本研究において、等温増幅法により HLA-DRB1 の low resolution タイピングが可能であることを示した。さらに、日本人集団で最も関節リウマチと強い関連を示す DRB1*04:05 も特異的に増幅可能であった。DRB1 の全アリルを増幅する条件と DRB1*04:05 の特異的増幅条件は同じであるので、同一プラットホーム上でタイピング可能である。これらは「関節リウマチの病型・病態進行予測ツール」に必要な HLA 情報を提供するためのプロトタイプになり得ると考えられる。

我々が用いている等温増幅法（改良 LAMP 法）は 4 種類のプライマーで 6 種類の多型領域を認識させることが可能である。そのため HLA アリルの多型領域のシス・トランスの位置関係も区別が可能である。しかし、この方法は singleplex であり、multiplex 化のためプラスチック基板上に固定したプローブの特性でタイピングを行う場合は、全 DRB1 アリルを増幅するプライマーを用いるので、シス・トランスの位置関係を区別できない。そのため “ambiguity” が生じる。例えば DRB1*15:01/15:02 のホモ接合体と DRB1*1501/15:02、DRB1*16:02 のヘテロ接合体はどちらも RG2P, 15cP のプローブに反応するので (Table 1) 区別できない。Ambiguity を少なくするためには、今後群特異的増幅の検討や、アリル特異的増幅との組み合わせの検討が必要である。

RA 感受性遺伝子の SNP タイピングについても、等温増幅法によるアリル特異的増幅が可能であり、それを可視化することも可能であった。さらに、等温増幅の条件は HLA と同一であるので同一のプラットホーム上でタイピングが可能である。プラスチック基板上での SNP タイピングの場合、プラスチックプレートの熱伝導率が良くないために非特異的増幅が起こり、すべての SNP で可能にはなっていない。今後の検討課題である。

等温増幅とプラスチック基板上でのプローブからの伸張反応を組み合わせ、多型を検出する本法は、HLA と SNP を同一のプラットホーム上でタイピング可能であり、特殊な機器を必要としないので、比較的広範囲の医療施設で使われ得ると考えられる。

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F.研究発表

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H. 知的財産権の出願・登録状況

なし

Table 1. DRB1 アリルのグループ分けと反応するプローブ

DRB1 Allele	Probe
DRB1*01:01	RG1P, dr1406P
DRB1*15:01/02	RG2P, 15cP
DRB1*16:02	RG2P
DRB1*13:01/02	RG356P, dr13P
DRB1*11:01	RG356P, dr1101P
DRB1*14:03	RG356P, dr08P
DRB1*03:01	RG356P
DRB1*14:06	RG356P, dr1406P
DRB1*14:01/05/07/12/29	RG356P, dr1401/05/07P
DRB1*04:01/03/04/05/06/07/10	RG4P
DRB1*12:01/02	RG512.8P
DRB1*08:02/03/09	RG512.8P, dr08P
DRB1*07:01	RG7P
DRB1*09:01	RG9P
DRB1*10:01	RG10P
Positive Control	GPC

