

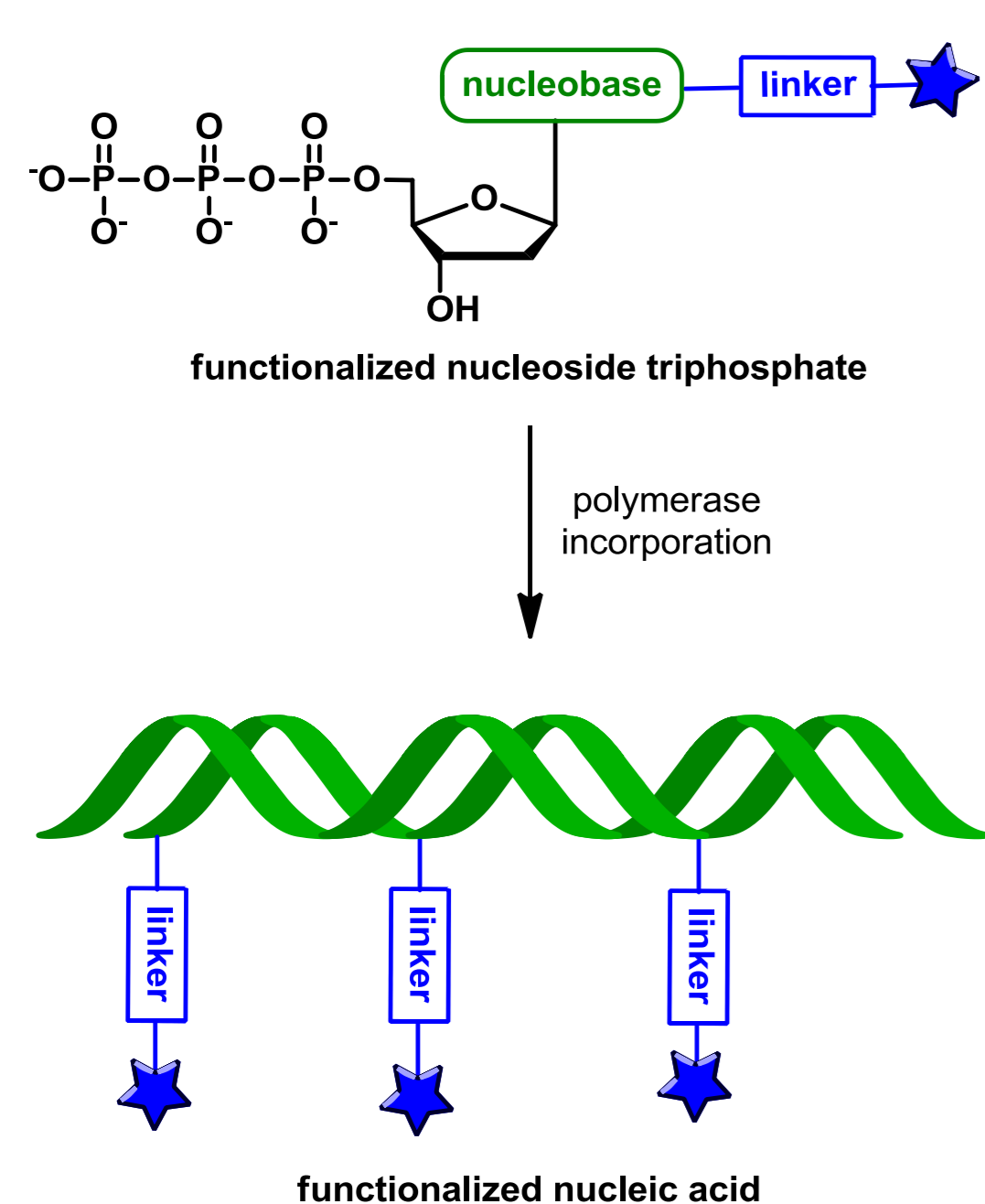
Preparation of Modified Oligonucleotides by Nicking Enzyme Amplification Reaction

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Introduction – Base-modified DNA^[1]

