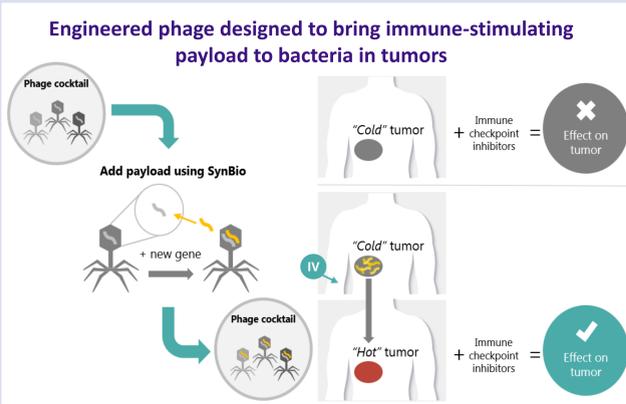


# Engineering phages for eukaryotic payload expression in cancer associated *Fusobacterium Nucleatum*

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## Background

*Fusobacterium nucleatum* (FN) is enriched in human colorectal tumors [1] and its presence is correlated with poor prognosis [2]. Bacteriophage ("phage") have high intrinsic safety and specificity and offer a promising treatment strategy to target FN associated with colorectal cancer (CRC). Phage engineering allows specific delivery of anti-tumor immune stimulating payloads. Phage targeting FN have been isolated. A major challenge in engineering these phage is to express eukaryotic payloads in non-model clinical prokaryotic hosts, such as FN.



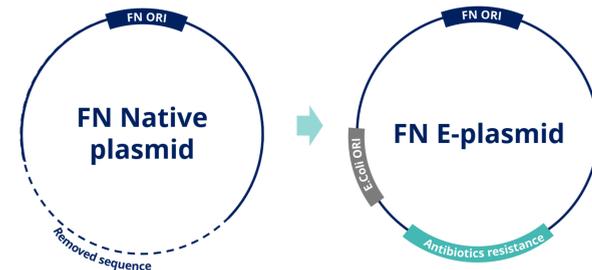
## Highlights

- We demonstrated the ability to express a eukaryotic protein in an unconventional bacterial host using a sequence designed by computational tools
- Our engineered phage can deliver a eukaryotic payload to its host with a similar pattern as an expression vector
- This methodology suggests that expression systems can be introduced by phage engineering into specific clinically relevant bacteria

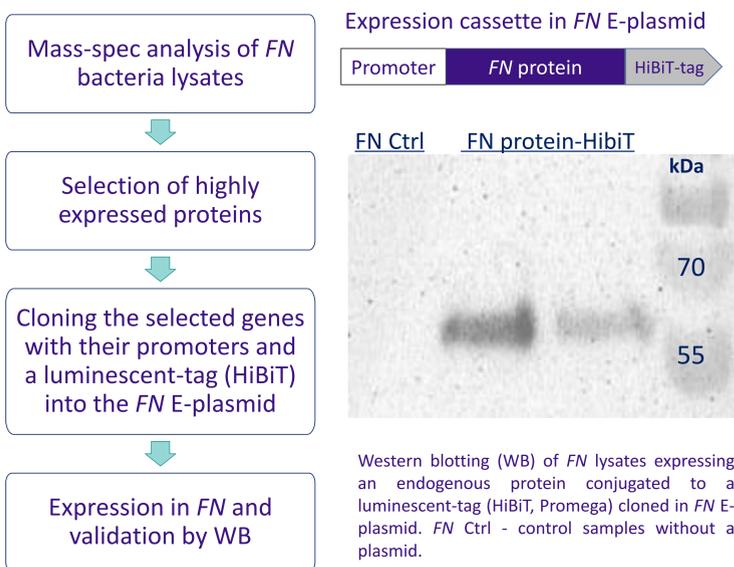
## 1 Generating a molecular system *de-novo*

### Designing a shuttle vector from a native plasmid

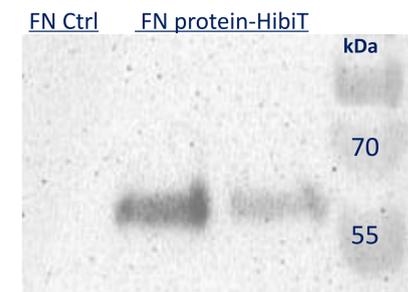
A shuttle vector was generated using a native FN plasmid, by adding an *E.coli* ORI and an antibiotic resistance gene for selection. Following this, the plasmid was shortened by knocking out unnecessary sequences.