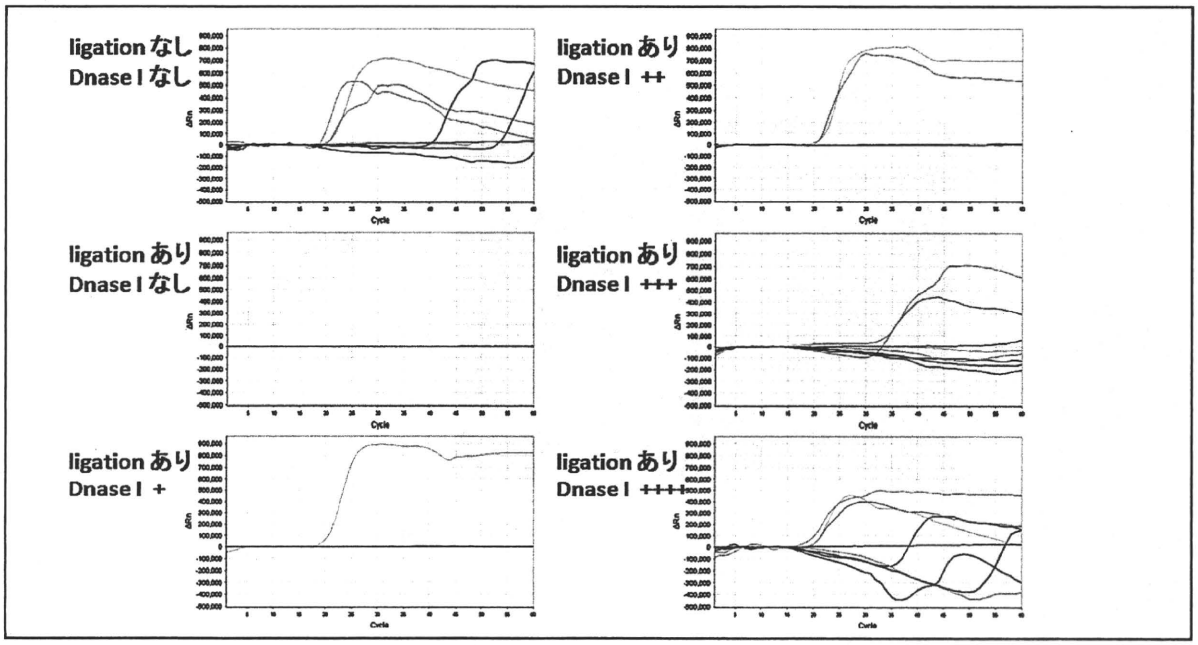
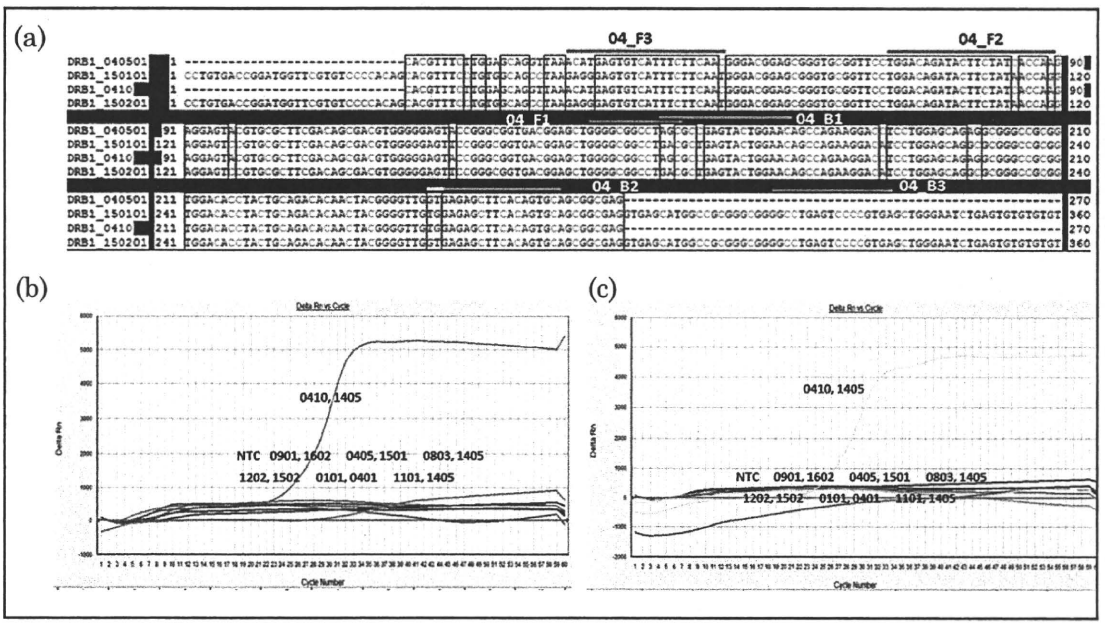


Figure 1. LAMP 反応に対する Ligation と DNase I の効果



Fiogure 2. LAMP による HLA-DRB1 のアリル特異的増幅には outer primer F3, B3 を必要としない。(a) に示したプライマーを用いて DRB1*0410 特異的増幅を行った。(b) は outer primer F3, B3 も使用し、(c) は F3, B3 を使用しないで反応を行った。ほぼ同様な増幅効率が得られている。

