

Flexible DNA sticky end formation technology

~ Three technologies can be selected depending on the application ~

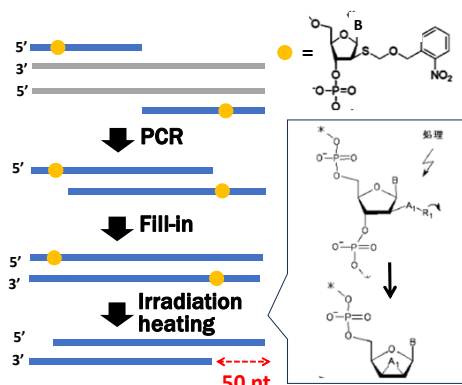
Key aspect of the invention

- Three types of chemical cohesive-end formation technologies enable;
 - 1.more flexible sequence design,
 - 2.highly-efficient DNA ligation,
 - 3.formation of a cohesive end on only one terminus
- Useful for construction of genetically modified vectors.

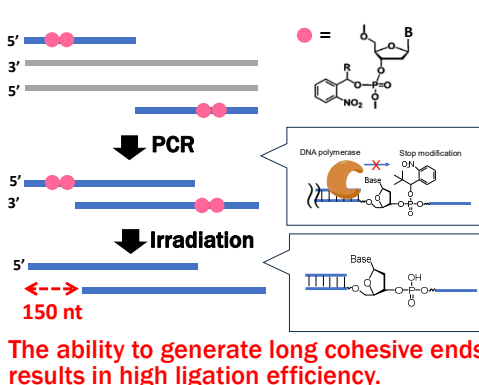
	Sticky end length	Sequence flexibility	Cost	Ligation efficiency	Characteristics
Conventional method: Enzyme(BsaI)	4 nt		✓✓	✓	Golden Gate Assembly
: Chemical(5-ethynyl U)	~50 nt		✓	✓	Severe cleavage condition
Invention A: DNA cleavage primer	~50 nt	✓	✓	✓	Cleavable in vivo
Invention B: Stop primer	~150 nt	✓✓	✓	✓✓	Applicable to long DNA ligation
Invention C: Silver nanoparticle	~50 nt	✓		✓	Less DNA damage, high yield

Summary of the invention

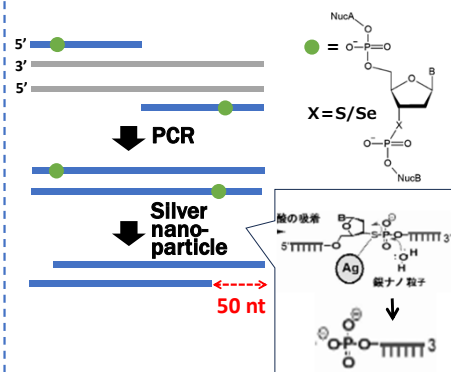
A: DNA cleavage primer
contains nucleic acids with the 2' position substituted with a photolabile group via a sulfur- or selenium-based linker



B: Stop primer
contains nucleic acids modified with a photolabile group via a phosphate linkage

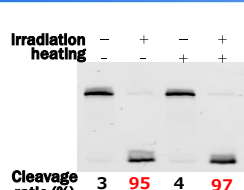


C: Silver nanoparticle
cleaving products amplified using primers containing thio-modified oligonucleotides, with silver nanoparticles.



Advantage over conventional methods

Sticky end formation



Quantitative cleavage via Irradiation

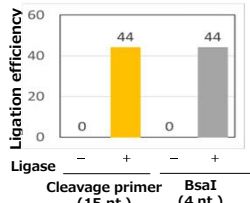
Primer	5' Fluorescein-ACCGAGCTCGAATTCGCG
Template	OT 1T 2T
Time (min)	0 10 30 60 0 10 30 60 0 10 30 60

Incorporation of two photolabile nucleotides results in extension termination.

