

次世代シーケンサーを用いた HLA遺伝子の配列決定

細道一善

情報・システム研究機構

国立遺伝学研究所人類遺伝研究部門

統合データベース講習会: AJACS富山

2013年8月30日(金)

富山大学 杉谷キャンパス講義実習棟 3F情報処理室

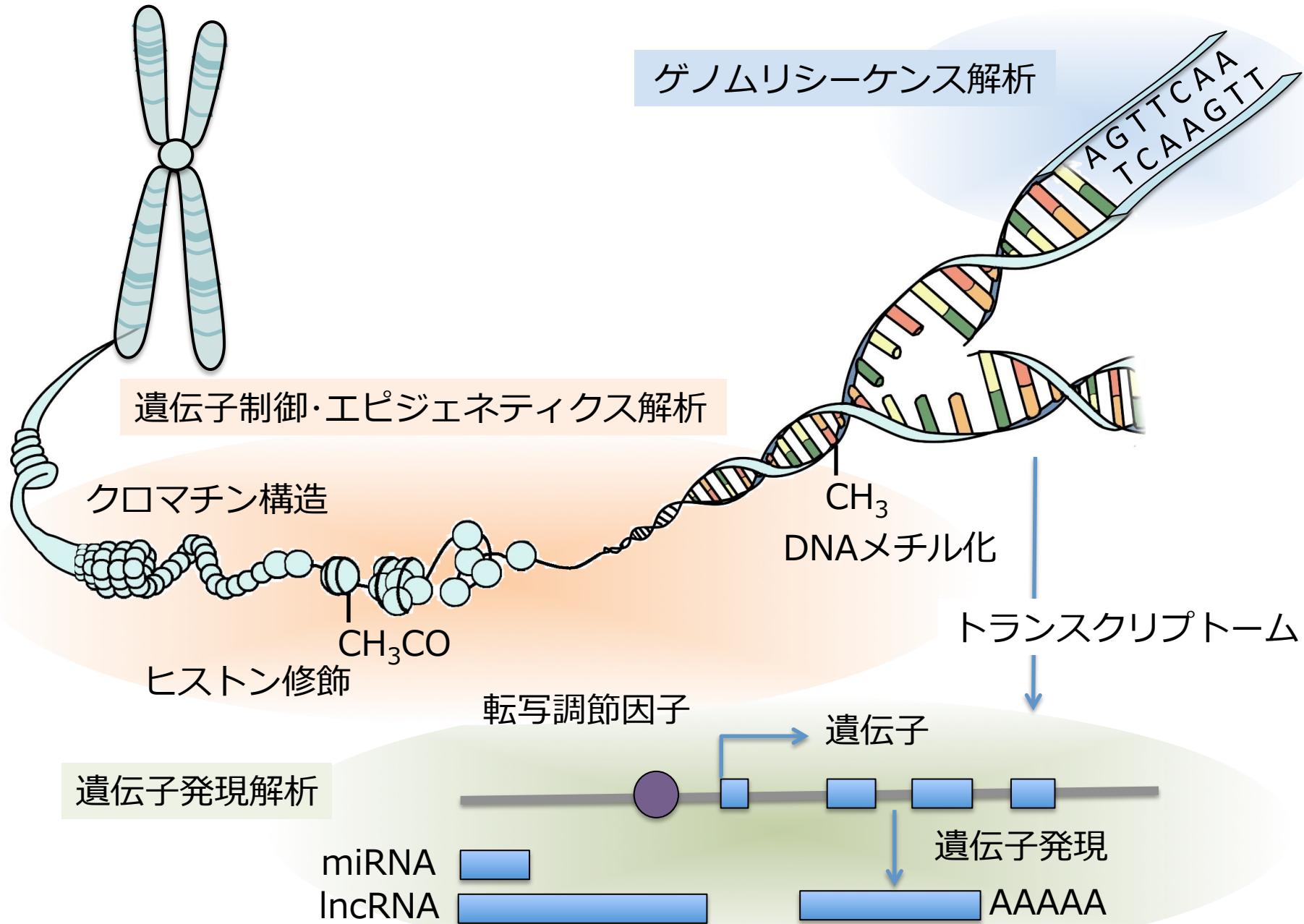
トピックス

- 次世代シークエンサーについて
- ロングPCRとNextera Kitによるシークエンス概要
- ライブラリ調整における改良事例
- HLA遺伝子解析の実例
- HLA遺伝子のデータ解析およびタイピング

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次世代シークンサーによる解析デザインと解析対象



次世代シーケンサーの比較

アプリケーション	イルミナ		ライフテクノロジーズ		ロシュ	
	ハイスループット HiSeq	ベンチトップ MiSeq	ハイスループット Ion Proton	ベンチトップ Ion PGM	ハイスループット GS FLX+	ベンチトップ GS Junior
ヒト全ゲノム解析	◎		○			
全エクソン解析	◎	○	○			
ターゲットリシークエンス	○	○	○	○	○	○
トランスクリプトーム	○	○	○	○		○
RNAシークエンス	○	○	○	○		
small RNAシークエンス	○	○	○	○		
ChIPシークエンス	○	○	○			
リード長(bp)	2x100 bp	2x250 bp	1x200 bp	1x200 bp 1x300 bp	700 bp	400 bp
仕様	データ量(bp)	600 Gb	8.5 Gb	60 Gb	1 Gb	700 Mb
	リード数	60億	3400万	2億	500万	100万
論文実績	2009年	2	0	0	0	
	2010年	66	1	0	0	2309
	2011年	244	13	0	5	71

ターゲットリシークエンス法

- ハイブリダイゼーションによる濃縮
 - TruSeq Custom Enrichment Kit (イルミナ)
 - SureSelect DNA Capture (アジレント)
 - Haloplex (アジレント)
 - SeqCap EZ choice library (ロシュ) など
- PCRアンプリコン
 - TruSeq カスタムアンプリコン (イルミナ)
 - Long-range PCR & Nextera kit

ロングPCRとNextera Kitによるシークエンス

次世代シーケンサー MiSeq



Nextera™ DNAサンプル調製キット



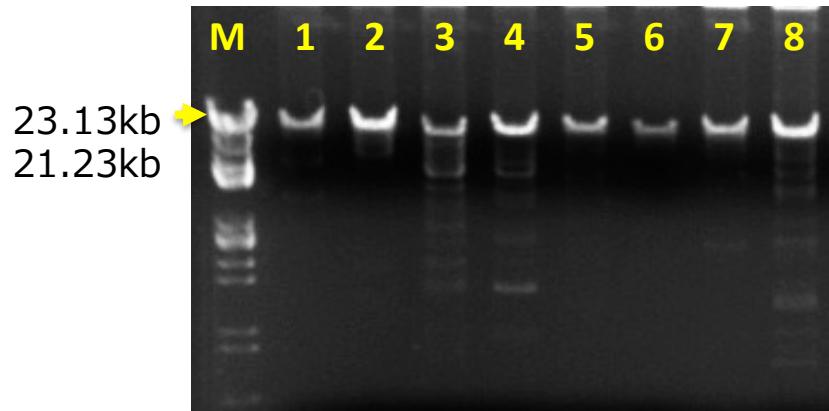
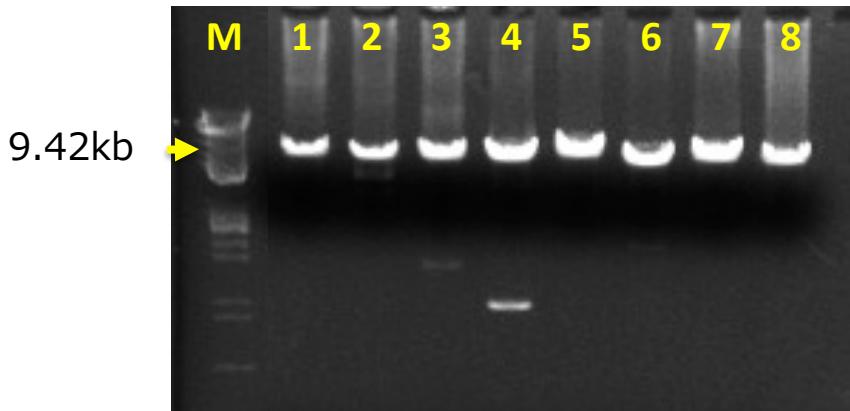
手軽に次世代シーケンサーを使う

- ・数十kb程度の領域(遺伝子)をシークエンスしたい
- ・数十検体のシークエンスをしたい
- ・PCR産物をクローニングしてシークエンスするような解析

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long-range PCRの增幅長 約10kb 約20kb



	増幅長(bp)	
1	11,513	23,986
2	11,078	21,459
3	10,071	20,623
4	10,196	23,870
5	12,327	23,406
6	10,127	21,972
7	10,438	22,776
8	10,335	21,306

long-range PCR Nextera 解析

～ 実験の流れ ～

1. ロングPCRプライマー設計
2. ロングPCR増幅
3. Nextera kitによるライブラリー調整
4. MiSeqによるラン

ロングPCRプライマー設計

- プライマー設計の条件
 - Tm値 68°C
 - 塩基数 26mer
 - プライマー配列がゲノム内でユニーク
 - プライマー設計位置にSNPが無い
- ロングPCR産物の長さ
 - 10kb-20kb (DNAの質にもよる)

PCR条件

DNA(20ng)	0.5	μl	94 °C	2 min	
5 x Buffer	2	μl	98 °C	10 sec	30cycles
dNTP Mixture	0.8	μl	68 °C	5 min*	
Primer F 5uM	0.4	μl	4 °C	∞	
Primer R 5uM	0.4	μl			
PrimeSTAR GXL	0.4	μl	※ 増幅長約20kbの場合は10min		
H ₂ O	5.5	μl			
total	10.0	μl			

PCR酵素

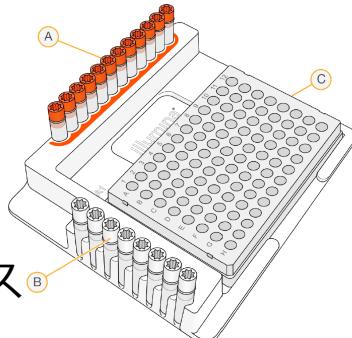
TaKaRa PrimeSTAR® GXL DNA Polymerase R050A
TOYOBO KOD FX KFX-101 など

Nextera DNA Sample Preparation Kit によるライブラリ調整

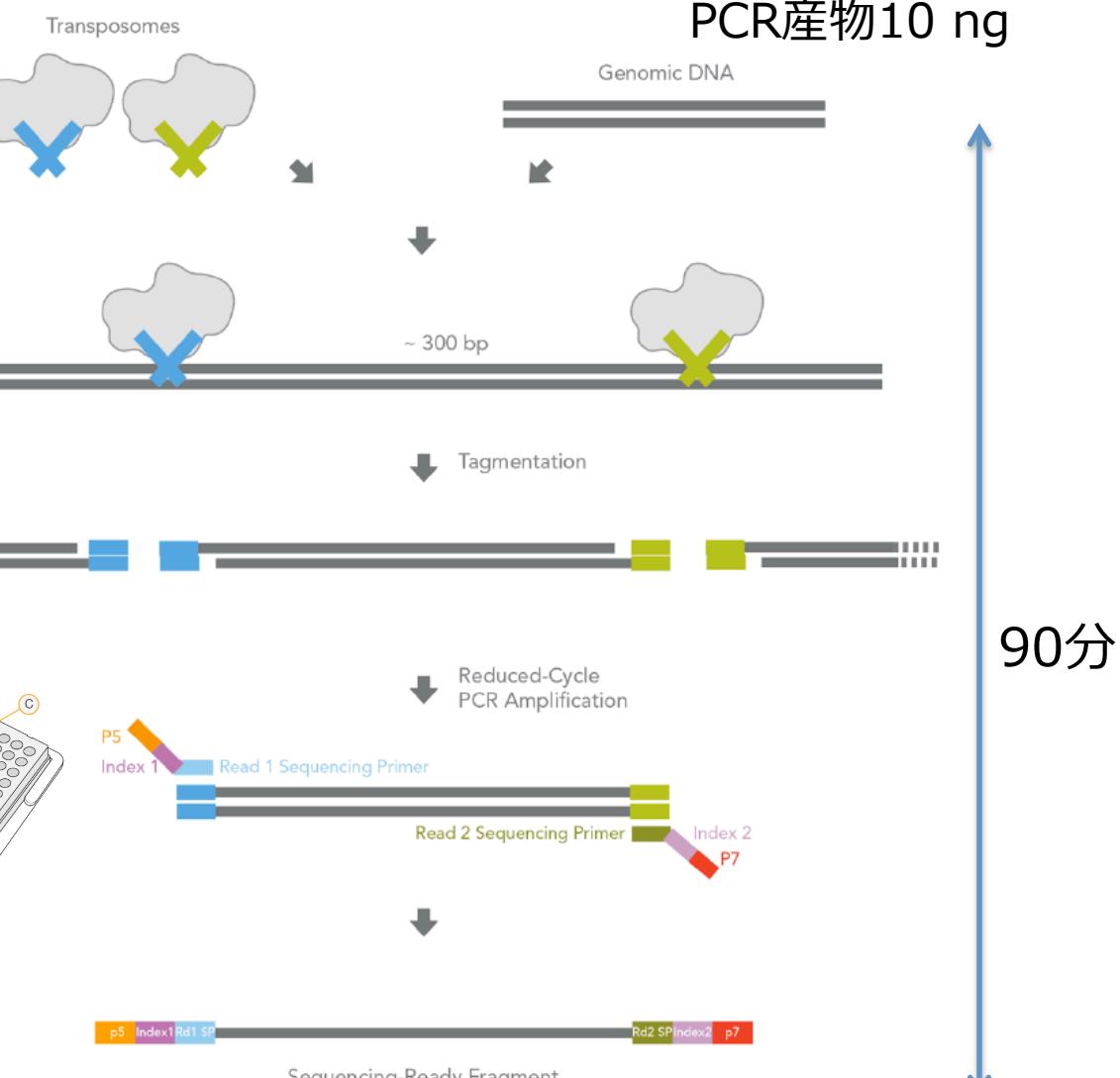
Nextera transposome と
アダプターのテンプレート
DNAへの結合

断片化とアダプターの結合
(Tagmentation)

最大96サンプル
のマルチプレックス



PCR によるIndexと
アダプター配列の追加



Library length : 300bp ~ >1.2kb

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Nextera kitによるライブラリー調整 (標準プロトコール)

ロングPCR増幅



Tagmentation

PCR産物(50ng)	20ul	55°C	5min
TD	25ul	10°C	∞
TDE1	5ul		

精製 Zymo-Spin1-96plate



ライブラリーのPCR増幅

Index1 primer	5ul	72°C	3min
Index2 primer	5ul	98°C	30sec
NPM	15ul	98°C	10sec
PPC	5ul	63°C	30sec
Tagmentation DNA	20ul	72°C	3min
		10°C	∞

5cycles

精製 AMPure XP bead

Nextera kitによるライブラリー調整 (1/5量プロトコール)

ロングPCR増幅



Tagmentation

PCR産物(10ng)	4ul	55°C	5min
TD	5ul	10°C	∞
TDE1	1ul		

精製 Zymo-Spin1-96plate



ライブラリーのPCR増幅

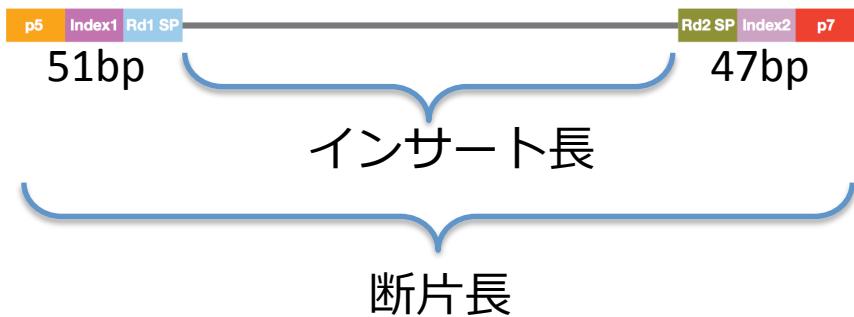
Index1 primer	1ul	72°C	3min
Index2 primer	1ul	98°C	30sec
NPM	3ul	98°C	10sec
PPC	1ul	63°C	30sec
Tagmentation DNA	4ul	72°C	3min
		10°C	∞

7cycles

精製 AMPure XP bead

Nexteraライブラリー長の最適化

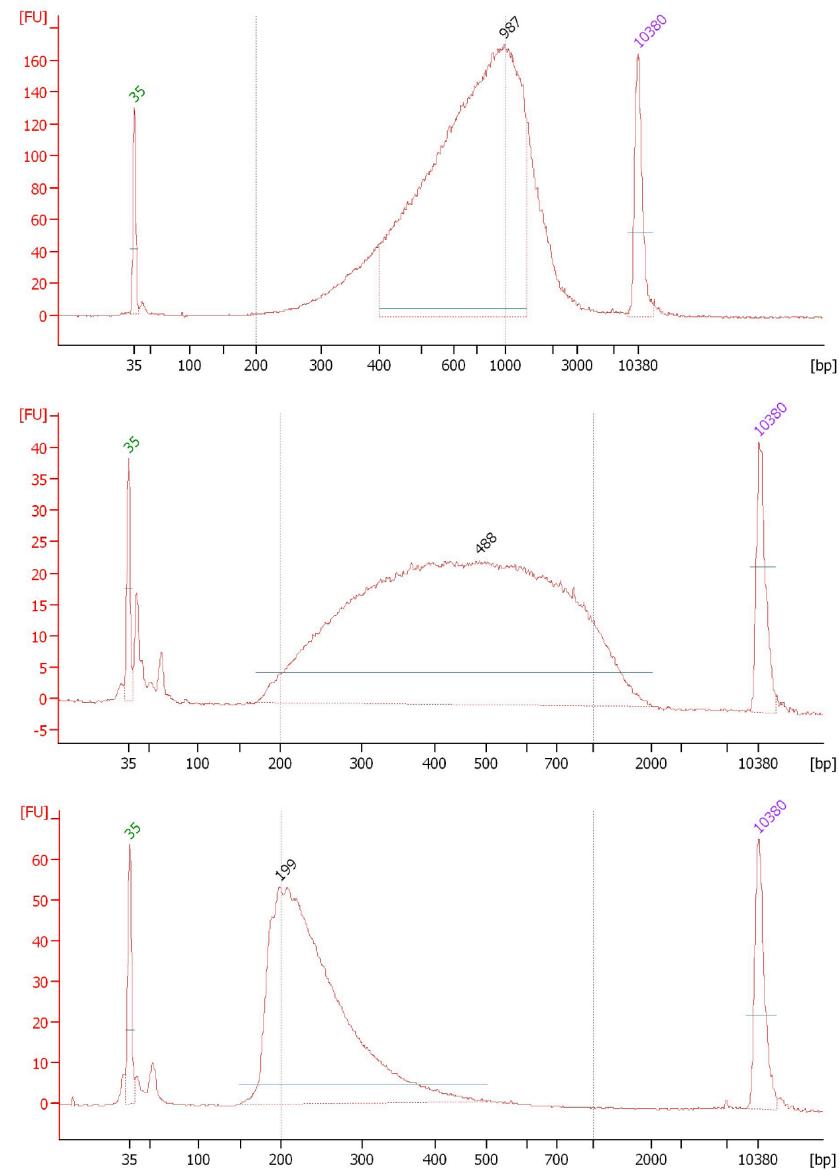
使用するPCR産物の量によりNextera
ライブラリーの長さが異なるため実験
デザインに応じた条件を決定