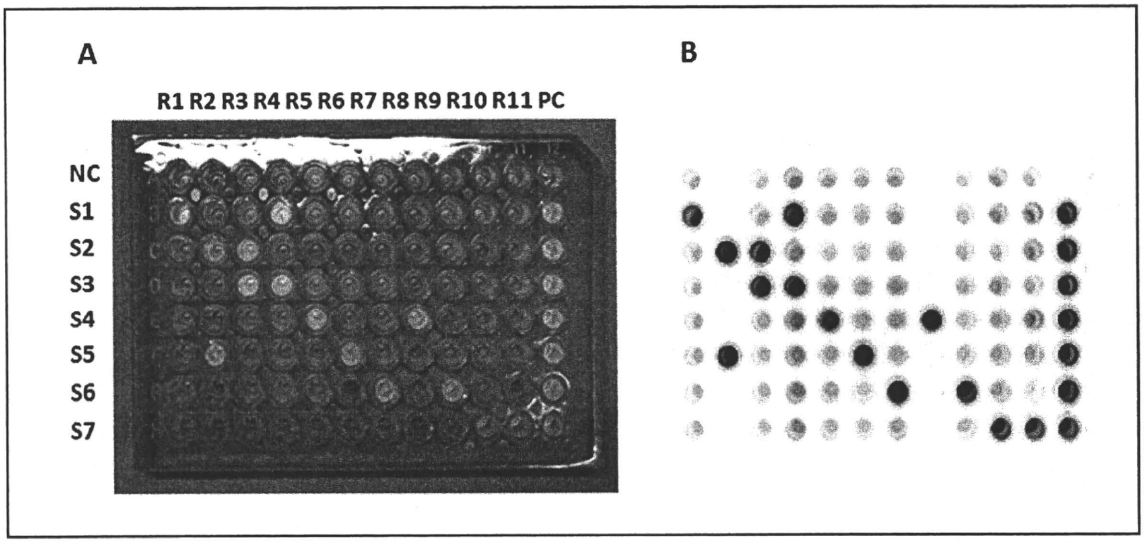


Figure 3. HLA-DRB1 の low-resolution typing. A は通常の写真、B は同じものを LAS（富士フイルム）を用いて SYBR Green I の蛍光を撮影したもの。NC: negative control (DW), S1-S7: サンプル 1 ～サンプル 7 , R1-R11: HLA-DRB1 アリルの群特異的増幅を行うプライマーセット 1～11 を含むもの、PC: positive control（すべての DRB1 アリルを増幅するプライマーセットを含むもの）。1 サンプルには R1～R11 および PC のプライマーセットを用いるので、12 well での反応が必要となる。各 well での増幅の有無により DRB1 のタイピングを行う。A では白い well、B では黒い well が増幅した well（プライマーセット）。