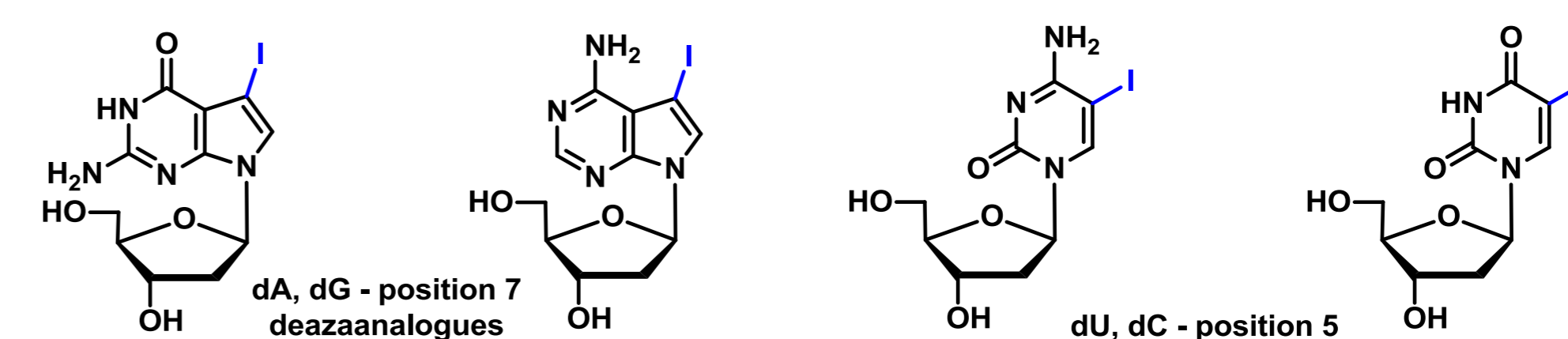
- Functionalized nucleic acids have found applications in chemical biology, bioanalysis, catalysis and nanotechnology.
- Modifications can be introduced into the phosphate backbone, sugar moiety and, most often, onto a nucleobase.
- Base-modified DNA can be synthesized chemically or enzymatically.
- Chemical synthesis is carried out on solid support and utilizes phosphorimidate building blocks.
- Enzymatic synthesis of (base-modified) DNA is based on the incorporation of (functionalized) nucleoside triphosphates by a DNA polymerase.



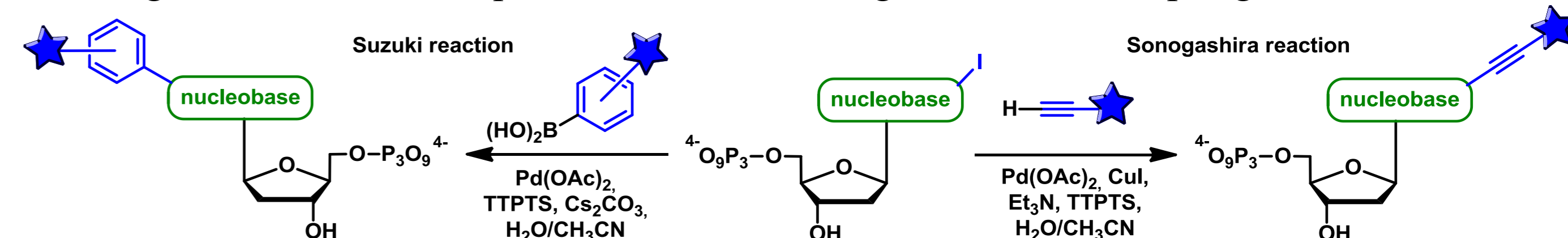
- Methods for enzymatic synthesis:
 - PEX – primer extension
 - PCR – polymerase chain reaction
- DNA polymerases: Vent(exo-), KOD XL, Pwo, Bst large fragment...

Introduction – Base-modified Nucleoside Triphosphates^[1]

