

## Abstract

Malaria is an infectious disease caused by the protozoan of the genus *Plasmodium*. It is a major problem in third-world countries, with hundreds of millions of infections and millions of fatalities annually. Current attempts at controlling this disease, which include pesticides and drugs, are unsatisfactory. New techniques of malaria prevention and treatment are currently in development, including vaccines. We propose a technique that combines two different technologies that are under development. The first aspect of this technology involves the use of antibodies against the enzyme aminopeptidase, which exists in the stomach of the *Anopheles* mosquito and is essential in the lifecycle of the parasite. The second aspect is the genetic engineering of algae, a food source of mosquito larvae, to make it produce these antibodies so that they will be introduced into the digestive system of the mosquito.



## Utilizing Algal Genetic Recombination to Prevent the Spread of Malaria

### Present Technology

Malaria is caused by a parasitic protist of the genus *Plasmodium* that is found in tropical climates throughout the world. The four species of the *Plasmodium* parasite (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) infect approximately 200-300 million people worldwide each year. Of these infections, between 700,000 and 1,000,000 are fatal, making it the 5<sup>th</sup> deadliest infectious disease<sup>1</sup>. Therefore, it will be useful to find multiple ways to tackle this devastating issue.

The current technology that is related to countering malaria can be separated into prevention and treatment. The major forms of prevention, pesticides and bed nets treated with pesticides, target mosquitoes rather than *Plasmodium*<sup>2</sup>. Examples of pesticides that are effective against mosquitoes are *Bacillus thuringiensis israelensis* (a bacteria-based pesticide), Methoprene, oil-based products, Pyrethrines, and Organophosphates. DDT was once used, but it is now banned in many countries throughout the world due to health and environmental concerns.

*Bacillus thuringiensis israelensis* consists of bacteria that are often mixed with clay. It is added to stagnant water sources, which often contain mosquito larvae. After the larvae eat the bacteria, it damages their internal organs. Methoprene, a chemical pesticide, is mixed with clay or used in a liquid form. A chemical that is similar to methoprene is found in mosquito larvae, but it is not found in adult mosquitoes. When methoprene is absorbed by the larvae, it interferes with their growth, so they never develop into adults and do not become carriers of *Plasmodium*. Oil-based products are added to the surface of stagnant water. They suffocate the mosquito larvae that live in these water sources, which come to the surface to breathe. Pyrethrines are derived from chrysanthemums, which are poisonous to mosquitoes. They block the nerve singles

that are transmitted between the brain and the heart of the mosquito, causing the mosquito's heart to stop. Organophosphates block the nerve transmissions between the brain and the heart and respiratory system, stopping the mosquito's heart and respiration<sup>3</sup>.

Current treatment of malaria involves one of several different drugs or a combination of these drugs. Some of the most popular antimalarial drugs are Malarone, Quinine, Chloroquine, Doxycycline, Mefloquine, and Primaquine<sup>4</sup>. Malarone consists of the compounds atovaquone and proguanil hydrochloride. These compounds interfere with the parasite's production of certain nitrogenous bases, which are necessary for the replication of DNA<sup>5</sup>. Quinine, Chloroquine, Mefloquine and Primaquine build up in the food vacuole of the parasite. Once there, they inhibit the enzyme heme polymerase. This enzyme breaks down a compound that is toxic to the parasite, known as heme, which is produced when the parasite attacks hemoglobin within red blood cells. Without the properly-functioning heme polymerase, heme builds up within the protist cell and kills it<sup>6,7</sup>. Doxycycline stops the parasite from producing certain essential proteins, which weakens it and allows the immune system to fight it off<sup>8</sup>.

Another option for treating malaria is Artemisinin Combination Therapy. Artemisinin comes from Chinese Wormwood and is a natural, safe and effective medicine, but the specifics of how this herb functions are not known. However, it only works temporarily, so it is often used with Chloroquine, Primaquine, Mefloquine, or Quinine. This combination technique is believed to prevent the malaria parasite from developing resistance against drugs<sup>9</sup>.

Although various drugs are currently effective at treating malaria, there is always the concern that the parasite will develop immunity to these drugs. The parasite has already become resistant to certain drugs in different parts of the world<sup>1</sup>. Also, the areas in which malaria is most

prevalent are usually very poor, and it is therefore difficult to administer the drugs in these areas. It's evident that alternative solutions to controlling the malaria parasite are needed.

## History

*Plasmodium* is believed to have originated in Africa about 30 million years ago. However, the first mention of a malaria-like disease was by Hippocrates in 4<sup>th</sup> century BCE. The next mention of malaria was in the *Nei Ching*, a Chinese book of medicine written in 2700 B.C.E. Later, in second century B.C.E. China, the Qinghao plant was mentioned in a medical treatise called *52 Remedies*. It was not until 340 CE that Ge Hong described why it works as a remedy; the plant has anti-fever properties and is used in antimalarial drugs today<sup>10</sup>.