### Finding a strong promoter for expression



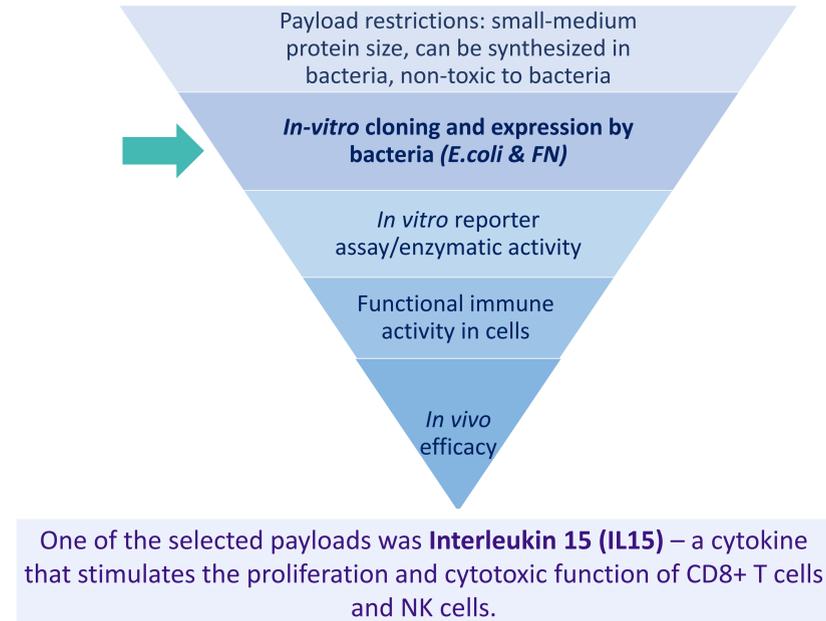
Expression cassette in FN E-plasmid



Western blotting (WB) of FN lysates expressing an endogenous protein conjugated to a luminescent-tag (HiBiT, Promega) cloned in FN E-plasmid. FN Ctrl - control samples without a plasmid.

Following validation of expression, the endogenous protein's gene can be replaced with a payload of interest

## 2 Selection process for potential payloads

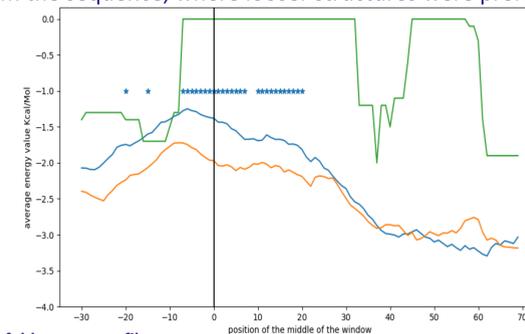


## 3

Working with non-model clinical bacteria is a challenge due to the lack of data on codon usage and non-coding genetic information. To address these challenges, two main computational approaches were utilized:

**1. Mimicking host transcriptome:** The final construct is built out of a string of large stretches of adjacent codons which can also be found in the host transcriptome.

**2. mRNA fold energy at translation initiation vicinity:** Codon selection in the area of translation initiation was guided by the local mRNA secondary structure resulting from the sequence, where looser structures were preferred.

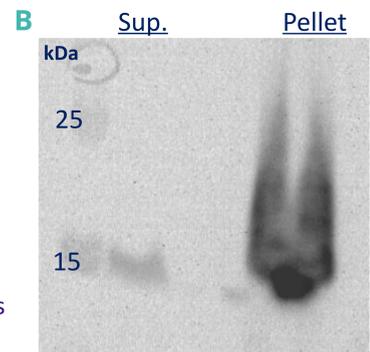


The average RNA fold energy profile. X axis is the position on the coding sequence of genes and the Y axis is the RNA folding energy. Blue and orange lines show the energy profile for the 100 highest and least expressed genes in FN (respectively). The Green line is the average RNA fold energy profile of the optimized IL15 gene. Blue stars mark significant difference between the energy of the highest and least expressed genes.

## 4

To test expression of the optimized IL15 gene sequence in FN, the IL15 gene was introduced on our E-plasmid and validated by WB and luminescence assay.

Sample	Lumi. signal (RLU)
FN+IL15	112,117
FN Ctrl	37



The luminescent signal was obtained from the HiBiT-tag conjugated to the IL15 protein using a commercial kit (Promega).

The payload was observed in both fractions of the FN lysate. Following this, the payload was ready for engineering into an FN phage.

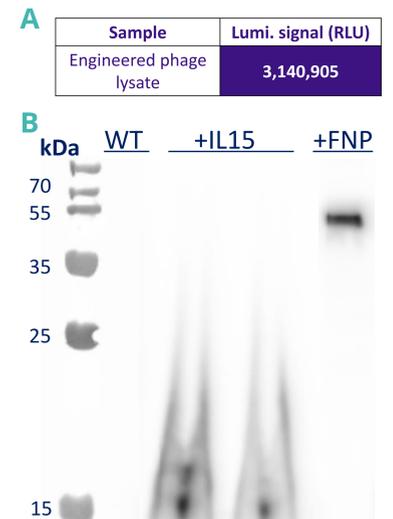
(A) Luminescence assay and (B) WB of FN expressing IL15 conjugated to a luminescent-tag (HiBiT) cloned in FN E-plasmid.

## 5

Generating engineered phage via a synthetic biology approach

Isolating the engineered phage

Propagating the engineered phage on FN host



(A) Luminescence assay and (B) WB of FN lysate after engineered phage infection. WT – wild type phage, IL15 – phage genome contains IL15, FNP – phage genome contains same endogenous FN protein as previously shown.

## References

- [1] Ito M, et al. Association of *Fusobacterium nucleatum* with clinical and molecular features in colorectal serrated pathway. *Int J Cancer*. 2015; 137:1258–1268.  
 [2] Yu T, et al. *Fusobacterium nucleatum* Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. *Cell*. 2017; 170:548–563.