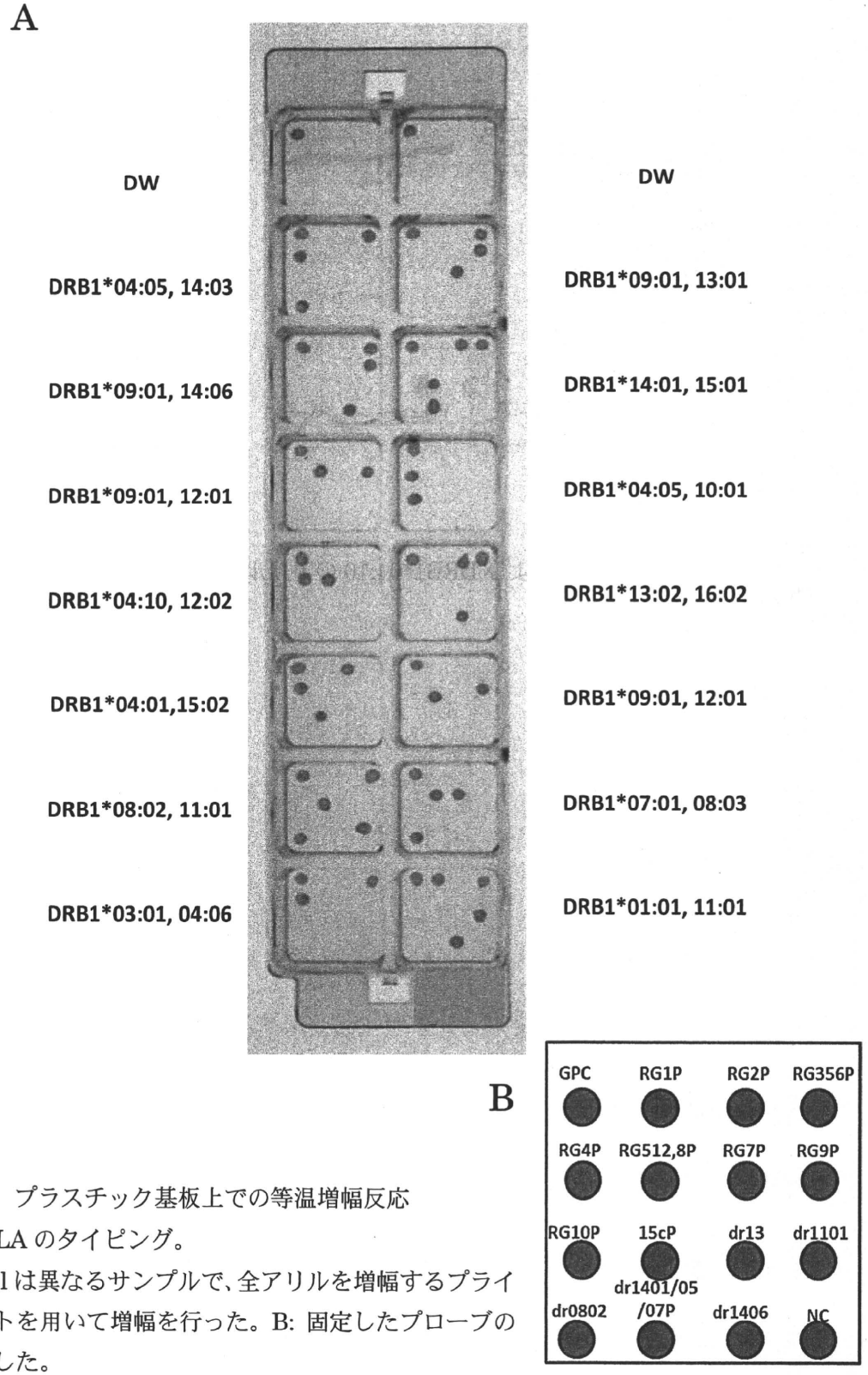


Figure 4. プラスチック基板上での等温増幅反応による HLA のタイピング。
A: 各 well は異なるサンプルで、全アリルを増幅するプライマーセットを用いて増幅を行った。B: 固定したプローブの位置を示した。