The first modern antimalarial drug, Quinine, was developed in the early 1600s. It is derived from the bark of the Cinchona tree, which Spanish missionaries discovered in the New World<sup>1</sup>. However, at this point in history, very little was known about malaria. The pathogen that actually causes the disease, a parasitic protist, was first discovered in 1880 by Louis Alphonse Laveran, who was interested in finding the source of the black pigment in the blood of those suffering from malaria. Certain characteristics in the patient's blood led him to suspect that parasites were involved<sup>11</sup>. In 1886, Camillo Golgi observed that there are different species of the malaria parasite, and that each of these species causes different symptoms. *Plasmodium vivax* and *Plasmodium malariae* were named in 1890 by Giovanni Batista Grassi and Raimondo Filetti. The most dangerous species of the parasite, *Plasmodium falciparum*, was named in 1897 by William H. Welch. During the same year, Ronald Ross discovered that the malaria parasite is spread by mosquitoes. The complete lifecycle of the *Plasmodium* parasite was first observed in 1898 by Giovanni Batista Grassi and his team of researchers<sup>1</sup>.

The antimalarial compound resochin was discovered by Hans Andersag in 1934. In 1946, British and American scientists built off of this discovery and created the second modern antimalarial drug, Chloroquine. Another important advancement against malaria in modern times occurred in 1939, when Paul Müller found that DDT could be used as a pesticide. This was vital in the control of malaria during WWII, while U.S. and European soldiers fought in malaria-infested countries<sup>1</sup>.

### **Future Technology**

Our idea for a future technique of eliminating the malaria parasite involves targeting mosquito larvae. Larvae live in water for between four days and two weeks. During this time period, they eat voraciously in order to develop into adult mosquitoes. They eat various types of microorganisms, including algae<sup>12</sup>. Our idea is centered on algae and recent scientific advancements that could be applied in different ways.

Scientists have recently discovered that there is an interaction between a type of aminopeptidase enzyme and the malaria parasite. This enzyme naturally exists within the mosquito's digestive system. When the mosquito consumes the *Plasmodium* parasite, the parasite seeks out this enzyme and binds to it. If it cannot bind to the enzyme, it will be digested by the mosquito. Scientists have taken advantage of this by developing a vaccine that contains a segment of the aminopeptidase enzyme. It can allow a person to produce antibodies against the enzyme. If a mosquito then drinks the person's blood, it will consume the antibodies as well, which can bind to the enzyme within the insect's digestive tract and stop the parasite from binding to the enzyme. Therefore, any *Plasmodium* parasite that the mosquito contracts will die.

We theorize that the concept of this new vaccine could be combined with the concept of genetically engineered algae. Algae have been genetically manipulated in order to allow them to

produce higher amounts of lipids for biofuels, as well as to increase the biomass that is produced. The genomes of several algae species have been sequenced. Multiple techniques are currently being studied, including homologous gene replacement, gene knockout and RNA interference<sup>13</sup>.

After further advancements in genetic engineering, recombinant DNA could be used to place a human gene into the DNA of algae. Recombinant DNA involves removing a portion of the DNA from one organism and inserting it into the DNA of another organism. One way to perform this process is called Transformation. A specific gene is selected and can be removed from the entire DNA molecule with the use of a specialized enzyme. This small segment of DNA is then inserted into a segment of the DNA of a different organism, known as a vector. This process has been used successfully many times in bacteria, which allows them to produce proteins that can be used in medicine, such as insulin and growth hormones<sup>14</sup>.

In order to target malaria, the human gene that produces antibodies against the aminopeptidase enzyme would be inserted into the DNA of algae. An algae species that is native to Africa should be chosen to prevent the spread of invasive species and to ensure that it is a viable food source of the *Anopheles* larvae. The DNA could also be altered to make it grow more rapidly or to make it more resilient, as is being done with biofuel-producing algae. This would allow for a greater chance of larvae consuming the genetically-altered strain of algae, and therefore a greater chance of eliminating the malaria parasite in certain areas.

Ideally, this technology would be combined with other new technologies that are currently being developed. It's unlikely that a single technique will be able to completely eliminate the malaria parasite from third-world countries. A combination of new vaccines, pesticides, and novel genetic approaches will likely lead to the most success.

## **Breakthroughs**

Although we believe this technology could be practically produced in the near future, there are several technological advances that need to be made first. The envisioned technology has two components: engineering algae to carry antibodies for amino peptidase and using the engineered algae as a vehicle to administer the antibodies to the mosquitoes. Each of these components presents their own problems that need to be solved in order to implement our technology.

More research would need to be done on the algae species of malaria infested regions of the world and the genomes of these species would need to be mapped. Once a genome is mapped we can isolate genes for replacement and work out how new genes would be inserted. At first, this technology is likely to be expensive because genetic engineering is not a very mainstream technology yet and we have ways to go in increasing the efficiency and bringing down the costs of creating genetically enhanced species. Another difficulty with the inception of our technology is that multiple species of algae will have to be genetically altered depending upon which region of the world they will be introduced to avoid introducing invasive species. Altering and testing so many species may be expensive.

Also, since the algae is trying to elicit an immune response from the mosquito more research on the immune system of mosquito larvae will be necessary to make sure that the technology will work before spending so much money. Finally, scientists must ensure that the algae actively produce the desired antibodies once the gene has been inserted. Humans only produce this antibody when exposed to the aminopeptidase enzyme, so it may be necessary to expose the algae to the enzyme, and further studies on gene activation in algae may be required.