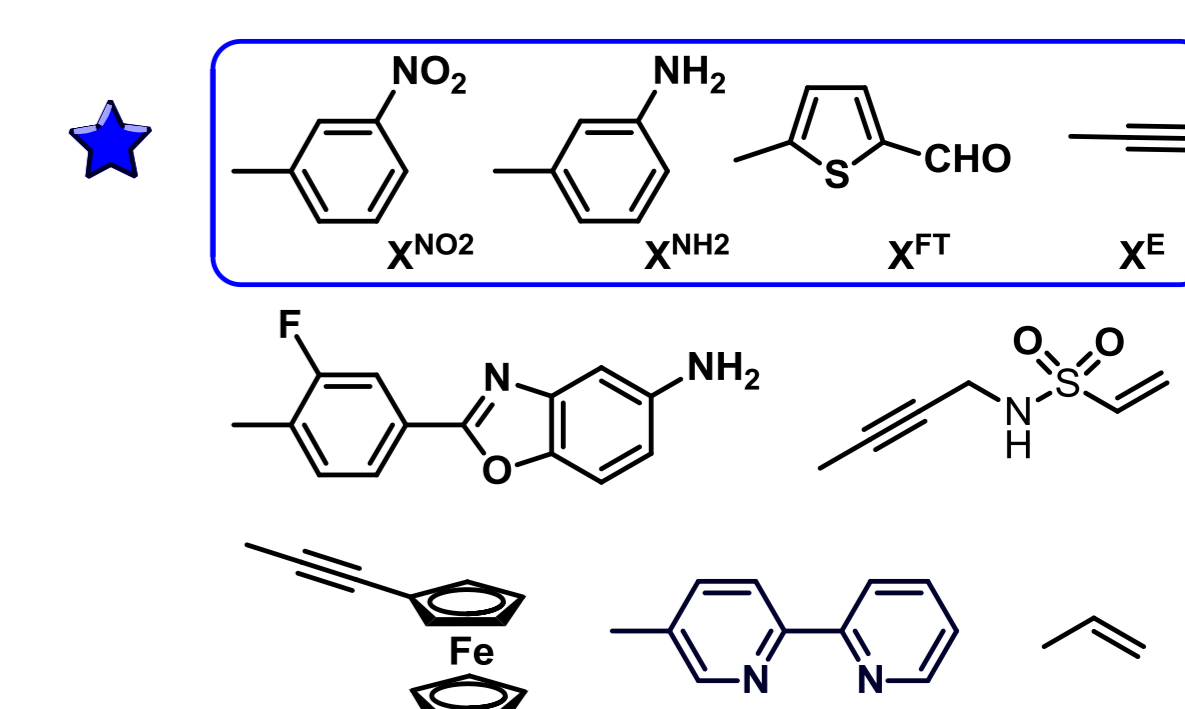
- Starting from 7-iodo-7-deazapurines and 5-iodopyrimidines



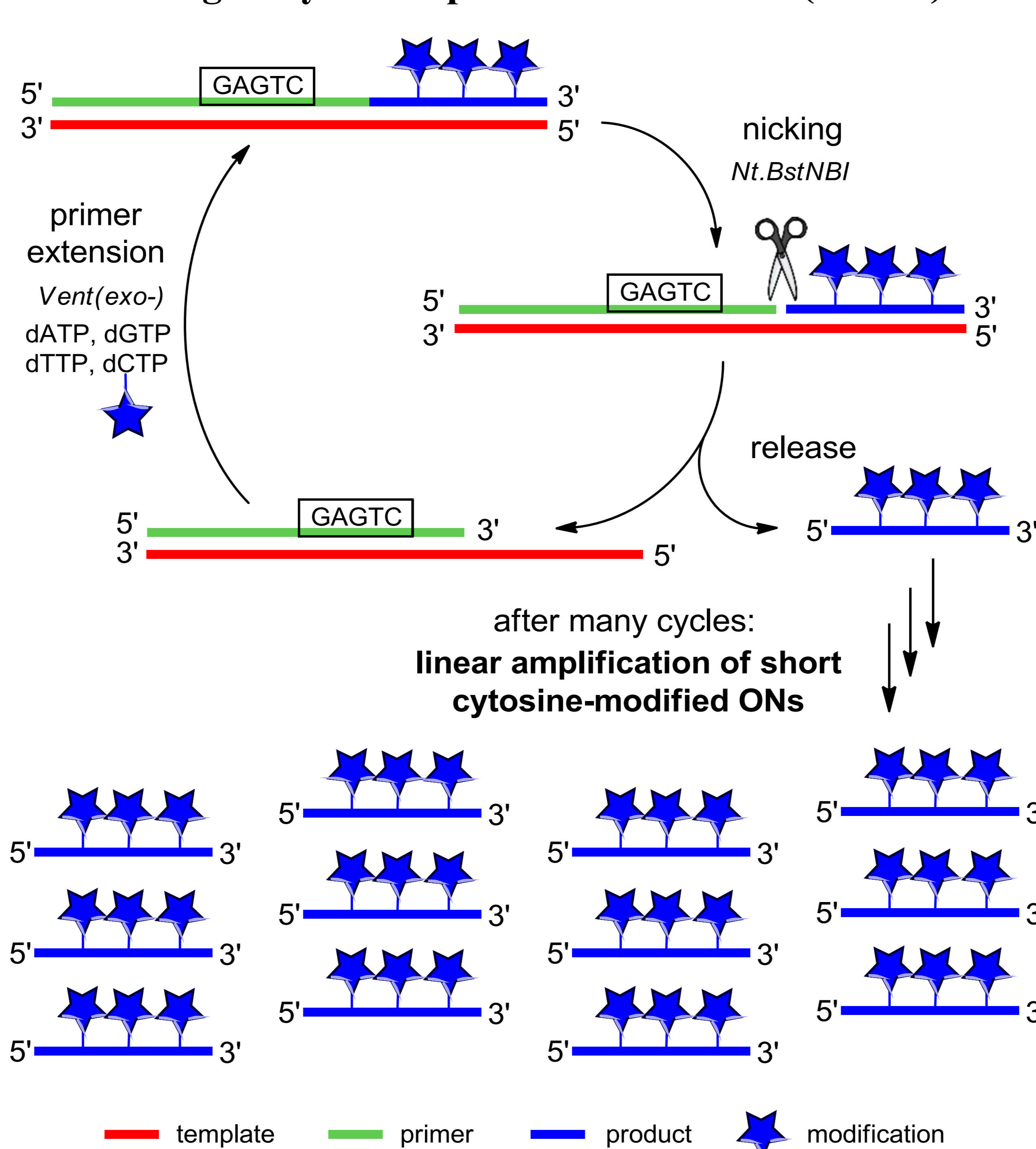
- Triphosphorylation
- Introducing a modification – aqueous Suzuki or Sonogashira cross-coupling reaction



