Optimizing Methods for Analysis of Novel Corona-Virus SARS-CoV-2 Receptor ACE2 Single Nucleotide Polymorphisms.

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Purpose of this study

- SARS-CoV-2 attaches to the ACE2 receptor.
- Understanding the interactions between SARS-CoV-2 and its receptor could help with creation of treatments and knowledge of vulnerable populations.
- This project seeks to optimize the procedures that will be used to gather human data on single nucleotide polymorphisms (SNPs) found in the local population.

ACE2 Receptor

- SARS-CoV-2 is a novel corona virus that binds to the 2nd angiotensin-converting enzyme (ACE2).
- ACE2 is expressed in blood vessels including those around the brain and most organs.
- The large range of tissues that express ACE2 makes the receptor a suspect for causing many of the COVID-19 long haul symptoms due to those symptoms being found in tissues known to express ACE2.

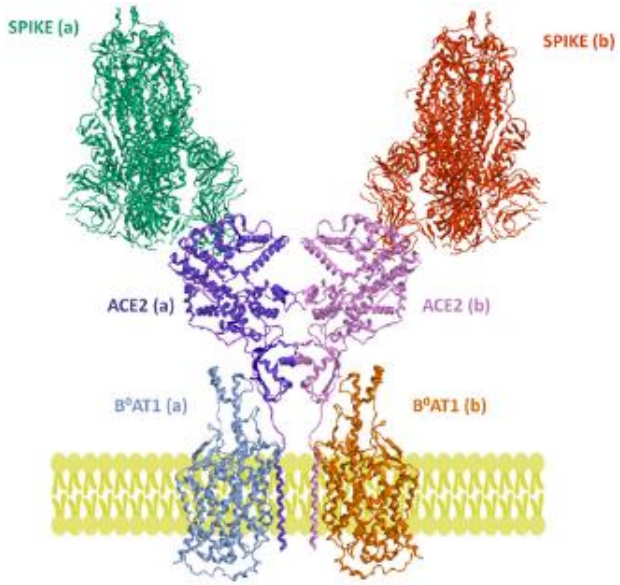

Figure 1

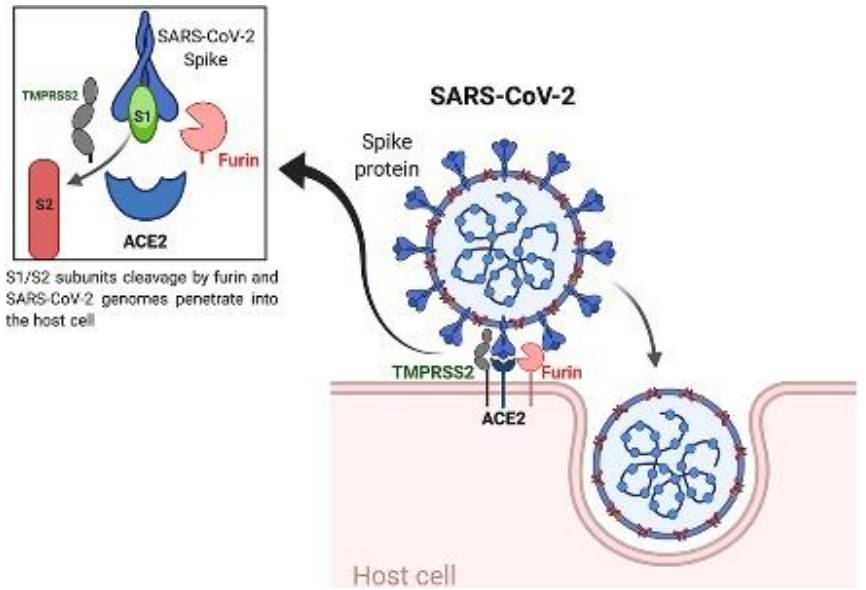

Figure 2

Figures 1 and 2 retrieved from

Nesci, S. (2021, June 21). *SARS-COV-2 first contact: Spike–ace2 interactions in COVID-19*. Wiley Online Library. Retrieved October 20, 2021, from https://onlinelibrary.wiley.com/doi/10.1111/cbdd.13898.

Lippi, G., Lavie, C. J., Henry, B. M., & Sanchis-Gomar, F. (2020, September 1). *Do genetic polymorphisms in angiotensin converting enzyme 2 (ACE2) gene play a role in coronavirus disease 2019 (covid-19)?* De Gruyter. Retrieved October 20, 2021, from https://www.degruyter.com/document/doi/10.1515/cclm-2020-0727/html.

