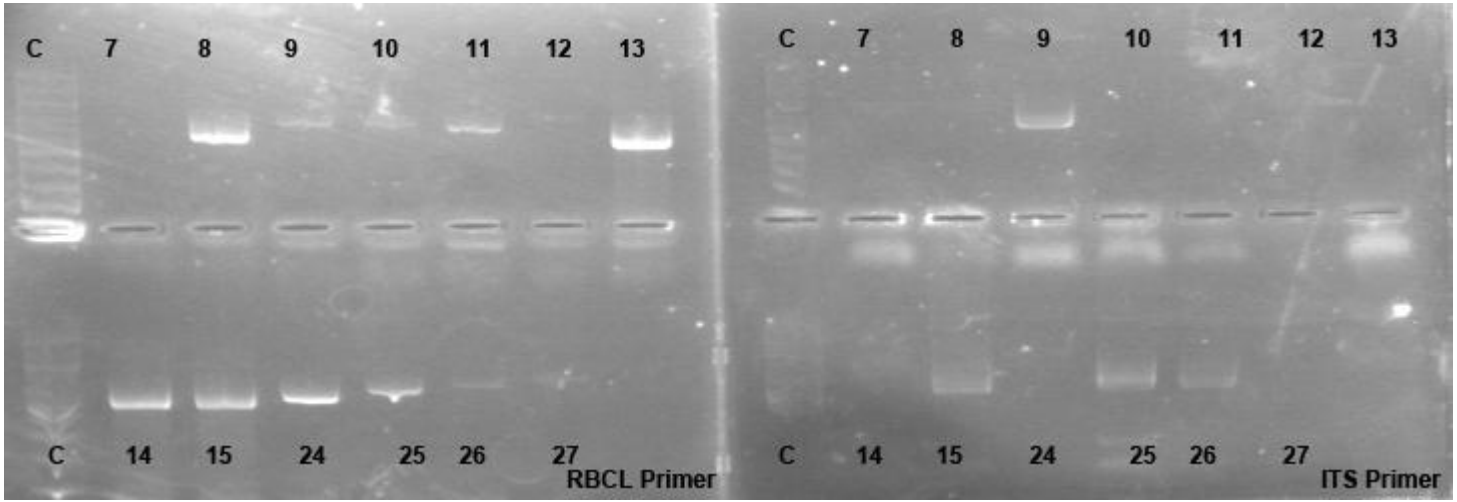


PCR with rbcl primers
and ITS primers

First Gel Electrophoresis result from PCR products with rbcl primers

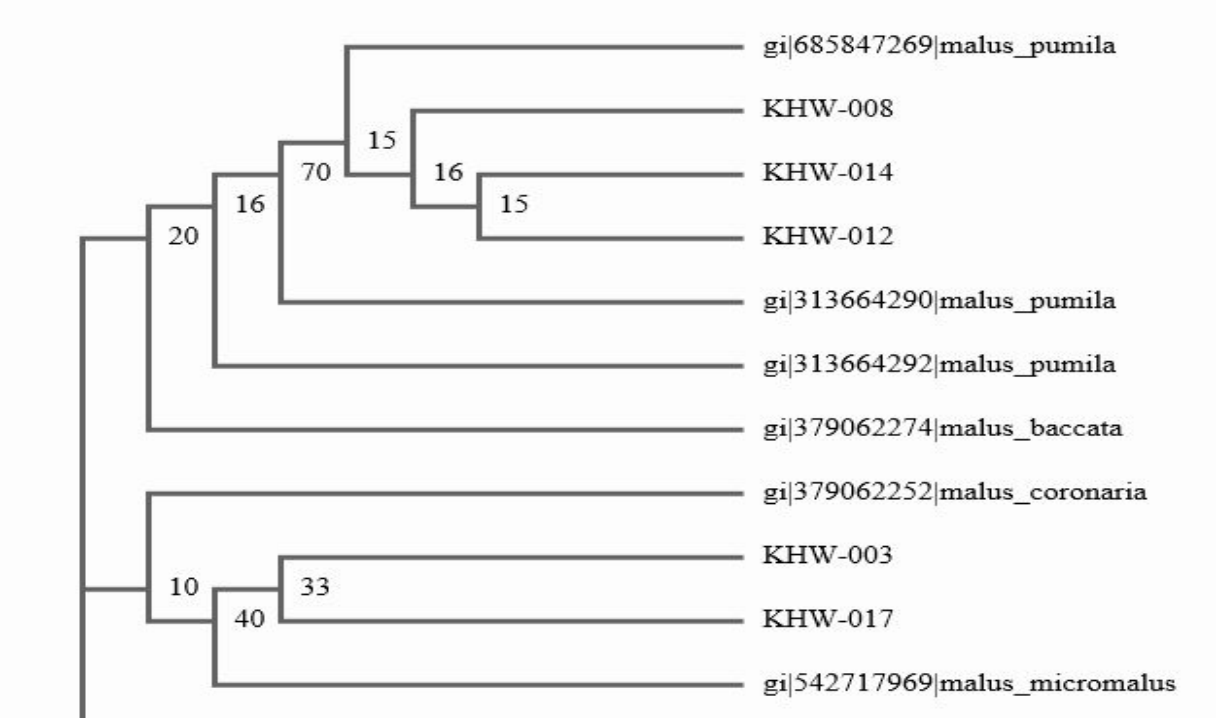


Second Gel Electrophoresis result from PCR products with rbcl and ITS primers



Results

Our result indicated that all the samples from the Governors Island and NYC areas belong to ***Malus baccata, Malus micromalus, Malus domestica(Apple Tree), Malus***



