

HIV-1 のジェノタイピングによる 薬剤耐性変異の同定

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✓次世代シーケンサー（NGS）を使用したウイルスゲノムのシーケンスにより、高効率でウイルスのジェノタイピングおよび薬剤耐性変異の同定を行うことが可能になった。また、従来のサンガー法では検出しにくかった低頻度の変異の発見が可能になった。

✓一方で、NGSから出力されたデータの解析には、さまざまなプロセスがあり、多少の技能が必要。

<NGS解析の一般的なプロセス>

- Fastqデータの処理
- リファレンスへのリードマッピング
- 変異検出
- ジェノタイピング
- 各種ガイドラインに基づいた薬剤耐性変異の同定

✓ウイルスジェノタイピング用ソフトウェアDeepChek® では、NGSから出力されたFastqファイルを指定するだけで、以下のような解析プロセスを一度に実行可能。

出力結果の例

✓サブタイピング結果

✓ゲノム中の各領域について、各ガイドラインに基づいて
Resistance, **Possible Resistance**, **Susceptible**
の3段階で薬剤耐性の評価が可能

✓ガイドラインは、
HIVdb/Stanford、ANRS等主要なガイドラインに対応

✓Geno2phenoに基づき、向性もレポート

✓上記のようなタイピング結果の他、
NGSデータの統計も含んだPDFレポートを出力

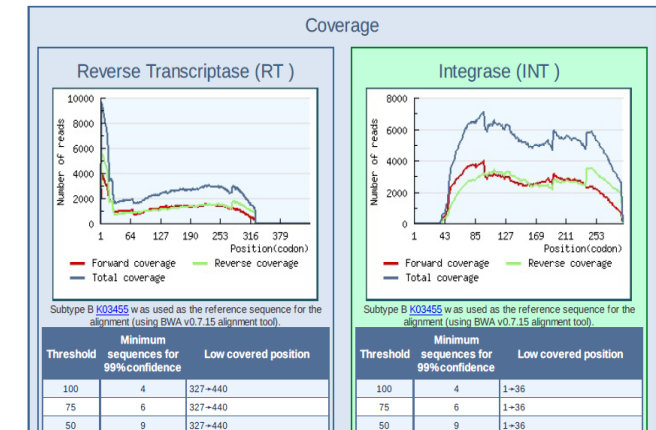
Nucleocapsid			
Genotype	Prevalence (1)	Number of reads	Similarity (2)
08_BC	42.52%	344	94.89%
08_BC or C	7.42%	60	94.48%
62_BC	7.05%	57	94.41%
Total count : 809			

Protease			
Genotype	Prevalence (1)	Number of reads	Similarity (2)
108_BC	37.41%	4696	93.91%
C	15.76%	1978	95.28%
86_BC	11.01%	1382	95.26%
86_BC or C	7.08%	889	95.15%
Total count : 12554			

Reverse Transcriptase			
Genotype	Prevalence (1)	Number of reads	Similarity (2)
C	17.34%	1573	95.06%
86_BC	14.9%	1352	95.33%
31_BC	10.58%	960	96.32%
86_BC or C	7.19%	652	95.32%
88_BC	5.55%	504	94.13%
Total count : 9073			

Integrase			
Genotype	Prevalence (1)	Number of reads	Similarity (2)
65_cpx	13.15%	860	95.74%
108_BC	11.16%	730	97.05%
10_CD	10.82%	708	95.98%
49_cpx	7.86%	514	94.7%
C	5.53%	362	95.55%
Total count : 6541			

HIV Nucleoside Reverse Transcriptase Inhibitors						
	Algorithm	100	75	50	25	5
Abacavir	ANRS	S	S	S	S	S
	Grade	S	S	S	S	S
	Rega institute	S	S	S	S	S
	RIS	S	S	S	S	S
	HIVdo	S	S	S	S	S
	RenaGeno	S	S	S	S	S
Didanosine	ANRS	NA	NA	NA	NA	NA
	Grade	NA	NA	NA	NA	NA
	Rega institute	S	R	R	R	R
	RIS	NA	NA	NA	NA	NA
	HIVdo	S	S	S	S	S
	RenaGeno	S	I	I	I	I
Emtricitabine	ANRS	S	S	S	S	S
	Grade	S	S	S	S	S
	Rega institute	S	S	S	S	S
	RIS	S	S	S	S	S
	HIVdo	S	S	S	S	S
	RenaGeno	NA	NA	NA	NA	NA



The screenshot shows the DeepChek-HIV v2.0 web interface. The browser address bar displays <https://distributor.ablisa.com/deepchk2/HIV/home>. The page title is "DeepChek[®]-HIV v2.0 : The HIV Genotyping Software & Database Solution".

The interface includes a sidebar on the left with the user's name "Jumpel Ikenaga" and last login time "17/10/2023 09:12:52". The sidebar lists various analysis modules: DeepChek[®] CMV, HBV, HCV, HIV, HPV, HSV, BacterioChek[®] TB, ViroScore, User Settings, and Logoff.

