

# E. COLI DERIVED NANOBODIES FOR P53 DETECTION IN SALIVA



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Introduction	Experimental Design	Results
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- Oral squamous cell carcinoma (OSCC) – malignant tumor with 640,000 new cases annually in the world
- Saliva testing can detect potential biomarkers for OSCC
- Elevated level of p53 protein was identified in OSCC patients at different stages of disease

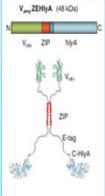
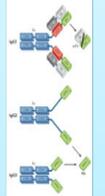
A)  B) 

Figure 1. A) Haemolysin transport system (HlyA) and zipper-containing polypeptide (ZEHlyA); B) Antibodies in sera of camels: conventional antibodies (IgG1) and two types of homodimeric heavy-chain antibodies

- Zipper-containing polypeptide (ZEHlyA) was secreted by *E. coli* carrying HlyBD transporter & accumulated in culture media as stable dimer (Figure 1.A)
- Camelid antibodies only with variable regions, nanobodies (VHH) & single-chain variable regions (scFv) with VH and VL, are becoming popular in many biological studies including diagnostic applications (Figure 1. B)

## Hypothesis

*E. Coli* having functional type I secretion system will be able to express and secrete Vhp53 nanobody that contains HlyA secretion signal

## Aim

To purify Vhp53 nanobody secreted by *E. coli* in order to check its affinity for p53 protein.

1. Transforming *E. coli* Dh5 *alpha* hlyB hlyD genes in pVDL9.3 (Cm) with pUC57 vector (Figure 2, 1)
2. Induce expression of the protein with 1uM IPTG
3. Collect both lysate of the cells and the medium
4. Purify proteins with Ni/NTA columns
5. SDS PAGE gel
6. Check the affinity of obtained nanobodies to p53 protein

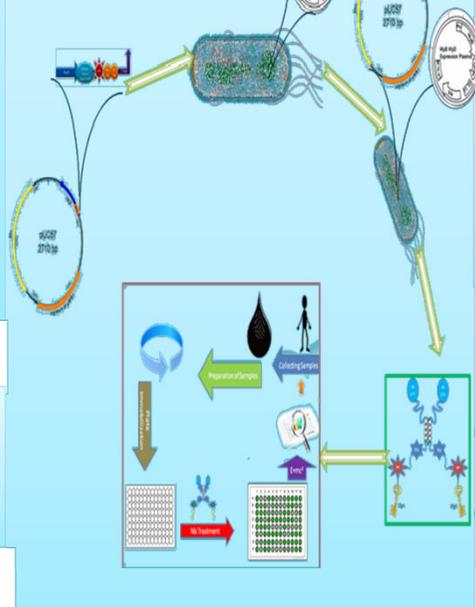


Figure 2. Schematic representation of experimental design

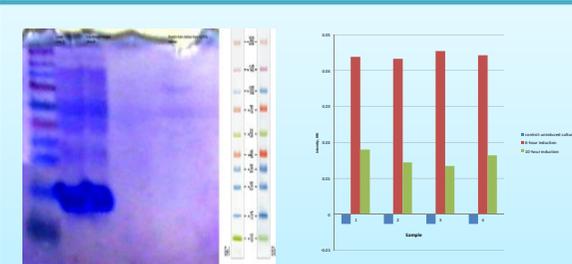


Figure 3. SDS PAGE of purified Vhp53  
Protein concentration after elution:  
1<sup>st</sup> elution: 46.95 ng/ul  
2<sup>nd</sup> elution: 123.16 ng/ul

## Conclusion

Vhp53 nanobody was purified from both medium and bacterial lysate. We plan to test the affinity of this nanobody for cancer biomarker p53. In future, this protein-nanobody interaction can be used to design a biosensor, based on available sequence of antibodies, in particular, to detect p53 in saliva samples for OSCC diagnosis

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