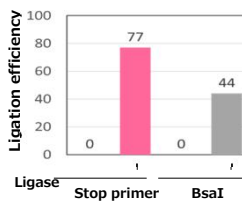
Reagent	Cleavage	Recovery
AgNO ₃	98.7%	14.4%
Ag nano-particle	89.6%	98.4%

• Comparable cleavage to AgNO₃
• Higher recovery to AgNO₃

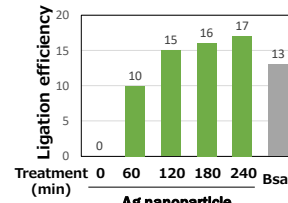
Ligation efficiency



Equivalent to restriction enzyme method



Higher ligation efficiency to enzyme



Comparable ligation efficiency to AgNO₃

Expected use

- DNA synthesis tool alternative to enzymatic synthesis with excellent scalability and efficiency
- Applicable to vector construction for recombinant products and genome synthesis

Lead Inventor: Hiroshi Abe (Professor, Graduate School of Science, Nagoya University)

Title of Licensable Patent Invention: Primer, apparatus for producing double-stranded DNA using same, and method for producing double-stranded DNA

Publication Number: WO2021/020561, WO2021/020562, WO2023/54391

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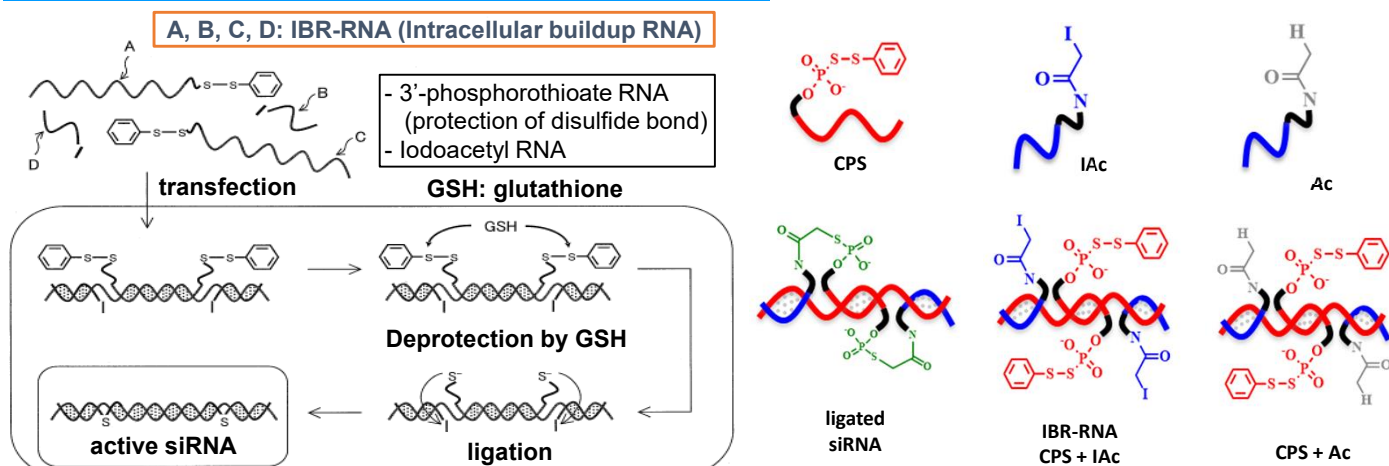
Building-up of Nucleic Acids in Cells

