Characteristics of the Southern Flying Squirrel (Glaucomys volans) population at Brookhaven National Laboratory

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Abstract

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Even though the Southern Flying Squirrel (*Glaucomys volans*) are prevalent in hardwood forests in the eastern half of the United States, the fact that they are nocturnal means they rarely cross paths with any human. Through this experiment, we sought to find out if, due to geographic isolation, the Flying Squirrel populations on Long Island differ genetically from those found on the mainland. We caught squirrels with baited Sherman traps and took genetic samples via cheek swabs. The DNA was then extracted using a Qiagen DNeasy Blood and Tissue Kit, and sent to Brookhaven National Laboratory's Biology facility for DNA sequencing. We were able to capture 83 squirrels out of 600 total trap nights (13.83% return). And from the 83 squirrels, 69 DNA samples were extracted (some extractions were duplicate sample from one individual). Out of the 69 DNA samples, 43 went through PCR successfully (62.32%). There are still samples left to be processed.

Introduction

The southern flying squirrel (*Glaucomys volans*) is prevalent in hardwood forests in the eastern half of the United States [1], east of 100W latitude, and in pockets in Mexico and Central America [2]. The Southern flying squirrels prefer to inhabit hardwood forests that contain seed producing trees such as oak and maple. They are omnivores and have a wide array of food in their diet including: seeds, bark, insects, and bugs. And because of this large diet and their fairly large home range, flying squirrels play an important role since they spread seeds of hardwoods and spores of fungi. Flying squirrels are very common but the fact that they are nocturnal, they rarely cross paths with humans. Flying squirrels have a fairly large home range and there is a possibility that the flying squirrels found on Long Island are genetically different from those found on the mainland. Long Island was formed over 400 million years ago due to pre-glacial geological events, and it is completely seperated from the mainland by the Hudson River on the island's west coast. The Long Island Sound is found along the northern coast of the island and this flows directly into the Atlantic Ocean, which surrounds the remainder of Long Island (Figure 1) [3]. Therefore, the squirrels are physically isolated from each other, and it would be rare that the two populations could come in contact with each other and mate. Even though they are called flying squirrels, they are incapable of extended flight, and can only glide due to the loose skin between their wrists and ankles called a patagium. Glide distance is directly proportional to launch height. For example, a glide from the top of an oak tree, approximately 18-meters, a flying squirrel can glide nearly 50-meters [1]. Previous studies over the past two years at Brookhaven National

Laboratory reveal that there is a large and stable population of southern flying squirrel within the borders of the laboratory proper.

Materials and Methods

Sherman traps were used to catch squirrels and trap locations were spread out to determine whether a geographically diverse population exists. Nestlettes (cotton squares) were also used to provide the squirrels with nesting material. The Sherman traps were mounted on a tree at an average height of 1.87 meters. They were attached to the tree by a modified C-Clamp. The C-Clamp was screwed onto the tree using a screw and washer. Trap location points were recorded using ESRI ArcPad V.8 on a Trimble GeoXT unit. The following information were recorded, giving a brief summary of the nearby habitat characteristics: (a) overstory; (b) understory; (c) tree species (to which trap was affixed); (d) tree breast height diameter; and (e) current weather. A range of eight to twenty traps were placed in a wide array of locations. A greater number of traps were placed at locations where no squirrels had been previously caught (i.e., Gamma Forest with twenty traps). Traps were spread out in these areas, generally several meters from each other, but this differed based on the size and density of the forest. Sherman traps were opened during the late afternoon in order to reduce the chance of diurnal animals from eating the bait or setting off the traps. Since squirrels are nocturnal, there is a very rare chance that they would be trapped for more than twelve hours. If there was a high chance of overnight rain (above 40% based on NOAA and weather.com for Upton,NY), traps were not opened to ensure that the squirrels would not be at risk of hypothermia.

