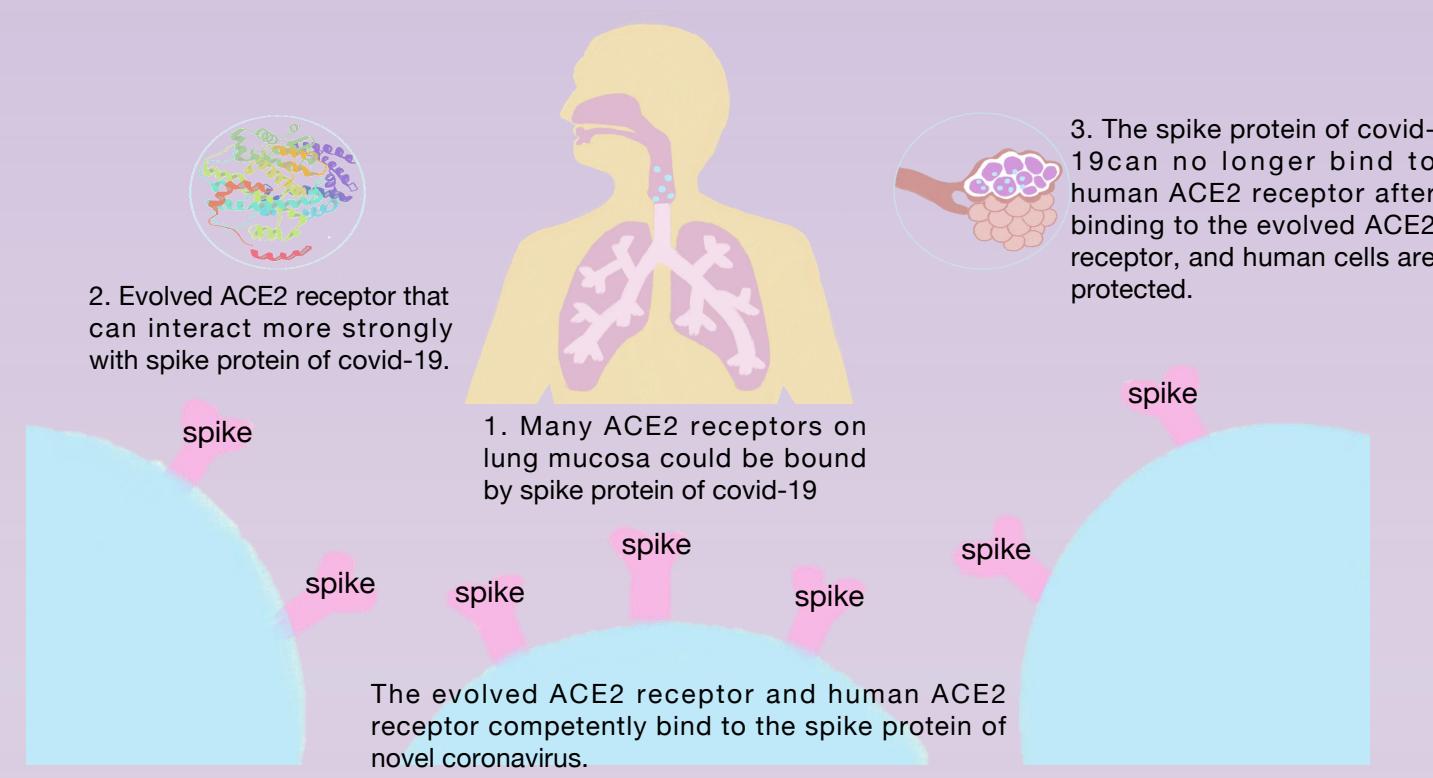


# proLib

## BACKGROUND

Since the outbreak of covid-19, our study and life have been greatly affected. With the passage of time, novel coronavirus has also evolved many mutant strains. In order to make our detection speed keep up with the mutation speed of the virus as much as possible, our FAFU team used modeling and random peptide library to screen the ACE2 protein that can strongly interact with the spike protein of novel coronavirus, and developed a platform for rapid screening of target protein interacting peptides. According to the specific somatic receptor binding sites of different viruses (such as spike's RBD), small peptides with stronger binding ability than receptor (such as ACE2) proteins can be quickly screened. Using these evolutionary proteins or small peptides, we can apply them to air purifiers and real-time virus monitoring devices, so as to prepare for the next outbreak of human infectious diseases. It also provides a new idea for competitive inhibition or targeted therapy against spike protein of novel coronavirus.