~ Efficient intracellular delivery and low side effect of RNA drugs ~

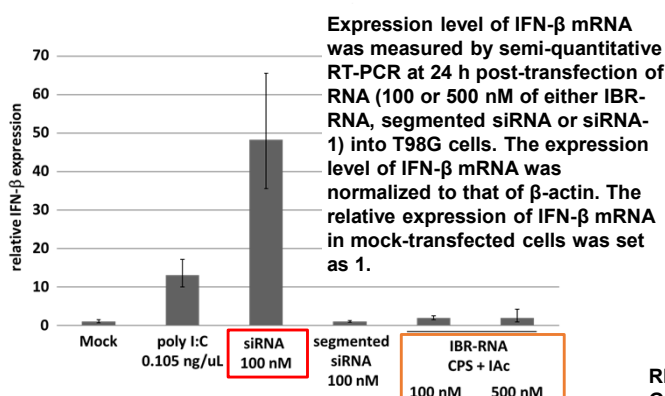
Key aspect of the invention

- Introduction of nucleic acid strands, such as those constituting siRNA, into cells and chemical binding of the strand enables to construct larger functional nucleic acids within cells
- Advantage of enhancing membrane permeability and avoiding immune activation.

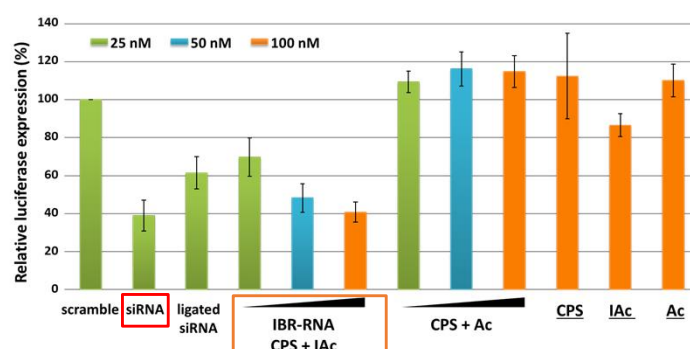
Intracellular ligation of nucleic acid strands (1)



Avoiding immune activation

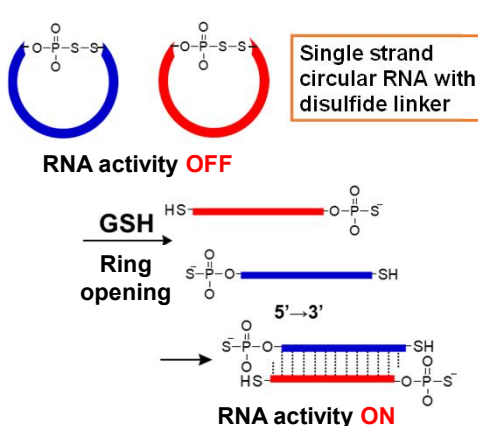


RNA interference effect

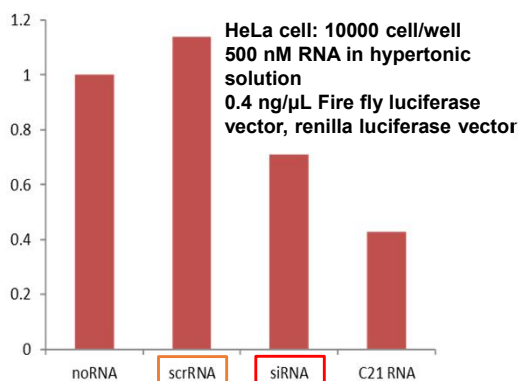


RNAs were transfected into HeLa cells stably expressing luciferase with GeneSilencer and luciferase expression was monitored at 24 h post-transfection.

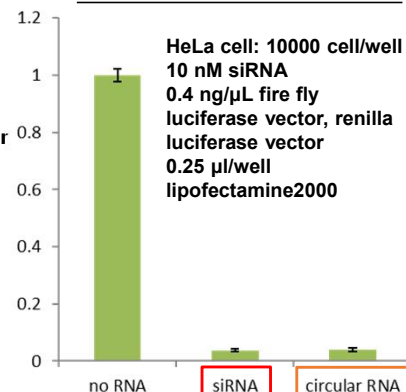
Intracellular ligation of nucleic acid strands (2)



Enhanced membrane permeability



RNA interference effect



Expected use

- Nucleic acid therapeutics and nucleic acid reagents

Lead Inventor: Hiroshi Abe (Professor, Graduate School of Science, Nagoya University)

Title of Licensable Patent Invention: Method for constructing functional nucleic acid molecule, and nucleic acid combination to be used in said method