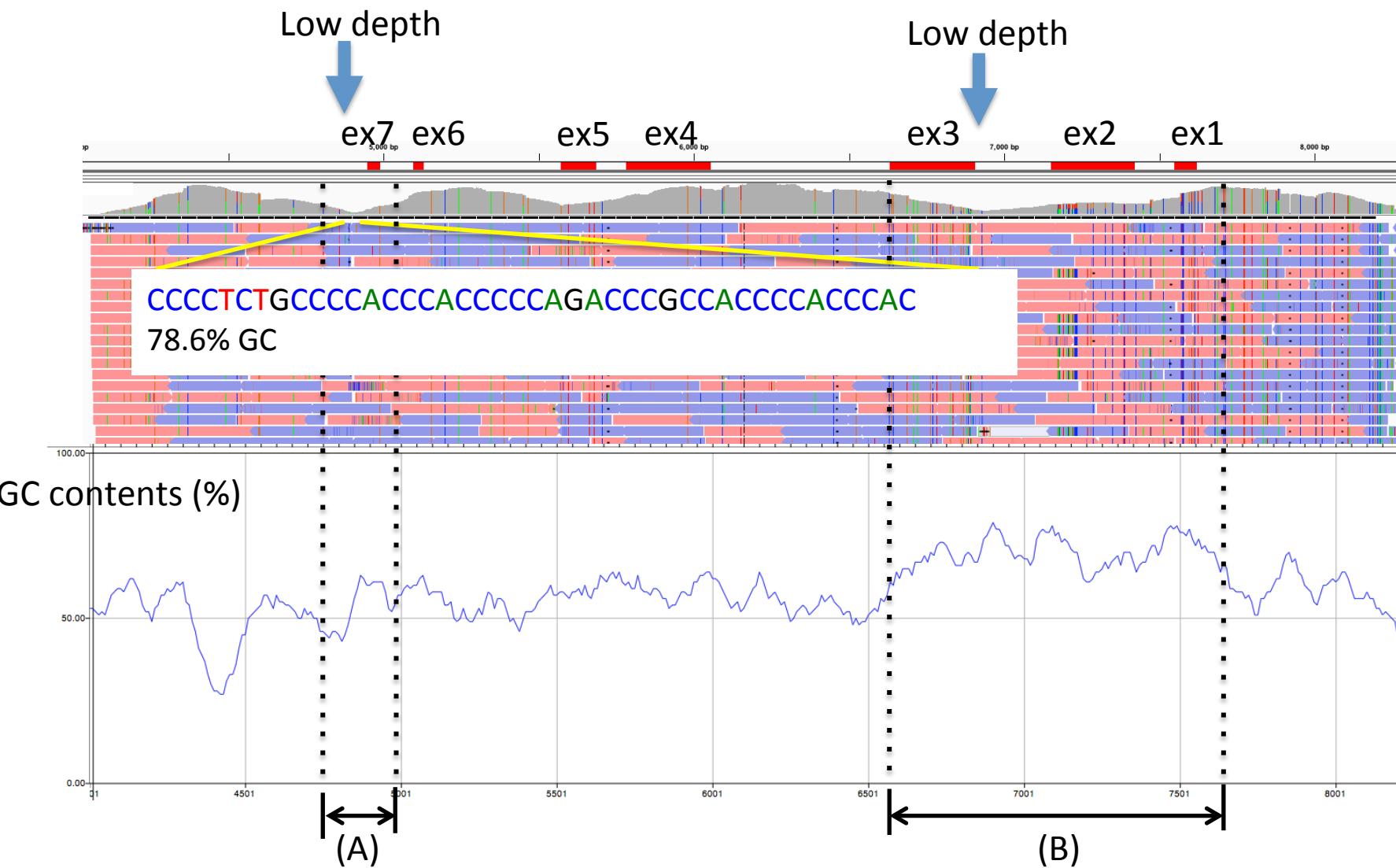
Index primer 1 (i7) 39bp + 8bp
5' CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

Index primer 2 (i5) 43bp + 8bp
5' AATGATAACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

$$\text{断片長} - 98\text{bp} = \text{インサート長}$$



GC含量の高い遺伝子



Nextera kitによるライブラリー調整 (高GC%プロトコール)

ロングPCR増幅



Tagmentation

PCR産物(10ng)	4ul	55°C	5min
TD	5ul	10°C	∞
TDE1	1ul		

精製 Zymo-Spin1-96plate



ライブラリーのPCR増幅

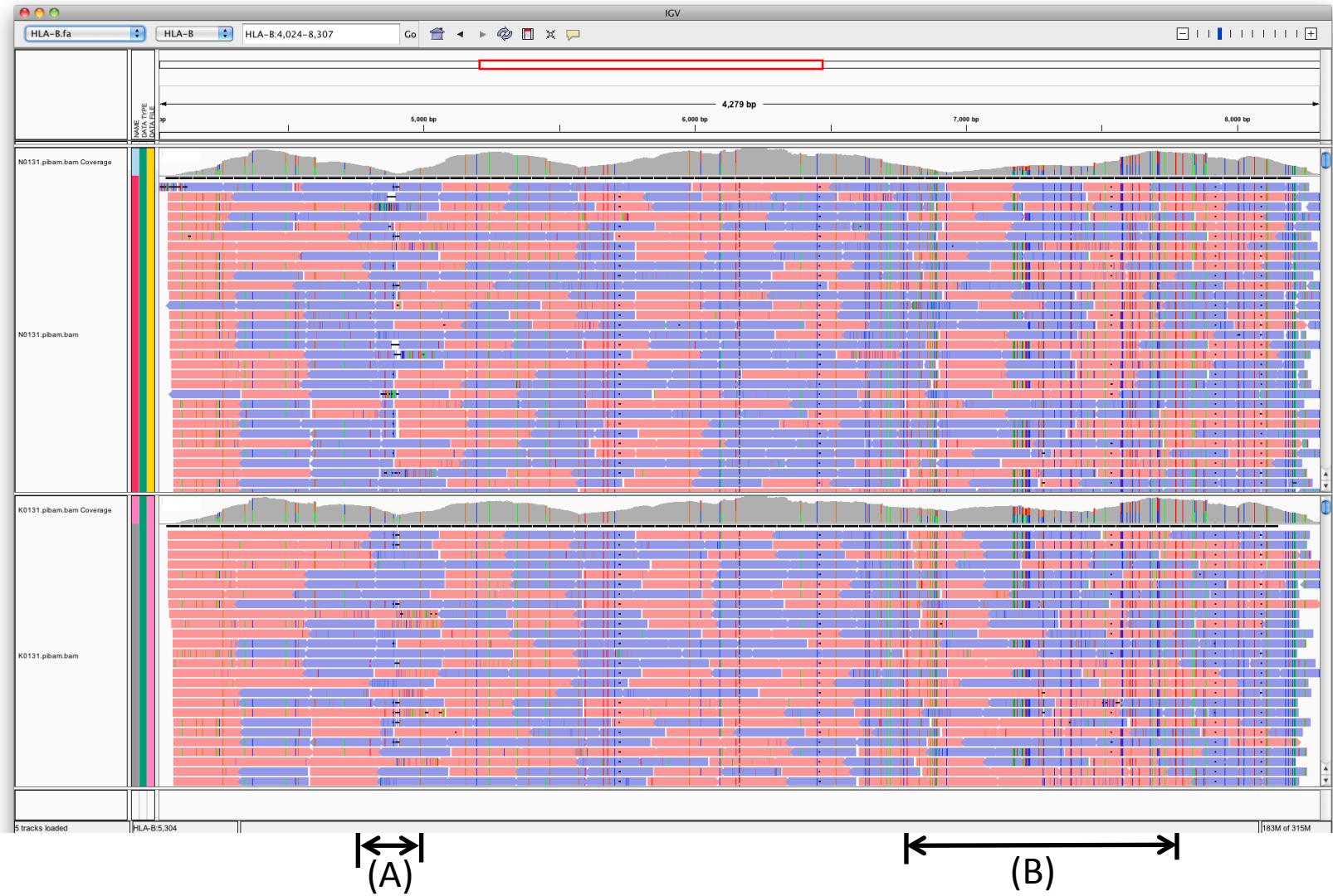
Index1 primer	1ul	72°C	3min
Index2 primer	1ul	98°C	30sec
2xKapa HiFi HS RM	5ul	98°C	10sec
PPC	1ul	63°C	30sec
Tagmentation DNA	2ul	72°C	3min
		10°C	∞

7cycles

精製 AMPure XP bead

GC含量の高い遺伝子

Nextera



PCR酵素

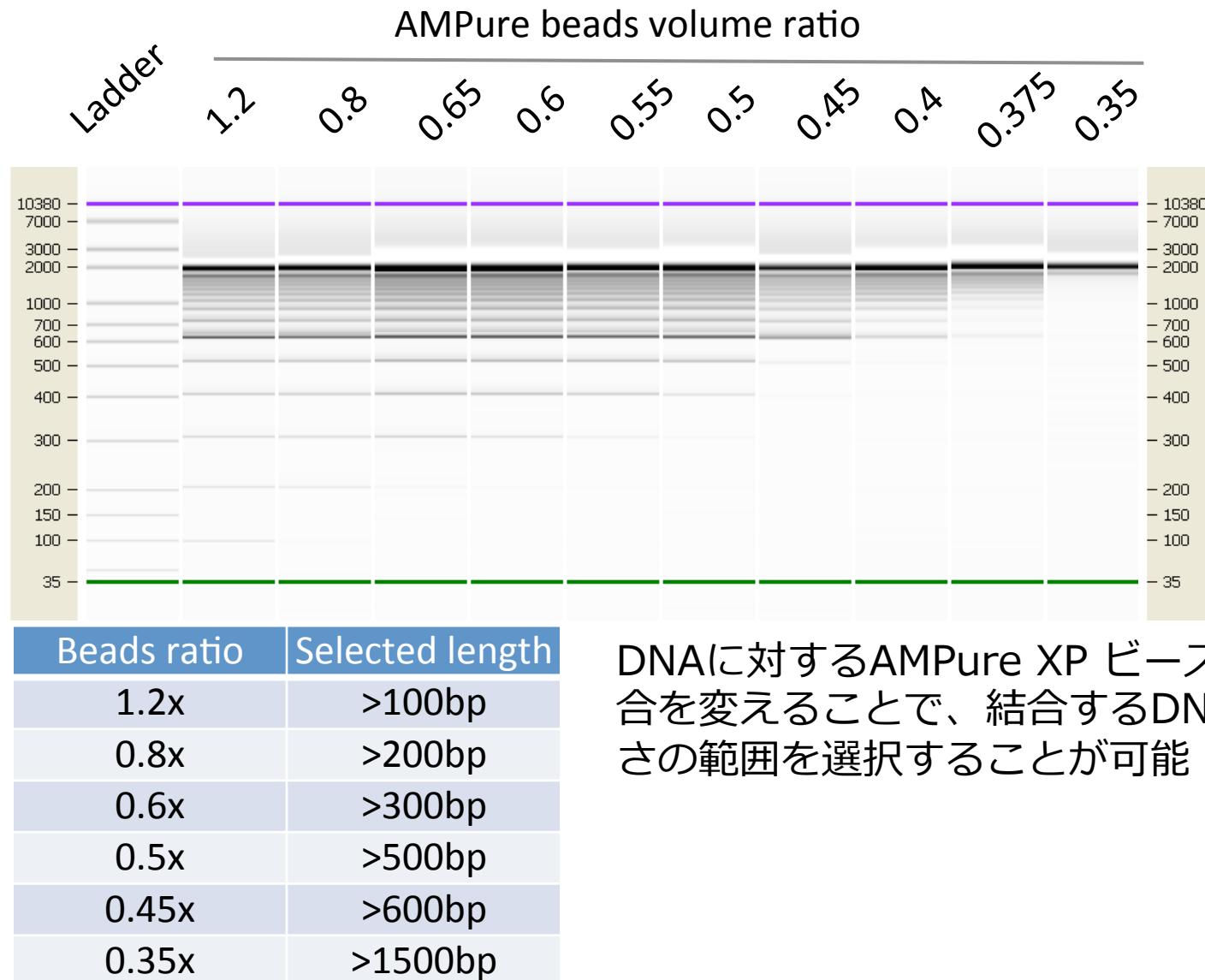
KAPA BIOSYSTEMS KAPA Library Amplification Kit

KAPA BIOSYSTEMS Kapa HiFi Hotstart mastermix

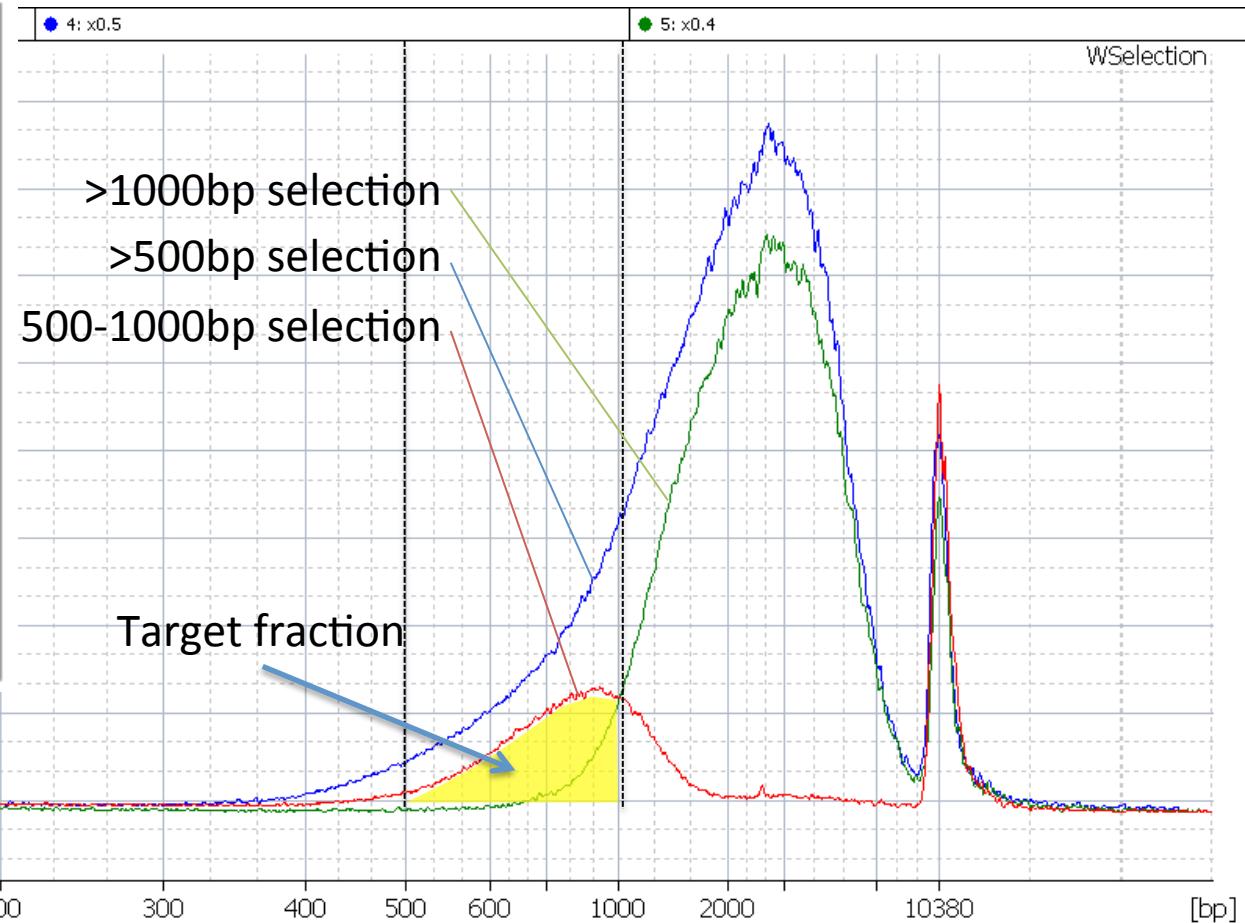
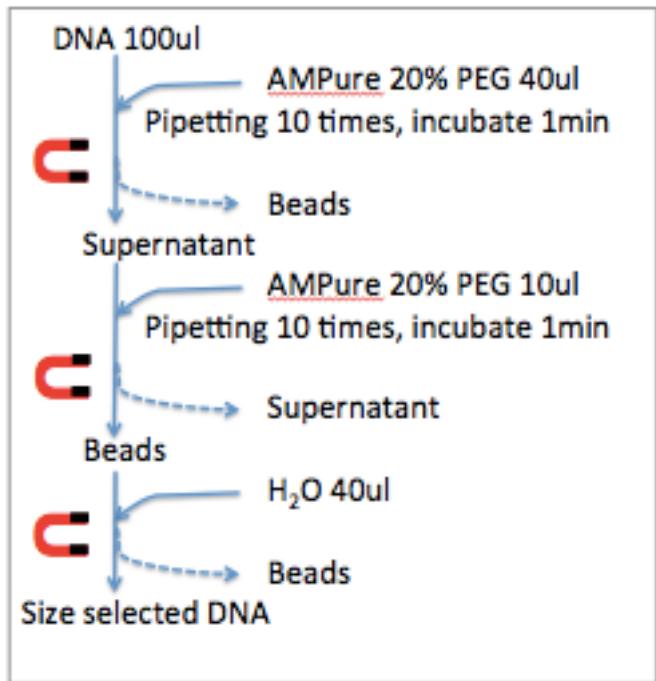
KK2611