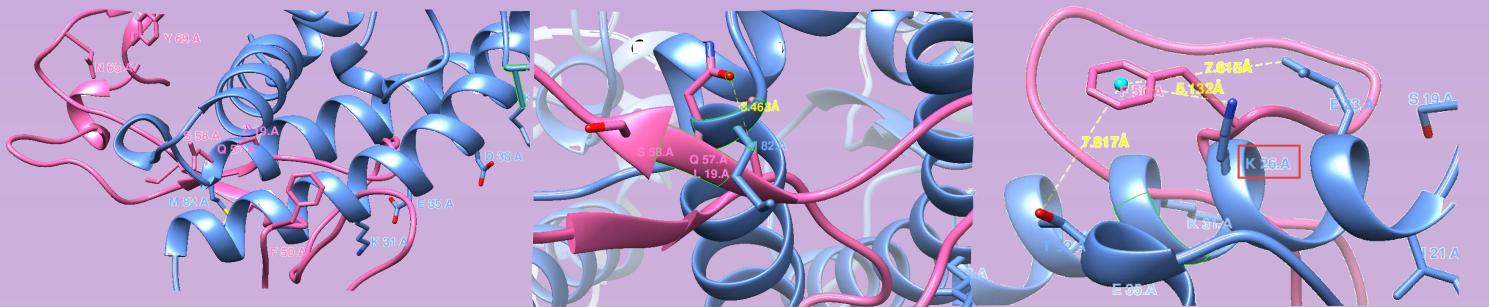


## SEWAGE EPIDEMIC DETECTION

As an economical real-time monitoring method, sewage epidemic detection can be widely used in the detection of pathogens in sewage. The Jaume I University in Spain earlier launched a pilot program to successfully detect trends in the third round of COVID-19 in Spain using sewage epidemiology [1]. In France, researchers conducted relatively evenly spaced samples in the Paris area [2] and compared them with the number of confirmed COVID-19 cases in that month. Results showed a strong positive correlation between them. All these prove that WBE can effectively represent the dynamic trend of infectious diseases. However, the current sewage detection is still strongly dependent on manual. To this end, we wanted to design a real-time detection device based on WBE and our products. Achieve early warning of local outbreaks.

First of all, we interviewed relevant sewage treatment enterprises in the local area, and modeled the sewage treatment network in the local urban and rural areas. And the binding process of our target protein and spike protein is transformed into electrical signals. The electrical signals will be processed and reported to local quarantine authorities. Due to the specificity of the target protein and spike protein, we can learn early on what subtypes of infectious diseases are breaking out there. And indicate the trend of outbreaks throughout the period of localized outbreaks. To provide basis for local epidemic prevention departments to formulate epidemic policies.