Publication Number: WO2013/129663

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Non-enzymatic oligonucleotide ligation method

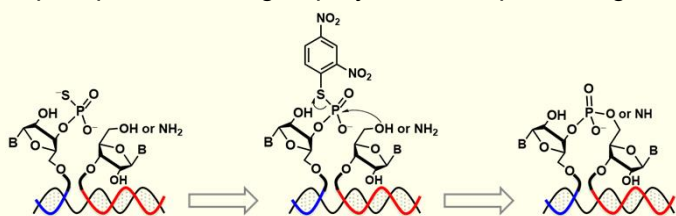
~ Chemical synthesis of DNA/RNA with repetitive sequences ~

Key aspect of the invention

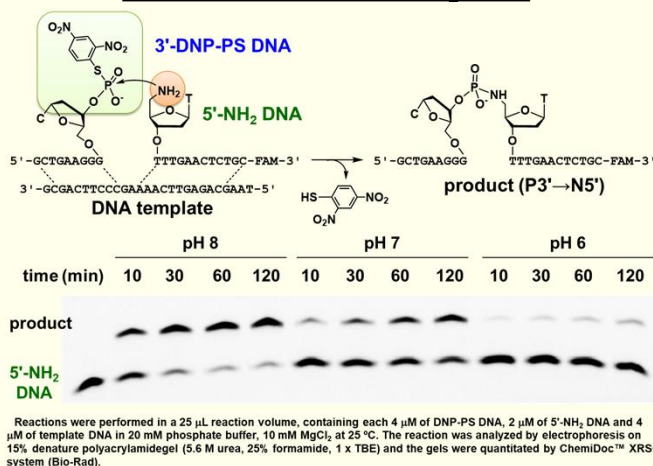
- An organic chemical method for nucleic acid strand ligation employing a phosphorothioate-containing nucleic acid strand together with an electrophilic reagent
- Enables the low-cost production of various functional nucleic acids and proteins

The key reaction underlying the technology

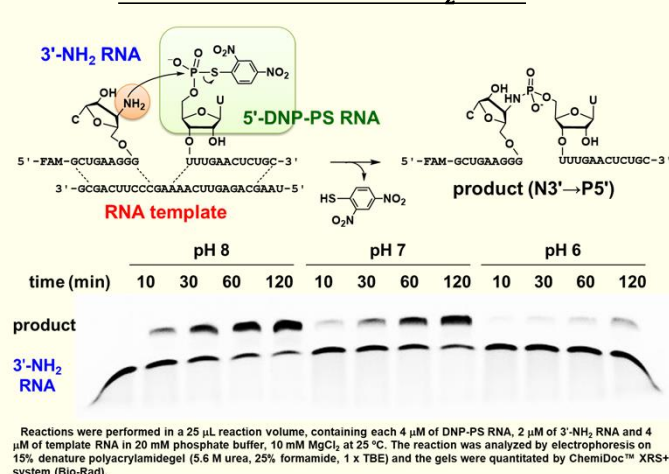
A chemical ligation reaction utilizing activation of a phosphorothioate group by an electrophilic reagent