### **Design Process**



After we decided to focus our project on malaria, we thought of three initial ideas before we settled on our final idea. The first idea was to immunize mosquito larvae against the malaria parasite. We considered the process of vaccine production, and instead of using this process to immunize humans, we wondered if it would be possible to instead use it directly on mosquitoes. In this process, the antigens of the parasite *Plasmodium falciparum* would be added to stagnant bodies of water, possibly within the food sources of the mosquito larvae or encased within a substance that would resemble a food source. In theory, the larvae should consume the antibodies, and they would then be immunized against malaria for life, never able to spread it to humans. However, there are many flaws in this idea. First is the fact that antigens are proteins, and they could therefore be easily digested by the mosquito larvae and would never elicit an immune response. Also, we learned that scientists have been working for years to develop similar technology for vaccines, but they haven't been successful until recently<sup>15</sup> (Scientific American). If scientists have been experiencing such difficulty immunizing people against malaria, it's unlikely that they would be able to immunize mosquitoes using the same process.

The second idea that we had concerned a type of dinoflagellate algae, named *Chromera*. *Chromera*'s genome has been found to link the lineages of parasites like *Plasmodium* to the lineages of other algae<sup>16</sup>. Since *Chromera* is so similar to *Plasmodium*, we had the idea of engineering *Chromera* with a cure for *Plasmodium* and introducing the engineered *Chromera* to stagnant water. The mosquito larvae would get infected by the *Chromera* in similar fashion to how *Plasmodium* infects. The *Chromera* should infect the same cells that *Plasmodium* would, thus giving the cells a cure for an existing or future *Plasmodium* infection. We rejected this idea because it may not be effective to introduce *Chromera* into stagnant water. *Chromera* is normally found in oceans, and we are not sure if it could survive in fresh water. Even if *Chromera* did

survive, we would be introducing an invasive species which could pose a potential threat to the local ecosystem.

Our third idea was to use RNA interference technology (RNAi) to target certain genes within the mosquito that allow it to contract and transmit malaria. RNAi involves using double-stranded RNA molecules to block the transmission of a gene. When this molecule enters a cell, it causes the cell to activate a primitive immune response, which stops the cell from producing the specific protein that is encoded in the RNA<sup>17</sup>. We theorized that RNAi could be produced to target a certain gene that is vital to the mosquito's ability to carry malaria. This RNAi could then be incorporated into the food sources of mosquito larvae and placed into stagnant water. The larvae would consume the RNAi along with the food source, and the molecule would then go to work within the bodies of the insects, preventing them from ever contracting or transmitting malaria. However, there are once again major flaws with this theory. First of all, the RNAi could be digested by the larvae, or it could be destroyed by the immune system before it enters any target cells. Scientists have faced this same problem in humans. RNAi has to be administered differently depending on what part of the body that it is meant to target, or else it will be destroyed before it gets there<sup>16</sup>. Also, we didn't have the slightest idea what gene or what part of the larvae's body to target with this technology. We decided that this method was impractical and that we didn't have a broad enough understanding of it to use it as the central idea of our project.

### **Consequences**

Once this technology is perfected, ideally the positive consequences would outweigh the negative consequences. An example of a positive consequence would be diminishing the devastating effects of malaria in third-world countries. This method of targeting malaria prevents

mosquitoes from contracting the parasite in the first place, so the spread of malaria would be greatly reduced without the hassle of administering drugs or vaccines and without the risk of the parasite developing resistance.

However, there are some negative consequences of this technology that should be considered. For example, the introduction of algae into warm, stagnant water sources within tropical climates could result in algae overgrowth, which would greatly disrupt the ecosystem. If the algae are genetically altered to be more resilient as we suggest, it would be difficult to control this overgrowth problem.

It is also uncertain what effect the genetically-engineered algae would have on organisms other than mosquito larvae that feed on algae. If these organisms consume the genetically-engineered algae, they could possibly be harmed by the antibodies that are produced by the algae.

We predict that any effects on the ecosystem would be minor. The genetically-engineered algae would be limited to small stagnant water sources throughout malaria-infested countries, not major water sources. The potential of limiting the spread of malaria greatly outweighs the consequences of minor harm to local ecosystems.

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<sup>1</sup> "CDC - Malaria - About Malaria - History." *Centers for Disease Control and Prevention*. Web. 14 Oct. 2010. <<http://www.cdc.gov/malaria/about/history/>>.

<sup>2</sup> "Malaria: Prevention - MayoClinic.com." *Mayo Clinic Medical Information and Tools for Healthy Living - MayoClinic.com*. Web. 02 Dec. 2010. <<http://www.mayoclinic.com/health/malaria/DS00475/DSECTION=prevention>>.

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<sup>3</sup> "What You Should Know About Mosquito Control Pesticides." *California West Nile Virus Website*. California Department of Public Health. Web.  
<[http://westnile.ca.gov/website/mosq\\_control/Mosquito\\_Control\\_Pesticides.pdf](http://westnile.ca.gov/website/mosq_control/Mosquito_Control_Pesticides.pdf)>.

<sup>4</sup> "CDC - Malaria - Diagnosis & Treatment (United States) - Treatment (U.S.)." *Centers for Disease Control and Prevention*. Web. 02 Dec. 2010.  
<[http://www.cdc.gov/malaria/diagnosis\\_treatment/treatment.html](http://www.cdc.gov/malaria/diagnosis_treatment/treatment.html)>.

