

**MARILIA deliverable: Report on generation of peptide-DNA conjugates**  
**Deliverable number: D2.1**

**MARILIA**

**MARA-BASED INDUSTRIAL LOW-COST IDENTIFICATION ASSAYS**

Project nr:	952110	Call reference:	H2020-EIC-FETPROACT-2019
Start date:	September 1 <sup>st</sup> , 2020.	Duration:	30 months

**Deliverable identification**

Leading beneficiary:	RBI	Planned delivery date:	M15
Related WP:	WP 2	Actual delivery date:	2.3.2022
Dissemination level:	Confidential		

**Contributors**

Beneficiary name	Contributor(s)' name(s)
RBI	Ivo Piantanida
RBI	Željka Ban
RBI	Ivo Crnolatac

**Deliverable Reviewers**

Version	Reviewer	Date
1.0	Ivan Barišić	1.3.2022

## Table of content

1. Context and objectives .....	2
2. Description of the performed tasks and obtained results .....	2
2.1 Modification of peptide thiol group .....	2
2.2 Modifications of peptide amino groups .....	4
2.2.1 NHS ester modification .....	5
2.2.2 Azido modification of amino group: diazotransfer reaction .....	5
2.2.3 Acrylate modification of amino group .....	9
2.3 Generation of BBP peptide-DNA conjugates.....	10
2.3.1 Activation of peptide cysteine with bifunctional crosslinker maleimide-DBCO followed by reaction with azido-oligonucleotide (Figure 7).....	10
2.3.2 Activation of peptide C-terminus carboxyl group with EDC/sulfo-NHS followed by reaction with amino-oligonucleotide .....	11
3. Conclusion .....	12
4. EXPERIMENTAL PROCEDURES FOR PEPTIDE MODIFICATIONS .....	14

### 1. Context and objectives

In MARILIA, we planned the development of horseradish peroxidase (HRP)-DNA barcoded components and conjugating them to bacteria-binding proteins (BBP), aiming toward selective targeting of particular pathogens in water. In parallel, a methodology for peptide (for instance BBP) – DNA conjugates had to be developed.

Thus, we had to optimize the DNA methodology on a simple peptide-DNA conjugate level, to have a good starting point for HRP-DNA tethering, as well as BBP-DNA conjugates with retained antibacterial activity and selectivity.

There are several strategies for the covalent connection of peptides and/or proteins to DNA oligonucleotides. The two most attractive amino acid residues available for modification are cysteine and lysine because of their high reactivity.

Therefore, we investigated:

2.1. Modification of peptide thiol groups (cysteine residues)

2.2. Modification of peptide amino groups (lysine residues and/or N-terminus)