Ligation reaction between DNP-PS DNA and 5'-NH₂ DNA

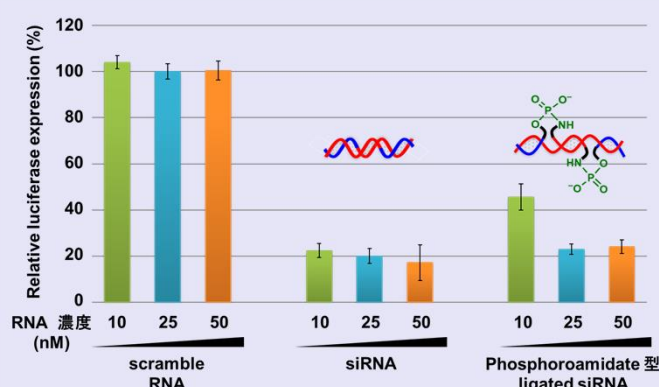


Ligation reaction between DNP-PS RNA and 3'-NH₂ RNA



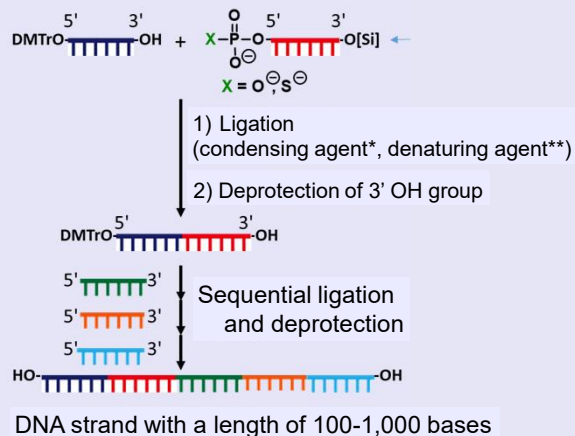
Application of the technology

RNA interference mediated by phosphoramidate-type siRNA



Comparable efficacy conventional technology

Ligation of repetitive sequences



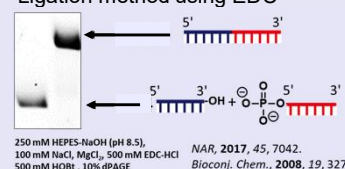
*condensing agent:

EDC, BrCN, TNCB

**denaturing agent:

Urea, formaldehyde

Ligation method using EDC



Enables to synthesize nucleic acids with repetitive or higher-order structure-forming sequences

Expected use

- Cost-effective production of nucleic acids and proteins (in-house and contract manufacturing), gene function analysis, and novel drug discovery and development

Lead Inventor: Hiroshi Abe (Professor, Graduate School of Science, Nagoya University)

Title of Licensable Patent Invention: Method for nonenzymatic combination of nucleic acid chains

Publication Number: WO2016/031247

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Nucleic acid probe

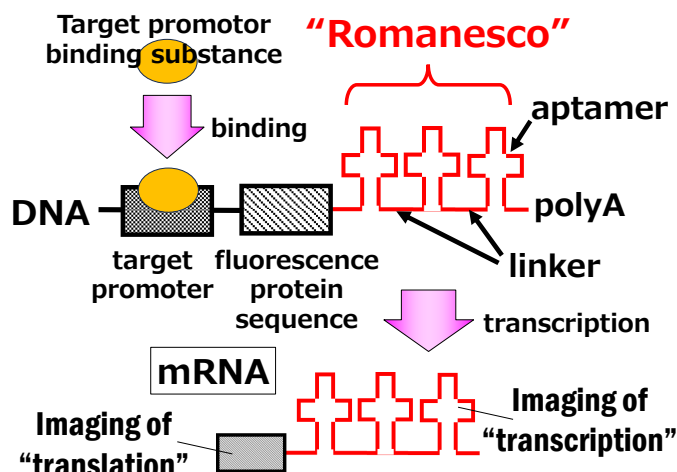
~Imaging of mRNA, DNA dynamics in a living cell~