## MODEL



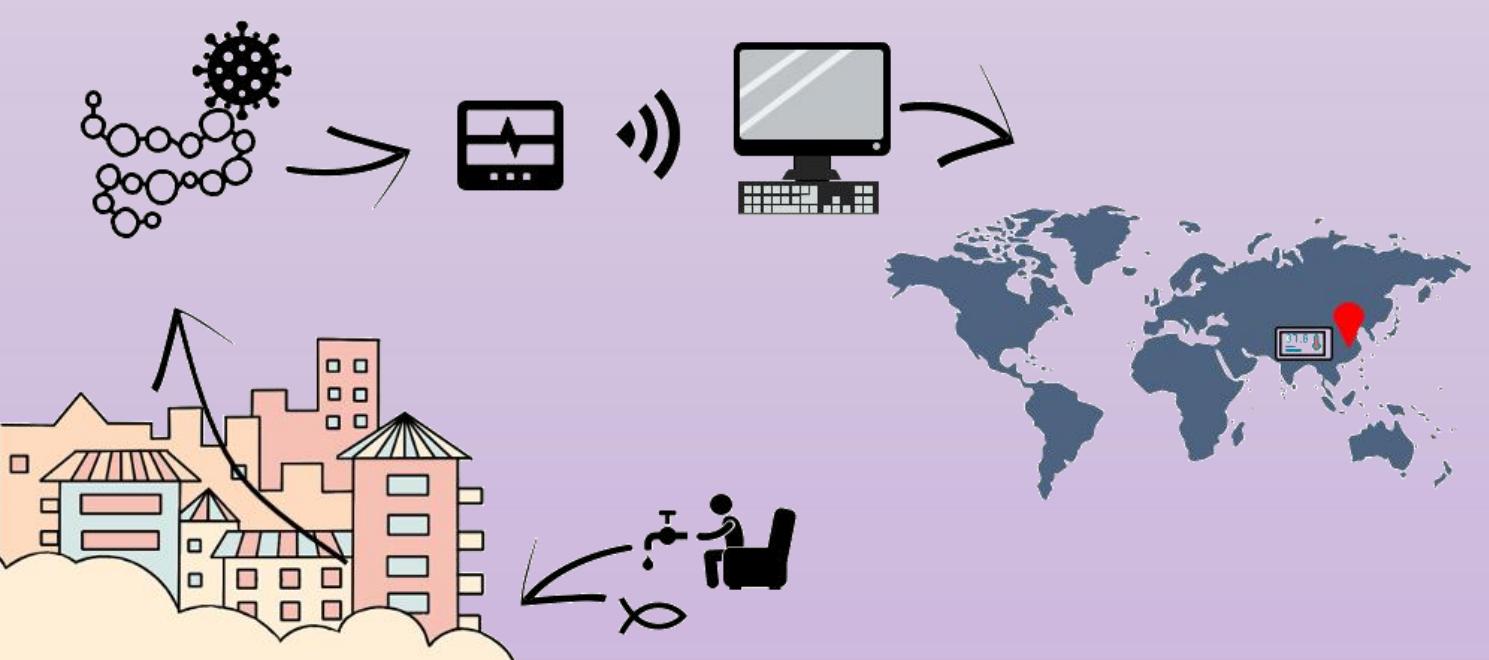
Mammalian ACE2 sequences are highly homologous, and there are also high similarities between SARS-CoV and SARS-CoV-2. ACE2 in humans, pangolins, cats, bats, and mice has 80% sequence identity. In addition, RDB proteins have 85% similarity in the sequence between SARS-CoV and SARS-CoV-2. Simulation was carried out using the Chimera software Ver 1.16. Simulates the structure of hACE2 based on RBD (PDB:) with SARS-CoV-2. The key AAs in hACE2 that interact with RBM are K31, E35, D38, M82, and K353. Among them, K31 and K353 in hACE2 are the most critical residues for RBM identification. According to the literature, the amino acids that interact with humans in SARS-CoV-2 are L455, F486, Q493, S494, N501, and Y505.

```

 1 MSSSSWLSSLVAVTAQASTIEEQATFLDKFNHEAEDLFYQSSLASWNY
 51 NTNTI TEENVGNMNNAGDKWSAFLKEESTLAQMYPLQEIQNLTVKLQLQAL
101 QQNGSSVLSLEDKSKRLLNTLNTMSITYSTGKVCNPDNPQECCLLEPGLNE
151 IMANSLDYNERLWAWE SWRSEVGKOLRPLYEEYYVVLKNEMARANHYEDYG
201 DYWRGDYEVNGVDGYDSRGQLIEDVEHTEEEIKPLPYEHLHAYVRAKLMN
251 AYPSYI SPICGLPAHL LGDMWGRFWTNLYSLTVPFQGKPNIDVTDAMVDQ
301 AWDARIFKEAEKFFVS VGLPNMTQGFWEWSMLTDPGNVQAKAVCHPTAWD
351 LGKGDFRILMCTKVMDFTLAHHEMGHQYDMAVYAAQPFLLRNGANEKF
401 HEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTL
451 PFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVVEPVPHDETYCDP
501 ASLFHVSNDYSFIRYTYTRTLYQFQFQEALCQAAKHEGPLHKCDINSNEA
551 GQKLFNMRLRGKSEPWTLALENVVGAKNMNRPLLNYYFEPFLTWLKDQNK
 601 NCEVGMSTNSDQYADQSTKURKLSKALGDPYQWQDQHRYLFGQGVALA
  ACE2.pdb (#1) ARG 26-A Out Hide Help

```

In particular, the human ACE2 variants S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P, and H378R are expected to increase susceptibility. The T92I variant is part of a consistent NxS/T N-glycosylation motif that confirms the role of N90 glycosylation in non-human coronavirus immunity. Other ACE2 variants K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L, and D509Y are considered protective variants with weakened binding to the SARS-CoV-2 S protein. Through UCSF Chimera software homology modeling, the F486 distance between K26 and the spike protein before the ACE2 K26 site was unmutated was 5.132A.