Once captured, a plastic container was charged with anesthetic isoflourene using an

open vial filled with cotton balls that had been saturated in the isoflourene. Once the box had been charged for at least two minutes, the squirrel was then placed into the charged box while still trapped within the Sherman trap. Once the squirrel was successfully anesthetized, it was placed into a plastic bag and it's weight was taken using a spring scale. Once the squirrel was weighed, one person equipped with leather gloves grabbed the squirrel and ensured it could not escape just in case the squirrel came to. Another person, also wearing gloves, recorded body length, hind foot length, hind foot pad length, and sex and making note of any scars/marks or signs of breeding. The squirrel was then ear tagged (National Band and Tag Co.) and its number was recorded. A genetic sample was then taken using a sterile cotton swab (Pur-Wraps, Puritan medical products). The cotton swab was swirled against the inner-cheek of the squirrel's mouth. An additional swab was used to increase the success rate and to get both cheeks, left and right.

The DNA from the genetic swabs was extracted following the Buccal Swab Spin protocol provided by Qiagen QIAamp DNA Mini and Blood Mini Handbook. The DNA was then processed using polymerase chain reaction (PCR). PCR was performed using standard protocol. The following primers were used: forward: 5'-AAACATCCGCAAAACTCACC-3' and reverse: 5'-GTAGGGGTGGAATGGGATTT-3' (Integrated DNA Technologies). The temperature profile for denaturing and extension followed a general protocol with the following temperatures: 93°C for 3 minutes, 93ºC for 30seconds, 56ºC for 1 minute and 30 seconds, 72°C for 2 minutes and 30 seconds, 72°C for 5 minutes, and a final hold at 10°C after 30 complete cycles. Once PCR had been finished, the samples were tested using 15ul in a 2% agarose E-gel . The samples

that displayed bright bands were then sent to Brookhaven National Laboratory Biology department for sequencing at a volume of 30ul.

Results

Out of 600 trap nights, eighty-three total squirrels were captured (29 recaptures). This yields a success rate of 11.83% (Figure 1). Forty-nine mice were also captured (forty-six *Peromyscus leucopus* and three *Mus musculus*). A rate of 8.26%. According to the figure 3, there is a ratio of 41% to 54% of females to males respectively. This ratio changes when only the recaptured squirrels are considered, 59% female and 41% male.

Out of 69 PCR products, 43 were successful and able to be sequenced (There are still more samples left to be processed.). This yields a success rate of 62.32%. Two samples were contaminated and twenty-four did not show bright bands on the 2% agarose E-gel (Figure 6).

Discussion

The use of nestlettes in the baited Sherman traps was a novel idea that hopefully would allow the squirrels to nest and stay warm during cold nights. Unfortunately, many of the squirrels did not make any sort of nests, but they were used as enrichment with the nestlette. This enrichment has been previously studied in laboratory mice [4].

The fairly large number of captured squirrels (83), in a ten-week time period, shows that these squirrels are prevalent in a variety of forests throughout Brookhaven National Laboratory. The results support the fact that squirrels have a large diet and can strive on a broad range of forest types. The southern flying squirrels are present in areas

that have varying understory and overstory. Many of the locations have a mixture of Oak and Pine trees. Some locations have an oak dominant overstory (i.e., Along Peconic River) and some have a pine dominant overstory (i.e., White Pine Forest along West Fifth). Throughout all these locations, it has been noted that varying understories also exist.

There is a possibility that many of the 17 recaptured females (compared to 12 males) are breeding or taking care of young. The average weight of these females is 64.05g, with a standard deviation of 11.49. With these calculations, this hypothesis may not be true, but it could also be because females are more loyal to one area and tend to depend on a constant food source, unlike males who tend to have a larger home and forage area/range [1].

Future Study – Mice & Forage times

Along South Boundary and Upton Road, where fourteen mice were captured, an additional research project could be setting cameras in order to get a time stamp to when the mice forage. Based on that time, researchers may able to find the time to open the traps the night before. Since mice are known to forage earlier and more often than southern flying squirrels, it may benefit the research by opening the traps once mice are not as active. There should be a high probability of catching squirrels in that area because four squirrels were caught in similar forest structure (Pitch-Pine/ White Oak) along the south end of Canopy Road. This method can also be used in the Crescent Road section where eleven mice were captured and in the Upton/West Princeton location where 4 mice were captured.