The main content area has a navigation bar with "Results List", "Start", "Tools", "Support", and "Settings". Below this, there are buttons for "New Analysis", "New Largescale", and "New UltraGene".

The "Results List" table contains the following data:

Performed date	Sample date	Pool	Patient data	SampleID	Status	
07/09/2023 09:19:47	07/09/2023	Filgen-Demo pool		a1	Uncomplete	
06/09/2023 03:31:17	05/09/2023	Filgen-Demo pool		2030850725_S6_L001_R1_001.fastq.gz	Done	
06/09/2023 03:31:17	05/09/2023	Filgen-Demo pool		3023390646_S4_L001_R1_001.fastq.gz	Done	
06/09/2023 03:16:08	05/09/2023	Filgen-Demo pool		c	Uncomplete	
06/09/2023 03:04:35	05/09/2023	Filgen-Demo pool		ab	Uncomplete	
06/09/2023 02:56:00	05/09/2023	Filgen-Demo pool		a	Done	
29/06/2023 04:31:53	29/06/2023	Filgen-Demo pool		6	Uncomplete	
28/06/2023 06:55:52	28/06/2023	Filgen-Demo pool	File ID: 1 Full name:	5	Done	
28/06/2023 06:43:37	28/06/2023	Filgen-Demo pool		4	Done	
28/06/2023 06:36:15	28/06/2023	Filgen-Demo pool		3	Done	

At the bottom of the table, there is a pagination control: "Go to page: 1 Row count: 10" and "Showing 1-10 of 12".

The footer of the page contains the URL <https://distributor.ablisa.com/deepchk2/HIV/analysis/new> and the copyright notice: "All content © 2023, ABL, SA and TherapyEdge, Inc. - Terms of use - Privacy and security policy - Version v3.30.112-hdr".

Start > New Analysisを選択

The screenshot shows the DeepChek HIV v2.0 web interface. The browser address bar indicates the URL is <https://distributor.ablisa.com/deepchek2/HIV/analysis/new>. The page title is "DeepChek[®]-HIV v2.0 : The HIV Genotyping Software & Database Solution". The navigation menu includes "Results List", "Start", "Tools", "Support", and "Settings". The main content area is titled "DeepChek[®] HIV / Create new analysis" and shows a progress bar with four steps: "Input Configuration" (active), "Sample information", "Setup report", and "Results".

The "Input Configuration" step is divided into two main sections:

- Your Dataset:** A search bar and a list of datasets. The "Filgen-Demo pool" is selected, showing a "Raw Seq" entry: "2030850725_S6_L001_R1_001.fastq.gz". This section is highlighted with a red box and labeled "データプール" (Data Pool).
- Analysis configuration:** An "Input" field with a dashed blue box and the instruction "Select an item from your dataset & Drag here to analyse". Below this are checkboxes for "Add a Sanger comparative analysis" and "Remove files from library after analysis". The "Features" section includes dropdown menus for "Subtyping characterization" (Local similarity testing), "Manual subtype" (01_AE), "Genotyping analysis" (Variant population analysis), "NGS Alignment Engine" (BWA v0.7.15), and "Settings profile" (None). A "Homopolymer correction" checkbox is also present.

A green arrow points from the "Your Dataset" section to the "Input" field in the "Analysis configuration" section.

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データプールの+ボタンをクリックし、Fastqなどのデータを登録する

データのフォーマットの指定

データ形式
Fastq/Fasta/BAMなど
シーケンスプラットフォーム

カバー領域

登録するデータに応じて、フォーマットなどをプルダウンメニューから
選択し、そのデータがカバーする領域を指定する

アルゴリズムの指定

DeepChek[®]-HIV v2.0 : The HIV Genotyping Software & Database Solution

Results List Start Tools Support Settings

DeepChek[®] HIV / Create new analysis

ドラッグ&ドロップ

**サブタイピングアルゴリズム
Comet/BLASTなど
マッピングアルゴリズム
BWA/TMAPなど**

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データプールから、右のエリアにデータをドラッグ&ドロップし、サブタイピング、リードマッピング等のアルゴリズムを選択する

The screenshot shows the DeepChek®-HIV v2.0 web interface. The browser address bar displays the URL: <https://distributor.abl.com/deepchek2/HIV/analysis/next/03-d72ef1f5-6d71-11ee-85c5-0242ac170002>. The page title is "DeepChek®-HIV v2.0 : The HIV Genotyping Software & Database Solution".

The interface features a navigation bar with "Results List", "Start", "Tools", "Support", and "Settings". A progress indicator shows four steps: "Input Configuration", "Sample information" (current step), "Setup report", and "Results".