- Modifications: redox labels, fluorescent labels, conjugation precursors etc.



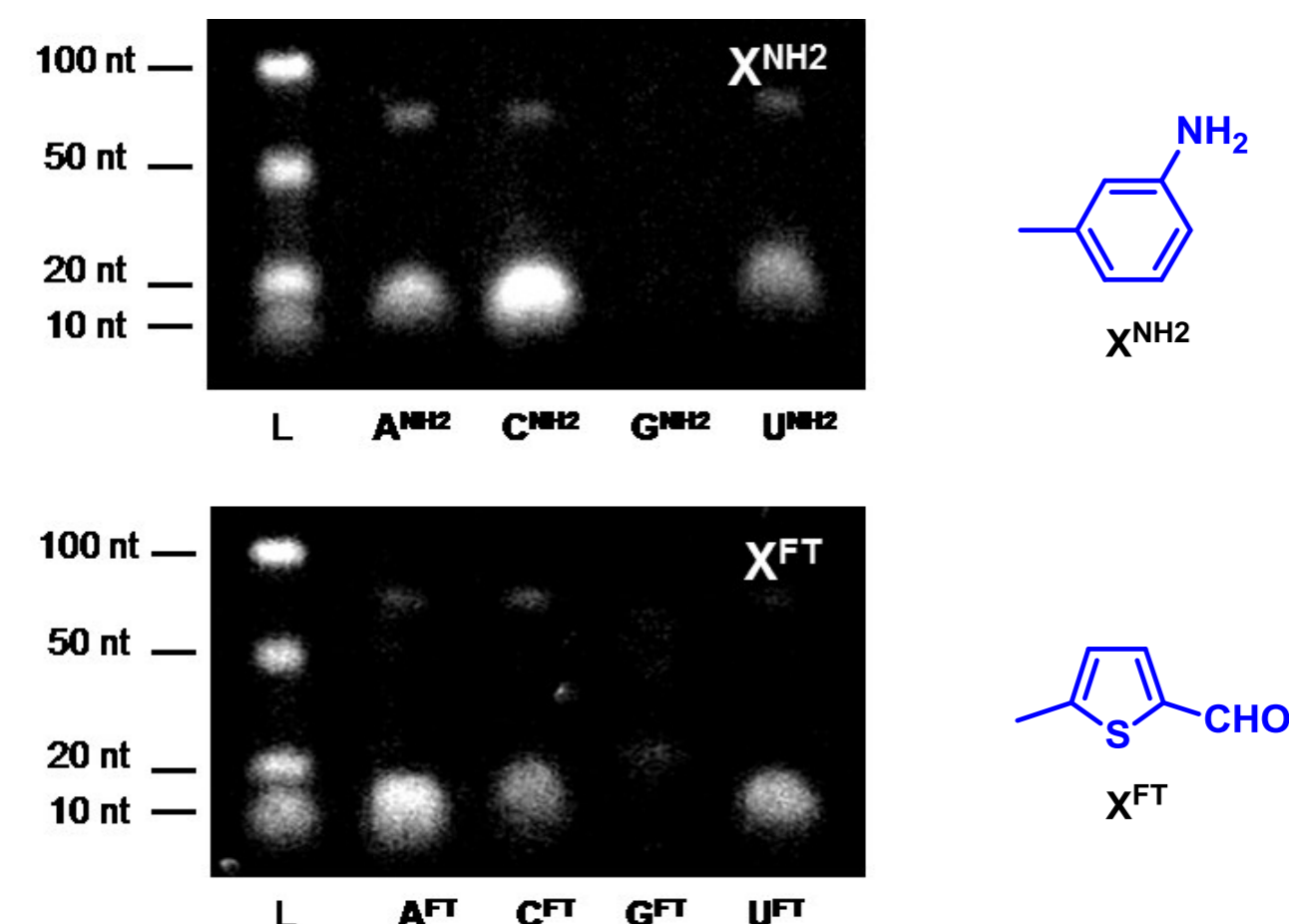
Nicking Enzyme Amplification Reaction (NEAR)^[2]