KK2602 など

AMPure XP ビーズによるサイズ選択

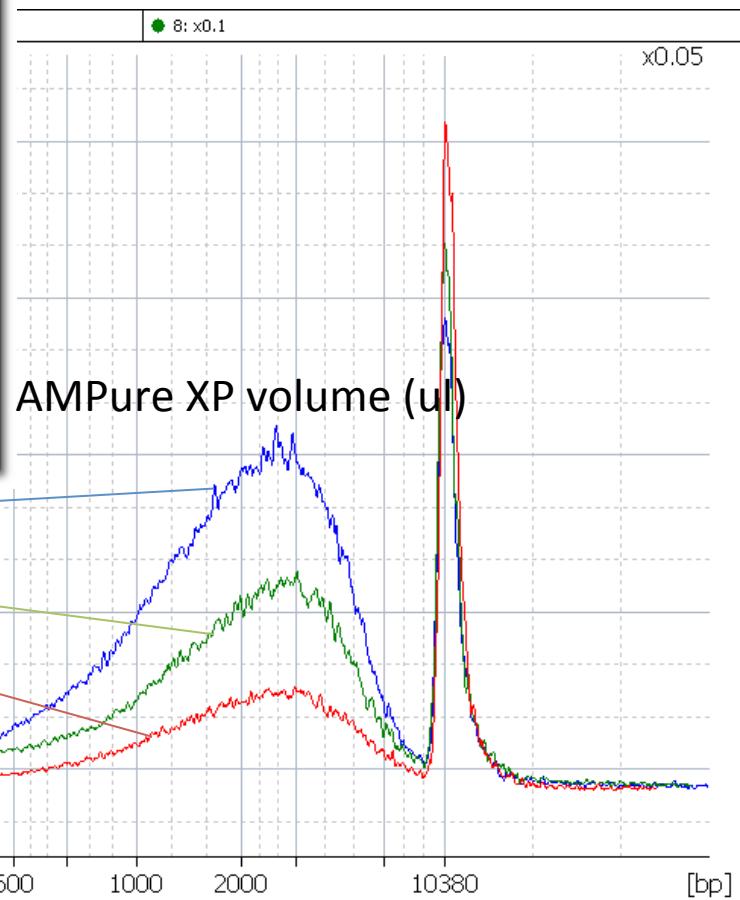
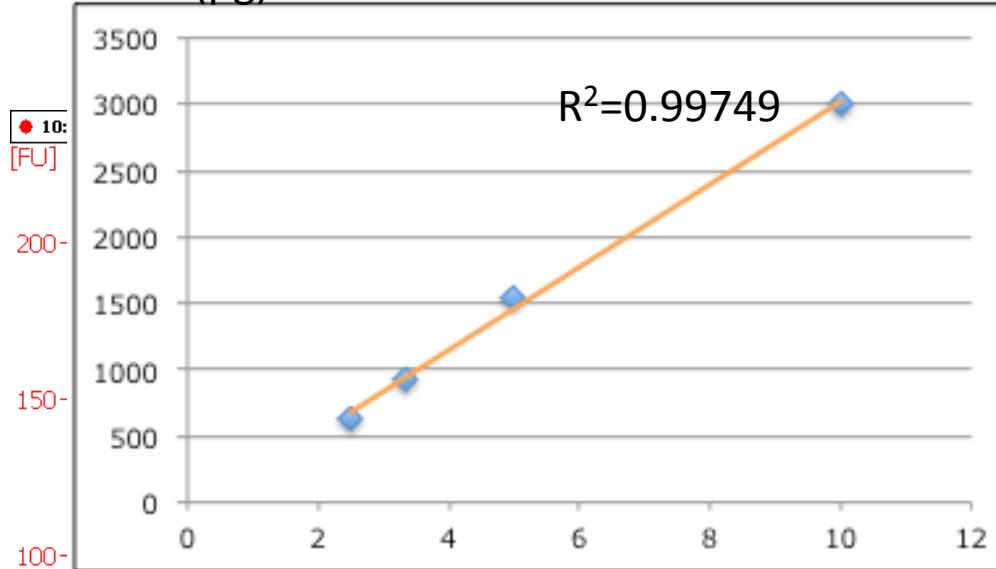


AMPure XP ビーズによるNextera ライブラリのサイズセレクション



AMPure XPビーズによるDNA濃度のノーマライゼーション

Bound DNA (pg)



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long-range PCR Nextera 解析 ～ HLA遺伝子の解析～

Hosomichi et al. BMC Genomics 2013, 14:355
<http://www.biomedcentral.com/1471-2164/14/355>



METHODOLOGY ARTICLE

Open Access

Phase-defined complete sequencing of the HLA genes by next-generation sequencing

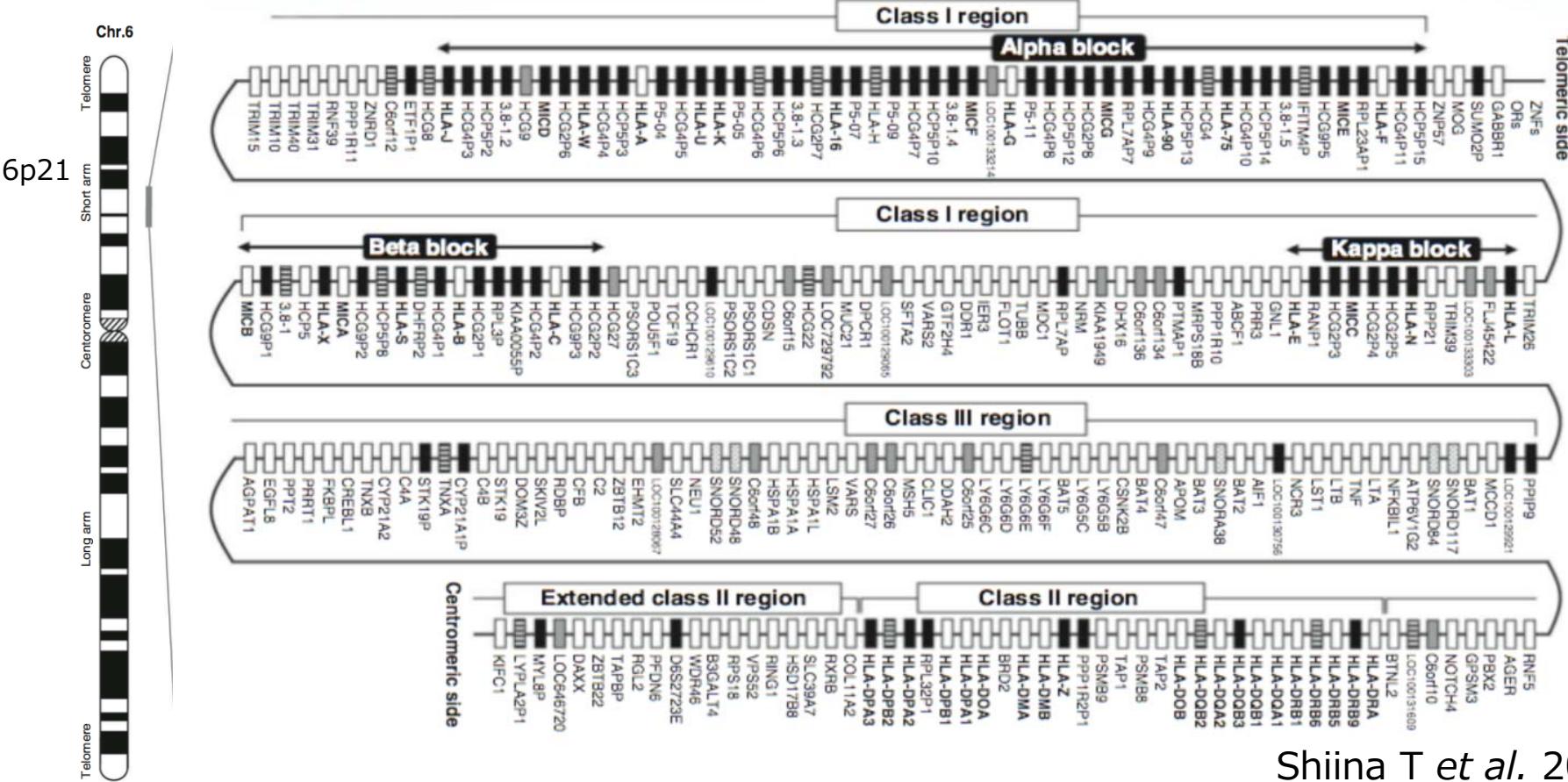
Kazuyoshi Hosomichi¹, Timothy A Jinam¹, Shigeki Mitsunaga², Hirofumi Nakaoka¹ and Ituro Inoue^{1*}

1. HLA遺伝子シークエンスの意義
2. PCRからシークエンス
3. 物理的情報に基づく相の決定

HLAとは

- HLA (Human Leukocyte Antigen
= ヒト白血球抗原)
 - 1954年、白血球の血液型として発見
- 組織適合性抗原
(MHC; Major Histocompatibility Complex)
- ヒトに関しては MHC = HLA

HLA領域のゲノム配列



Shiina T et al. 2009

HLA領域の特徴

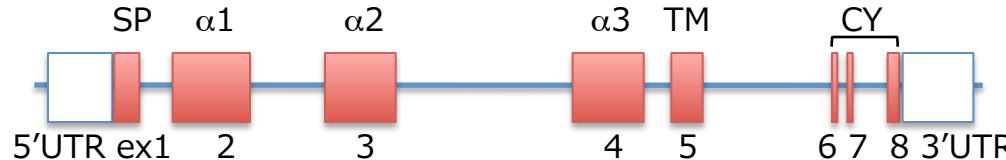
252の遺伝子、6つの古典的HLA遺伝子と少なくとも132のタンパク質をコードする遺伝子を含む
極めて高度な多型性を示す
100以上の疾患および薬剤副作用と関連する

MHC分子

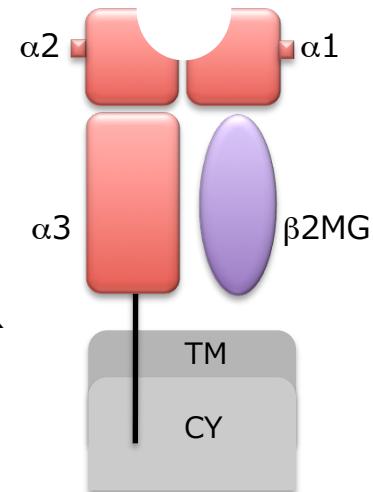
MHCクラスI

HLA-A, -C, -Bなど

内在抗原提示
細胞性免疫



HLAクラスI分子

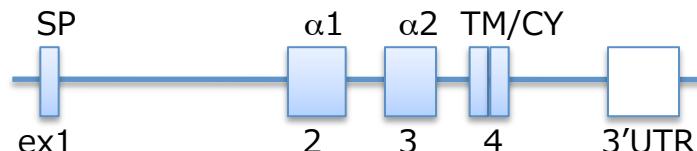


MHCクラスII

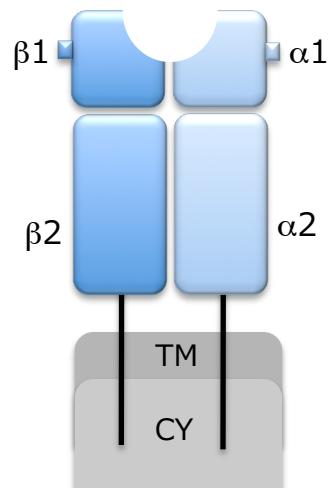
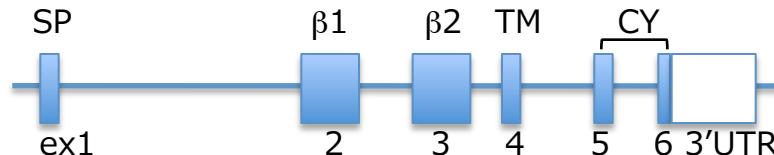
α鎖遺伝子
HLA-DRA1, -DQA1など

β鎖遺伝子
HLA-DRB1, -DQB1, -DPB1など

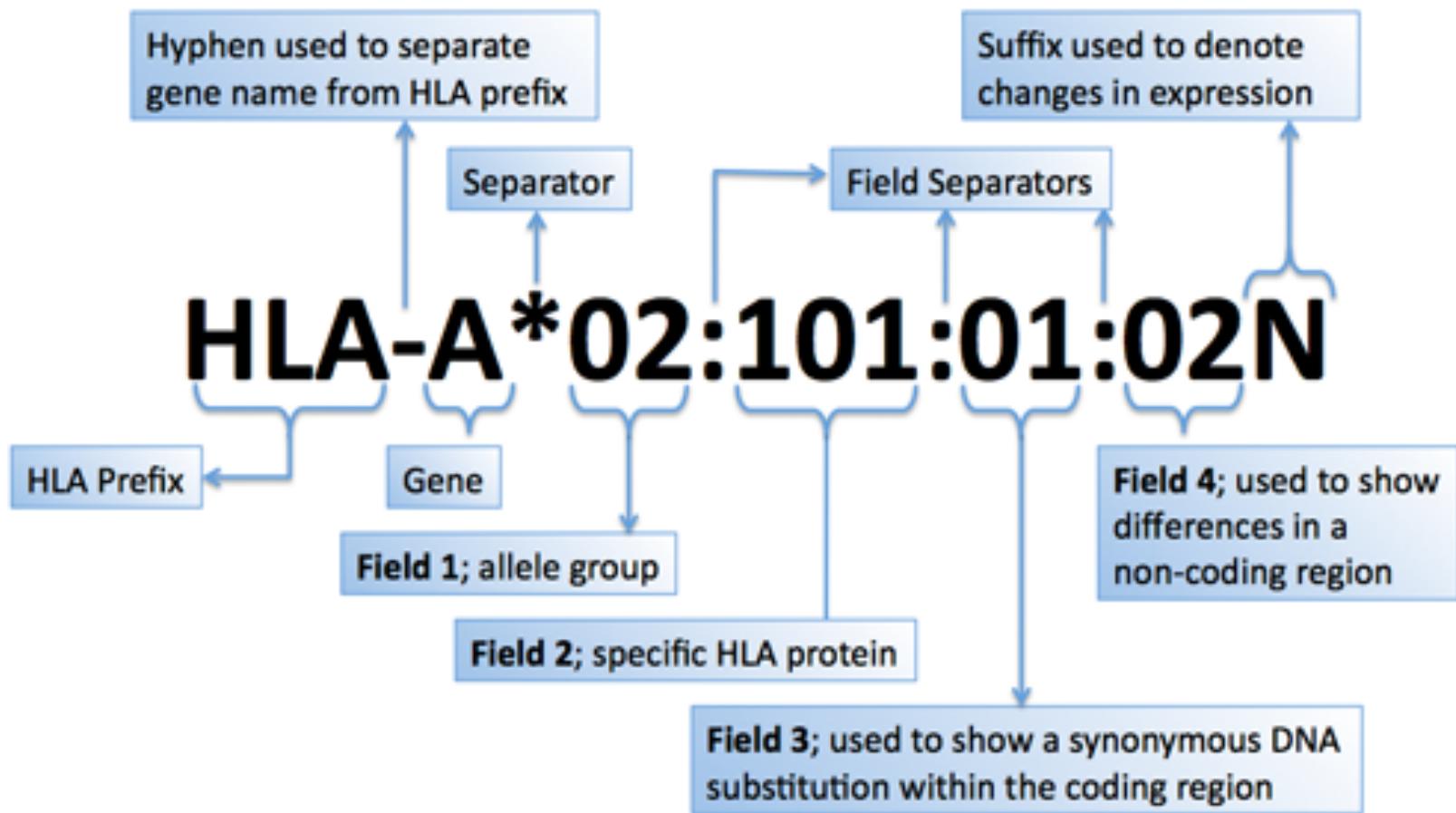
外来性抗原提示
液性免疫



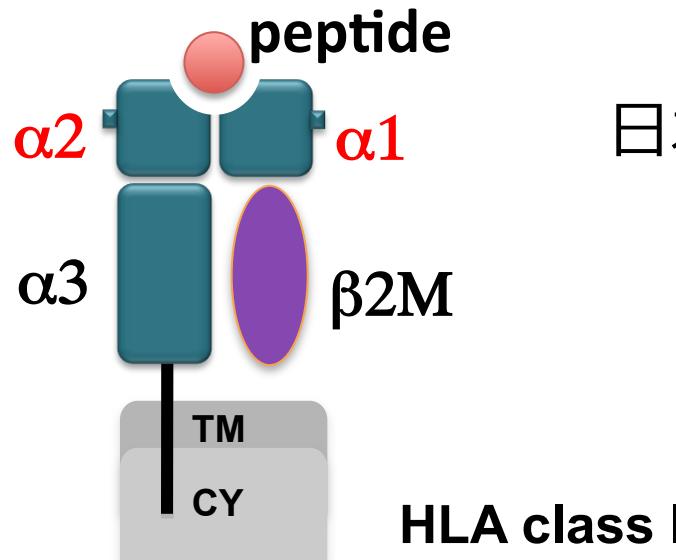
HLAクラスII分子



HLAアレルの命名法



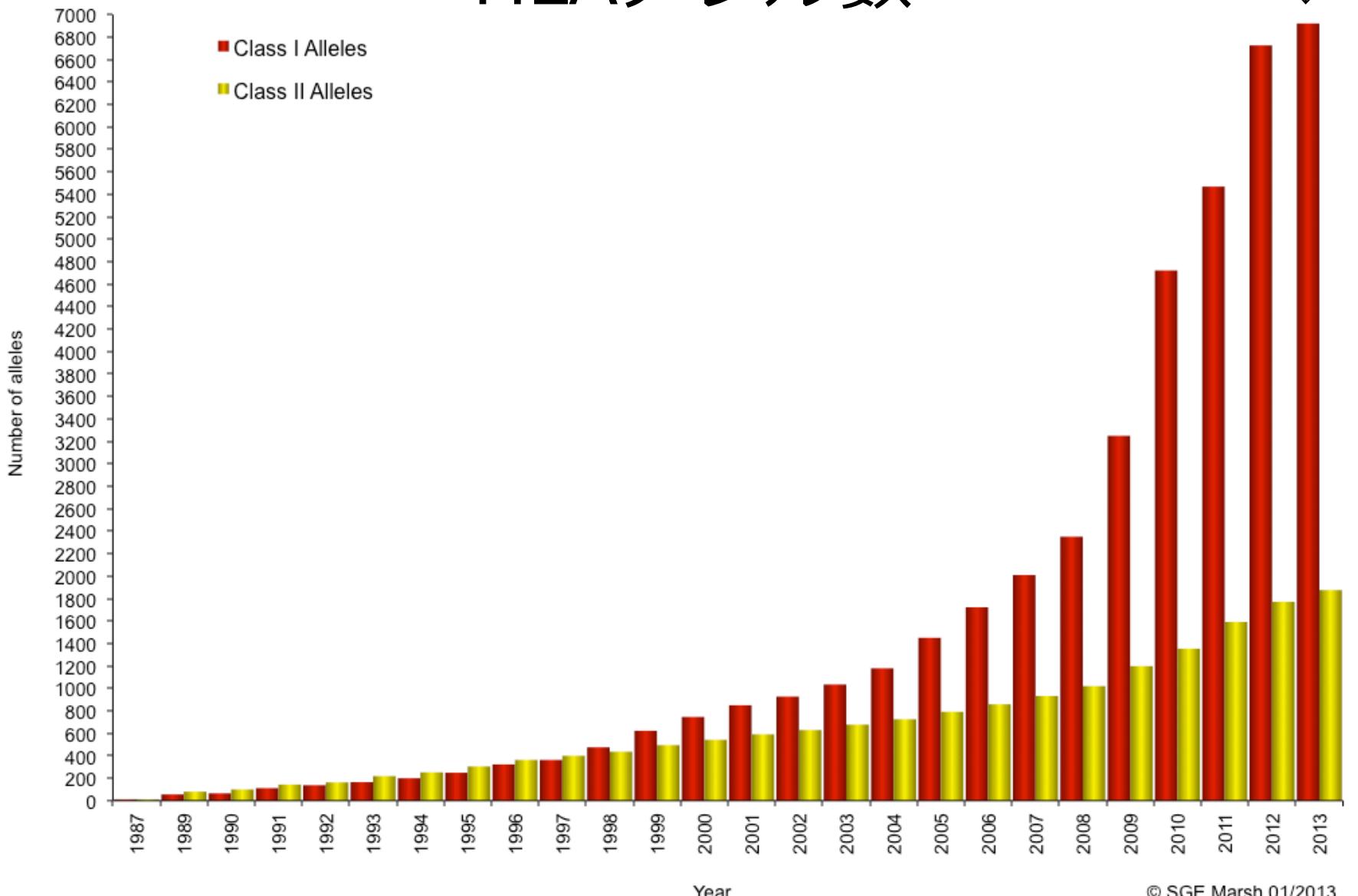
HLA クラスI α 1および α 2ドメインの 多様性



IMGT/HLAデータベースに登録されている HLAアレル数

Numbers of HLA Alleles			
HLA Class I Alleles			7,089
HLA Class II Alleles			2,065
HLA Alleles			9,154
HLA Class I			
Gene	A	B	C
Alleles	2,244	2,934	1,788
Proteins	1,612	2,211	1,280
Nulls	109	97	47
HLA Class II			
Gene	DRB	DQB1	DPB1
Alleles	1,418	323	185
Proteins	1,051	216	153
Nulls	32	7	6