The "Sample information" section is divided into three tabs: "Identification", "Clinical data", and "HealthCare providers". The "Identification" tab is active and contains three sub-sections:

- General identifiers:** Includes a "Pool *" dropdown menu set to "Filgen-Demo pool" and a "Project" dropdown menu.
- Sample information:** Includes "Your sample ID *" (text input: "Webinar1"), "Alternative ID" (text input), "Date of sample *" (text input: "18/10/2023"), "Type of sample *" (dropdown: "Other"), "Reason for genotyping analysis" (dropdown: "Missing data"), and "Other type of sample" (text input).
- NGS details:** Includes "Date of sequencing *" (text input: "18/10/2023"), "Sequencing platform" (dropdown: "Missing data"), "NCS Method" (dropdown), "Plate ID" (text input), "Reagent expiration date" (text input: "dd/mm/yyyy"), "Processing software" (dropdown: "Missing data"), "Assay version" (text input), and "Cartridge S/N" (text input).

A "Notes" section with a text area is located at the bottom of the "Identification" tab. At the bottom of the page, there are navigation arrows and a copyright notice: "All content © 2023, ABL, SA and TherapyEdge, Inc. - Terms of use - Privacy and security policy - Version v3.30.112-ivr".

データのIDや検体のIDを登録する

変異検出パラメーターの指定

The screenshot shows the DeepChek HIV v2.0 web interface. The browser address bar indicates the URL: <https://distributor.ablisa.com/deepchek2/HIV/analysis/edit/03-d72ef1f5-6d71-11ee-85c5-0242ac170002#group-GeneralInformation>. The page title is "DeepChek[®]-HIV v2.0 : The HIV Genotyping Software & Database Solution".

The interface includes a sidebar with the user's name "Junpei Ikenaga" and a list of menu items: DeepChek[®] CMV, DeepChek[®] HBV, DeepChek[®] HCV, DeepChek[®] HIV, DeepChek[®] HPV, DeepChek[®] HSV, BacterioChek[®] TB, ViroScore, User Settings, and Logout.

The main content area is titled "DeepChek[®] algorithms version" and shows a dropdown menu set to "13.2". Below this is the "Expert system options" section, which contains several configuration options:

- Noisy filtering threshold: 3 %
- Forward/Reverse unbalanced coverage filtering:
- Forward/Reverse unbalanced frequency filtering:
- Q-Score filtering:
- Confidence level defining the coverage limit *: 99 %
- Minimal read count *: 100 read
- Minimal coverage % *: 50 %
- Range of amino acid positions defined for validating drug resistance assessment: DeepChek[®] Genotyping - Whol...

Below these options is a table of amino acid positions with their respective minimum and maximum values:

Position	min	max
P17	1	132
P24	1	231
P7	1	55
PROT	1	99
RT	1	440
INT	1	288
GP120	1	511
GP41	1	345

Red annotations are present on the right side of the interface:

- "バリアントの最低頻度" (Minimum frequency of variants) points to the "Noisy filtering threshold" field.
- "ペアリード間でのバランスに関するフィルター" (Filter related to balance between paired reads) points to the "Forward/Reverse unbalanced coverage filtering" and "Forward/Reverse unbalanced frequency filtering" checkboxes.
- "最低カウント" (Minimum count) points to the "Minimal read count" field.
- "最低カバレッジ" (Minimum coverage) points to the "Minimal coverage" field.

At the bottom of the page, there is a copyright notice: "All content © 2023, ABL, SA and TherapyEdge, Inc. - Terms of use - Privacy and security policy - Version v3.30.112-ivr".

リードのフィルタリングのThreshold等を指定

ガイドラインの指定

DeepChek[®]-HIV v2.0 : The HIV Genotyping Software & Database Solution

Results List Start Tools Support Settings

Algorithms

Algorithms

- ANRS
- Grade
- HIVDb
- Rega institute
- RegaGeno
- RIS
- V3loop Tropism determination (G2P)

Normalized interpretation (SIR) Original interpretation (5 levels)

ANRS
Grade
HIVdb
Rega institute
RegaGeno
RIS
geno2pheno (向性の判定)

Mutations options

Display: All Mutations

Highlight Mutations of Interest:

Mutations of interest definition: RIS 2020

Report Sections

Display sections on report

- Drug Resistance Determination
- Mutation Analysis
- Subtyping
- Expert System
- Mutation Notes

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薬剤耐性判定のガイドラインを指定（複数指定可）

DeepChek[®]-HIV v2.0 : The HIV Genotyping Software & Database Solution

Jumpel Ikemaga
last login : 18/10/2023
08:47:29

- DeepChek[®] CMV
- DeepChek[®] HBV
- DeepChek[®] HCV
- DeepChek[®] HIV
- DeepChek[®] HPV
- DeepChek[®] HSV
- BacterioChek[®] TB
- ViroScore
- User Settings
- Logout