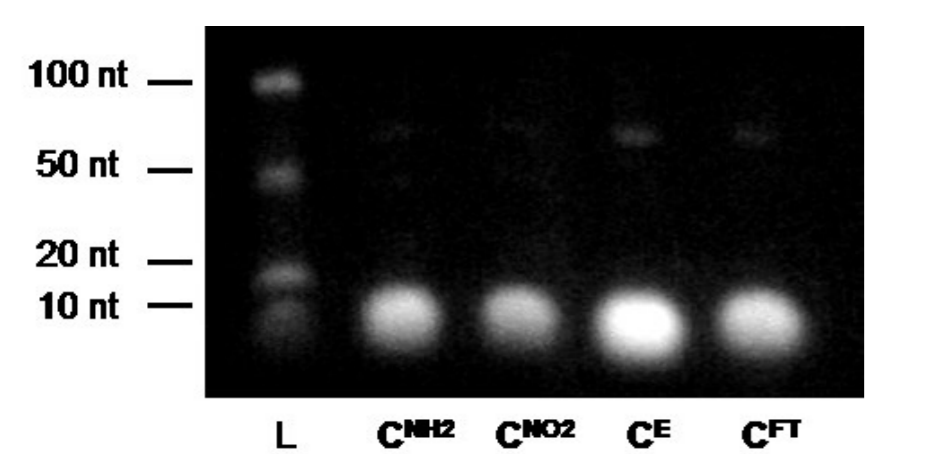
— template — primer — product — modification

Scope^[3]