Acknowledgements

I would like to thank Brookhaven National Laboratory and the SULI program for this research opportunity. I would especially like to thank Jen Higbie, Maria Brown, Dan Williams, and Tim Green for the excellent guidance and help. I am also very grateful for the constant help from Courtney Buckley and Tamer Marshood. I appreciate all the help from Rich Lagattolla and everyone from 528.

References

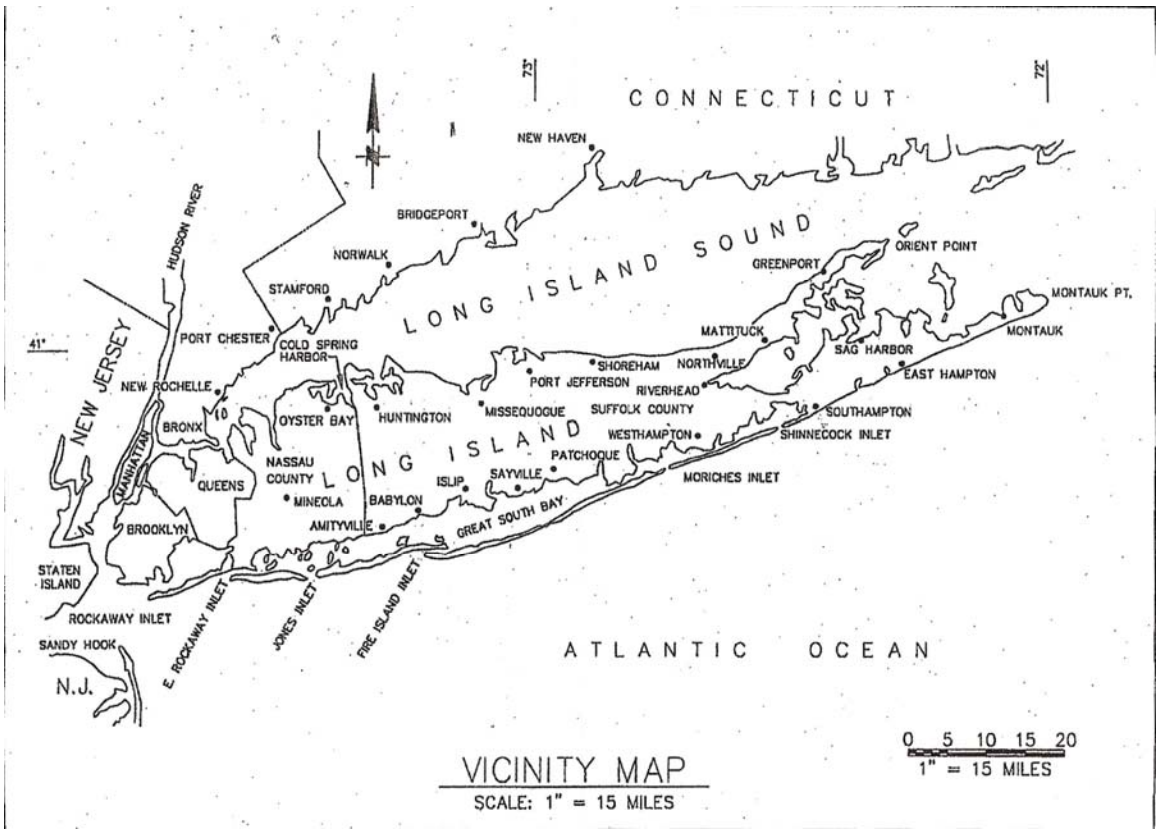
[1] Fox, David L., and Michael Mulheisen. "Glaucomys Volans." *Animal Diversity Web*. University of Michigan, 2008. Web. 02 Aug. 2011. <http://animaldiversity.ummz.umich.edu/site/accounts/information/Glaucomys_volans.html>.