SNPs studied

- p.Asn720Asp, c.1665-7delT, and p.Lys26Arg as found in the genomAD database.
- All polymorphisms used were found as significant in SARS-CoV-2 interactions in other research and/or were analyzed by a webserver that can predict changes in a protein caused by polymorphisms.
- c.1665-7delT was chosen because it is a deletion in a splice cite. Splice cites are parts of DNA that allow the cell to know what are introns (non-coding “junk” strands) and what are exons (coding strands). Changes in splice cites could have massive changes to the protein, but they are capable of being tested in the webserver.

Parts of study

- Primer design
  - Small segments of DNA that interact with human DNA and tell PCR and LCR proteins where to operate
- DNA extraction
- Polymerase chain reaction (PCR)
  - Duplication of select strands of DNA
- Ligation chain reaction (LCR)
  - Fuses two primers together if they lie down on DNA properly
- Analysis through gel electrophoresis.

Primer Design

Primers are designed with a specific annealing temperature in mind. (Annealing is the process of primers attaching to DNA).

Two primers are made for PCR ~300 base pairs apart. One primer is right after the SNP of interest.

One primer is made for LCR and the primer close to the SNP from PCR is also used.

Polymorphism name	Primer 1	Primer 1L	Primer 2C	Primer 2CL	Primer 2	Primer 2L	Primer 3
p.Asn720Asp	GATGCTTTCCGTC TGAATGAC	GTCATTCAGACG GAAAGCATC	AACAGCCTAGAGT TTCTGGGGATAC	GTATCCCCAGAAA CTCTAGGCTGTT	GACAGCCTAGAGTTT CTGGGGATAC	GTATCCCCAGAAA CTCTAGGCTGTC	GCCTCTTTGTAG GAGGGAAATGA C
c.1665-7delT	CAAAAGGCCCTG AACCCCCTTTTTT	AAAAAAGGGGG TTCAGGGCCTTTT G	TGTGTAGCAATAT GCTGAGGCTTGG	CCAAGCCTCAGCA TATTGCTACACA	GTGTAGCAATATGCT GAGGCTTGG	CCAAGCCTCAGCA TATTGCTACAC	CTTTCTCTGCTAT GGTGACAGCTG
p.Lys26Arg	AGTCCACCATTG AGGAACAGGCCA	TGGCCTGTTCCTC AATGGTGGACT	AGACATTTTTGGAC AAGTTTATCCAC	GTGGATAAACTTG TCCAAAAATGTCT	GGACATTTTTGGACA AGTTTATCCAC	GTGGATAAACTTG TCCAAAAATGTCC	TCAATAACTTAT GGCATTTCCATG GA

Table 1: shows the various primers made and the SNPs they are associated with. SNP location is highlighted in yellow.

DNA extraction

- A combination of “10 mmol tris(hydroxymethyl)aminomethane (Tris)-Cl (pH 8.0), 2 mmol ethylene diamine tetraacetic (EDTA) (pH 8.0), 0.2 mol NaCl and 200 μg/mL Proteinase K.” is combined with DNA and incubated for 30 minutes at 55°C and then for 5 minutes at 95°C (Li H et al. 2011).
- This procedure is used instead of traditional DNA extraction procedures because it takes considerably less time.

PCR and LCR

Polymerase chain reaction

- Saw success with the following conditions:

Primer set	Annealing Temp (C)	MaCl amount (µL)
p.Asn720Asp	45-60	3
c.1665-7delT	48	1
p.Lys26Arg	48	4

Table 2: identifies the optimal conditions for PCR reactions for each SNP being studied.

Ligation chain reaction

- No evidence of a successful LCR was seen throughout the study.
- Adjuvants (added chemicals to improve annealing) will be tested in the near future.

Gel electrophoresis analysis

- Success in PCR should be seen at around 300 nucleotides or just under the third bar in the leftmost column.
- Success in LCR should be seen if some primers are twice as long as others in polyacrylamide gels implying they fused together.