Results List Start Tools Support Settings

Results analysis
Result batch
Result UltraGene Refresh results

Performed date	Sample date	Pool	Patient data	SampleID	Status	
18/10/2023 07:12:34	18/10/2023	Filgen-Demo pool		Webinar3	Uncomplete	🔍 🗑️
18/10/2023 07:07:20	18/10/2023	Filgen-Demo pool		webinar2	Done	🔍 🗑️
18/10/2023 07:00:23	18/10/2023	Filgen-Demo pool		Webinar1	Done	🔍 🗑️
18/10/2023 02:05:41	18/10/2023	Filgen-Demo pool		2030850725_S6_L001_R2_001.fastq.gz	Done	🔍 🗑️
18/10/2023 02:05:41	18/10/2023	Filgen-Demo pool		2030850725_S6_L001_R1_001.fastq.gz	Done	🔍 🗑️
17/10/2023 09:50:41		Filgen-Demo pool			Uncomplete	🔍 🗑️


<< 1 >> Go to page: 1 Row count: 10 Showing 1-6 of 6







https://distributorabisa.com/deepchek2/HIV/result/list/analysis All content © 2023, ABL, SA and TherapyEdge, Inc... - Terms of use - Privacy and security policy - Version v3.30.112-ivr

解析終了後、Results Listから解析結果のレポートを確認する

Result ✕

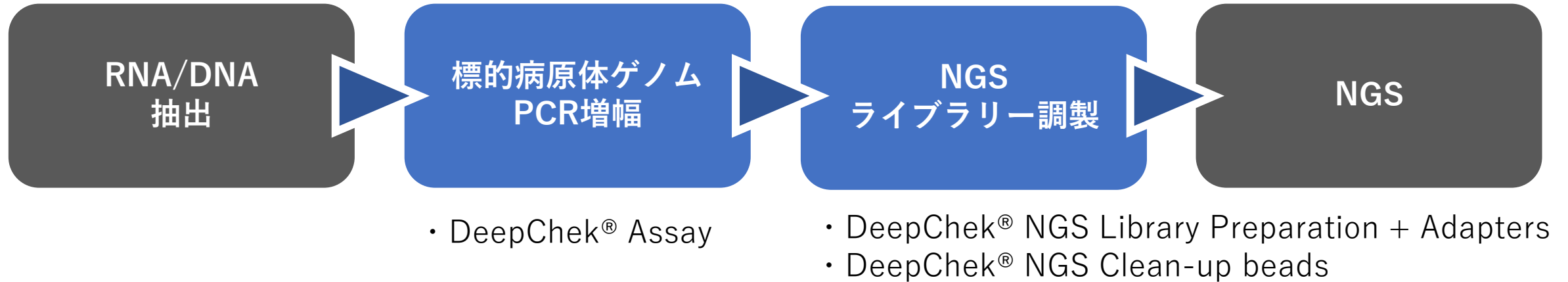
General Information | Data entry | **Reports**

 Upload a report

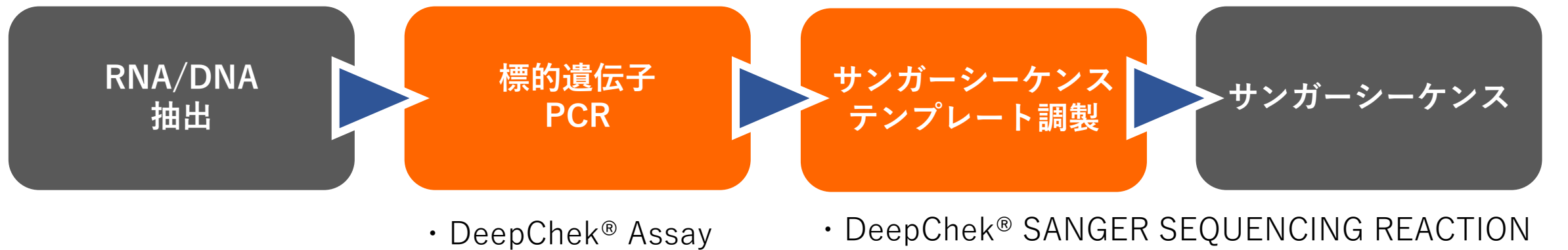
Created Date	Description	
18/10/2023 07:07:20	Quality information report (Nucleotide)	
18/10/2023 07:07:20	Quality information report (Amino Acid)	
18/10/2023 07:07:20	DeepChek report 2.0	 
18/10/2023 07:07:20	Clinical summary report (Onepage)	 

- ✓ ジェンタイピングや薬剤耐性変異を検出するためのNGSライブラリーの調製が可能
- ✓ Deepchek[®]ソフトウェアを用いたジェンタイピングや薬剤耐性変異の検出に最適
- ✓ HIV、HCV、HBV、CMV、HSV、BKV、インフルエンザウイルス、新型コロナウイルスに対応
- ✓ 細菌および真菌の菌叢解析も可能
 - ワークフロー
 - キット構成
 - シークエンス対応機種
 - 製品ラインアップ