[2] "Range Maps." *Flying Squirrels*. Glaucomys.org. Web. 02 Aug. 2011. <http://www.flyingsquirrels.com/Rangemaps/nfs_map_can.html>.

[3] Grande, Anthony. "Long Island Geography." The Geography of Long Island. CUNY Hunter, 17 May 2011. Web. 11 Aug. 2011. <http://www.geo.hunter.cuny.edu/courses/geog383.33/index.html>.

[4] Hobbs, Ann B., Walter Kozubal, and F. Feuno. "Evaluation of Objects for Environmental Enrichment of Mice." *Journal of the American Association for Laboratory Animal Science* 36.3 (1997): 69-71. Print.

Figure 1: Vicinity Map of Long Island



http://www.geo.hunter.cuny.edu/courses/geog383.33/Outline%20map%20of%20Long%20Island.jpg

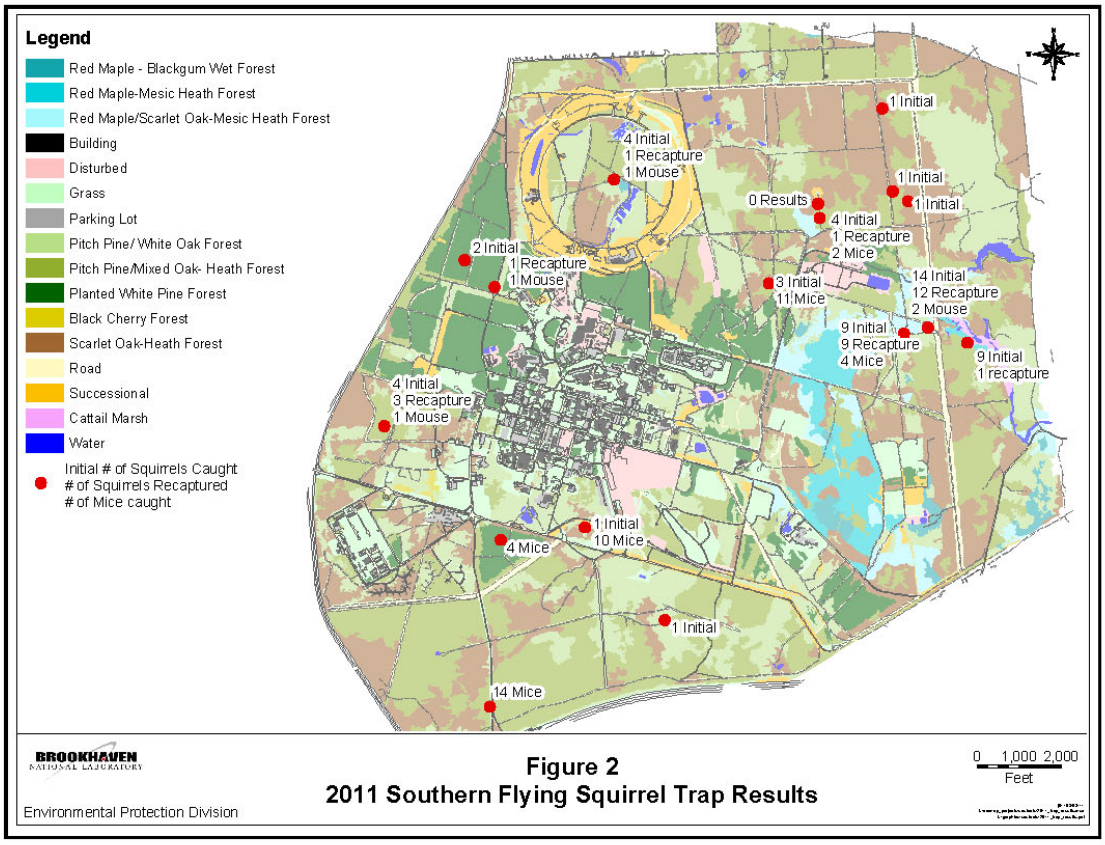


Figure 2
2011 Southern Flying Squirrel Trap Results

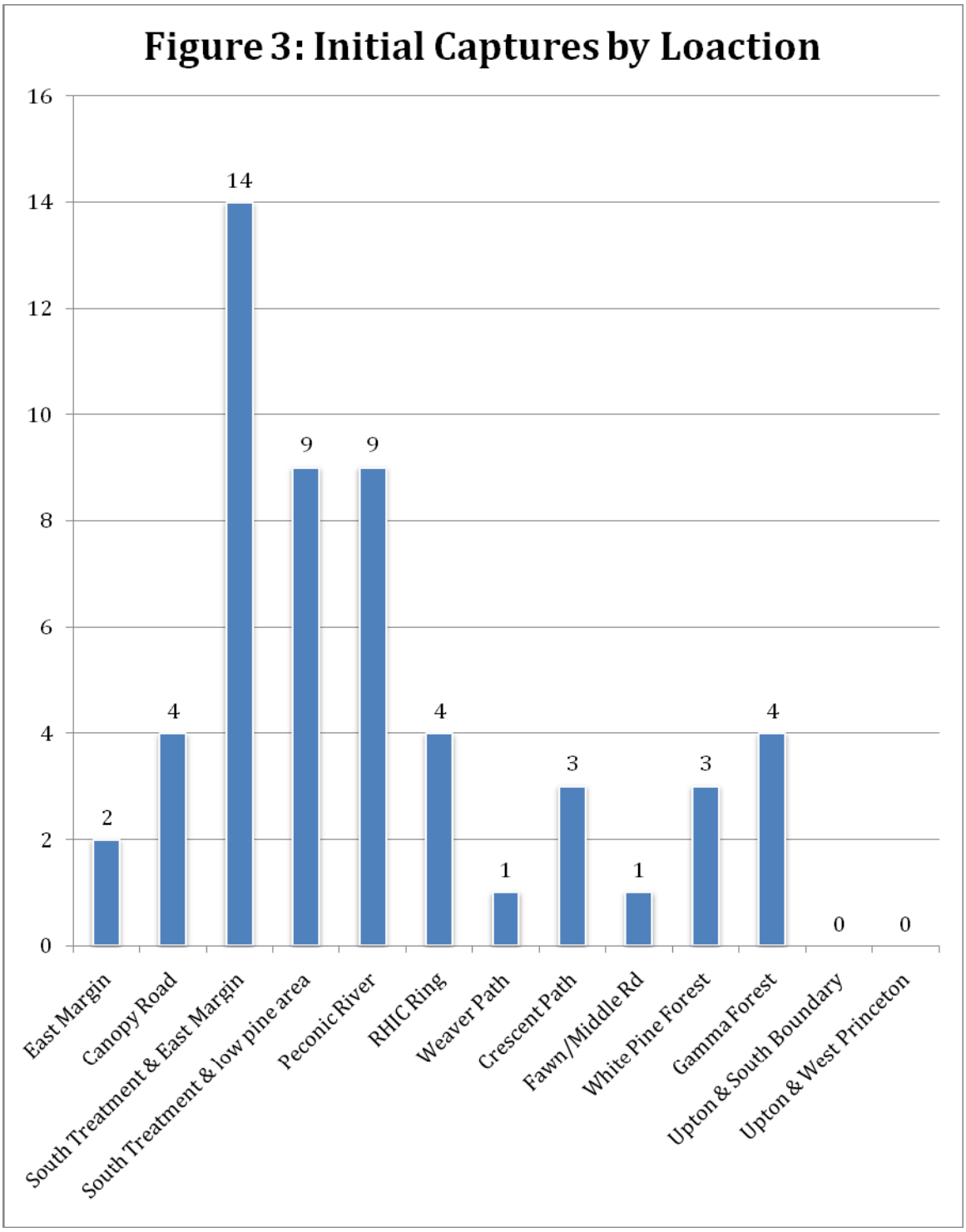


Figure 3: Initial Captures by Loaction
16
14
12
10
8
6
4
2
0
2
4
14
9
9
4
1
3
1
3
4
0
0
East Margin
Canopy Road
South Treatment & East Margin
South Treatment & low pine area
Peconic River
RHIC Ring
Weaver Path
Crescent Path
Fawn/Middle Rd
White Pine Forest
Gamma Forest
Upton & South Boundary
Upton & West Princeton