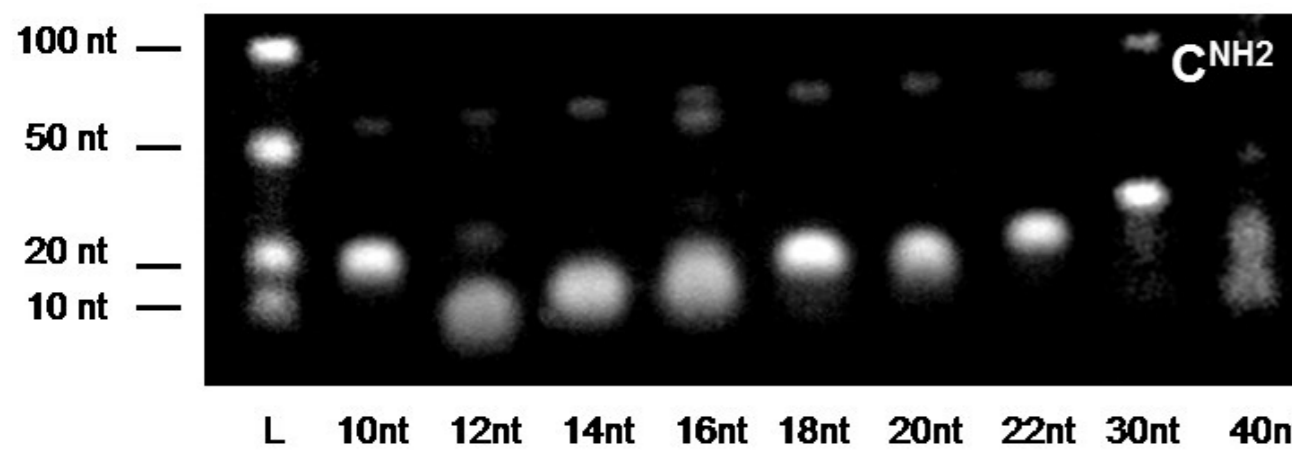
- Various modified nucleobases



- Modified cytidines proved to be the best substrates
- Various modifications on dCTP

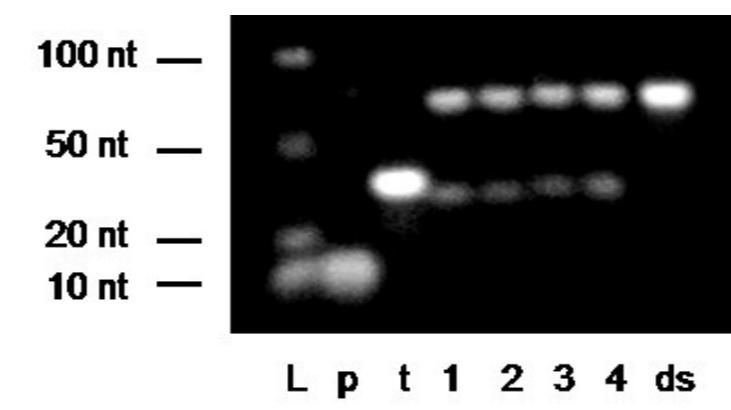
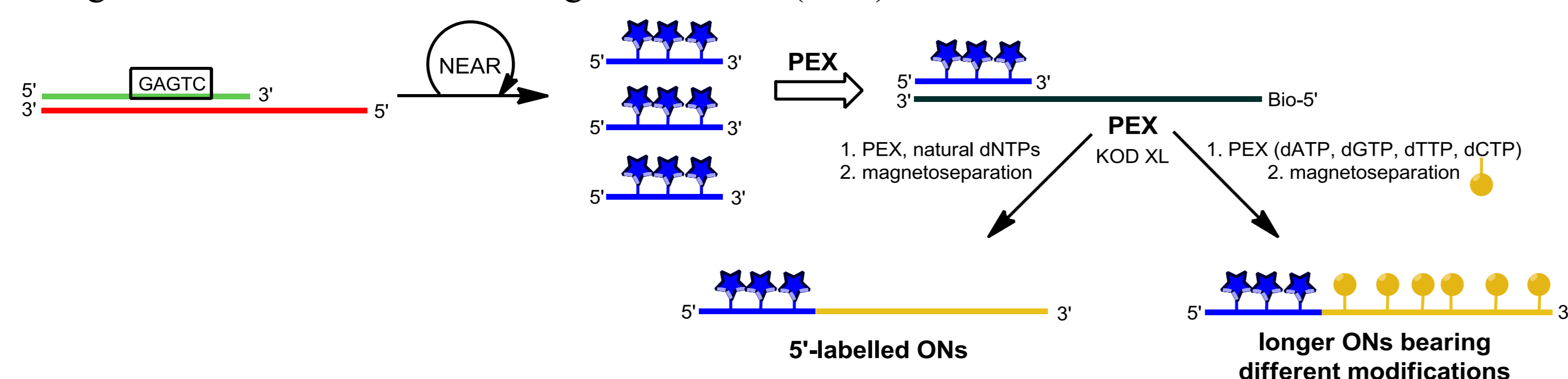