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BLASTN

KHW-002

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	gi|291201182|emb|FN689370	Crataegus monogyna - rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, country Italy:Umbria	599	1072	0.0	2
2(2).	gi|424949962|gb|JX848520	Amelanchier alnifolia - large subunit (rbcL) gene, partial cds; chloroplast	599	1067	0.0	3
3(3).	gi|285020601|gb|GU363810	Malus baccata - voucher PS1107MT01 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1045	0.0	0
4(4).	gi|285020613|gb|GU363816	Malus micromalus - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1045	0.0	0
5(5).	gi|817992124|gb|KM360872	Malus domestica - large subunit (rbcL) gene, partial cds; plastid	579	1045	0.0	0
6(6).	gi|285020559|gb|GU363789	Sorbus discolor - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1036	0.0	2
7(7).	gi|285020591|gb|GU363805	Chaenomeles sinensis - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1036	0.0	2
		Cotoneaster horizontalis - voucher				

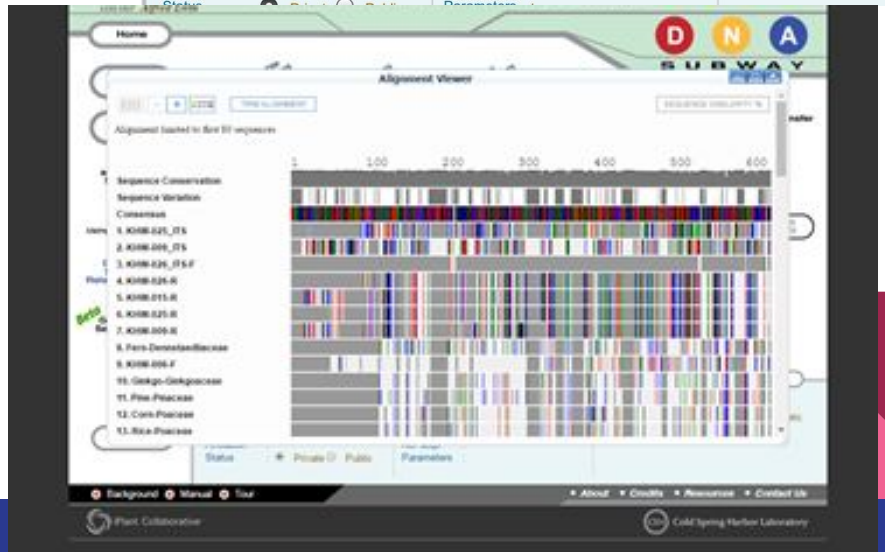
KHW-011

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	gi|291201182|emb|FN689370	Crataegus monogyna - rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, country Italy:Umbria	599	1072	0.0	2
2(2).	gi|424949962|gb|JX848520	Amelanchier alnifolia - large subunit (rbcL) gene, partial cds; chloroplast	599	1067	0.0	3
3(3).	gi|285020601|gb|GU363810	Malus baccata - voucher PS1107MT01 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1045	0.0	0
4(4).	gi|285020613|gb|GU363816	Malus micromalus - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1045	0.0	0
5(5).	gi|817992124|gb|KM360872	Malus domestica - large subunit (rbcL) gene, partial cds; plastid	579	1045	0.0	0
6(6).	gi|285020559|gb|GU363789	Sorbus discolor - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1036	0.0	2
7(7).	gi|285020591|gb|GU363805	Chaenomeles sinensis - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1036	0.0	2
		Cotoneaster horizontalis - voucher				

BLASTN

KHW-005

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	gi|291201182|emb|FN689370	Crataegus monogyna - rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, country Italy:Umbria	599	1072	0.0	2
2(2).	gi|424949962|gb|JX848520	Amelanchier alnifolia - large subunit (rbcL) gene, partial cds; chloroplast	599	1067	0.0	3
3(3).	gi|285020601|gb|GU363810	Malus baccata - voucher PS1107MT01 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1045	0.0	0
4(4).	gi|285020613|gb|GU363816	Malus micromalus - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1045	0.0	0
5(5).	gi|817992124|gb|KM360872	Malus domestica - large subunit (rbcL) gene, partial cds; plastid	579	1045	0.0	0
6(6).	gi|285020559|gb|GU363789	Sorbus discolor - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1036	0.0	2
7(7).	gi|285020591|gb|GU363805	Chaenomeles sinensis - ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	579	1036	0.0	2
		Cotoneaster horizontalis - voucher				



Discussion:

The results of our research supported our hypothesis that there is genetic variation among the species of crabapples found on Governors Island and New York City Area. During our gel electrophoresis for the PCR product of 27 samples, sample number 18, 22, 23, 26 and 27 did not come out well, while the rest were successful. We wondered why we did not have high quality DNA from those samples, and assumed that there may have been an error in our DNA extraction or the primers used. We conferred with scientists from the DNA Learning center and they suggested that we should run the PCR of those DNA samples using two different primers, rbcl & ITS primers.

We found out the result of the second PCR came out best with Rbcl. The result indicated that all the samples belong to different *Malus* species. Because of the limitation of DNA Barcoding, and time constraint and small sample sizes, we cannot determine if there is subspecies level variations among those crab apples. In order to do so requires other molecular tools. Since there are many cultivars from hybrids, it is not easy to verify the genetic diversity. We suggest that further research need to be done with larger sample size. Even though our sample size was small, we hope that our research may contribute to some degrees to the DNA Database of Biodiversity New York City.

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