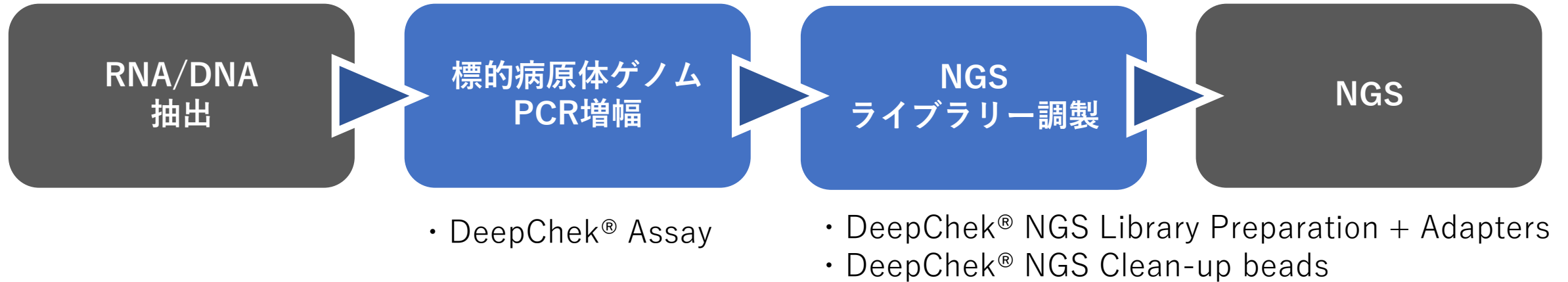
NGS



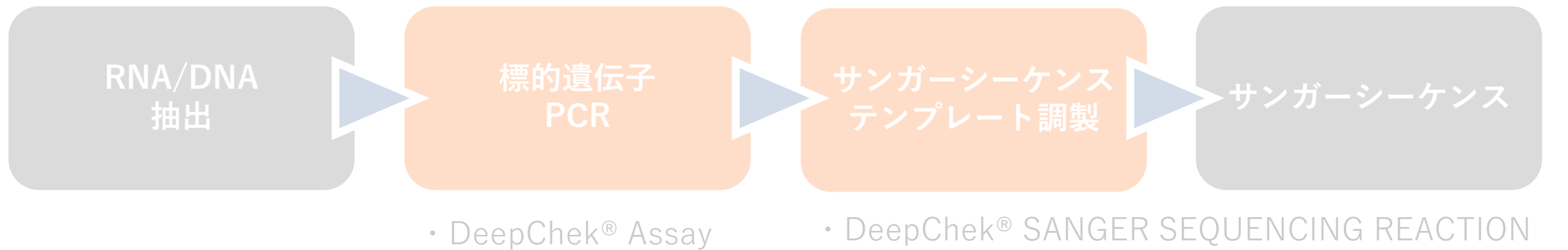
サンガーシーケンス



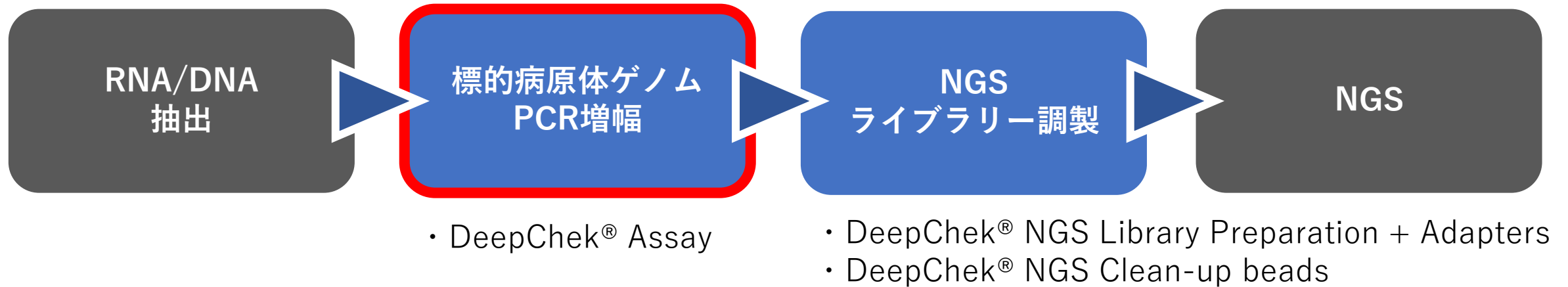
NGS



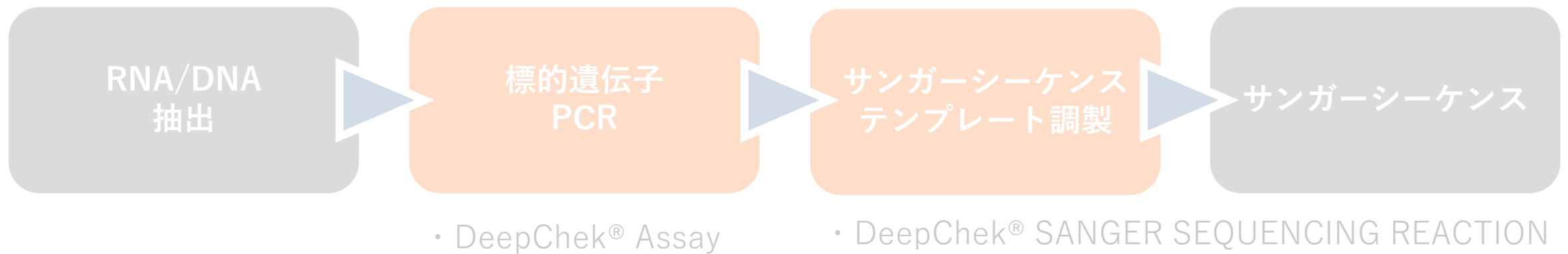