IMGT/HLAデータベースに登録されている HLAアレル数



HLAと疾患

- 生活習慣病
- 自己免疫疾患
- がん
- 造血幹細胞移植に伴う移植片対宿主病
- ウイルス感染症における防御と重症化
- 薬剤副作用

日本人における疾患とHLAの関連

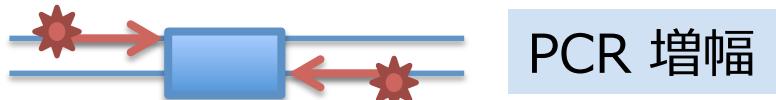
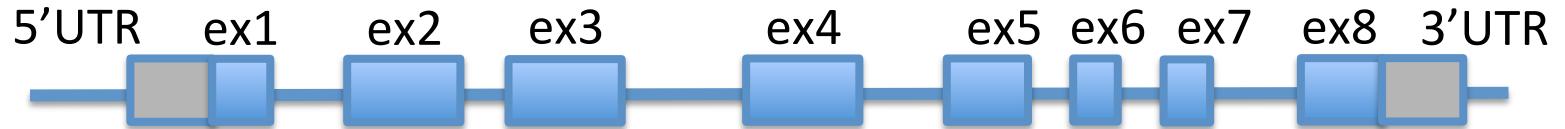
疾患	関連を示すHLA型	患者集団中の頻度(%)	一般集団中の頻度(%)	オッズ比
ナルコレプシー	HLA-DRB1*15:01 HLA-DQB1*06:02	100	12.4	1372.7
強直性脊椎炎	HLA-B27	83.3	0.5	1056.3
ベーチェット病	HLA-B51:01	59.4	13.6	9.3
関節リウマチ	HLA-DRB1*04:05 HLA-DQB1*04:01	58.8	24.7	4.4
1型糖尿病	HLA-B*54:01 HLA-DRB1*04:05 HLA-DQB1*04:01	44.1 56.6 58.3	14 24.7 24.7	4.8 4 4.3
グレーヴス病	HLA-DPB1*05:01	87.2	61.8	4.2
橋本病	HLA-DRB4*01:01	88.7	63.7	4.5
多発性硬化症(大脳、小脳型)	HLA-DRB1*15:01	30.7	12.4	3.1
多発性硬化症(眼神経、脊髄型)	HLA-DPB1*05:01	93.6	61.8	9
潰瘍性大腸炎	HLA-B*52:01 HLA-DRB1*15:02 HLA-DPB1*09:01	56.4 59.3 55.6	24.1 24.4 20.6	4.1 4.5 4.8
原発性胆汁性肝硬変	HLA-DRB1*08:03	3.4	0.6	5.9
SLE	HLA-B39 HLA-DRB1*15:01	16.7 29.6	3.1 12.4	6.3 3
混合結合組織病(MCTD)	HLA-DRB1*04:01	18.8	4.4	5
亜急性甲状腺炎	HLA-B*35:01 HLA-B*67:01	71.4 16.1	12.2 1.7	18 11.2
高安動脈炎(高安病)	HLA-B*52:01 HLA-B*39:02	50.5 4.1	24.1 0.5	3.2 8.5
バージャー病	HLA-DRB1*15:01	6	0.6	10.7
川崎病	HLA-DPB1*02:02	17.2	5.3	3.7

HLAと薬剤副作用との関連性

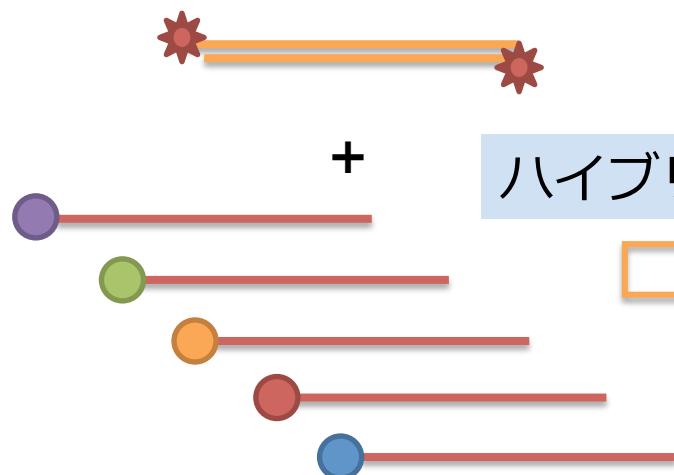
薬剤副作用	関連を示すHLA型	陽性率(%)	オッズ比
○ Tiopronin(重金属)と肝内胆汁うつ滯	HLA-A*33:03	93	41.5
○ Carbamazepine(抗痙攣剤)と Stevens-Johnson 症候群	HLA-B*15:02	100	895.5
Abacavir(抗HIV剤)と胃腸障害、嗜眠、低血圧による致死副作用	HLA-B*57:01	78	117.5
○ Allopurinol(抗痛風、抗尿酸血症剤)と 薬疹	HLA-B*58:01	100	393.5
Ticlopidine(抗血小板剤)と肝障害	HLA-A*33:03	86	36.5
Amoxicillin-clavulanate potassium (抗生素質)と肝障害	HLA-DRB1*15:01	57	35.6
Flucloxacillin(抗生素質)と肝障害	HLA-B*57:01	84	80.6

原因がHLA遺伝子そのものであるか、連鎖不平衡によりHLA遺伝子と関連しているようにみえるかはAbacavirを除き不明

現行のHLAタイピング法 PCR-SSO (sequence specific oligonucleotide)



PCR 增幅



ハイブリダイズ

+

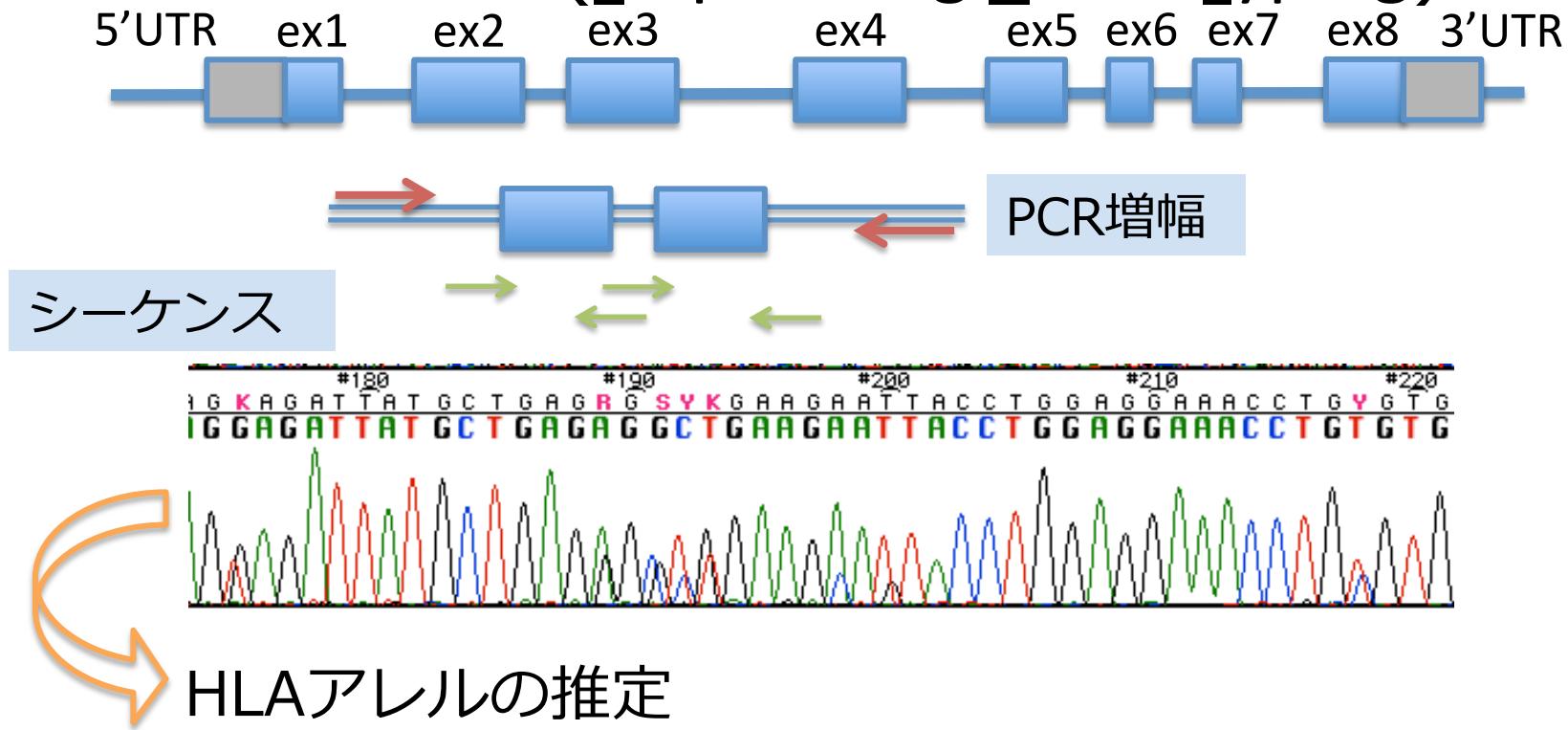


HLAアレルの決定

*B*51:02:01 B*58:01:01*

現行のHLAタイピング法

PCR-SBT (sequencing based typing)



複数の組み合わせ候補

Combination 1 $B^*07:021 + B^*35:011$

Combination 2 $B^*07:18 + B^*35:05$

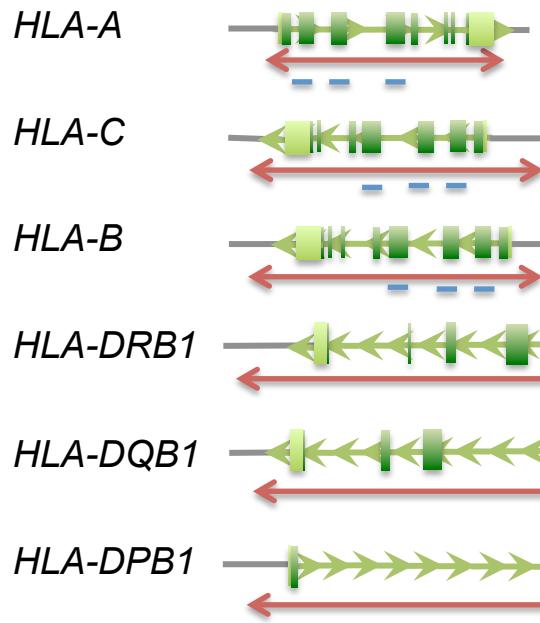
Combination 3 $B^*07:09 + B^*35:34$

Combination 4 $B^*07:24 + B^*35:15$

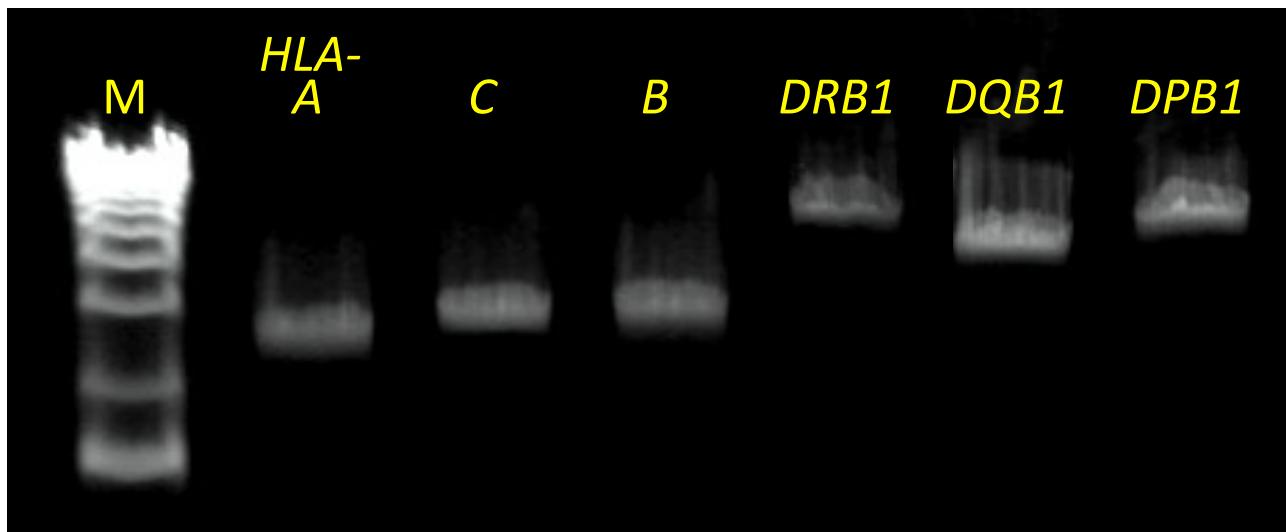
Ambiguity

HLA遺伝子のロングPCR

PCR



Locus	length (bp)
<i>HLA-A</i>	3,398
<i>HLA-C</i>	4,296
<i>HLA-B</i>	4,440
<i>HLA-DRB1</i>	11,899
<i>HLA-DQB1</i>	7,118
<i>HLA-DPB1</i>	13,605



NexteraとMiSeqによる HLA遺伝子のシークエンシング

PCR増幅

locus	Length (bp)
HLA-A	3,398
HLA-B	4,296
HLA-C	4,440
HLA-DRB1	11,899
HLA-DQB1	7,118
HLA-DPB1	13,605



ライブラリ調整

Nextera DNA Sample Preparation Kit
(illumina)



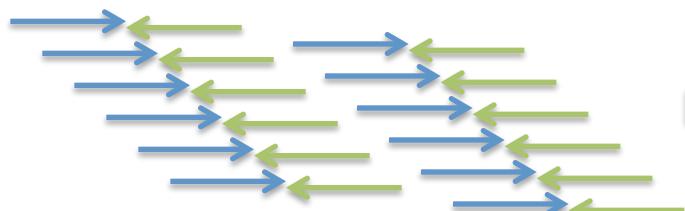
シークエンシング MiSeq v2 (illumina)

Read length 2 x 250 bp
Output 7.5-8.5 Gb
Total time 39 hr

データ解析

HLA遺伝子配列決定の概要

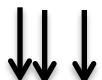
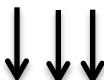
Sequence reads



Alignment

HLA-B

SNVs



Diplotype sequence

ATG

TAC

250bp

CAA

TTG

CAA

TTG

600bp

TAC

AGG

TAC

AGG

800bp

1000bp

1000bp

T

C

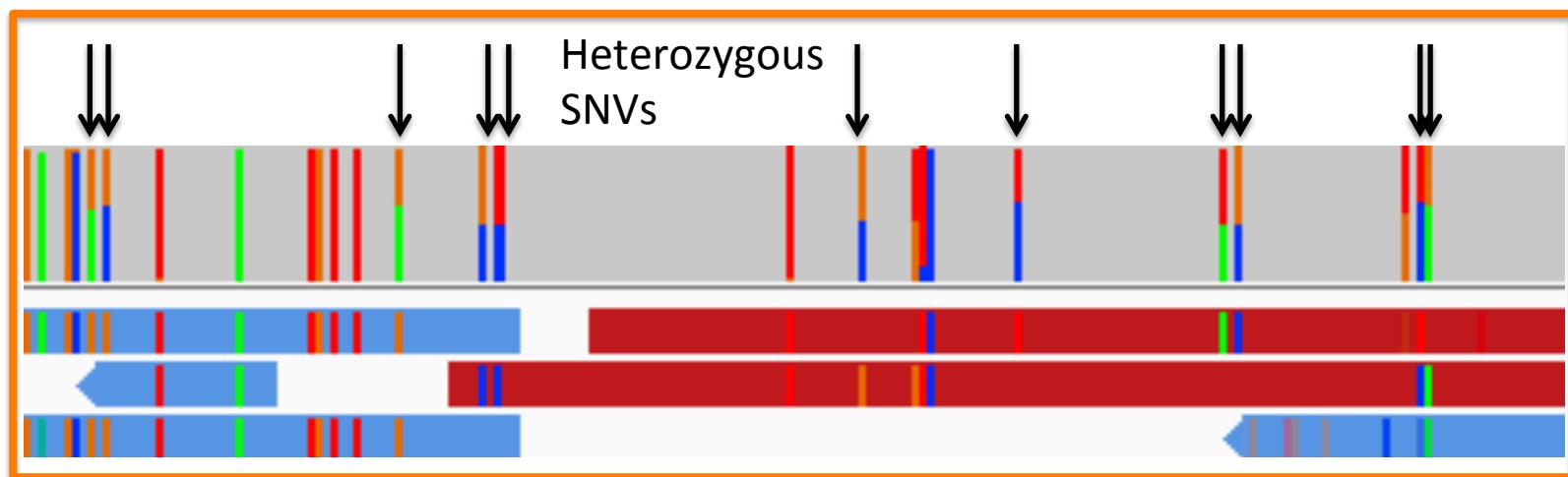
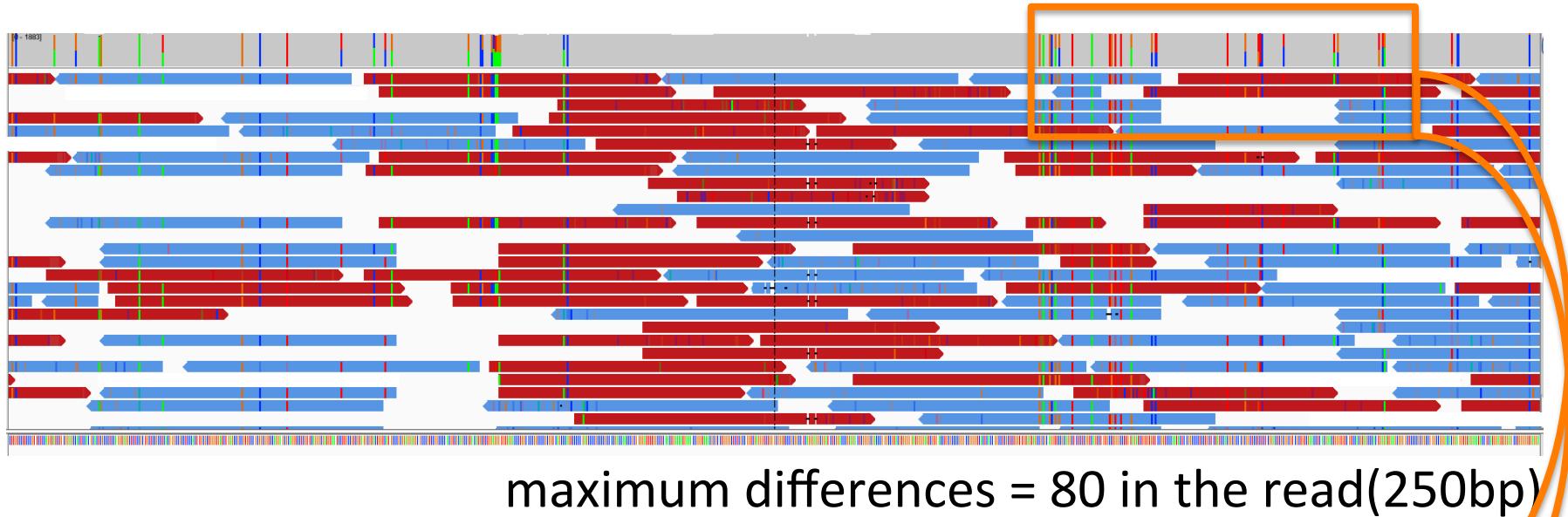
250bp

