<!doctype html>

<html>

<head>

<meta charset="UTF-8">

<title>Experiment | Team Sendai - Biomod 2014</title>

<link href="styles/common.css" rel="stylesheet" type="text/css">

<link href='http://fonts.googleapis.com/css?family=Roboto' rel='stylesheet' type='text/css'>

<link href='http://fonts.googleapis.com/css?family=Roboto+Slab' rel='stylesheet' type='text/css'>

<script src="scripts/jquery-1.11.1.min.js"></script>

<script src="scripts/retina.js"></script>

<script type="text/javascript">

window.onload=$(function() {

// ナビゲーションのリンクを指定

var navLink = $('#contentsnav li a');

// 各コンテンツのページ上部からの開始位置と終了位置を配列に格納しておく

var contentsArr = new Array();

for (var i = 0; i < navLink.length; i++) {

// コンテンツのIDを取得

var targetContents = navLink.eq(i).attr('href');

// ページ内リンクでないナビゲーションが含まれている場合は除外する

if(targetContents.charAt(0) == '#') {

// ページ上部からコンテンツの開始位置までの距離を取得

var targetContentsTop = $(targetContents).offset().top;

// ページ上部からコンテンツの終了位置までの距離を取得

var targetContentsBottom = targetContentsTop + $(targetContents).outerHeight(true) - 1;

// 配列に格納

contentsArr[i] = [targetContentsTop, targetContentsBottom]

}

};

$('a[href^=#]').click(function(){

var speed = 500;

var href= $(this).attr("href");

var target = $(href == "#" || href == "" ? 'html' : href);

var position = target.offset().top;

$("html, body").animate({scrollTop:position + 1}, speed, "swing");

return false;

});

// 現在地をチェックする

function currentCheck() {

for (var i = 0; i < navLink.length; i++) {

// コンテンツのIDを取得

var targetContents = navLink.eq(i).attr('href');

// ページ内リンクでないナビゲーションが含まれている場合は除外する

if(targetContents.charAt(0) == '#') {

// ページ上部からコンテンツの開始位置までの距離を取得

var targetContentsTop = $(targetContents).offset().top;

// ページ上部からコンテンツの終了位置までの距離を取得

var targetContentsBottom = targetContentsTop + $(targetContents).outerHeight(true) - 1;

// 配列に格納

contentsArr[i] = [targetContentsTop, targetContentsBottom]

}

};

// 現在のスクロール位置を取得

var windowScrolltop = $(window).scrollTop();

for (var i = 0; i < contentsArr.length; i++) {

// 現在のスクロール位置が、配列に格納した開始位置と終了位置の間にあるものを調べる

if(contentsArr[i][0] <= windowScrolltop && contentsArr[i][1] >= windowScrolltop) {

// 開始位置と終了位置の間にある場合、ナビゲーションにclass="current"をつける

navLink.removeClass('current');

navLink.eq(i).addClass('current');

i == contentsArr.length;

}

};

}

// ページ読み込み時とスクロール時に、現在地をチェックする

$(window).on('load scroll', function() {

currentCheck();

});

});

</script>

</head>

<body>

<div id="wrapper">

<header id="globalheader">

<nav id="globalnav">

<a href="index.html"><img src="images/logo3.png" height="60px" width="300px" style="margin-right:40px; margin-left:20px" align="left"></a>

<ul>

<li><a href="./project.html">OUR PROJECT</a></li>

<li><a href="./design\_enzyme.html">DESIGN</a></li>

<li><a href="./simulation.html">SIMULATION</a></li>

<li class="currentpage"><a href="./experiment.html">EXPERIMENT</a></li>

<li><a href="./protocol.html">MATERIALS&amp;METHODS</a></li>

<li><a href="./discussion.html">DISCUSSION</a></li>

<li><a href="./team.html">TEAM</a></li>

</ul>

</nav>

</header>

<div id="main">

<nav id="contentsnav">

<ul>

<li><a href="#contents01"><b>Enzymatic device</b></a></li>

<li><a href="#contents02">Functionality</a></li>

<li><a href="#contents03">Testing component processes</a></li>

<li><a href="#contents04"><b>Enzyme-free device</b></a></li>

<li><a href="#contents05">Functionality</a></li>

<li><a href="#contents06">Testing component reactions</a></li>

</ul>

</nav>

<div id="maincol">

<article>

<h1>Experiment</h1>

<p>

Experimental results of electrophoresis and other chemical observation methods proved our devices to work properly as we designed. This page explains the experimental results of Enzymatic device and Enzyme-free device, both of which include detailed analyses of component reactions.<br>

