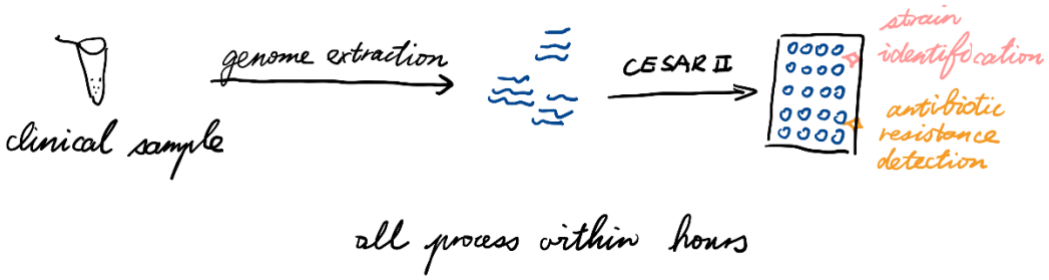
Problem to solve:

Through field research we found that currently the antibiotic resistance test in clinical lab usually takes days. The main reason for the time consumption is strain identification through selective culture.

However, in clinical practice, doctors care more about **whether the patient shows antibiotic resistant** to help them decide prescription rather than **the antibiotic resistance is caused by which bacteria**. Therefore, CESAR II focus on examining the antibiotic resistance and has the potential to shorten the time duration from days to hours.

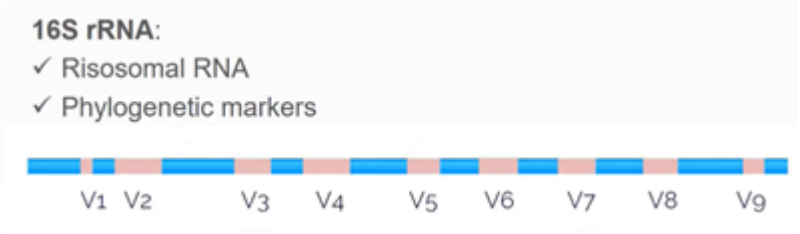
Pipeline:



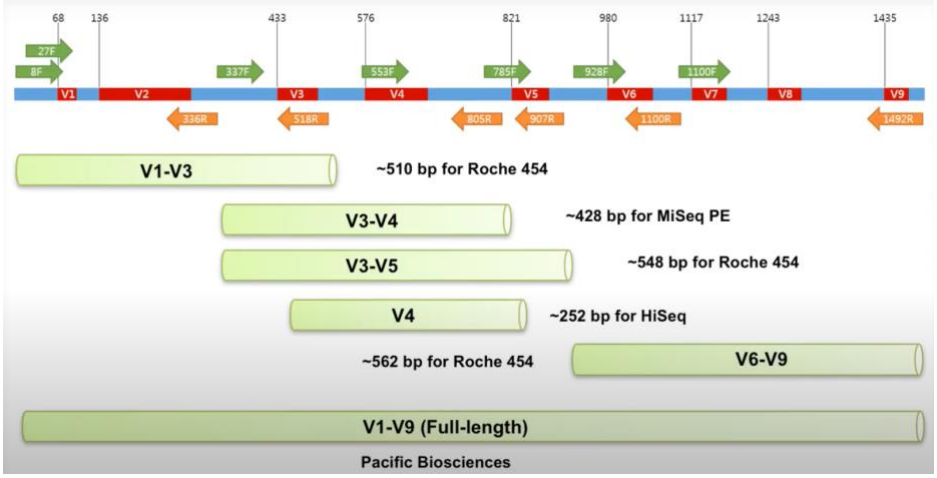
CESAR II pipeline includes only three steps: genome extraction, sample loading to plates of CESAR equipment and coincubation. After hours, tester could infer whether the sample shows antibiotic resistance as well as bacterial existence of the sample through fluorescent signals.

Background:

16s rRNA, Pol I, RuBisCo and RNR (ribonucleotide reductase) gene are among the most expressed genes in bacteria. 16s rRNA not only exists in genomes of all bacteria, but also contains specific regions (V1~V9) interspaced by invariant regions that can tell different species apart. These properties make 16s rRNA appropriate for bacterial taxonomy.



With the reduction of sequencing costs, 16s rRNA sequencing has become a popular method for scientist to study microbiome. Primers have been designed by platforms according to knowledge about invariant regions of 16s rRNA of all species.