600bp

800bp

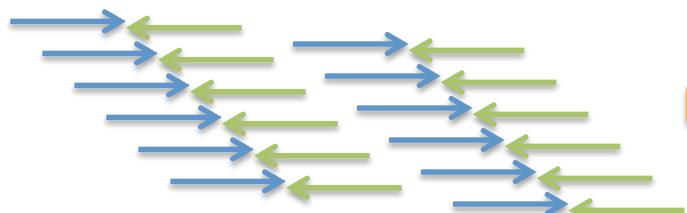
1000bp

HLA-B エクソン2および3



HLA遺伝子配列決定の概要

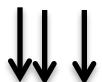
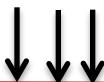
Sequence reads



Alignment

HLA-B

SNVs



Diplotype sequence

ATG

TAC

250bp

CAA

TTG

CAA

TTG

600bp

800bp

TAC

AGG

TAC

AGG

1000bp

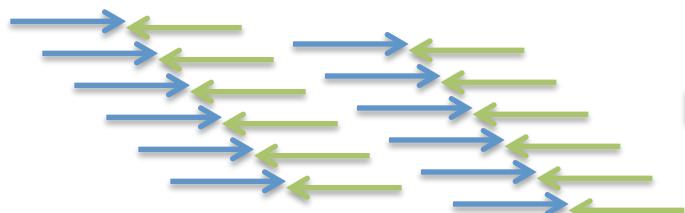
T

C

1000bp

HLA遺伝子配列決定の概要

Sequence reads



Alignment



SNVs



Diplotype sequence

ATG → CAA

CAA →

TAC →

TAC →

TTG →

TAC →

250bp

600bp

TTG →

AGG →

TTG →

AGG →

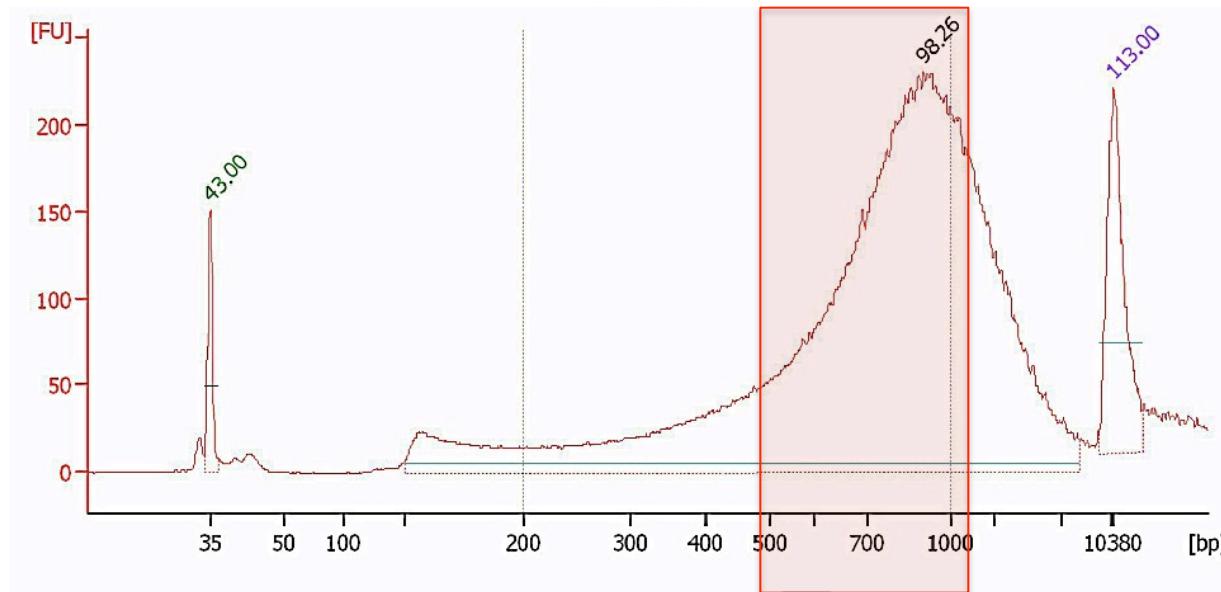
800bp

1000bp

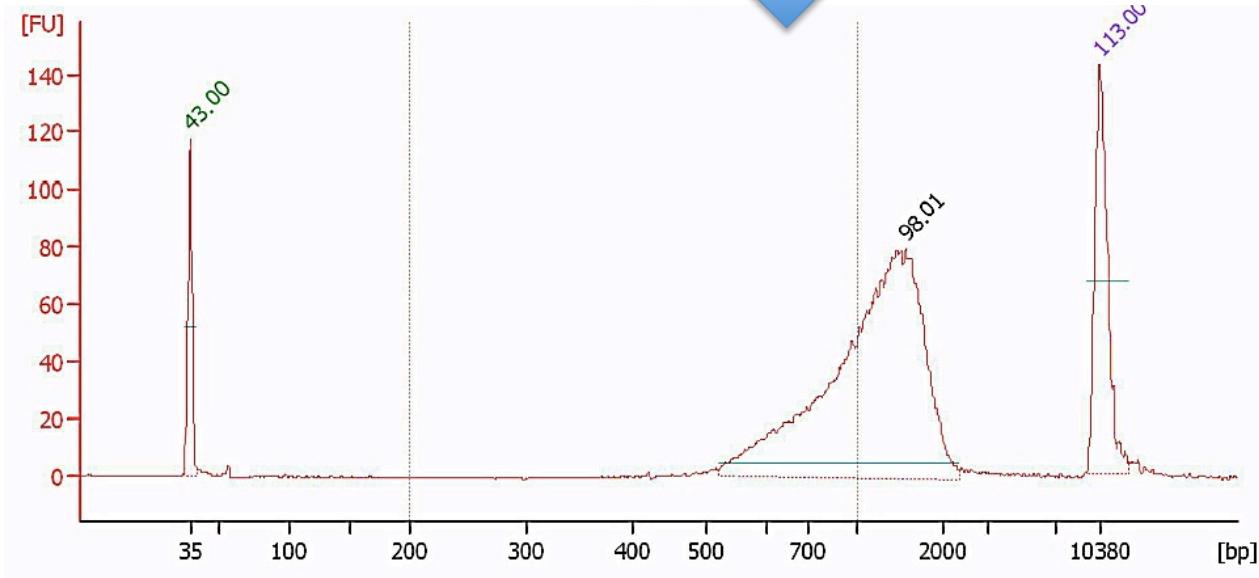
T

C

Nextera DNA ライブライリのサイズ選択

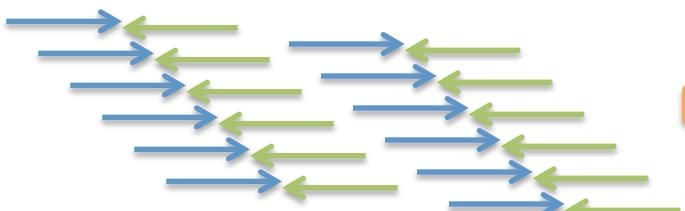


Agarose gel size selection

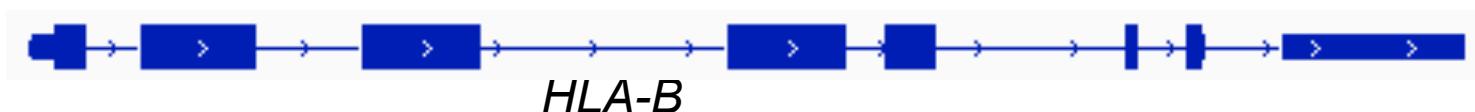


HLA遺伝子配列決定の概要

Sequence reads

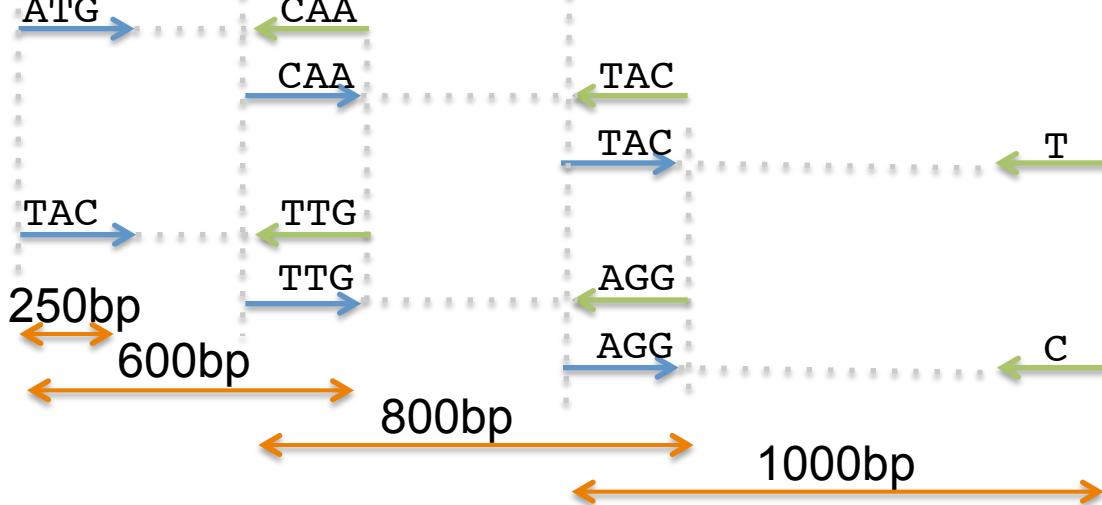


Alignment



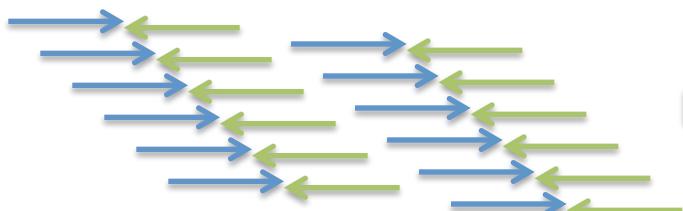
SNVs

Diplotype sequence

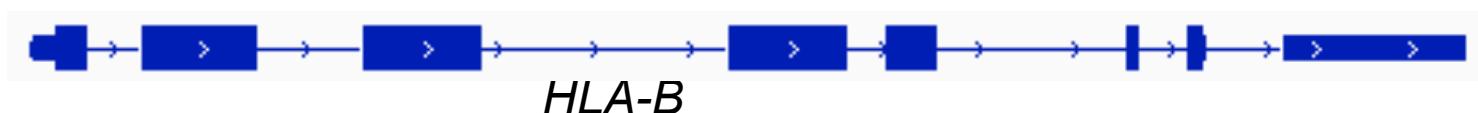


HLA遺伝子配列決定の概要

Sequence reads



Alignment



SNVs



Haplotype sequences

ATG CAA TAC T

TAC TTG AGG C

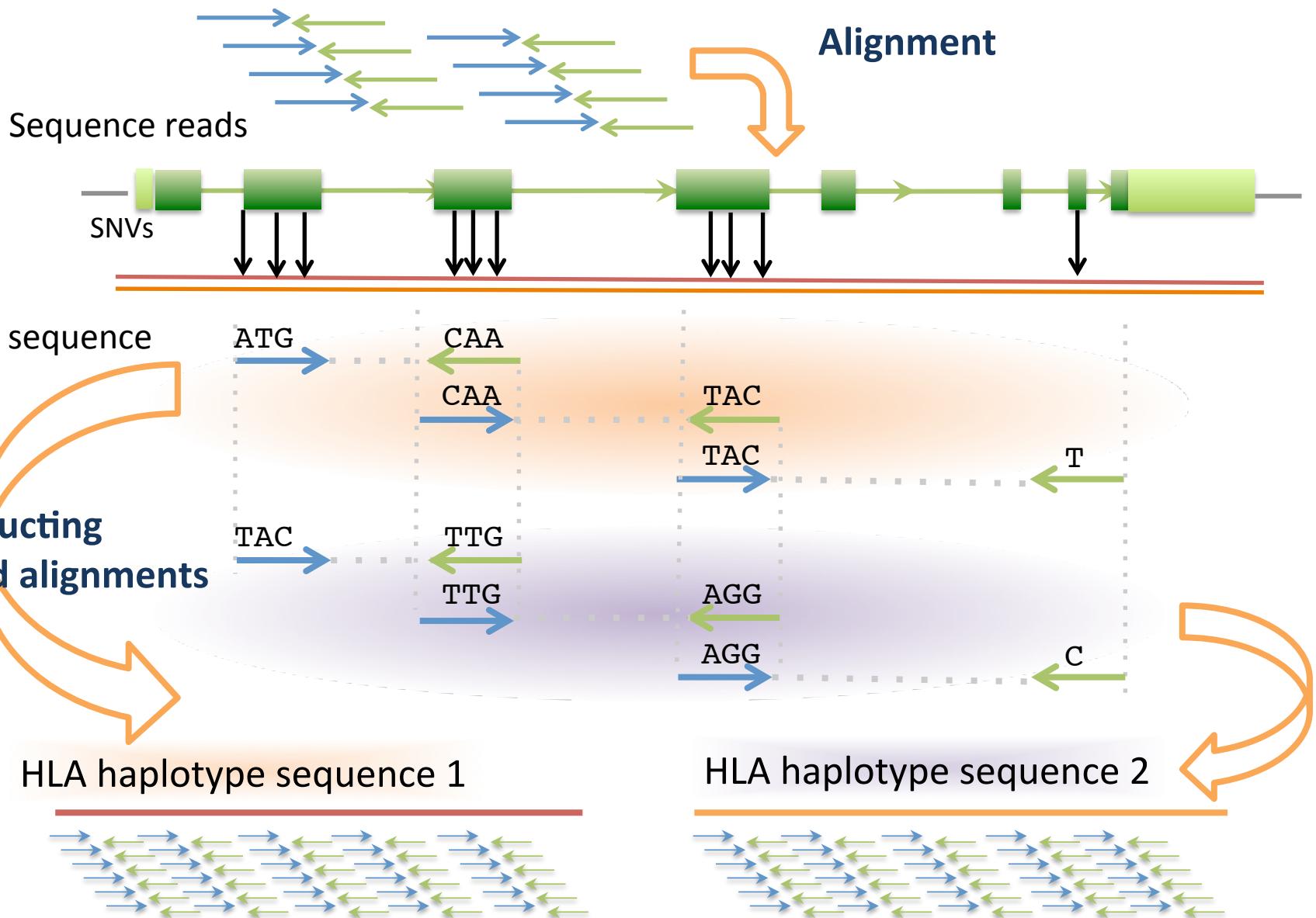
250bp

600bp

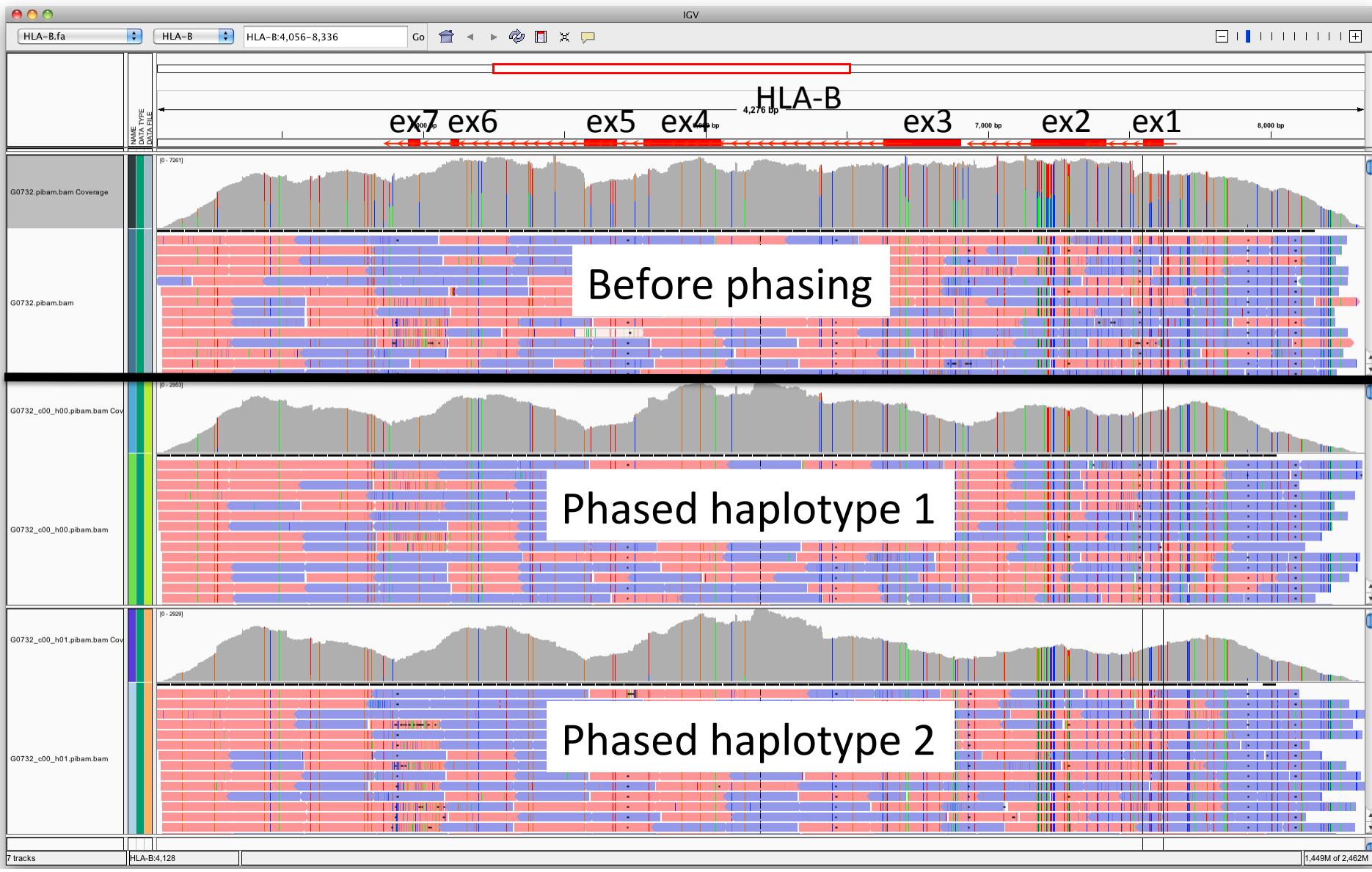
800bp

1000bp

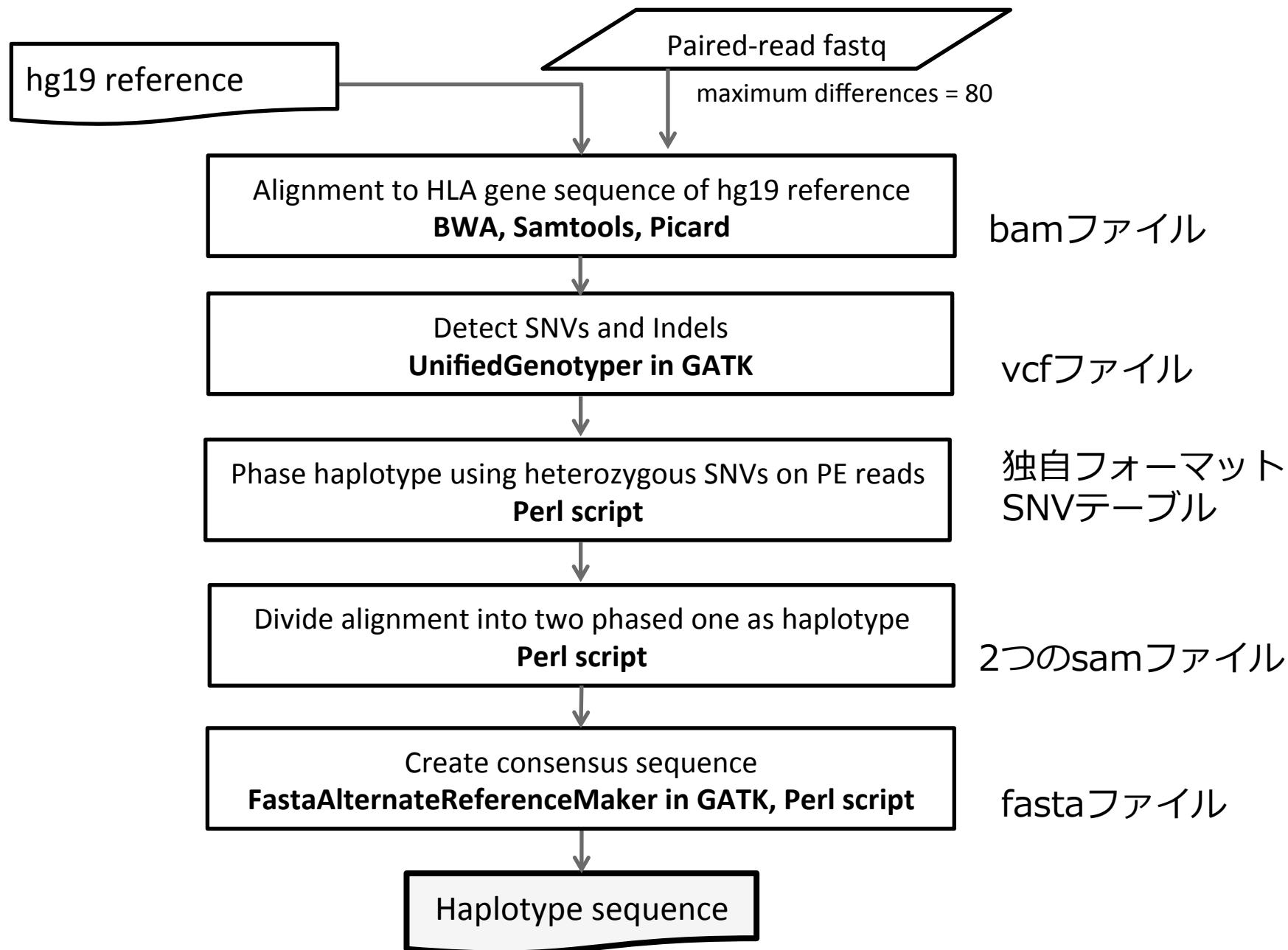
相を特定した2つのHLA遺伝子配列のアライメントの構築



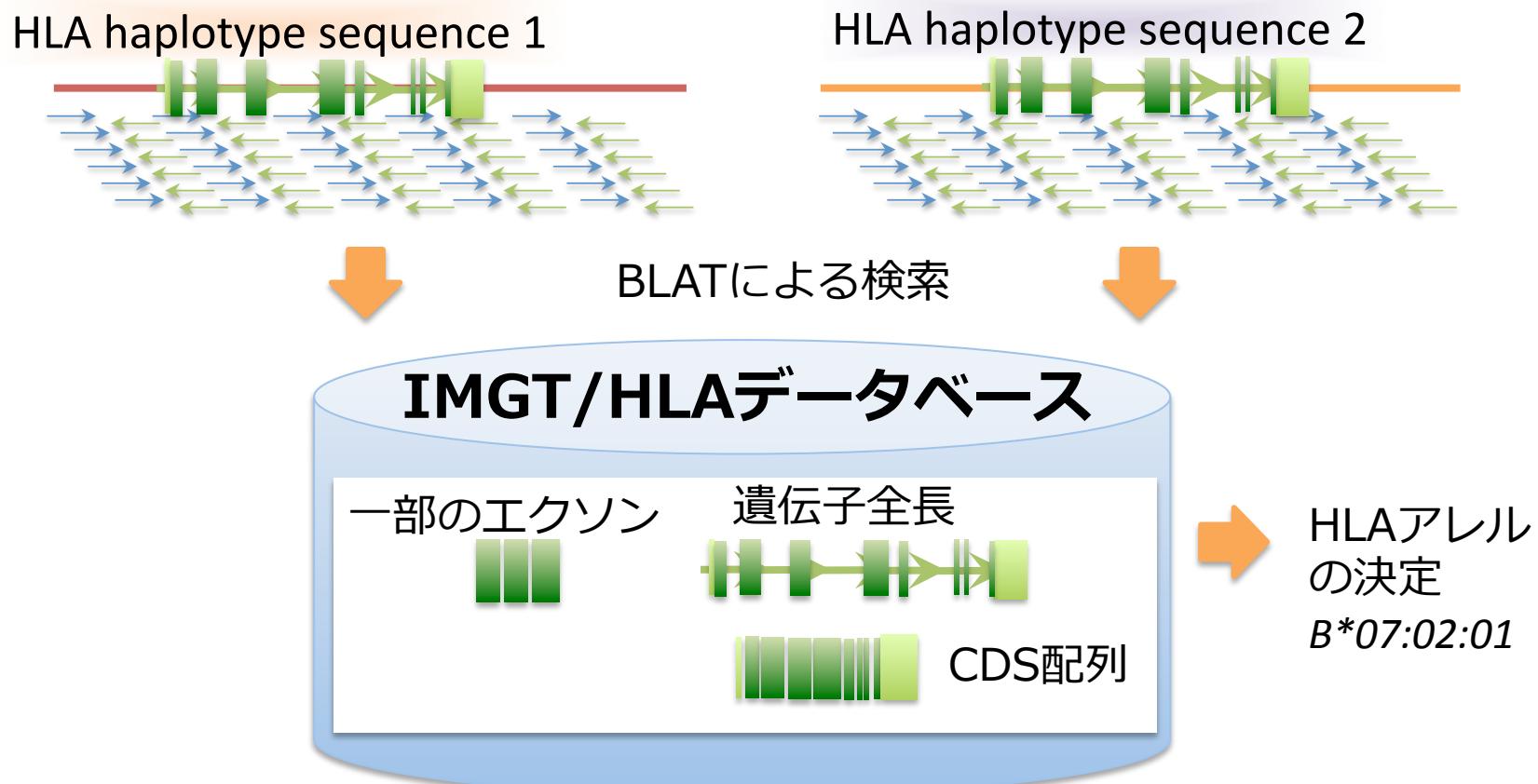
ハプロタイプに分類したアライメント



HLAハプロタイプシーケンス決定のワークフロー



決定したHLA遺伝子完全配列の HLAアレル決定



HLAアレルを決定することよりもHLA遺伝子の塩基配列を完全に決定することが本質

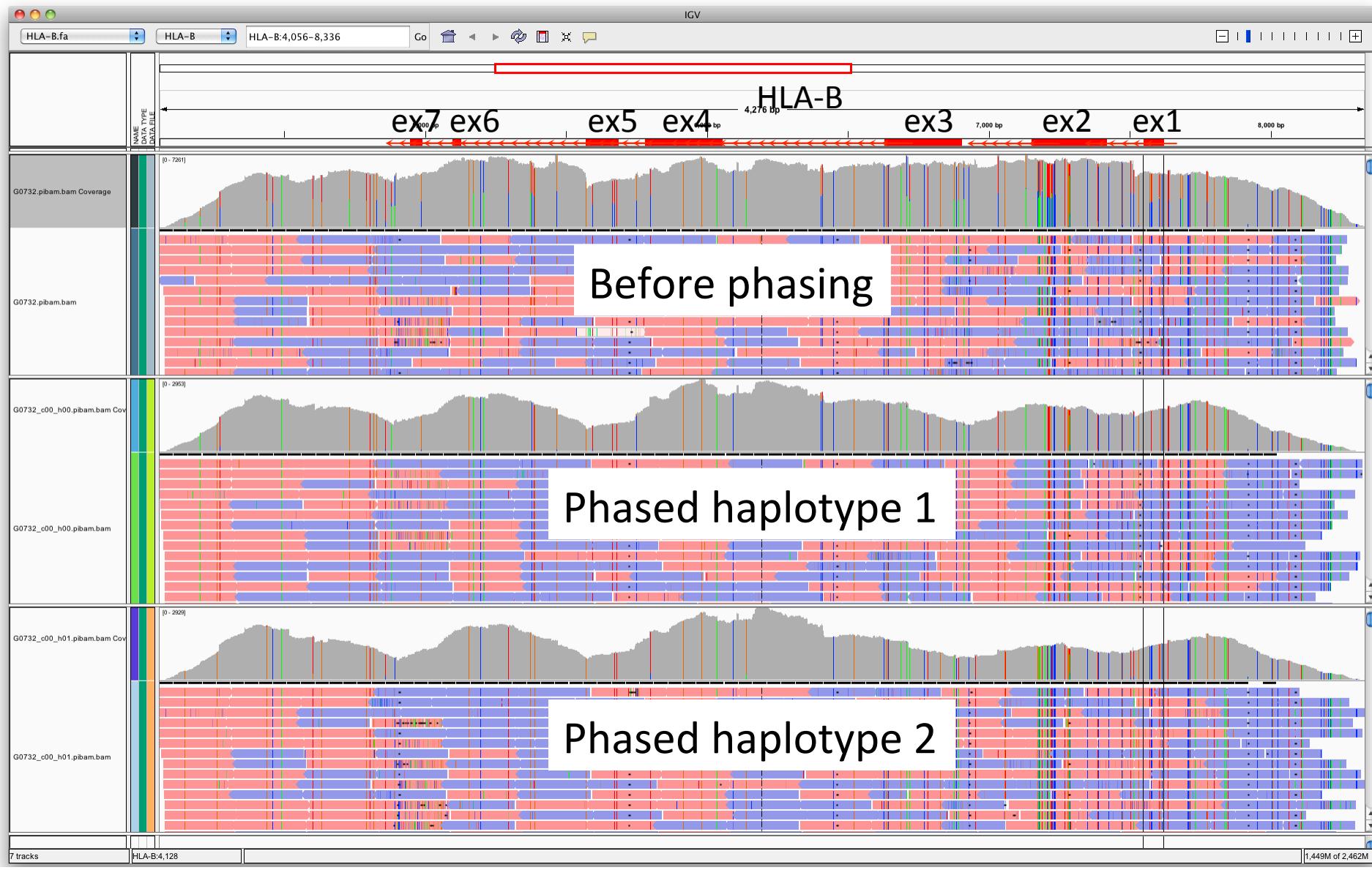
決定したHLA遺伝子配列

Sample ID	PCR-SSO		Next-gen SBT		Average read depth (allele1 / allele2)	Determined sequence (%) (allele1 / allele2)
1	B*51:01:01	B*15:02	B*51:01:01	B*15:02:01	2593.5 / 2431.3	100 / 100
2	B*07:02:01	B*15:02	B*07:02:01	B*15:02:01	3391.2 / 4206.2	100 / 100
3	B*35:01:01	B*15:02	B*35:01:01:01	B*15:02:01	2096.2 / 2431.8	100 / 100
4	B*15:21	B*15:02:01	B*15:21	B*15:02:01	4308.2 / 4693.6	100 / 100
5	B*35:05	B*15:02:01	B*35:05:01	B*15:02:01	4264.3 / 3620.2	100 / 100
6	B*15:18	B*15:02	B*15:18:01	B*15:02:01	1239.9 / 1277.3	100 / 100
7	B*40:06:01:01	B*15:02	B*40:06:01:01	B*15:02:01	5552.1 / 5139.5	100 / 100
8	B*52:01:01	B*15:02	B*52:01:01:01	B*15:02:01	4651.7 / 5085.9	100 / 100
9	B*15:01:01:01	B*57:01:01	B*15:01:01:01	B*57:01:01	2326.4 / 2195.1	100 / 100
10	B*54:01	B*57:01:01	B*54:01:01	B*57:01:01	1999.6 / 2034.6	100 / 100
11	B*40:06:01:01	B*57:01:01	B*40:06:01:01	B*57:01:01	3087.4 / 2833.9	100 / 100
12	B*15:11:01	B*57:01:01	B*15:11:01	B*57:01:01	1948.4 / 1721.9	100 / 100
13	B*15:01:01:01	B*57:01:01	B*15:01:01:01	B*57:01:01	1660.0 / 1482.0	100 / 100
14	B*44:03:01	B*57:01:01	B*44:03:01	B*57:01:01	806.0 / 668.2	100 / 100
15	B*55:07	B*58:01	B*55:07	B*58:01:01	771.6 / 1000.2	100 / 100
16	B*38:01:01	B*58:01	B*38:01:01	B*58:01:01	1565.1 / 2139.7	100 / 100
17	B*48:01:01	B*58:01	B*48:01:01	B*58:01:01	5555.3 / 4610.8	100 / 100
18	B*35:01:01	B*58:01:01	B*35:01:01:01	B*58:01:01	819.5 / 778.1	100 / 100