サンガーシーケンス



NGS

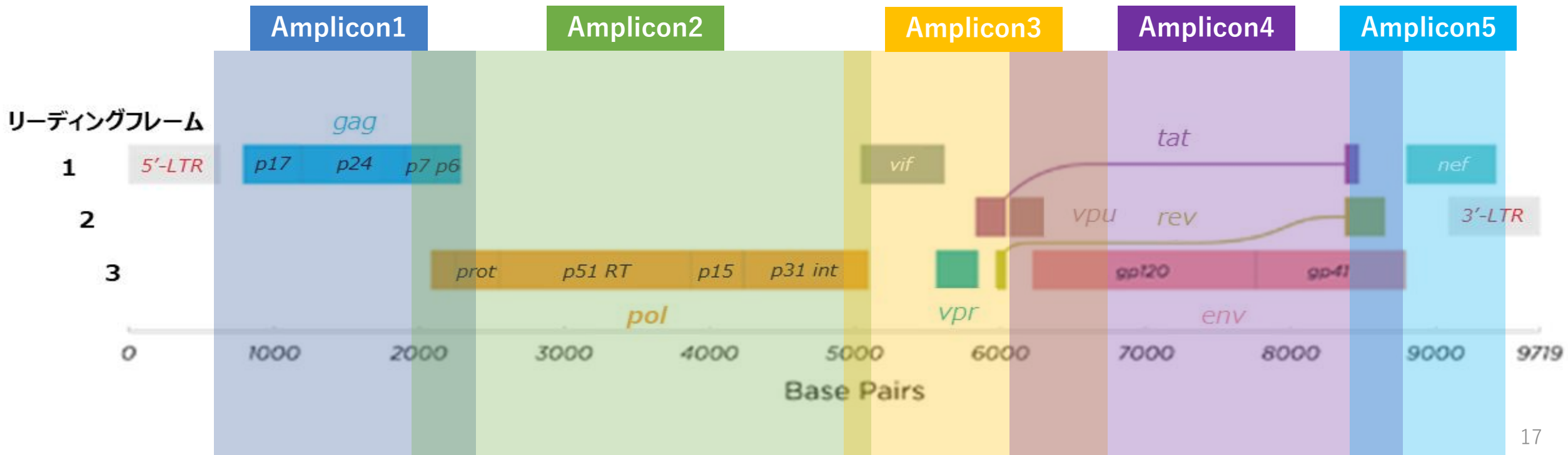


サンガーシーケンス

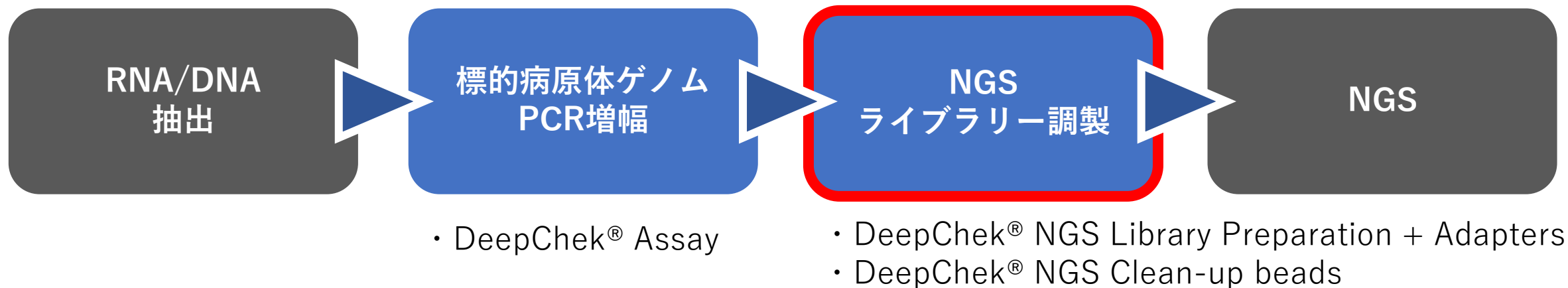


▶ DeepChek[®] Assay Whole Genome HIV-1 (対応解析法：NGS)