<sup>5</sup> GlaxoSmithKline. Web.  
<[http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2008/021078s014lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021078s014lbl.pdf)>.

<sup>6</sup> "Chloroquine - Drug Information from Medic8.com." *Medical Search Engine | Cosmetic Surgery, Dentistry Health Insurance Guides - Medic8*. Web. 10 Jan. 2011.  
<<http://www.medic8.com/medicines/Chloroquine.html>>.

<sup>7</sup> "Malaria Site: Chloroquine." *Malaria Site: Comprehensive Malaria Website*. Web. 10 Jan. 2011. <<http://www.malariasite.com/malaria/chloroquine.htm>>.

<sup>8</sup> "Doxycycline." *Antibiotics Home Page*. Web. 10 Jan. 2011.  
<<http://antibiotics.emedtv.com/doxycycline/doxycycline.html>>.

<sup>9</sup> "Discovery Health "How Malaria Drugs Work"" *Discovery Health "Health Guides"* Web. 06 Jan. 2011. <<http://health.howstuffworks.com/medicine/medication/malaria-drug.htm/printable>>.

<sup>10</sup> Marcus, Bernard A. *Malaria*. Philadelphia: Chelsea House, 2004. Print.

<sup>11</sup> Lambert, Paul H. "Malaria - Past and Present." *Nobelprize.org*. 9 Dec. 2003. Web. 14 Oct. 2010. <<http://nobelprize.org/educational/medicine/malaria/readmore/history.html>>.

<sup>12</sup> "Mosquito Information: Larvae - WUVCD." *WUVCD - West Umatilla Vector Control District*. Web. 06 Jan. 2011. <<http://www.wuvcd.org/mosquito/larvae.html>>.

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<sup>13</sup> Hildebrand, Mark. "Development of Algal Genetic Tools." Scripps Institution of Oceanography. Web. 26 Dec. 2010. <<http://www.nrel.gov/biomass/pdfs/hildebrand.pdf>>.

<sup>14</sup> Merriam, John. "Recombinant DNA." *Biology*. Ed. Richard Robinson. Vol. 4. New York: Macmillan Reference USA, 2002. 38-46. *Gale Virtual Reference Library*. Web. 7 Jan. 2011.

<sup>15</sup> Carmichael, Mary. "Halting the World's Most Lethal Parasite." *Scientific American* Nov. 2010: 69-75. Print.

<sup>16</sup> "Malaria And Algae Genetically Related - Genetics - an ELab Article at Scientist Live." *Scientist Live - the Latest Science News and Opinion*. Web. 06 Jan. 2011. <[http://www.scientistlive.com/European-Science-News/Genetics/Malaria\\_and\\_algae\\_genetically\\_related/24585/](http://www.scientistlive.com/European-Science-News/Genetics/Malaria_and_algae_genetically_related/24585/)>.

<sup>17</sup> Binns, Corey. "An Rx for Every Disease." *Popular Science* Sept. 2010: 35-37. Print.