19	B*15:25:01	B*58:01:01	B*15:25:01	B*58:01:01	3441.2 / 3852.0	100 / 100
20	B*54:01	B*58:01:01	B*54:01:01	B*58:01:01	1308.3 / 1454.1	100 / 100
21	B*51:02:01	B*58:01:01	B*51:02:01	B*58:01:01	1464.0 / 1132.7	100 / 100
22	B*51:01:01	B*58:01:01	B*51:01:01	B*58:01:01	462.9 / 636.3	100 / 100
23	B*51:02:01	B*58:01:01	B*51:02:02	B*58:01:01	637.0 / 663.7	100 / 100
24	B*39:23	B*58:01:01	B*39:23	B*58:01:01	2112.6 / 2553.3	100 / 100

決定したHLA遺伝子配列

Sample ID	PCR-SSO		Next-gen SBT		Average read depth (allele1 / allele2)	Determined sequence (%) (allele1 / allele2)
1	<i>B*51:01:01</i>	<i>B*15:02</i>	<i>B*51:01:01</i>	<i>B*15:02:01</i>	2593.5 / 2431.3	100 / 100
2	<i>B*07:02:01</i>	<i>B*15:02</i>	<i>B*07:02:01</i>	<i>B*15:02:01</i>	3391.2 / 4206.2	100 / 100
3	<i>B*35:01:01</i>	<i>B*15:02</i>	<i>B*35:01:01:01</i>	<i>B*15:02:01</i>	2096.2 / 2431.8	100 / 100
4	<i>B*15:21</i>	<i>B*15:02:01</i>	<i>B*15:21</i>	<i>B*15:02:01</i>	4308.2 / 4693.6	100 / 100
5	<i>B*35:05</i>	<i>B*15:02:01</i>	<i>B*35:05:01</i>	<i>B*15:02:01</i>	4264.3 / 3620.2	100 / 100
6	<i>B*15:18</i>	<i>B*15:02</i>	<i>B*15:18:01</i>	<i>B*15:02:01</i>	1239.9 / 1277.3	100 / 100
7	<i>B*40:06:01:01</i>	<i>B*15:02</i>	<i>B*40:06:01:01</i>	<i>B*15:02:01</i>	5552.1 / 5139.5	100 / 100
8	<i>B*52:01:01</i>	<i>B*15:02</i>	<i>B*52:01:01:01</i>	<i>B*15:02:01</i>	4651.7 / 5085.9	100 / 100
9	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	2326.4 / 2195.1	100 / 100
10	<i>B*54:01</i>	<i>B*57:01:01</i>	<i>B*54:01:01</i>	<i>B*57:01:01</i>	1999.6 / 2034.6	100 / 100
11	<i>B*40:06:01:01</i>	<i>B*57:01:01</i>	<i>B*40:06:01:01</i>	<i>B*57:01:01</i>	3087.4 / 2833.9	100 / 100
12	<i>B*15:11:01</i>	<i>B*57:01:01</i>	<i>B*15:11:01</i>	<i>B*57:01:01</i>	1948.4 / 1721.9	100 / 100
13	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	1660.0 / 1482.0	100 / 100
14	<i>B*44:03:01</i>	<i>B*57:01:01</i>	<i>B*44:03:01</i>	<i>B*57:01:01</i>	806.0 / 668.2	100 / 100
15	<i>B*55:07</i>	<i>B*58:01</i>	<i>B*55:07</i>	<i>B*58:01:01</i>	771.6 / 1000.2	100 / 100
16	<i>B*38:01:01</i>	<i>B*58:01</i>	<i>B*38:01:01</i>	<i>B*58:01:01</i>	1565.1 / 2139.7	100 / 100
17	<i>B*48:01:01</i>	<i>B*58:01</i>	<i>B*48:01:01</i>	<i>B*58:01:01</i>	5555.3 / 4610.8	100 / 100
18	<i>B*35:01:01</i>	<i>B*58:01:01</i>	<i>B*35:01:01:01</i>	<i>B*58:01:01</i>	819.5 / 778.1	100 / 100
19	<i>B*15:25:01</i>	<i>B*58:01:01</i>	<i>B*15:25:01</i>	<i>B*58:01:01</i>	3441.2 / 3852.0	100 / 100
20	<i>B*54:01</i>	<i>B*58:01:01</i>	<i>B*54:01:01</i>	<i>B*58:01:01</i>	1308.3 / 1454.1	100 / 100
21	<i>B*51:02:01</i>	<i>B*58:01:01</i>	<i>B*51:02:01</i>	<i>B*58:01:01</i>	1464.0 / 1132.7	100 / 100
22	<i>B*51:01:01</i>	<i>B*58:01:01</i>	<i>B*51:01:01</i>	<i>B*58:01:01</i>	462.9 / 636.3	100 / 100
23	<i>B*51:02:01</i>	<i>B*58:01:01</i>	<i>B*51:02:01</i>	<i>B*58:01:01</i>	637.0 / 663.7	100 / 100
24	<i>B*39:23</i>	<i>B*58:01:01</i>	<i>B*39:23</i>	<i>B*58:01:01</i>	2112.6 / 2553.3	100 / 100