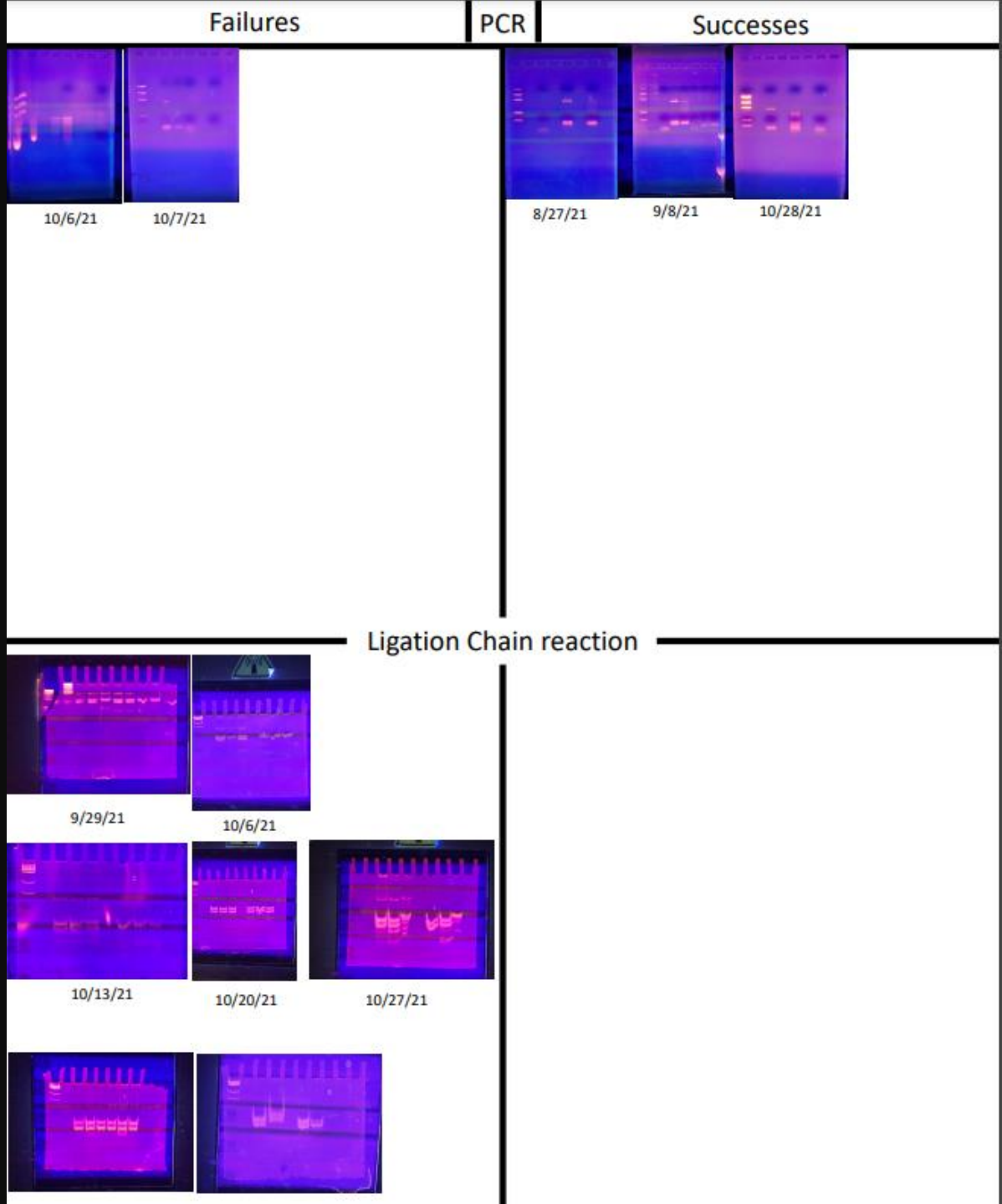

Figure 3: shows various gels from PCR and LCR reactions and the dates in which they were analyzed. Successful reactions are in the right column, unsuccessful are in the left column. PCR reactions are in the top row while LCR reactions are analyzed in the bottom row. Method of analysis is explained above. All pictures were taken by William Burris.

Discussion/summary

- A quick and easy DNA extraction procedure was found.
- A method of finding optimal PCR conditions was found.
- LCR was not successful. Adjuvants should be tested.
- Plans for quantitative PCR (qPCR) are being made.
  - A type of PCR machine that can identify successful annealing while the process is under way. This will make the LCR unnecessary.
- IRB certification is under progress (as of 2/18/22) and human testing will begin soon.
- This project is valuable because it can be easily be replicated by other labs including those that lack expensive qPCR machines.

References (graphics and pictures)

Nesci, S. (2021, June 21). *SARS-COV-2 first contact: Spike–ace2 interactions in COVID-19*. Wiley Online Library. Retrieved October 20, 2021, from https://onlinelibrary.wiley.com/doi/10.1111/cbdd.13898.

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