And microbiome 16s rRNA data are available in online databases such as EzbioCloud or SILVA.

Design:

We firstly analyzed open **EzBioCloud 16S database (https://www.ezbiocloud.net/resources/16s_download)** to gather sequences of 16s rRNA of bacterial of interests.

From bacterial occurrence statistics in samples given by **EzBioCloud** (https://www.ezbiocloud.net/resources/human_microbiome) and clinical experiences told by doctors, we design crRNA for 7 bacteria that are carefully selected out of database due to common appearance in clinical labs to demonstrate feasibility and general applicability of CESAR II.

Bacteria	Gram Staining
staphylococcus aureus	positive
enterococcus faecalis	
staphylococcus epidermidis	
pseudomonas aeruginosa	*negative*
Klebsiella pneumoniae	

Escherichia coli

Acinetobacter baumannii

(gram-positive: *staphylococcus aureus, enterococcus faecalis, staphylococcus epidermidis*; gram-negative: *pseudomonas aeruginosa, klebsiella pneumoniae, escherichia coli, acinetobacter baumannii*)

From MSA (Multiple Sequence Alignment) using Clustal, we calculated out the invariant and variant regions of these 7 bacteria. Next, we want to select an appropriate 23bp-length sequence out of variant regions for crRNA design. crRNA acts as the probe for templates and is the only segment in the whole CESAR system that can potentially tell different species apart. Therefore, the template that is to be probed by CESAR must be specific enough. In other words, we want to find a template that serves as the fingerprint of the species (no one else have the same fingerprint) and design the crRNA that can scan and identify the fingerprint.

In regarding the choice of variant regions, it is reported that V3 to V6 sequencing often carries out sufficient information to classify species; V6 was the most accurate at differentiating species between all CDC-watched pathogens tested. Meanwhile, considering the specificity of the sequences (calculating the distances between 7 sequences and find combination with maximum distances overall) and genome wide off-target effect (cut-off principle examined through blastn), It is V1 chosen out of which the template is come from. More specifically the sequences below:

BACTERIAL	SEQUENCES
ACINETOBACTER BAUMANNII	GGAAGGTAGCTTGCTACCGGACC
ENTEROCOCCUS FAECALIS	CGAGTGCTTGCACTCAATTGGAA
ESCHERICHIA COLI	CACCGGAGCTTGCTCCACCGGAA
KLEBSIELLA PNEUMONIAE	GTAACAGGAAGCAGCTTGCTGCT
PSEUDOMONAS AERUGINOSA	GTAGCACAGAGAGCTTGCTCTCG
STAPHYLOCOCCUS AUREUS	GATGAAGGGAGCTTGCTCCTGGA
STAPHYLOCOCCUS EPIDERMIDIS	ACGGACGAGAAGCTTGCTTCTCT

(gram-positive: *staphylococcus aureus, enterococcus faecalis, staphylococcus epidermidis*; gram-negative: *pseudomonas aeruginosa, klebsiella pneumoniae, escherichia coli, acinetobacter baumannii*)

	scaffold	23 bp spacer	
5‘	**TTAAAGATGAGAACATCTA**	GGAAGGTAGCTTGCTACCGGACC	3’
		CGAGTGCTTGCACTCAATTGGAA	
		CACCGGAGCTTGCTCCACCGGAA	
		GTAACAGGAAGCAGCTTGCTGCT	
		GTAGCACAGAGAGCTTGCTCTCG	
		GATGAAGGGAGCTTGCTCCTGGA	
		ACGGACGAGAAGCTTGCTTCTCT	

The transcription template is given by table above, and the corresponding crRNA is given by table below:

Bacterial		scaffold	23 bp spacer	
acinetobacter baumannii	5‘	**AAUUUCUACUCUUGUAGAU**	CCUUCCAUCGAACGAUGGCCUGG	3’
enterococcus faecalis			GCUCACGAACGUGAGUUAACCUU	
escherichia coli			GUGGCCUCGAACGAGGUGGCCUU	
klebsiella pneumoniae			CAUUGUCCUUCGUCGAACGACGA	
pseudomonas aeruginosa			CAUCGUGUCUCUCGAACGAGAGC	
staphylococcus aureus			CUACUUCCCUCGAACGAGGACCU	
staphylococcus epidermidis			UGCCUGCUCUUCGAACGAAGAGA	