</p>

<section id="contents01">

<span><h1>Enzymatic device</h1></span>

<hr></hr>

</section>

<section id="contents02">

<span><h2 id="ed Whole device ex">Functionality</h2></span>

<h3>The order of releasing</h3>

<p>

Our device releases different Outputs in order depending on the information of Input. It was proved by two experimental results of electrophoresis (Fig.1) where we add two types of Inputs (<font color="DB0015">A</font> then <font color="96D050">B</font>, <font color="96D050">B</font> then <font color="DB0015">A</font>). Both Output-A and B were tagged with TAMRA, though the length of two Outputs is different. We conducted quantitative analyses of the band intensity of Outputs. Following graphs show the ratio of Outputs to the total intensity of 2 bands (Transducer, Output) on each lane. <br>

<br>

<div align="center">

<img src="images/experiment/experiment es second output electrophoresis after AB\_1.0.png" width="100%"><br>

Fig.1 Time evolution of Outputs with <font size="+2"><B>Input-<font color="DB0015">A</font><font color="96D050">B</font></B></font> and <font size="+2"><B>Input-<font color="96D050">B</font><font color="DB0015">A</font></B></font><br>

<font size="-1">

All enzymes and DNAs (Transducer, Gate) were mixed at 45 &#08451 , then the reactions start when Input is added. Reaction times were 0, 1/6, 1/2, 1, 2, 5, 10, 12, 15, 20, 25, 30, 40 min. After the reaction, the solution was heated up to 80 &#08451 to stop enzymatic activities, then annealed again. The length of Output-A and Output-B is 20 mer and 25 mer, respectively.

<br>

</font>

</div>

<br>

</p>

<p>

These experimental results proved that our device properly releases Outputs in order. The order of Outputs was controlled by the Input sequence. Both graphs show that saturated time of the first and the second Output was 2 and 20 minutes, respectively. More than 90 % of Outputs were released from Transducer.<br>

</p>

<p>

Detailed experimental conditions are given in <a href="protocol.html#ed Whole device">Materials and Methods</a><br>

</p>

<br>

<h3 id="ABC ex">3-instruction operation</h3>

<p>

In this experiment, we confirmed that our device properly releases 3 Outputs ~~properly~~ by Input coding "<font color="DB0015">A</font> then <font color="96D050">B</font> then <font color="0070C0">C</font>"

<!--

and "<font color="0070C0">C</font> then <font color="DB0015">A</font> then <font color="96D050">B</font>"

-->

.

All Outputs have ~~a~~ different lengths and modified with TAMRA. Following plot shows the ratio of Outputs in the total intensity of 2 bands (Transducer, Output) at each lane.<br>

<div align="center">

<img src="images/experiment/experiment es abc\_1.0.png" width="50%"><br>

Fig.2 Time development of Outputs with <font size="+2"><B>Input-<font color="DB0015">A</font><font color="96DO50">B</font><font color="0070C0">C</font></B></font>

<!--

and <font size="+2"><B>Input-<font color="0070C0">C</font><font color="DB0015">A</font><font color="96D050">B</font></B></font><br>

-->

<br>

<font size="-1">

All enzymes and DNAs (Transducer, Gate) were mixed at 45 &#08451. The reaction starts when Input is added. Reaction times were 0, 1/6, 1/2, 1, 2, 5, 10, 12, 15, 20, 22, 25, 30, 35, 40, 45, 50, 60 min. After the reaction, temperature was heated up to 80 &#08451 to stop enzymatic activities, then annealed again. The length of Output-A, Output-B, and Output-C is 20 mer, 25 mer, and 35 mer, respectively.

<br>

</font>

</div>

</p>

<p>

Our device released 3 Outputs in correct order. The saturation time of the first, second, and third Output were 2, 20, and 40 min, respectively.

</p>

<p>

In the <a href="discussion.html">discussion page</a>, we discuss the difference between this result and the simulation.<br>

<br>

</p>

<p>

<a href="protocol.html#ABC">Materials and Methods</a>

</p>

</section>

<section id="contents03">

<span><h2>Testing component processes</h2></span>

<!--

<p>

Reaction conditions such as temperature, concentration, and time were determined by preliminary experiment of components reactions in advance to the experiment in the previous section. DNA strands and enzyme were carefully designed and chosen (For details, see <a href="protocol.html">Method and Material page</a>).<br>

<br>

</p>

-->

<br>

<img src="images/experiment/experiment reading process eido\_1.0.png" width="35%"align="right" style="margin-left:10px">

<span><h3 id="Reading Process ex">Reading Process</h3></span>

<p>

When Input, Template, polymerase, nickase, and dNTP are all mixed together in the same solution, this process produces Signal.