- Various lengths of NEAR products



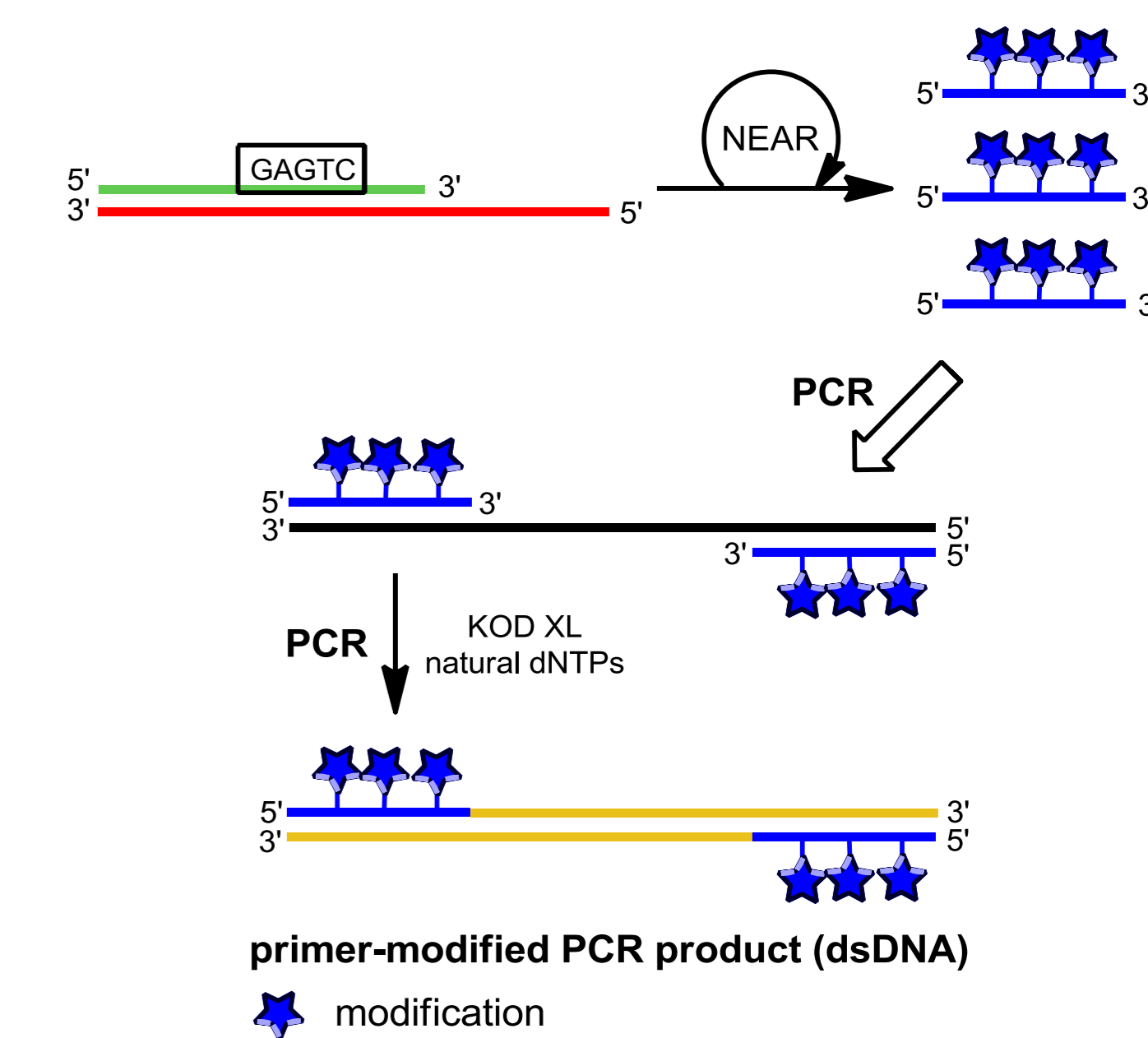
Application – Primers in PEX^[4]

- PEX = primer extension
- Combination with magnetoseparation – production of single-stranded functionalized oligonucleotides (ONs)