## Works Cited

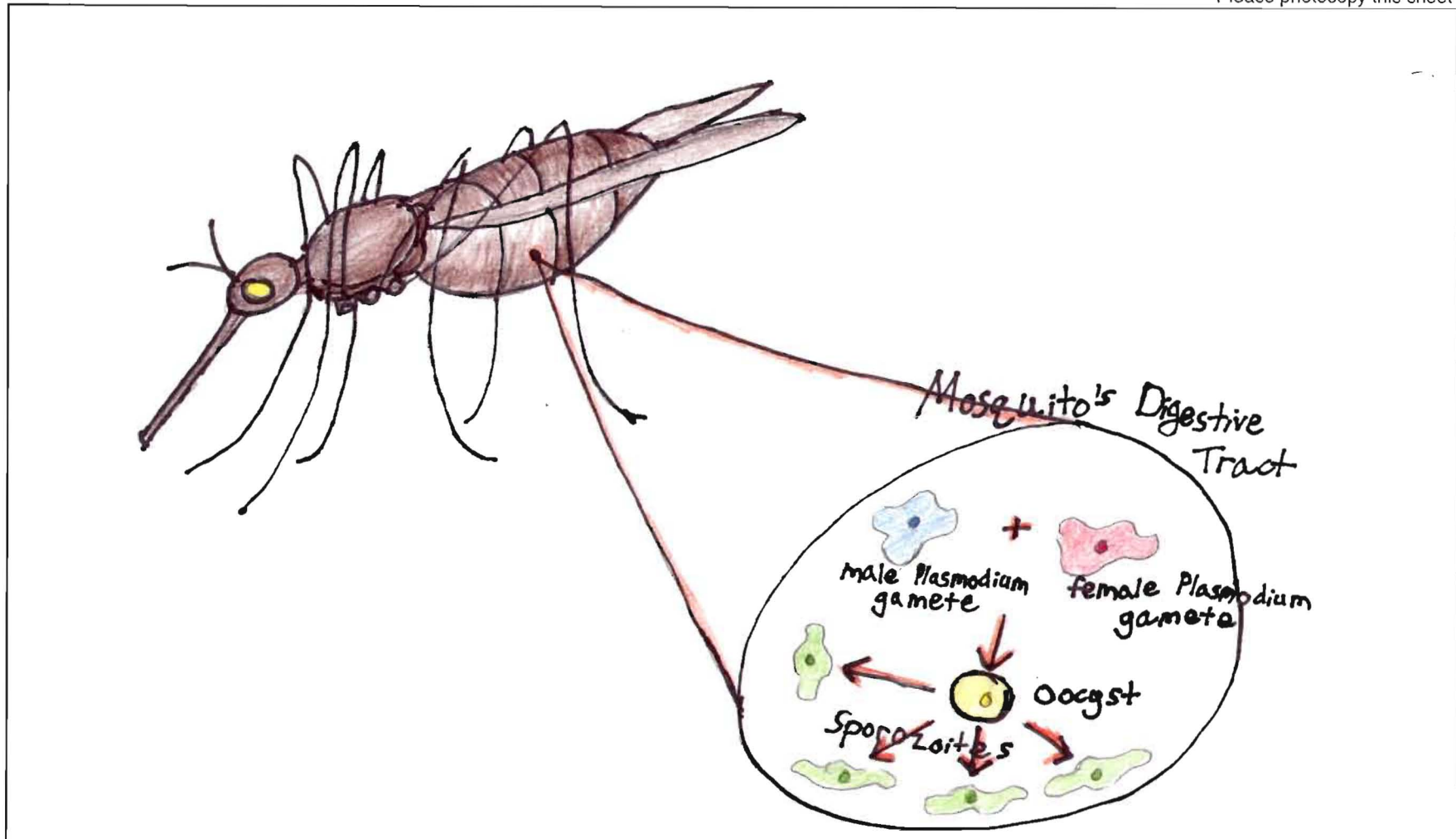
1. Binns, Corey. "An Rx for Every Disease." *Popular Science* Sept. 2010: 35-37. Print.
2. Carmichael, Mary. "Halting the World's Most Lethal Parasite." *Scientific American* Nov. 2010: 69-75. Print.
3. "CDC - Malaria - About Malaria - History." *Centers for Disease Control and Prevention*. Web. 14 Oct. 2010. <<http://www.cdc.gov/malaria/about/history/>>.
4. "CDC - Malaria - Diagnosis & Treatment (United States) - Treatment (U.S.)." *Centers for Disease Control and Prevention*. Web. 02 Dec. 2010. <[http://www.cdc.gov/malaria/diagnosis\\_treatment/treatment.html](http://www.cdc.gov/malaria/diagnosis_treatment/treatment.html)>.
5. "Chloroquine - Drug Information from Medic8.com." *Medical Search Engine | Cosmetic Surgery, Dentistry Health Insurance Guides - Medic8*. Web. 10 Jan. 2011. <<http://www.medic8.com/medicines/Chloroquine.html>>.
6. "Discovery Health "How Malaria Drugs Work"" *Discovery Health "Health Guides"* Web. 06 Jan. 2011. <<http://health.howstuffworks.com/medicine/medication/malaria-drug.htm/printable>>.
7. "Doxycycline." *Antibiotics Home Page*. Web. 10 Jan. 2011. <<http://antibiotics.emedtv.com/doxycycline/doxycycline.html>>.
8. GlaxoSmithKline. Web. <[http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2008/021078s014lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021078s014lbl.pdf)>.
9. Hildebrand, Mark. "Development of Algal Genetic Tools." Scripps Institution of Oceanography. Web. 26 Dec. 2010. <<http://www.nrel.gov/biomass/pdfs/hildebrand.pdf>>.

10. Lambert, Paul H. "Malaria - Past and Present." *Nobelprize.org*. 9 Dec. 2003. Web. 14 Oct. 2010.  
<<http://nobelprize.org/educational/medicine/malaria/readmore/history.html>>.
11. "Malaria And Algae Genetically Related - Genetics - an ELab Article at Scientist Live." *Scientist Live - the Latest Science News and Opinion*. Web. 06 Jan. 2011.  
<[http://www.scientistlive.com/European-Science-News/Genetics/Malaria\\_and\\_algae\\_genetically\\_related/24585/](http://www.scientistlive.com/European-Science-News/Genetics/Malaria_and_algae_genetically_related/24585/)>.
12. "Malaria: Prevention - MayoClinic.com." *Mayo Clinic Medical Information and Tools for Healthy Living - MayoClinic.com*. Web. 02 Dec. 2010.  
<<http://www.mayoclinic.com/health/malaria/DS00475/DSECTION=prevention>>.
13. "Malaria Site: Chloroquine." *Malaria Site: Comprehensive Malaria Website*. Web. 10 Jan. 2011. <<http://www.malariasite.com/malaria/chloroquine.htm>>.
14. Marcus, Bernard A. *Malaria*. Philadelphia: Chelsea House, 2004. Print.
15. Merriam, John. "Recombinant DNA." *Biology*. Ed. Richard Robinson. Vol. 4. New York: Macmillan Reference USA, 2002. 38-46. *Gale Virtual Reference Library*. Web. 7 Jan. 2011.
16. "Mosquito Information: Larvae - WUVCD." *WUVCD - West Umatilla Vector Control District*. Web. 06 Jan. 2011.  
<<http://www.wuvcd.org/mosquito/larvae.html>>.
17. "What You Should Know About Mosquito Control Pesticides." *California Westnile Virus Website*. California Department of Public Health. Web.  
<[http://westnile.ca.gov/website/mosq\\_control/Mosquito\\_Control\\_Pesticides.pdf](http://westnile.ca.gov/website/mosq_control/Mosquito_Control_Pesticides.pdf)>.