<br>

</p>

<p>

The result of electrophoresis is shown right. The band of Signal appeared in lane2 and lane3 by the function of polymerase and nickase. Reading P(p)rocess works properly at 37 &#08451; ~45 &#08451; .<br>

</p><br />

<p>

Detailed experimental conditions are given in <a href="protocol.html#Reading Process">Materials and Methods</a><br>

<br clear="right">

</p>

<div style="text-align:right">

Fig.3 Confirmation of Reading process<br>

<font size="-2">\* Other components include Input/Template complex, polymerase, nickase, and dNTP.</font>

</div>

<span><h3 id="Releasing Process ex" style="margin-top:15px">Releasing Process</h3></span>

<img src="images/experiment/experiment releasing process eido\_1.0.png" width="35%" align="right" style="margin-left:10px">

<p>

In this process, Signal hybridizes (at) with the toehold of transduction, and then it initiates polymerization, resulting in releasing of Output.<br>

</p>

<p>

The result of electrophoresis is shown in Fig.4. Output did not appear in lane1 when no Signal applied. Under the presence of Signal, Output appeared (lane2 and 3). From these results, we conclude that Releasing process works properly. Releasing P(p)rocess works at 37 &#08451; ~ 45 &#08451; .<br>

</p>

<p style="margin-bottom:100px">

Detailed experimental conditions are given in <a href="protocol.html#Releasing Process">Materials and Methods</a><br>

</p>

<clear="right">

<div style="text-align:right">

<br>

<br>

<br>

Fig.4 Confirmation of Releasing process<br />

<font size="-2">\* Other components include Output transducer, polymerase, and dNTP.</font>

</div>

<span><h3 id="Updating Process ex" style="margin-top:20px">Updating Process</h3></span>

<img src="images/experiment/experiment\_updating\_process\_eido\_1.0.png" width="35%" align="right" style="margin-top:60px; margin-left:10px;"><br />

<p>

In this process, Signal hybridizes (at)with the toehold of Gate, and then it initiates polymerization, resulting in the releasing of Updater. Then, recognition sequence is formed by the hybridization between Updater and Input, and then EcoRI cleaves the Input.<br>

</p>

<p>

The result of electrophoresis is in Fig.5. In lane1 and 2, we verified that Updated complex and Waste were generated when Signal is applied.<br>

</p>

<p>

Detailed experimental conditions are given in <a href="protocol.html#Updating Process">Materials and Methods</a><br>

</p>

<clear="right">

<br>

<br>

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<br>

<br>

<div style="text-align:right">

Fig.5 Confirmation of Updating process<br>

<font size="-2">Other components include Input, Gate, polymerase, EcoRI, and dNTP.</font>

</div>

<span><h3 id="Combination of 3 processes ex" style="margin-top:20px">Reaction speed measurement</h3></span>

<p>

Here, we evaluated the speed of releasing the first Output. The release of the first Output (Output-A) by Input-AB, was confirmed by a gel shift ass(e)ay. Output-A is modified with fluorescent molecule TAMRA. Before stained by SYBR Gold, a band contains Output-A (Transducer, Output) is observed.<br>

<br>

<div align="center">

<img src="images/experiment/experiment es first output electrophoresis 01\_1.0.png" width="60%"><br>

Fig.6 Result of electrophoresis to confirm the first output releasing (before SYBR Gold staining)<br>

<br>

</div>

<p>

Fluorescent intensity of Output was increased until it (is)was saturated around 10 minutes.<br>

<br>

</p>

<div align="center">

<img src="images/experiment/experiment es first output electrophoresis 02\_1.0.png" width="60%"><br>

Fig.7 Result of electrophoresis to confirm the first output releasing (after SYBR Gold staining)<br>

<br>

</div>

<p>

When reaction time is over 20 minutes, Signal band emerged in Fig.7 because Output is released by excess amount of Signal. This Signal will be used in the next Updating process. <br>

</p>

<p>

Detailed experimental conditions are given in <a href="protocol.html#Combination of 3 processes">Materials and Methods</a><br>

</p>

</section>

<section id="contents04">

<span><h1>Enzyme-free device</h1></span>

<hr></hr>

</section>

<section id="contents05">

<span><h2 id="efd Whole device ex">Functionality</h2></span>

<p>

To confirm the functionality of the Enzyme-free device, we have designed the following experiments. The first experiment measures the intensity of Output-A modified with FAM. We prepared Input-AB and Input-BA. The order of releasing is changed depending on the Inputs. Fig.8 shows the time development of Output-A.<br>

</p>

<div align="center">

<img src="images/experiment/experiment efs whole device spectro together\_1.0.png" width="60%"><br>

Fig.8 The intensity of Output-A (FAM) measured by spectrofluorometer.<br> <font size="-1">The fluorescence wavelength is 520nm. For quantitative analysis, the highest intensity data is defined as 100%, and the lowest was defined as 0 %.</font><br>

</div>

<br>

<p>

According to the plot, the intensity of Output-A was increased later when it was coded as the second section than when coded as the first. Therefore, this result shows our device releases Outputs based on the order coded on Input. The time difference between (the first and the second releasing) Output-A derives from Input-AB and Input-BA is about 40 minutes.<br>

We discuss this result along with simulation in <a href="discussion.html">Discussion page</a>.