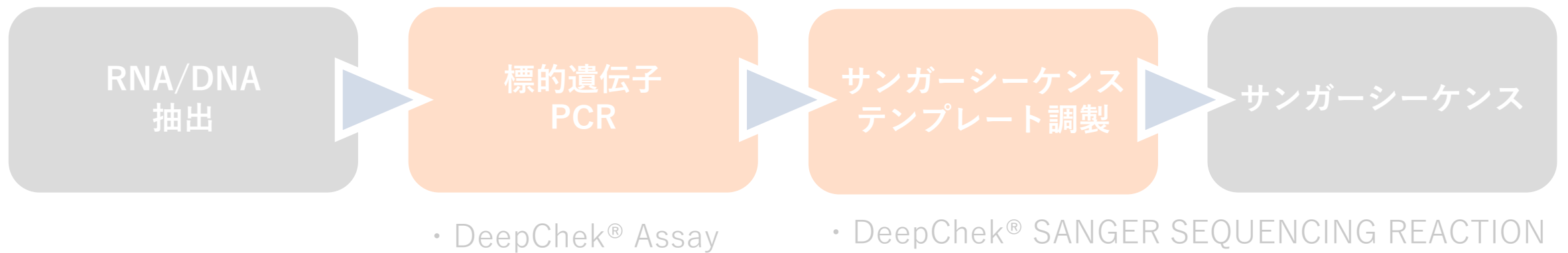
RT-PCR Buffer	RP Solution	BP Solution	RT Enzyme	H2O
PCR Master Mix	DMSO	Nested PCR Master Mix		
PCR FRAG 1	PCR FRAG 2	PCR FRAG 3	PCR FRAG 4	PCR FRAG 5
Nested PCR FRAG 1	Nested PCR FRAG 2	Nested PCR FRAG 3	Nested PCR FRAG 4	Nested PCR FRAG 5



NGS



サンガーシーケンス

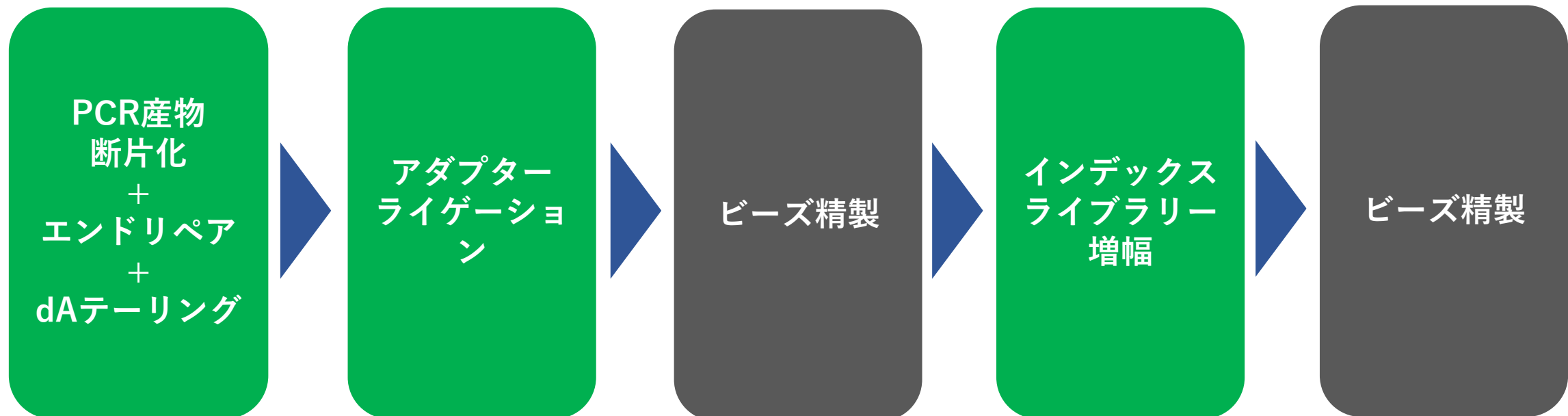


▶ DeepChek[®] NGS Library Preparation + Adapters

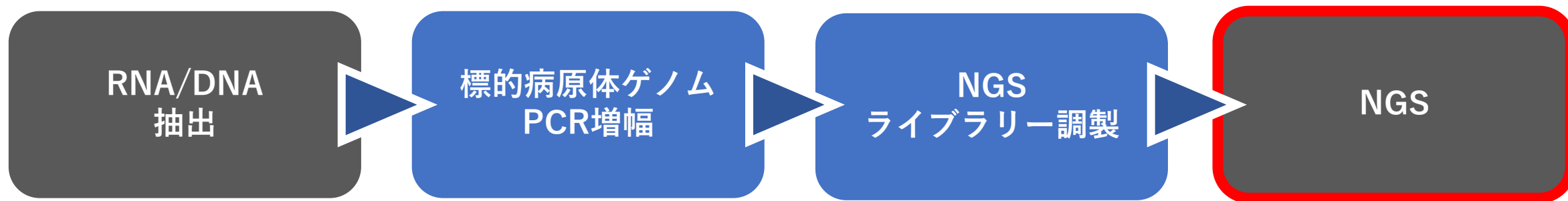
FAE Buffer	FAE Enzyme	Ligation buffer	DNA ligase	PCR Primer Mix
PCR Mix	DeepChek [®] ADAPTERS			