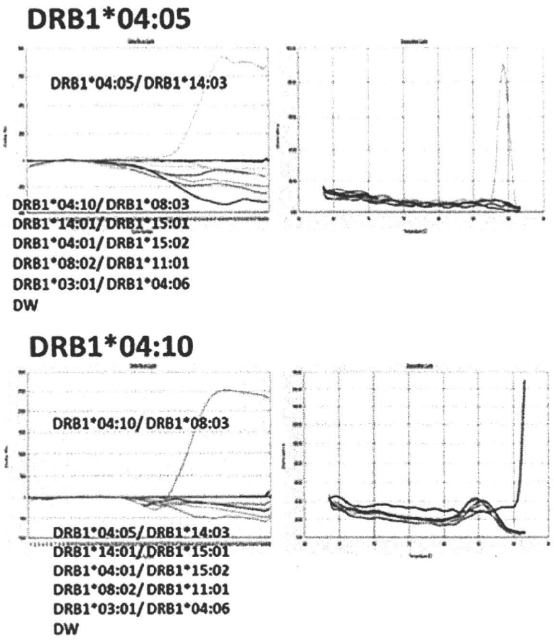


Figure 5. DRB1*04:05 および DRB1*04:10 のアリル特異的増幅。左側は増幅曲線。右側は同じものの解離曲線。

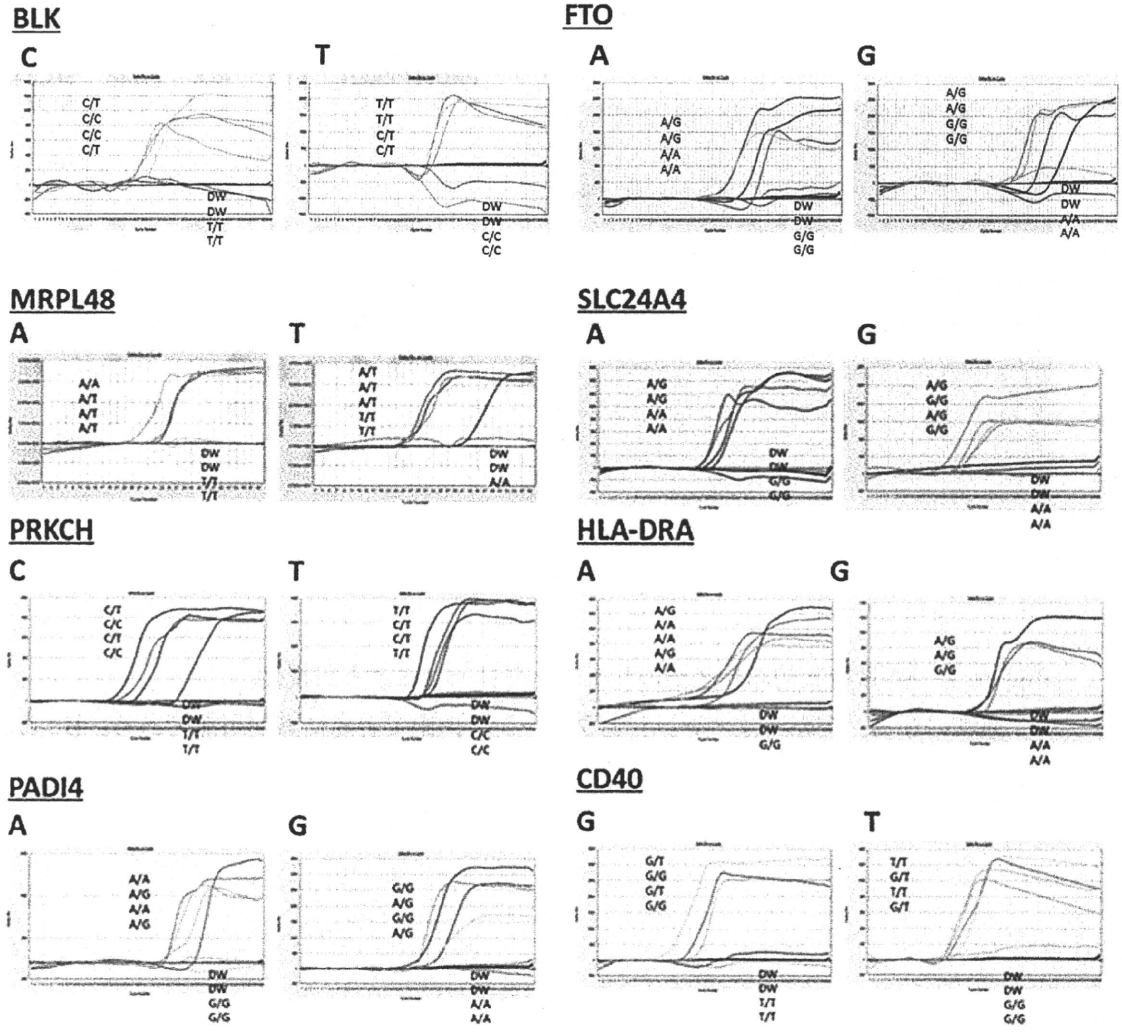


Figure 6. 等温増幅法による SNP のタイピング
各遺伝子の SNP を SNP ごとに特異的に増幅した。遺伝子名と各 SNP の塩基は図中に示した。

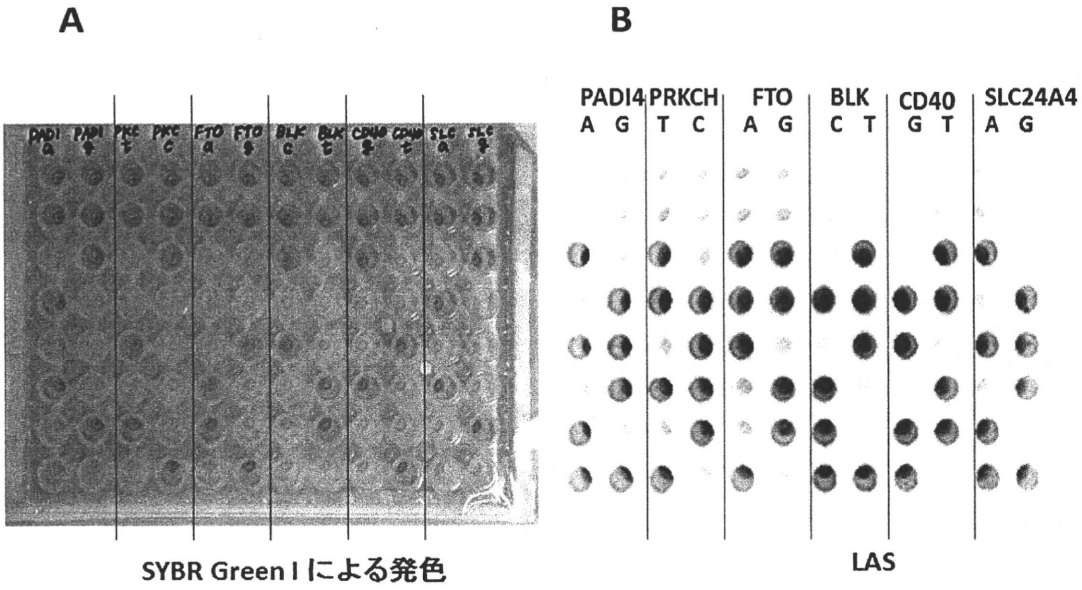


Figure 7. SNP 特異的増幅産物のサイバーグリーンによる可視化。
A：通常の写真。実際にはオレンジとグリーンで肉眼で容易に識別可能である。B：A と同じものを LAS（富士フイルム）を用いて SYBR Green I の蛍光を撮影したもの。A では白い well、B では黒い well が増幅した well。

II. 研究成果の刊行に関する一覧表

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