</p>

<p>

Experimental conditions are written in <a href="protocol.html#efd Whole device">Materials and Methods</a><br>

</p>

</section>

<section id="contents06">

<span><h2>Testing component reactions</h2></span>

<p>

We have checked the function of one Seesaw gate by fluorescence measurement and electrophoresis.<br>

<br>

</p>

<span><h3 id="Measurement of fluorescence ex">Measurement of fluorescence</h3></span>

<p>

We quantify Output released from Transducer. We prepared a DNA modified with BHQ that hybridizes with Output.

<!--

Click <a href>here</a> for more information about FRET.

-->

Input is A then B (is used). The final concentration of all components (Input, Transducer, Gate, Trigger) were 100nM.<br>

Time evolution of fluorescence is shown in Fig.9. We added Trigger into the solution at 0 [s].<br>

<br>

<div align="center">

<img src="images/experiment/experiment efs seesaw 1stoutputdetecting spectro\_1.0.png" width="65%"><br>

Fig.9 Time (change) evolutionに揃える of Output-A measured by spectrofluorometer<br>

</div>

</p>

<p>

For quantitative analysis, we define the fluorescence intensity of DNA modified FAM as 100%, and the level of the solution with Input, Transducer, and Gate as 0%. After adding Trigger, fluorescence increased←逆→ gradually. The increase of fluorescence stopped around 1700sec. The reaction ends at this point. About a half of Output strands were released according to the fluorescence measurement.<br>

</p>

<p>

Detailed experimental conditions are given in <a href="protocol.html#Measurement of fluorescence">Materials and Methods</a><br>

<br>

</p>

<span><h3 id="Electrophoresis ex">Electrophoresis</h3></span>

<p>

We also evaluated the ratio of first Output by electrophoresis to confirm the function of the Seesaw gate.<br>

The result of electrophoresis is shown in Fig.10.<br>

<div align="center">

<img src="images/experiment/experiment efs seesaw 1stoutputdetecting eido\_1.0.png" width="40%"><br>

Fig.10 Confirmation of Seesaw gate by electrophoresis<br>

</div>

<br>

Two bands appeared on lane1 and lane3. The upper one is Transducer, and the lower one is Output. The fluorescence intensity of Output in lane1 with Trigger is stronger than that in lane3. Fig.11 shows the fluorescence intensity of 2 bands in lane 1 and 3.<br>

</p>

<!--

<div align="center">

<img src="images/experiment/experiment efs seesaw 1stoutputdetecting eido intensity analysis lane2\_1.0.png" width="650"><br>

fig. Lane1??u?????x????<br>

<img src="images/experiment/experiment efs seesaw 1stoutputdetecting eido intensity analysis lane4\_1.0.png" width="650"><br>

fig. Lane3??u?????x????<br>

</div>

-->

<br>

<div align="center">

<img src="images/experiment/experiment efs seesaw 1stoutputdetecting eido intensity analysis table\_1.0.png" width="40%"><br>

Fig.11 Band intensity of Transducer and Output<br>

</div>

<br>

<p>

The intensity of Output increased by 83.0%, and Transducer decreased 83.0% when Trigger was added. We conclude that Output is released by adding Trigger.<br>

</p>

<p>

Detailed experimental conditions are given in <a href="protocol.html#Electrophoresis">Materials and Methods</a><br>

</p>

</section>

</article>

</div>

</div>

<script src="scripts/scroll-up-bar.js"></script>

<script>

$('#globalheader').scrollupbar();

</script>

<footer>

<div id="pageinfo">

<a href="mailto:teamsendai2014@gmail.com" id="info\_mail"><img src="images/icon\_mail.png" width="18" height="12" alt="mail">TeamSendai2014@gmail.com</a><br />

<span id="info\_copyright">Copyright c 2014 Biomod 2014 team Sendai All rights reserved.</span>

<span id="info\_lab"><a href="http://www.molbot.mech.tohoku.ac.jp/eng/index.html" target=?h\_blank?h><img src="images/Muratalab-icon2\_dark-01.png" height="60"></a></span>

</div>

</footer>

</div>

</body>

</html>