▶ DeepChek[®] NGS Clean-up beads (AMPure[®] XPで代用可能)

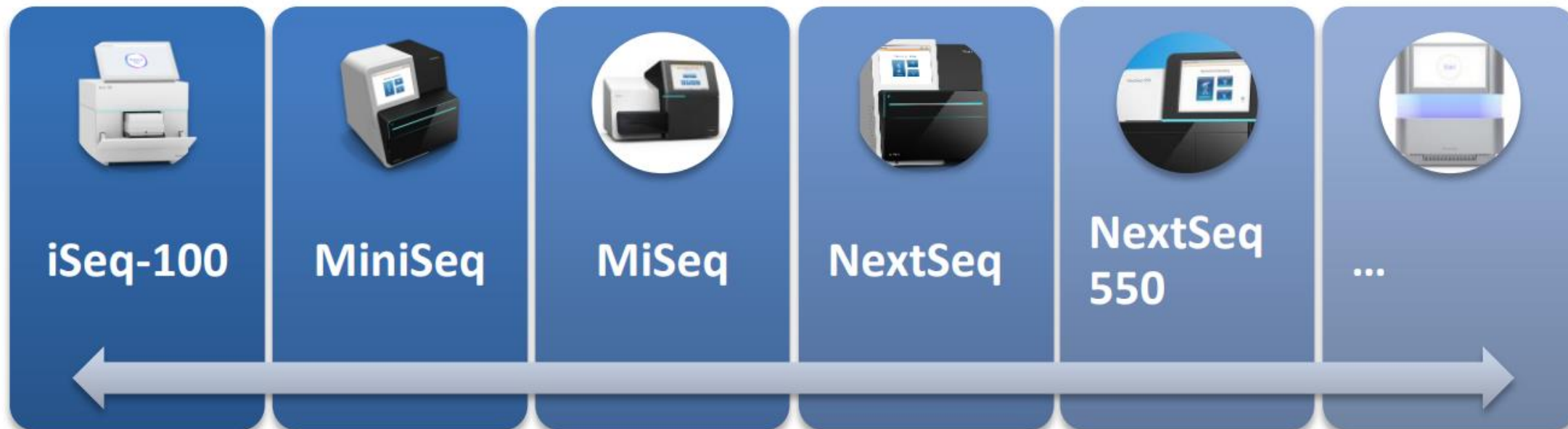
NGSライブラリー調製手順



NGS

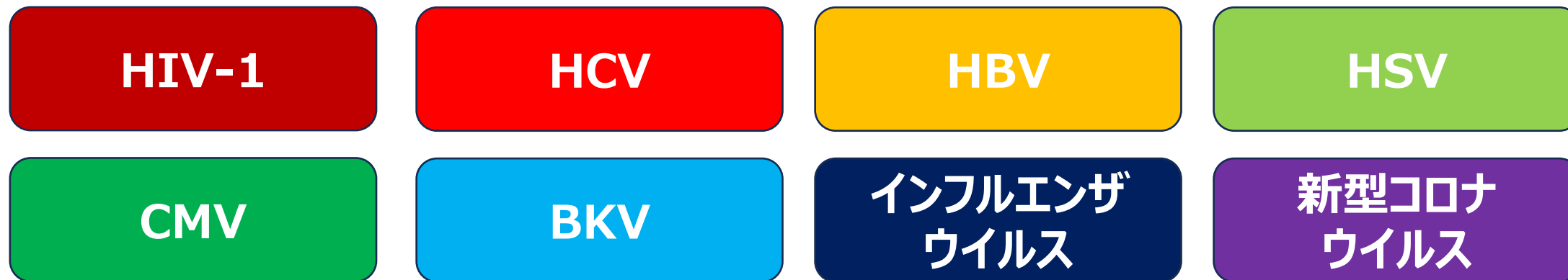


▶ illumina社製シーケンサー



※Ion Torrent、MGI、Nanopore用調製キットについても順次リリース予定

ジェノタイピング/薬剤耐性変異検出用 DeepChek[®] Assay



▶ ヒト免疫不全ウイルス1型 (HIV-1)

170A24	198A24	101B24 / 96	102C24 / 96	103A24	194A24 / 96
<ul style="list-style-type: none"> • Whole Genome 	<ul style="list-style-type: none"> • Protease • Reverse Transcriptase • Integrase 	<ul style="list-style-type: none"> • Protease • Reverse Transcriptase 	<ul style="list-style-type: none"> • Integrase 	<ul style="list-style-type: none"> • V3 Loop • Tropism 	<ul style="list-style-type: none"> • GP41 • GP120

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