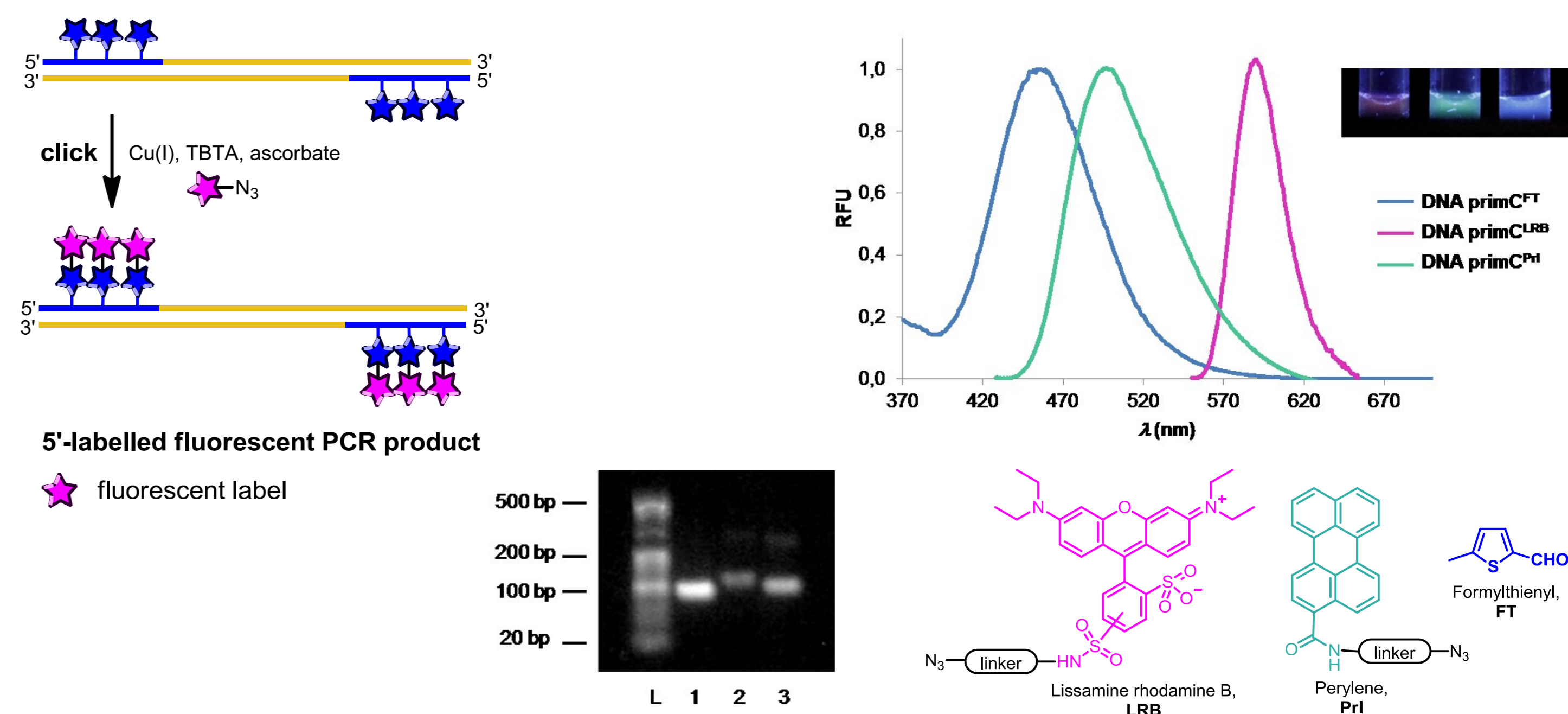


Application – Primers in PCR^[4]

- PCR = polymerase chain reaction
- Production of double-stranded functionalized oligonucleotides
- Direct fluorescent labelling formylthienyl-modified primers



- Indirect fluorescent labelling – click reaction on ethynyl-modified primer after PCR



References

- [1] a) M. Hocek, M. Fojta, *Org. Biomol. Chem.* **2008**, *6*, 2233–2241; b) M. Hocek, M. Fojta, *Chem. Soc. Rev.* **2011**, *40*, 5802–5814.
 [2] P. Měnová, M. Hocek, *Chem. Commun.* **2012**, *48*, 6921–6923.

- [3] J. Van Ness, L. K. Van Ness, D. J. Galas, *Proc. Nat. Ac. Sci.* **2003**, *100*, 4504–4509.

- [4] P. Měnová, V. Raindlová, M. Hocek, *Bioconjugate Chem.* **2013**, *24*, 1081–1093.

Acknowledgements

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