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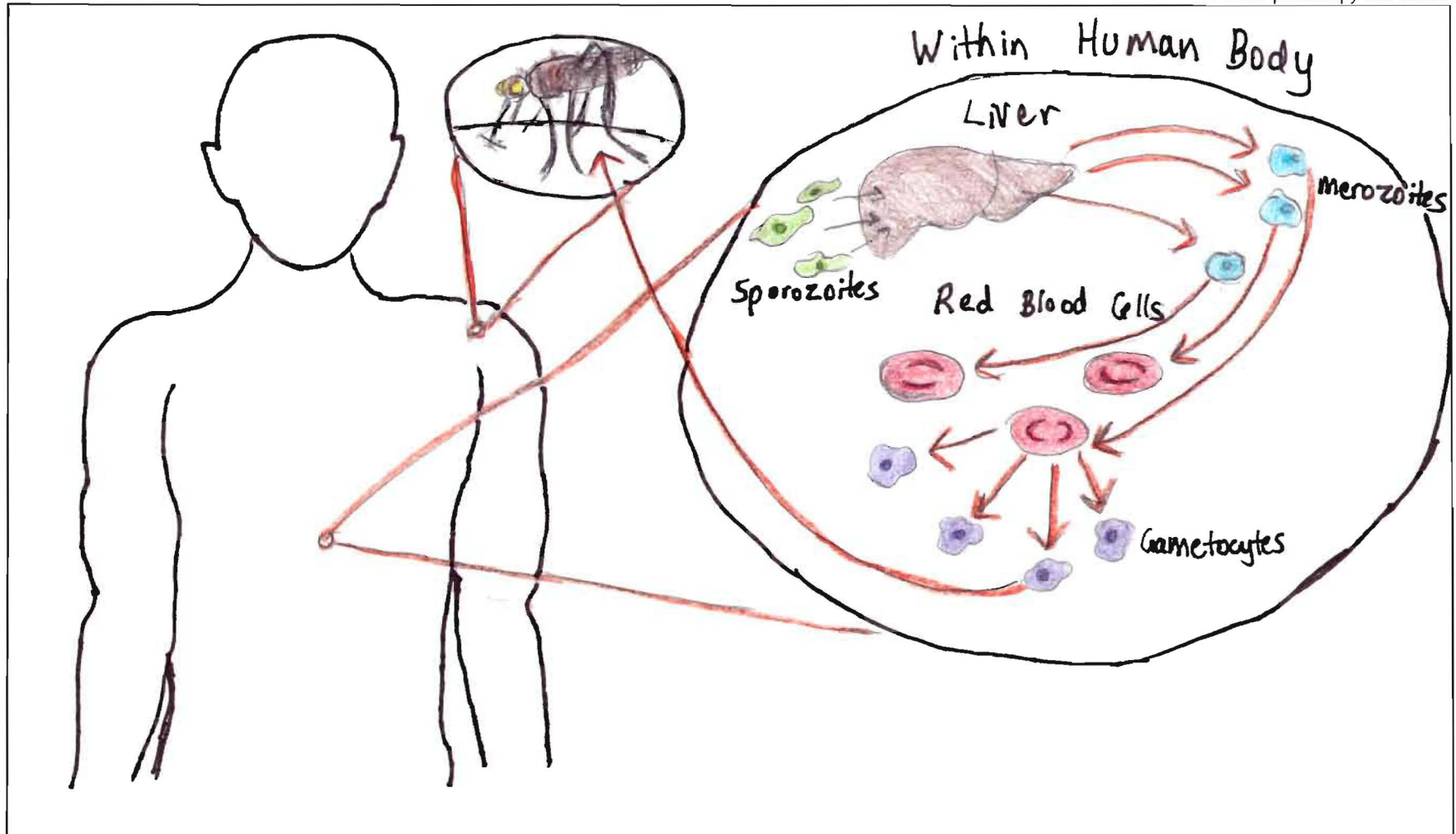
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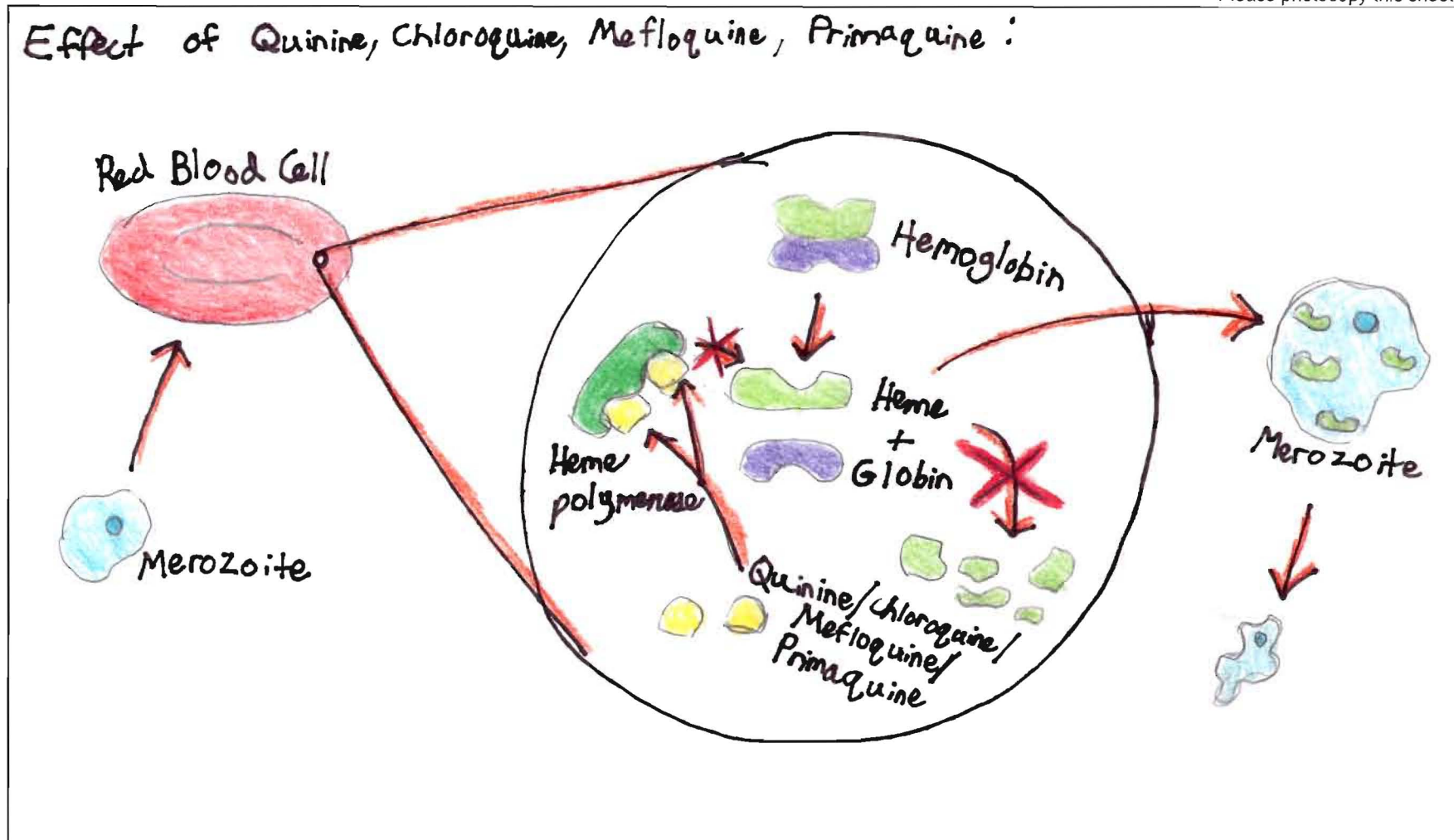