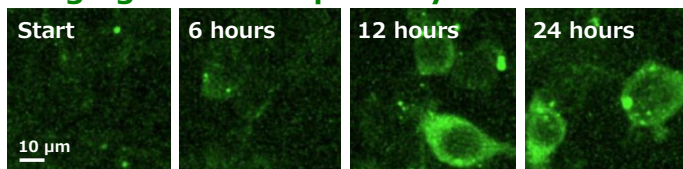
Overview and efficacy of the invention

mRNA analysis probe

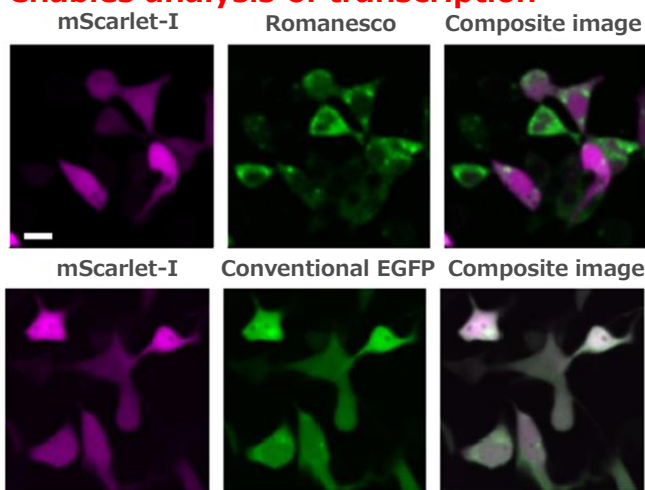
- Fluorescence emission intensity can be maximized by arranging multiple scaffold sequences containing fluorescent molecule-binding aptamers in tandem using a unique linker sequence.
- "Romanesco" sequence is introduced into the target promoter.



Imaging of transcription dynamics in a cell

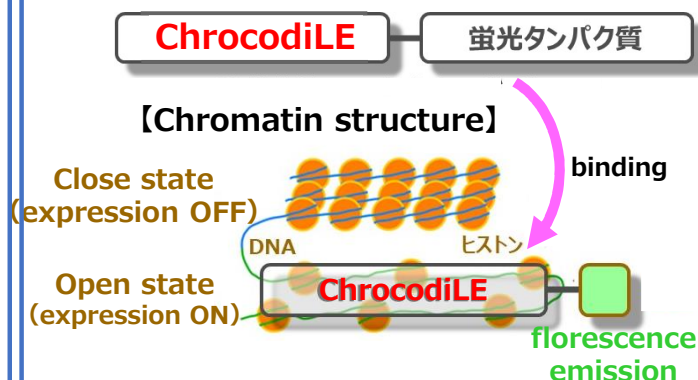


- ◎ 300-times higher intensity than Broccoli
- ◎ Simultaneous tracking of protein&mRNA enables analysis of transcription



DNA analysis probe

- "ChrocodiLE" protein capable of selectively and sequence-independently binding to DNA of open chromatin with minimal steric hindrance was created.
- Fluorescent protein emits light upon binding of ChrocodiLE to DNA.



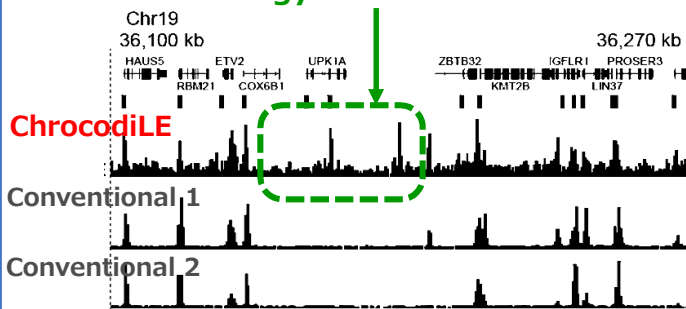
Example of tissue observation in knock-in mice



- ◎ Time-dependent changes in the three-dimensional structure of the genome in living cells is trackable.

Genome sequence analysis using DNA sequencers.

The sequences of open regions that could not be identified with conventional technology was confirmed.



Application

- ◎ Probes for tracking the in vivo behavior of drug-responsive genes
- ◎ Analysis of gene expression in living cells during neuronal differentiation and nerve tissue repair in specific neural tissues
- ◎ Imaging of gene expression in living cells for specific diseases

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PDF資料はこちら



ライセンス可能な特許（発明の名称－国際公開番号）

- ◎ Romanesco：蛍光発生核酸分子、及び標的RNAの蛍光標識方法－WO2020116446
- ◎ ChrocodiLE：核酸結合性タンパク質－WO2020209332

連絡先：JST知的財産マネジメント推進部 ライセンス担当

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