## EDUCATION

1. Education in the form of picture books in kindergartens
2. Co-produce manga with JNU, XMU, OUC, NEFU
3. Record micro-lessons and qie expand the influence of biology
4. Conduct live lectures with ten universities.

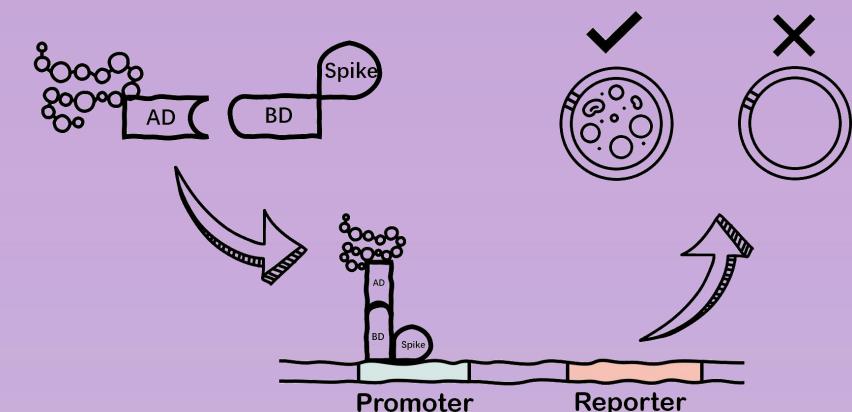


## REFERENCES

- [1] de Llano R, Cejudo-Marin R, Barneo M, Pérez-Cataluña A, Barberá-Riera M, Reboliato M, Bellido-Blasco J, Sánchez G, Hernández F, Bijlsma L. Monitoring the evolution of SARS-CoV-2 on a Spanish university campus through wastewater analysis: A pilot project for the reopening strategy. *Sci Total Environ*. 2022 Jul 13;845:157370. doi: 10.1016/j.scitotenv.2022.157370. Epub ahead of print. PMID: 35842154; PMCID: PMC9278994.
- [2] WURTZERS M, MARECHAL V, MOUCHEL JM, et al. Time course quantitative detection of SARS-CoV-2 in Parisian wastewater correlates with COVID-19 confirmed cases [J]. *medRxiv*, 2020. DOI: 10.1101/2020.04.12.20062679.
- [3] Yang E, Zha J, Jochel J, et al. Bad, a heterodimeric partner for Bcl-xL and Bcl-2, displaces bax and promotes cell death [J]. *Cell*. 1995;80(27):285-291.
- [4] Yang M, Wu Z, Fields S. Protein-peptide interact ions analyzed with the yeast two-hybrid system [J]. *Nucleic Acids Research*, 1995;23(7):1152-1156.
- [5] Cloas P, Brent R. The impact of two-hybrid and related methods on biotechnology[J]. *Trends Biotechnol*, 1998, 16:355-363.
- [6] Clontech In-Matchmaker gal4 two-hybrid system 3, 1999. PT3247-1.pdf://www.clontech.com/techinfo/manuals/PDF/PT3247-1.pdf
- [7] Fields S, Song O. A novel genetic system to detect protein-protein interactions[J]. *Nature*, 1989, 340(6230):245-246.

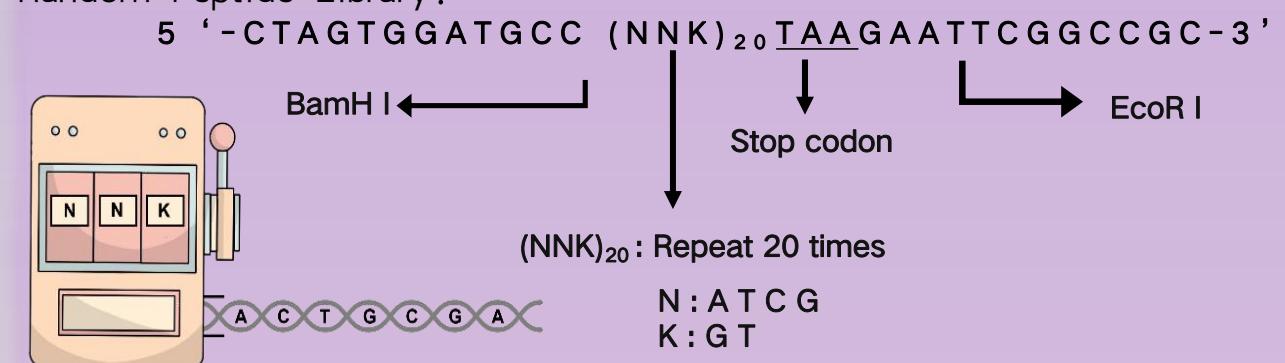
## LAB

Yeast two hybrid:



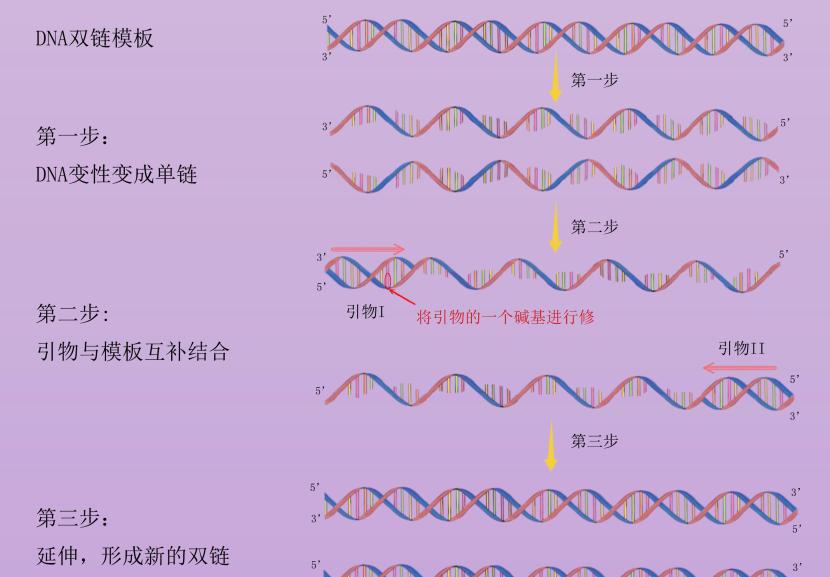
Yeast two hybrid system can determine the binding between proteins or between proteins and peptides in vivo, which is highly sensitive. Its principle is that transcription factors usually contain two independent domains: DNA binding domain (BD) and transcription activation domain (AD). Separate DNA binding proteins and transcription activation domain proteins are separated from each other in different positions in cells, and cannot activate the transcription of reporter genes. Only when these two domains work together can transcription proceed normally. Using this feature, we fused DNA binding protein and transcriptional activation domain protein with spike protein of covid-19 and evolved ACE2, respectively. If the evolved ACE2 can interact with the spike protein of covid-19, the DNA binding domain and the transcription activation domain can be fully close in space, thus activating the transcription of the reporter gene. Therefore, we can finally determine whether there is interaction between these two proteins by detecting whether the reporter gene is expressed [3].

## Random Peptide Library:



We know that the protein sequence is encoded by the triplet code. We take these three consecutive nucleotide sequences as a unit. Through the random change of the internal sequence of the codon, we can randomly produce an amino acid. Then we can produce a series of random polypeptides with 20 aa length by continuously performing the above actions [4]. Theoretically, we can cite  $20^{20}$  possibilities. We linked this 20 peptide to transcription activation domain (AD) and added two restriction sites at both ends. Fused into yeast that has fused spike protein and DNA binding domain (BD). If this random short peptide can specifically bind to spike protein, it can activate the transcription of the lower gene, so that the yeast can survive on the two deficient medium [5]. We propose the surviving yeast, conduct colony PCR and sequencing, and obtain the sequence of our target peptide.

## SOE PCR:



We use overlapping extension PCR to realize the site directed mutation of our ACE2 evolutionary protein. First, we synthesize two complementary primers with mutated gene bases at the position where mutation is needed [6]. One end of the sequence is complementary to its own target fragment, and the other end is another segment of target gene sequence. In this way, after PCR cloning, special connectors are added to one end of each product. After processing the PCR products, PCR amplification was carried out by using the specific complementarity of the connector, so that the two PCR products were connected together to form a hybrid gene fragment that was not connected by restriction endonuclease [7].