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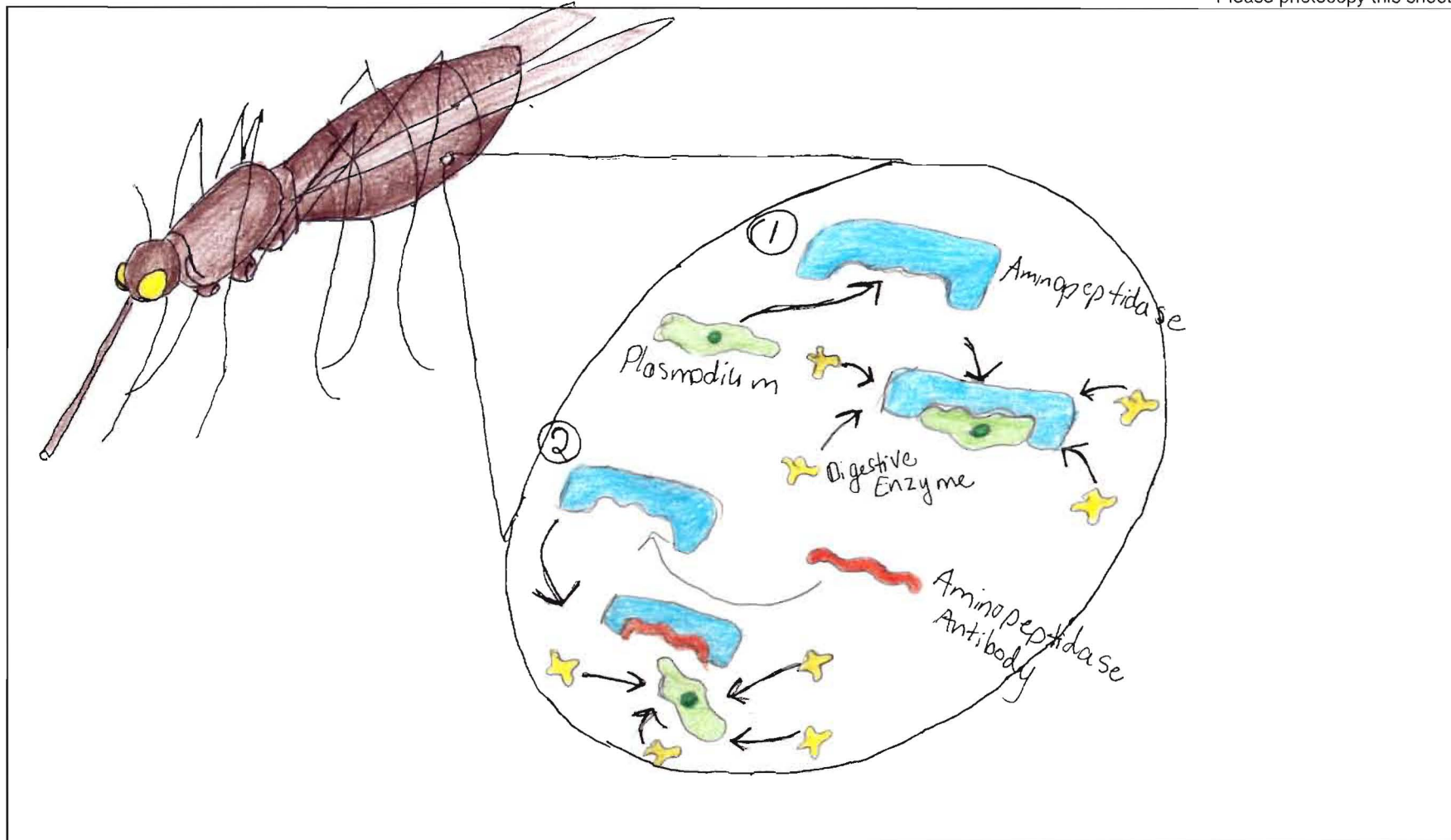