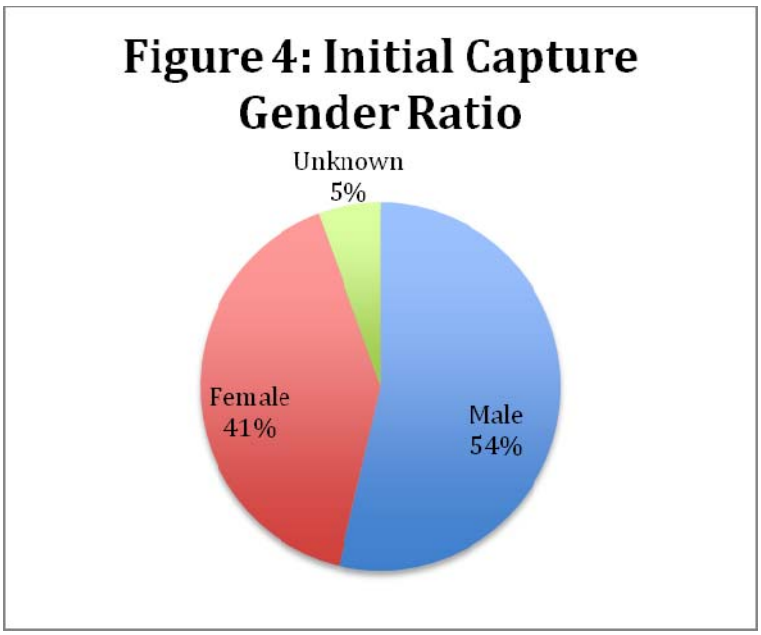


Figure 4: Initial Capture Gender Ratio
Unknown 5%
Female 41%
Male 54%

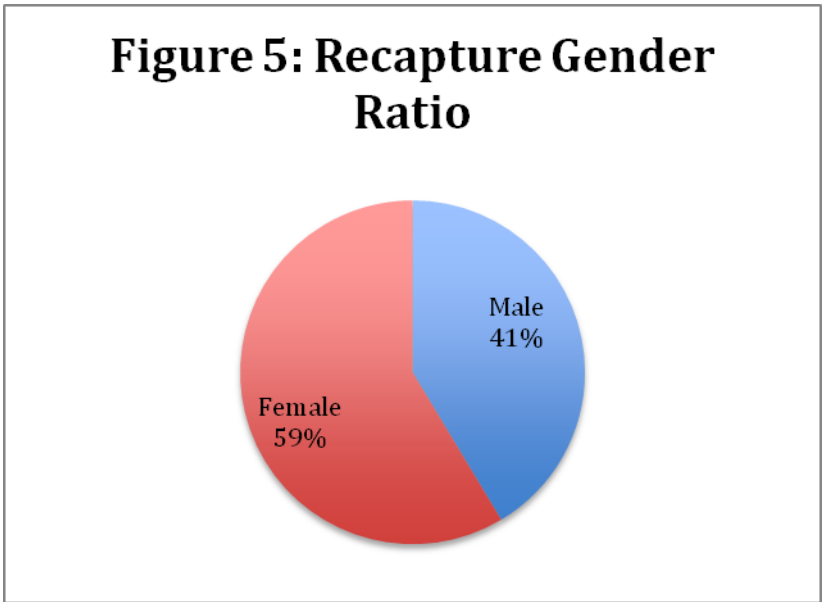


Figure 5: Recapture Gender Ratio
Male 41%
Female 59%

Figure 6: 2% Agarose E-Gel