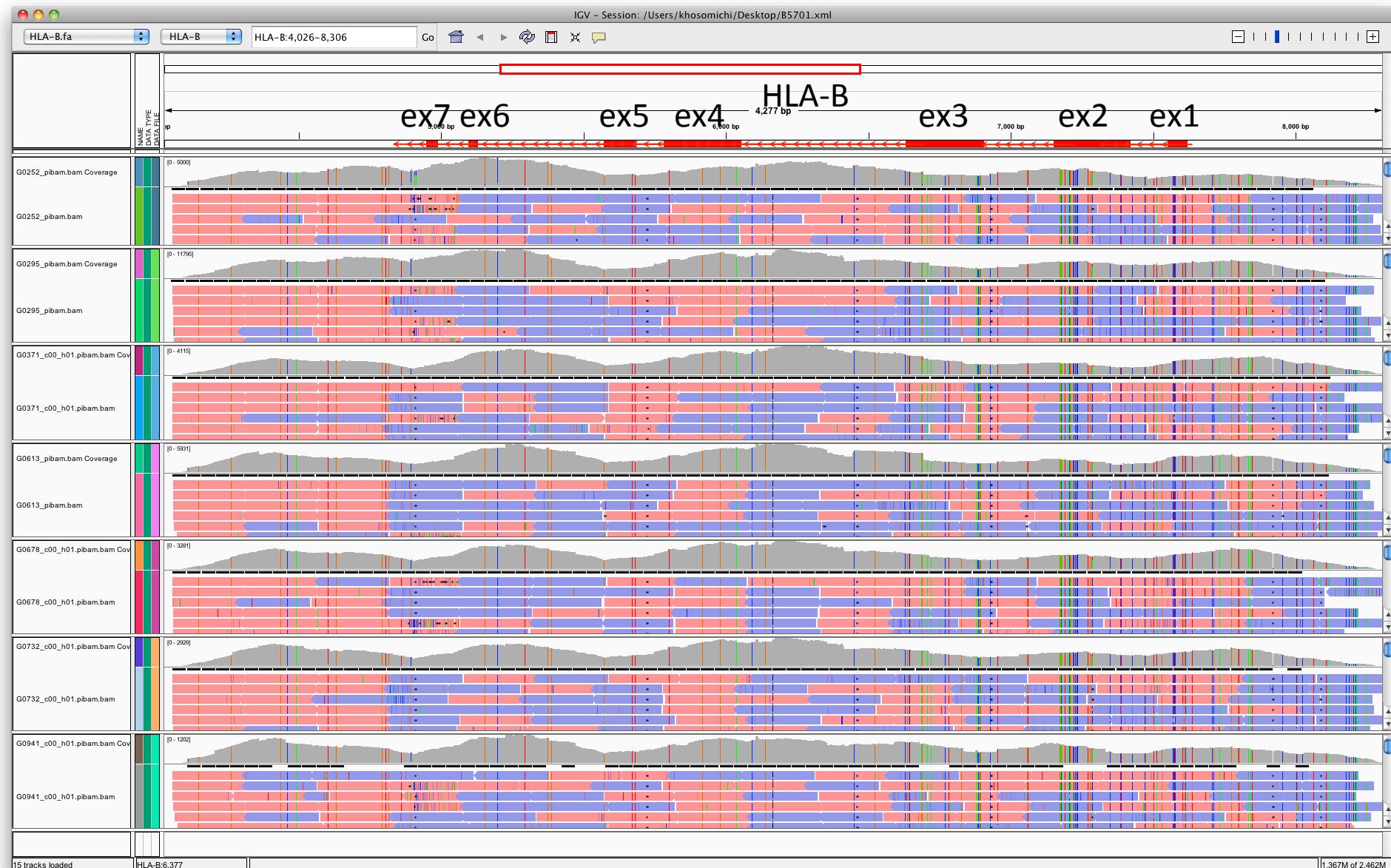
ハプロタイプに分類したアライメント



決定したHLA遺伝子配列

Sample ID	PCR-SSO		Next-gen SBT		Average read depth (allele1 / allele2)	Determined sequence (%) (allele1 / allele2)
1	<i>B*51:01:01</i>	<i>B*15:02</i>	<i>B*51:01:01</i>	<i>B*15:02:01</i>	2593.5 / 2431.3	100 / 100
2	<i>B*07:02:01</i>	<i>B*15:02</i>	<i>B*07:02:01</i>	<i>B*15:02:01</i>	3391.2 / 4206.2	100 / 100
3	<i>B*35:01:01</i>	<i>B*15:02</i>	<i>B*35:01:01:01</i>	<i>B*15:02:01</i>	2096.2 / 2431.8	100 / 100
4	<i>B*15:21</i>	<i>B*15:02:01</i>	<i>B*15:21</i>	<i>B*15:02:01</i>	4308.2 / 4693.6	100 / 100
5	<i>B*35:05</i>	<i>B*15:02:01</i>	<i>B*35:05:01</i>	<i>B*15:02:01</i>	4264.3 / 3620.2	100 / 100
6	<i>B*15:18</i>	<i>B*15:02</i>	<i>B*15:18:01</i>	<i>B*15:02:01</i>	1239.9 / 1277.3	100 / 100
7	<i>B*40:06:01:01</i>	<i>B*15:02</i>	<i>B*40:06:01:01</i>	<i>B*15:02:01</i>	5552.1 / 5139.5	100 / 100
8	<i>B*52:01:01</i>	<i>B*15:02</i>	<i>B*52:01:01:01</i>	<i>B*15:02:01</i>	4651.7 / 5085.9	100 / 100
9	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	2326.4 / 2195.1	100 / 100
10	<i>B*54:01</i>	<i>B*57:01:01</i>	<i>B*54:01:01</i>	<i>B*57:01:01</i>	1999.6 / 2034.6	100 / 100
11	<i>B*40:06:01:01</i>	<i>B*57:01:01</i>	<i>B*40:06:01:01</i>	<i>B*57:01:01</i>	3087.4 / 2833.9	100 / 100
12	<i>B*15:11:01</i>	<i>B*57:01:01</i>	<i>B*15:11:01</i>	<i>B*57:01:01</i>	1948.4 / 1721.9	100 / 100
13	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	<i>B*15:01:01:01</i>	<i>B*57:01:01</i>	1660.0 / 1482.0	100 / 100
14	<i>B*44:03:01</i>	<i>B*57:01:01</i>	<i>B*44:03:01</i>	<i>B*57:01:01</i>	806.0 / 668.2	100 / 100
15	<i>B*55:07</i>	<i>B*58:01</i>	<i>B*55:07</i>	<i>B*58:01:01</i>	771.6 / 1000.2	100 / 100
16	<i>B*38:01:01</i>	<i>B*58:01</i>	<i>B*38:01:01</i>	<i>B*58:01:01</i>	1565.1 / 2139.7	100 / 100
17	<i>B*48:01:01</i>	<i>B*58:01</i>	<i>B*48:01:01</i>	<i>B*58:01:01</i>	5555.3 / 4610.8	100 / 100
18	<i>B*35:01:01</i>	<i>B*58:01:01</i>	<i>B*35:01:01:01</i>	<i>B*58:01:01</i>	819.5 / 778.1	100 / 100
19	<i>B*15:25:01</i>	<i>B*58:01:01</i>	<i>B*15:25:01</i>	<i>B*58:01:01</i>	3441.2 / 3852.0	100 / 100
20	<i>B*54:01</i>	<i>B*58:01:01</i>	<i>B*54:01:01</i>	<i>B*58:01:01</i>	1308.3 / 1454.1	100 / 100
21	<i>B*51:02:01</i>	<i>B*58:01:01</i>	<i>B*51:02:01</i>	<i>B*58:01:01</i>	1464.0 / 1132.7	100 / 100
22	<i>B*51:01:01</i>	<i>B*58:01:01</i>	<i>B*51:01:01</i>	<i>B*58:01:01</i>	462.9 / 636.3	100 / 100
23	<i>B*51:02:01</i>	<i>B*58:01:01</i>	<i>B*51:02:01</i>	<i>B*58:01:01</i>	637.0 / 663.7	100 / 100
24	<i>B*39:23</i>	<i>B*58:01:01</i>	<i>B*39:23</i>	<i>B*58:01:01</i>	2112.6 / 2553.3	100 / 100

HLA-B*58:01 の塩基配列の比較



トピックス

- 次世代シークエンサーについて
- ロングPCRとNextera Kitによるシークエンス概要
- ライブラリ調整における改良事例
- HLA遺伝子解析の実例
- HLA遺伝子のデータ解析およびタイピング

HLA解析ツール (1)

- p-galaxy <http://p-galaxy.ddbj.nig.ac.jp>

The screenshot shows the P-GALaxy web interface. On the left, there is a sidebar with a red box highlighting the "HOSOMICHI HLA ANALYSIS" section. This section contains a list of tools:

- Trim By Quality
- Picks Up Fine Pairs From Paired Read Set.
- Map with BWA for Illumina
- samtools_HOSO
- GATK FastaAlternateReferenceMaker
- GATK UnifiedGenotyper
- awk Sam 300
- Picard add
- BAM to CDS
- Rewrite Vcf
- GrepVariant
- awk cds closest
- awk cds perfect
- Blat for haplo
- Haplo

Below this list are "Get Data" and "Send Data" links, followed by sections for ENCODE Tools, Lift-Over, and Text Manipulation.

The main workspace is titled "Map with BWA for Illumina (version 1.2.3)". It includes fields for selecting a reference genome (set to "HLA-B"), indicating if the library is mate-paired (set to "Paired-end"), and specifying forward and reverse FASTQ files. It also includes settings for BWA (set to "Commonly Used") and options to suppress the header in the output SAM file. A large blue "Execute" button is at the bottom of this form.

The right side of the interface shows a history panel titled "Unnamed history" with 0 bytes, containing a note about starting a new analysis. There are also standard browser controls like back, forward, and search.

HLA解析ツール（1）

HLA解析ツール (1)

Galaxy / TEST-PG

http://133.39.116.52:9080/workflow/editor?id=b472e2eb553fa0d1

Google

アップル Yahoo! Japan Google マップ Google 翻訳 YouTube Wikipedia NIG SSL VPN Service DDBJとか Galaxy / TEST-PG lbi.gov/WebApolloDemo

Galaxy / TEST-PG Analyze Data ワークフロー Shared Data Visualization P-galaxy Manual Admin Help User Using 55.8 GB

ツール Workflow Get Data Send Data ENCODE Tools Lift-Over Text Manipulation Filter and Sort Join, Subtract and Group Convert Formats Extract Features Fetch Sequences Fetch Alignments Get Genomic Scores Operate on Genomic Intervals Statistics Wavelet Analysis Graph/Display Data Regional Variation Multiple regression Multivariate Analysis Evolution Motif Tools

Workflow Canvas | imported: HLAwf

```
graph LR; A[Trim By Quality] --> B[Picks Up Fine Pairs From Paired Read Set]; C[Trim By Quality] --> D[Picks Up Fine Pairs From Paired Read Set]; B --> E[Map with BWA for Illumina]; C --> F[Map with BWA for Illumina]; E --> G[awk Sam]; F --> H[awk Sam];
```

Details

Edit Workflow Attributes

Name: imported: HLAwf

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール (1)

Galaxy / TEST-PG

http://133.39.116.52:9080/workflow/editor?id=b472e2eb553fa0d1

Google

アップル Yahoo! Japan Google マップ Google 翻訳 YouTube Wikipedia NIG SSL VPN Service DDBJとか Galaxy / TEST-PGlbl.gov/WebApolloDemo

Galaxy / TEST-PG Analyze Data ワークフロー Shared Data Visualization P-galaxy Manual Admin Help User Using 55.8 GB

ツール Workflow Get Data Send Data ENCODE Tools Lift-Over Text Manipulation Filter and Sort Join, Subtract and Group Convert Formats Extract Features Fetch Sequences Fetch Alignments Get Genomic Scores Operate on Genomic Intervals Statistics Wavelet Analysis Graph/Display Data Regional Variation Multiple regression Multivariate Analysis Evolution Motif Tools

Workflow Canvas | imported: HLAwf

```
graph LR; A[samtools_HOSO] --> B[samtools_HOSO]; A --> C[awk Sam 300]; B --> D[Picard add]; D --> E[GATK UnifiedGenotyper]; C --> D; E --> F[PiBam]
```

Details

Edit Workflow Attributes

Name: imported: HLAwf

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール (1)

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Analyze Data ワークフロー Shared Data Visualization P-galaxy Manual Admin Help User Using 55.8 GB

ツール Workflow Canvas | imported: HLAwf

Workflow Canvas (imported: HLAwf)

```
graph LR; A[GATK UnifiedGenotyper] --> B[Rewrite Vcf]; B --> C[GrepVariant]; C --> D[Haplo];
```

Details

Edit Workflow Attributes

Name: imported: HLAwf

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール (1)

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http://133.39.116.52:9080/workflow/editor?id=b472e2eb553fa0d1

Analyze Data ワークフロー Shared Data Visualization P-galaxy Manual Admin Help User Using 55.8 GB

ツール Workflow Canvas | imported: HLAwf

Workflow Canvas diagram:

```
graph LR; H[Haplo] -- Sam --> S1[samtools_HOSO]; H -- variant table --> S1; S1 -- Sam --> O1[output (bam)]; S1 -- output1 (sam) --> S2[samtools_HOSO]; S1 -- output2 (sam) --> S3[samtools_HOSO]; S2 -- Sam --> O2[output (bam)]; S3 -- Sam --> O3[output (bam)]; O2 -- Bam --> O4[output (bam)]; O3 -- Bam --> O5[output (bam)]; O4 -- Bam --> O6[output (bam)]; O5 -- Bam --> O6; O6 -- Bam --> Picard[Picard add]; Picard -- Bam --> O7[output (bam)];
```

Details

Edit Workflow Attributes

Name: imported: HLAwf

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール (1)

Galaxy / TEST-PG

http://133.39.116.52:9080/workflow/editor?id=b472e2eb553fa0d1

Analyze Data ワークフロー Shared Data Visualization P-galaxy Manual Admin Help User Using 55.8 GB

ツール Workflow Canvas | imported: HLAwf

Workflow Canvas (imported: HLAwf)

```
graph LR; A1[Picard add] --> B1[Bam output (bam)]; A2[Picard add] --> B2[Bam output (bam)]; B1 --> C1[GATK UnifiedGenotyper]; B2 --> C2[GATK UnifiedGenotyper]; C1 --> D1[PiBam output (vcf)]; C2 --> D2[PiBam output (vcf)]; D1 --> E1[Rewrite Vcf]; D2 --> E2[Rewrite Vcf]; E1 --> F1[Vcf output (vcf)]; E2 --> F2[Vcf output (vcf)]; F1 --> G1[GATK FastaAlternate]; F2 --> G2[GATK FastaAlternate]; G1 --> H1[Variant VCF output (fastq)]; G2 --> H2[Variant VCF output (fastq)];
```

Details

Edit Workflow Attributes

Name: imported: HLAwf

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール（1）

Galaxy / TEST-PG

Workflow Canvas | imported: HLAwf

Workflow Attributes:

- Name: imported: HLAwf
- Tags: Apply tags to make it easy to search for and find items with the same tag.
- Annotation / Notes: Describe or add notes to workflow Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール (1)

Galaxy / TEST-PG

http://133.39.116.52:9080/workflow/editor?id=b472e2eb553fa0d1

Analyze Data ワークフロー Shared Data Visualization P-galaxy Manual Admin Help User Using 55.8 GB

ツール Workflow Canvas | imported: HLAwf

Workflow Canvas diagram:

```
graph LR; A1[Fastq] --> B1[BAM to CDS]; A2[Fastq] --> B2[BAM to CDS]; B1 --> C1[Blat for haplo]; B2 --> C2[Blat for haplo]; C1 --> D1[awk cds closest]; C2 --> D2[awk cds perfect]; D1 --> E1[Blat psl]; D2 --> E2[Blat psl]; E1 --> F1[awk cds closest]; E2 --> F2[awk cds perfect]; F1 --> G1[Blat psl]; F2 --> G2[Blat psl]; G1 --> H1[Blat psl]; G2 --> H2[Blat psl]; H1 --> H2;
```

Details

Edit Workflow Attributes

Name: imported: HLAwf

Tags:

Apply tags to make it easy to search for and find items with the same tag.

Annotation / Notes:

Describe or add notes to workflow

Add an annotation or notes to a workflow; annotations are available when a workflow is viewed.