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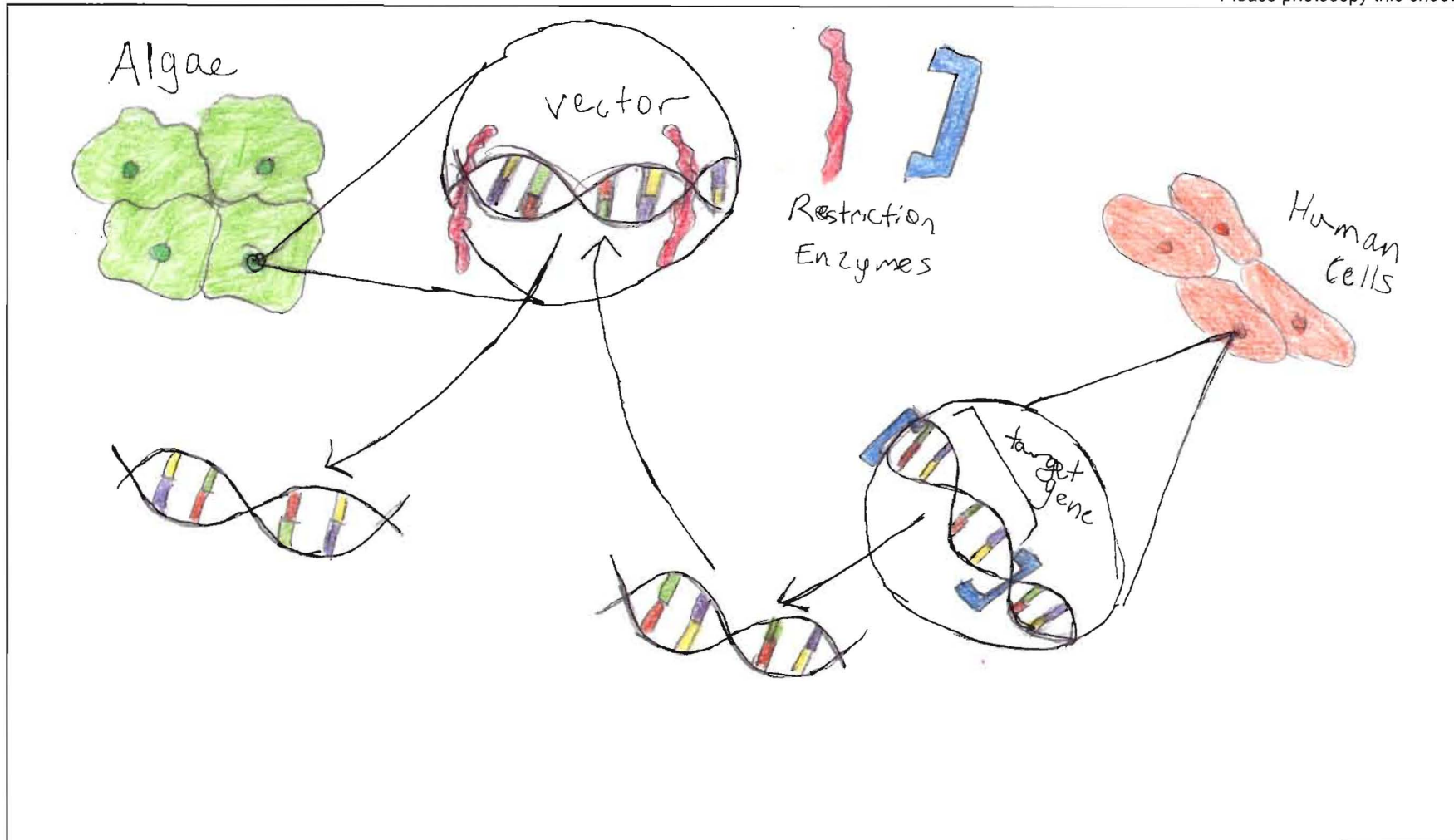
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