HLA解析ツール (2)

HLA analysis pipeline

English Login

HLA

221.186. 221.186. /HLA/

New Analysis Reanalysis Reanalysis(analysis Only) Search Reload making summary Download Jobs : Run [0] Wait [1] Delete

sequence file1 sequence file2 comment analysis registered start end status

Register a new analysis

1. target sequence files

single end paired end

File1: File2:

threads number: 4

2. sequence trimming

2.1. trimming of adaptor portions

exec trimming

adaptor sequence: TruSeq

min overlap length: 12 error rate: 0.1 min seqs length: 0

treatment of 'N': as wildcards

method: remove adaptor portions

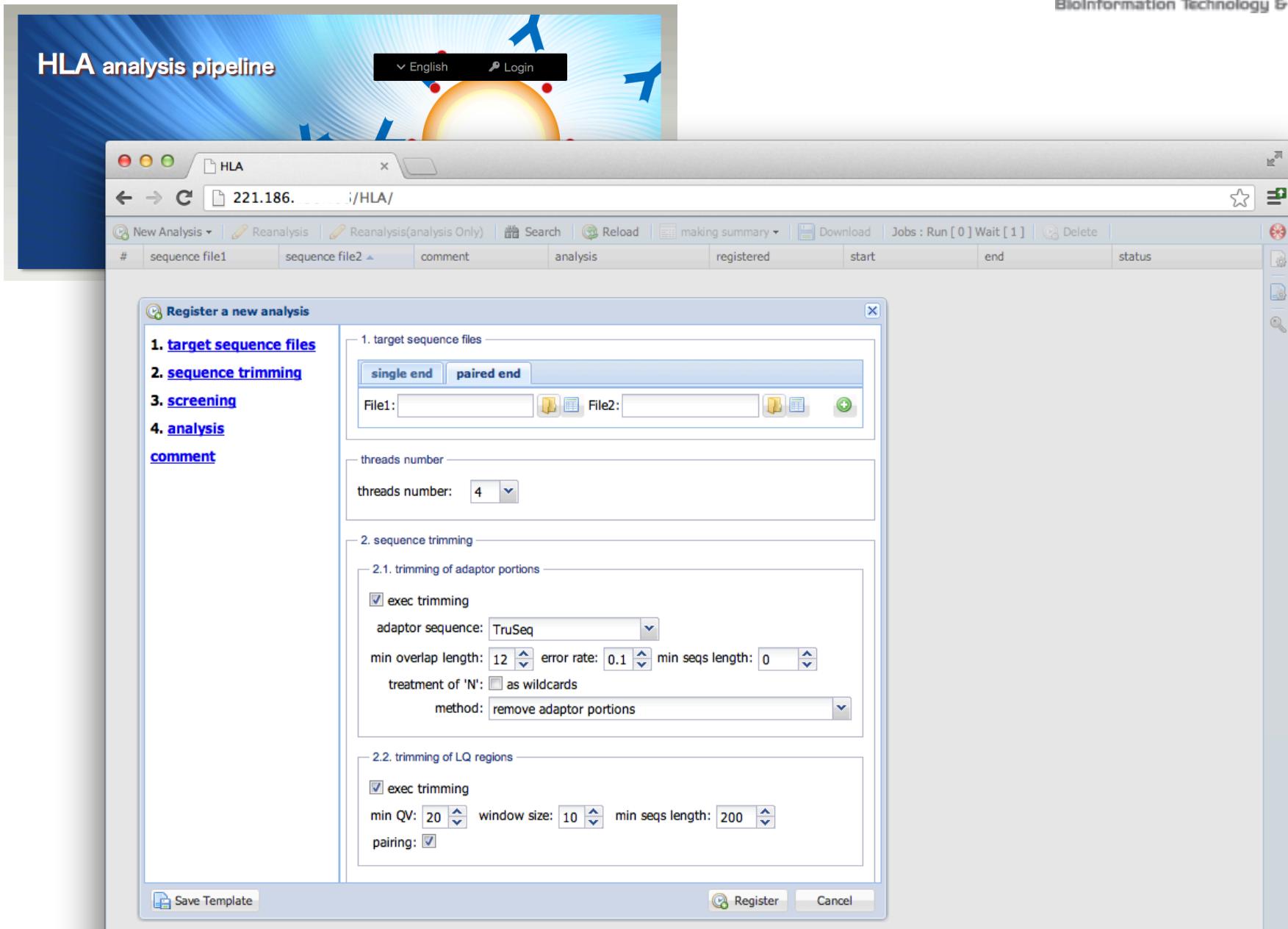
2.2. trimming of LQ regions

exec trimming

min QV: 20 window size: 10 min seqs length: 200

pairing:

Save Template Register Cancel



HLA解析ツール (2)

1. target sequence files

single end paired end

File1:   File2:   

threads number

threads number: 

HLA解析ツール (2)

1. target sequence files

single end paired end

2. sequence trimming

2.1. trimming of adaptor portions

exec trimming
adaptor sequence: TruSeq
min overlap length: 12 error rate: 0.1 min seqs length: 0
treatment of 'N': as wildcards
method: remove adaptor portions

2.2. trimming of LQ regions

exec trimming
min QV: 20 window size: 10 min seqs length: 200
pairing:



HLA解析ツール (2)

1. target sequence files

single end paired end

2. sequence trimming

2.1. trimming of adaptor portions

exec trimming
adaptor sequence: TruSeq
min overlap length: 12 error rate: 0.1 min seqs length: 0
treatment of 'N': as wildcards
method: remove adaptor portions

2.2. trimming of LQ regions

exec trimming
min QV: 20 window size: 10 min seqs length: 200
pairing:

4. analysis

Program: bwa (aln) DB: HLA-A Release: Apr. 2013

4.1. mapping, SNP call, phasing

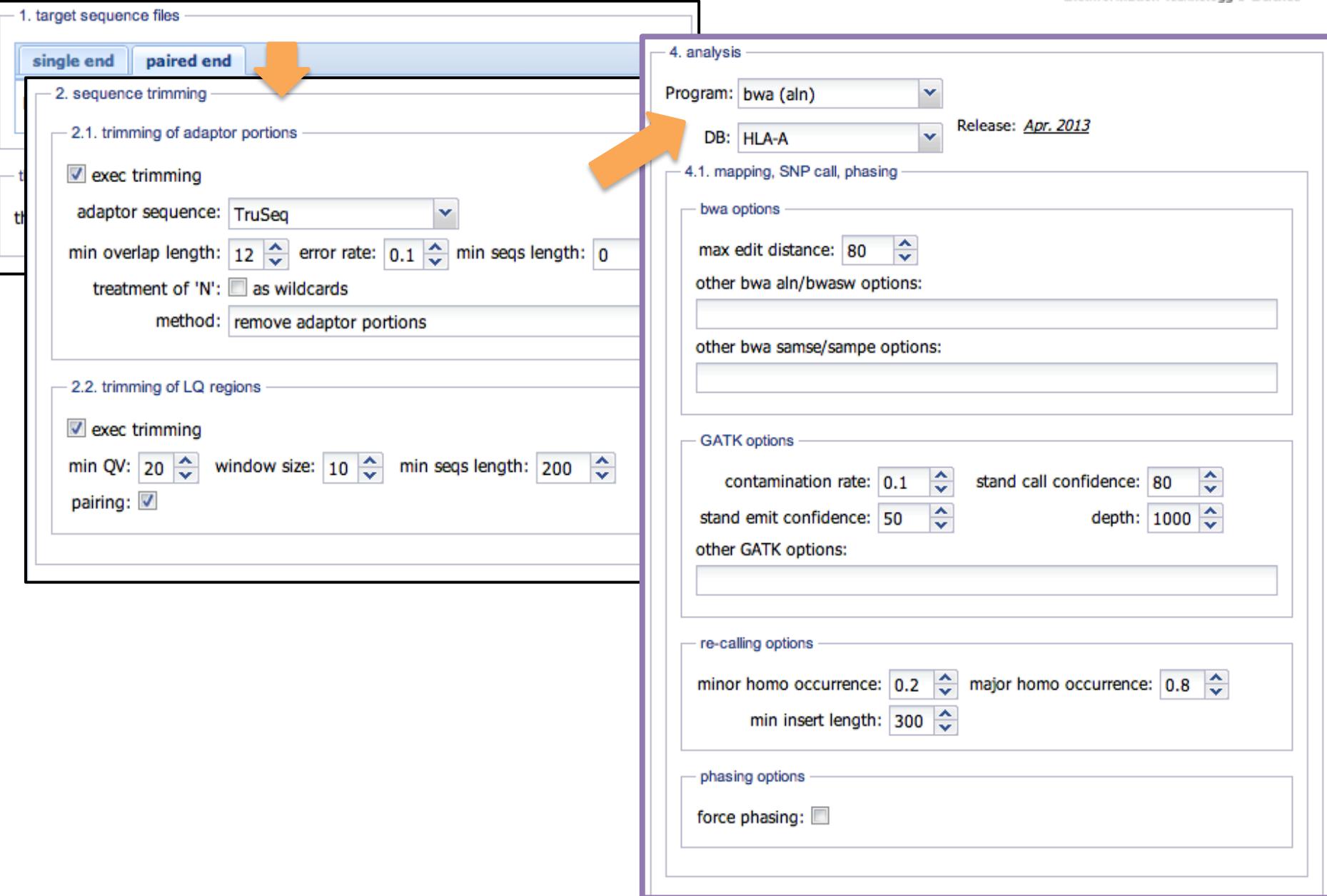
bwa options
max edit distance: 80
other bwa aln/bwasw options:

other bwa samse/sampe options:

GATK options
contamination rate: 0.1 stand call confidence: 80
stand emit confidence: 50 depth: 1000
other GATK options:

re-calling options
minor homo occurrence: 0.2 major homo occurrence: 0.8
min insert length: 300

phasing options
force phasing:



HLA解析ツール (2)

1. target sequence files

single end paired end **paired end**

2. sequence trimming

2.1. trimming of adaptor portions

exec trimming

adaptor sequence: TruSeq

min overlap length: 12 error rate: 0.1 min seqs length: 0

4.2. haplotype analysis

making haplotype options

GATK options

stand call confidence: 50 stand emit confidence: 10

depth: 1000

other GATK options:

re-calling options

minor homo occurrence: 0.4 major homo occurrence: 0.6

blat options

min identity: 99 tile size: 18 max mismatch: 2

other blat options:

4. analysis

Program: bwa (aln) DB: HLA-A Release: Apr. 2013

4.1. mapping, SNP call, phasing

bwa options

max edit distance: 80

other bwa aln/bwasw options:

other bwa samse/sampe options:

GATK options

contamination rate: 0.1 stand call confidence: 80

stand emit confidence: 50 depth: 1000

other GATK options:

re-calling options

minor homo occurrence: 0.2 major homo occurrence: 0.8

min insert length: 300

phasing options

force phasing:

The screenshot displays the HLA Analysis Tool's configuration interface. It includes sections for target sequence files (with 'paired end' selected), sequence trimming, and analysis parameters. The analysis section is expanded, showing details for the bwa (aln) program using the HLA-A database, released in April 2013. The 'GATK options' section is highlighted with orange arrows, specifically pointing to the 'stand call confidence' (50), 'stand emit confidence' (10), and 'depth' (1000) fields. Other sections shown include 'haplotype analysis' (making haplotype options, GATK options, re-calling options, blat options), 'mapping, SNP call, phasing' (bwa options, other bwa options, other bwa samse/sampe options), and 'phasing options'.

HLAタイピングソフトウェア



HLA Typing

Determine the HLA types
within sets of NGS sequencing data.



GENDX NGSengine®

エクソームシークエンスデータから のHLAタイプ

Analysis	Allele	HLA-A	HLA-B	HLA-C	HLA-E	HLA-F	HLA-G	HLA-DRA	HLA-DRB1	HLA-DRB2	HLA-DRB3	HLA-DRB4	HLA-	
E33_exome	Allele 1	A*24:02:01:01	B*40:02:01	C*12:02:02	E*01:03:01	F*01:01:02	G*01:04:03	DRA*01:02:02	DRB1*15:02:01		DRB3*02:02:01		DRB5*0	
		A*02:89												
E34_exome	Allele 1	A*02:01:01:01	B*13:01:01	C*03:04:01	E*01:01:01	F*01:01:01	G*01:01:01	DRA*01:02:02	DRB1*15:01:01		DRB3*03:01:01		DRB5*0	
	Allele 2	A*02:01:01:01	B*35:01:01	C*08:01:01	E*01:03:02	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*12:02:01		DRB3*03:01:01		DRB5*0	
E35_exome	Allele 1	A*02:06:01	B*15:01:01	C*07:04:01	E*01:03:01	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*04:01:01			DRB4*01:03:01		
	Allele 2	A*02:01:01:01	B*15:18:01	C*04:01:01:01	E*01:01:01	F*01:01:03	G*01:01:01	DRA*01:01:01	DRB1*04:06:01		DRB4*01:02			
E36_exome	Allele 1	A*33:03:01	B*54:01:01	C*01:02:01	E*01:03:01	F*01:01:02	G*01:04:01	DRA*01:01:01	DRB1*04:05:01		DRB3*03:01:01	DRB4*01:03:01		
	Allele 2	A*24:02:01	B*44:03:01	C*14:03	E*01:01:01	F*01:01:03	G*01:04:01	DRA*01:02:02	DRB1*13:02:01		DRB3*03:01:01	DRB4*01:03:01		
E37_exome	Allele 1	A*24:02:01:01	B*15:20	C*03:03:01	E*01:01:01	F*01:01:02	G*01:04:01	DRA*01:01:01	DRB1*04:03:01		DRB4*01:03:01			
	Allele 2	A*24:02:01:01	B*35:43:01	C*03:03:01	E*01:03:01	F*01:01:03	G*01:04:01	DRA*01:01:01	DRB1*09:01:02		DRB4*01:03:01			
E38_exome	Allele 1	A*24:11N	B*40:03	C*12:02:02	E*01:03:01	F*01:01:03	G*01:04:01	DRA*01:02:02	DRB1*15:02:01			DRB4*01:03:02	DRB5*0	
	Allele 2	A*24:02:01:01	B*52:01:01	C*03:04:01	E*01:03:01	F*01:01:02:01	G*01:01:02:01	DRA*01:01:01	DRB1*09:01:02		DRB4*01:03:02	DRB5*0		
E39_exome	Allele 1	A*24:02:01:01	A*24:11N	B*40:02:01	C*03:04:01	E*01:03:02	F*01:01:01:01	G*01:01:08	DRA*01:01:01	DRB1*04:05:01		DRB3*02:02:01	DRB4*01:03:01	
	Allele 2	A*02:01:01	B*59:01:01	C*01:02:01	E*01:03:02	F*01:01:03	G*01:04:03	DRA*01:01:01	DRB1*14:54:01		DRB3*02:02:01	DRB4*01:03:01		
E40_exome	Allele 1	A*11:01:01	B*54:01:01	C*03:03:01	E*01:01:01	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*04:05:01		DRB4*01:03:01			
	Allele 2	A*26:01:01	B*15:01:01	C*01:02:01	E*01:01:01	F*01:01:01	G*01:02:01	DRA*01:01:01	DRB1*04:05:01		DRB4*01:03:01			
E41_exome	Allele 1	A*24:02:01	A*24:11N	B*07:02:01	C*07:02:01:03	E*01:01:01	F*01:01:01	G*01:04:01	DRA*01:01:01	DRB1*15:01:01				DRB5*0
	Allele 2	A*26:02	B*35:01:01	C*03:03:01	E*01:01:01	F*01:01:03	G*01:01:02:01	DRA*01:01:01	DRB1*01:01:01				DRB5*0	
E42_exome	Allele 1	A*26:01:01	B*55:02:01	C*01:02:01	E*01:03:01	F*01:01:01:08	G*01:01:02:01	DRA*01:02:02	DRB1*04:05:01		DRB3*01:01:02	DRB4*01:03:01	DRB5*0	
	Allele 2	A*26:01:01	B*54:01:01	C*01:02:01	E*01:03:02:02	F*01:01:01:08	G*01:01:02:01	DRA*01:01:01	DRB1*12:01:01		DRB3*01:01:02	DRB4*01:03:01	DRB5*0	
E43_exome	Allele 1	A*02:06:01		B*07:02:01	C*07:02:01	E*01:03:02	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*01:01:01			DRB4*01:01:01	
	Allele 2	A*11:01:01	B*48:01:01	C*08:01:01	E*01:03:02	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*09:01:02			DRB4*01:03:02		
E44_exome	Allele 1	A*24:02:01:01	A*24:11N	B*48:01:01	C*01:02:01	E*01:03:02	F*01:01:03	G*01:01:08	DRA*01:01:01	DRB1*16:02:01			DRB4*01:03:01	DRB5*0
	Allele 2	A*02:06:01	B*59:01:01:02	C*08:40	E*01:03:02	F*01:01:01	G*01:04:03	DRA*01:01:01	DRB1*04:05:01			DRB4*01:03:01	DRB5*0	
E45_exome	Allele 1	A*24:02:01:01	B*07:02:01	C*01:02:01	E*01:01:01	F*01:01:03	G*01:04:01	DRA*01:01:01	DRB1*15:01:01					DRB5*0
	Allele 2	A*24:02:01:01	B*56:01:01	C*07:02:01	E*01:03:02:02	F*01:01:03	G*01:04:01	DRA*01:01:01	DRB1*01:01:01					DRB5*0
E46_exome	Allele 1	A*02:01:01:01	B*40:02:01	C*03:03:01	E*01:03:02	F*01:01:01	G*01:01:01	DRA*01:02:02	DRB1*09:01:02		DRB3*03:01:01	DRB4*01:03:01		
	Allele 2	A*02:01:01:01	B*15:11:01	C*03:03:01	E*01:03:01	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*12:02:01		DRB3*03:01:01	DRB4*01:03:01		
E47_exome	Allele 1	A*02:01:01:01	B*46:01:01	C*01:02:01	E*01:03:02	F*01:01:01	G*01:01:01	DRA*01:02:02	DRB1*15:01:01					DRB5*0
	Allele 2	A*02:06:01	B*15:18:01	C*03:04:01	E*01:03:01	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*08:03:02					DRB5*0
E48_exome	Allele 1	A*11:01:01	B*59:01:01	C*07:02:01	E*01:01:01	F*01:01:02	G*01:01:03	DRA*01:01:01	DRB1*15:01:01		DRB4*01:03:01	DRB5*0		
	Allele 2	A*02:06:01	B*39:01:03	C*01:02:01	E*01:01:01	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*04:05:01		DRB4*01:03:01	DRB5*0		
E49_exome	Allele 1	A*24:11N	B*40:01:02	C*03:04:01	E*01:03:01	F*01:01:02	G*01:04:01	DRA*01:01:01	DRB1*11:01:01		DRB3*02:02:01	DRB4*01:03:01		
	Allele 2	A*33:03:01	B*59:01:01:02	C*01:02:01	E*01:03:02	F*01:01:01	G*01:04:01	DRA*01:01:01	DRB1*04:05:01		DRB3*02:02:01	DRB4*01:03:01		
E50_exome	Allele 1	A*02:06:01	B*54:01:01	C*01:02:01	E*01:03:02	F*01:01:01	G*01:01:02:01	DRA*01:01:01	DRB1*04:05:01		DRB3*01:01:02	DRB4*01:03:01		
	Allele 2	A*31:01:02	B*51:01:01	C*14:02:01	E*01:03:02	F*01:01:03	G*01:01:08	DRA*01:02:02	DRB1*14:03:01		DRB3*01:01:02	DRB4*01:03:01		
E51_exome	Allele 1	A*02:06:01	B*54:01:01	C*14:02:01	E*01:01:01	F*01:01:01	G*01:01:01	DRA*01:01:01	DRB1*04:05:01		DRB3*02:02:01	DRB4*01:03:01		
	Allele 2	A*31:01:02	A*31:14N	B*51:01:01	C*01:02:01	E*01:03:02	F*01:01:03	G*01:03:01	DRA*01:01:01	DRB1*11:01:01		DRB3*02:02:01	DRB4*01:03:01	
E52_exome	Allele 1	A*24:02:01:01	A*24:11N	B*59:01:01	C*01:02:01	E*01:03:01	F*01:01:02	G*01:04:01	DRA*01:01:01	DRB1*15:02:01		DRB4*01:03:01	DRB5*0	
	Allele 2	A*26:01:01	B*52:01:01	C*12:02:02	E*01:03:01	F*01:01:01	G*01:01:02:01	DRA*01:02:02	DRB1*04:05:01		DRB4*01:03:01	DRB5*0		

ご質問等は

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までご連絡ください