Economical liquid phase dose assays in microplates: proofs of principle for sex testing and haptoglobin duplicon genotyping

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>1400 variable loci at last count

(Sanger Centre)

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Segmental duplication content of the human genome

Chromosome	% Intra	% Inter
1	2.1	1.2
2	2.0	1.4
3	1.1	0.8
4	1.4	1.1
5	1.9	0.8
6	1.4	0.4
7	5.5	1.7
8	1.6	0.8
9	5.5	3.6
10	3.9	1.0
11	2.6	1.2
12	1.4	0.7
13	0.8	1.1
14	0.5	0.8
15	4.6	2.4
16	6.0	2.7
17	6.0	0.8
18	0.7	0.9
19	4.5	1.2
20	0.9	1.4
21	1.1	2.9
22	3.6	2.9
X	1.8	2.0
Y	10.2	6.0

Cheung et al. *Genome Biology 2003 4:R25*

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Defensins

- **Important part of immune system**
- **Small peptides**
- **Hexamers – ion channels in pathogen membrane**
- **Permeabilisation → cell death**
- **DEFA1: 3 - 9 copies (majority 5)**
- **DEFA3: 0 – 4 copies (majority 1 or 2)**

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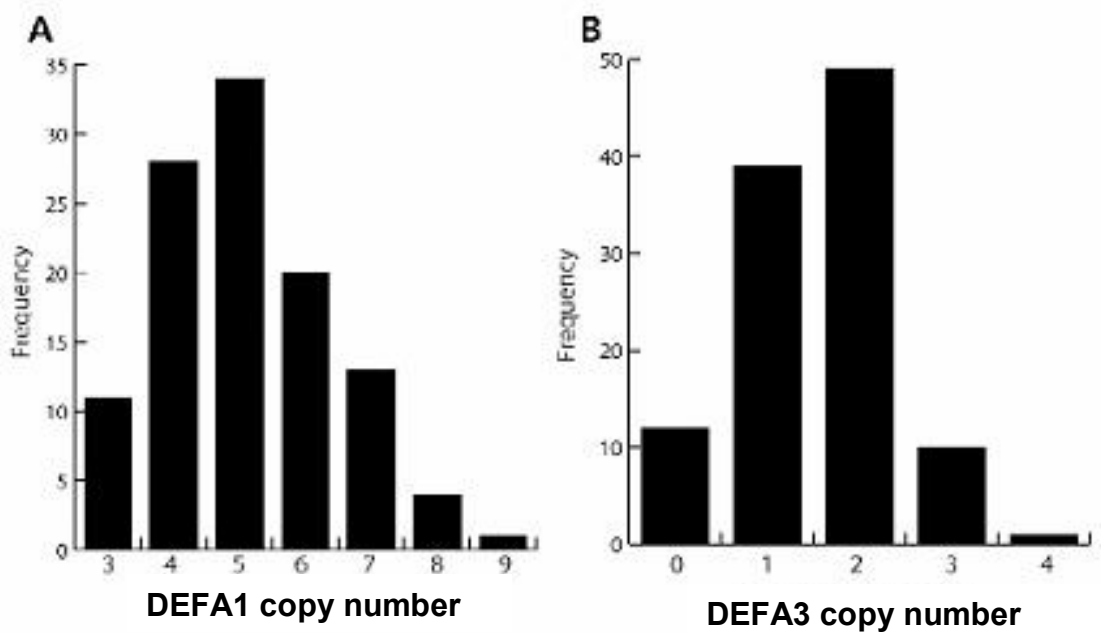
CNV in Defensins



Aldred et al. Human Molecular Genetics 2005 14(14):2045-2052

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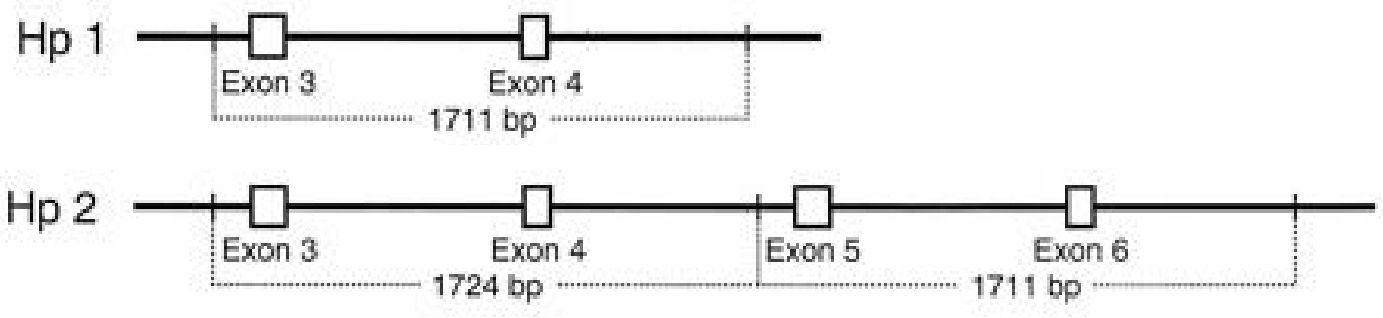
Cytochrome P450 2D6 (CYP2D6)

- Involved in metabolisation of up to 20% of all drugs in clinical use
- 0 copies – 'Poor' metabolisers
- 1 copy – 'Intermediate'
- 2 copies – 'Extensive'
- 3 copies – 'Ultra-rapid'
- Low copy number – high drug plasma concentrations
- High copy number – rapid drug degradation & therapeutic failure

Haptoglobin



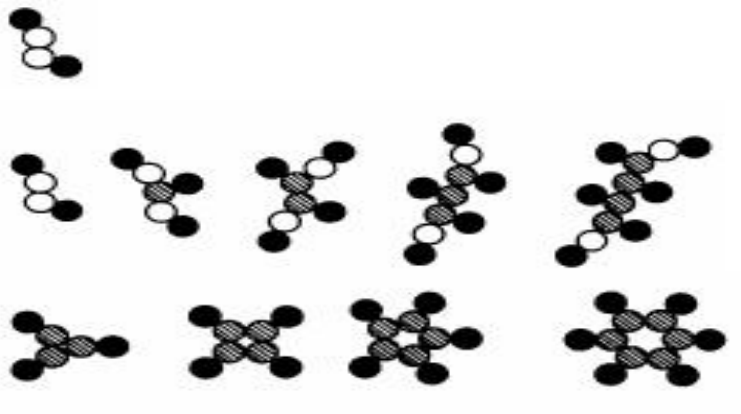
2/2 genotype:

- reduced vitamin C levels
- disease associations e.g. diabetic vascular complications

1/1

1/2

2/2



W Koch et al. Clin. Chem. 2002;48:1377-1382

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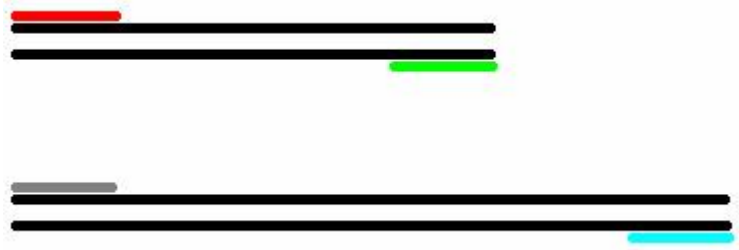
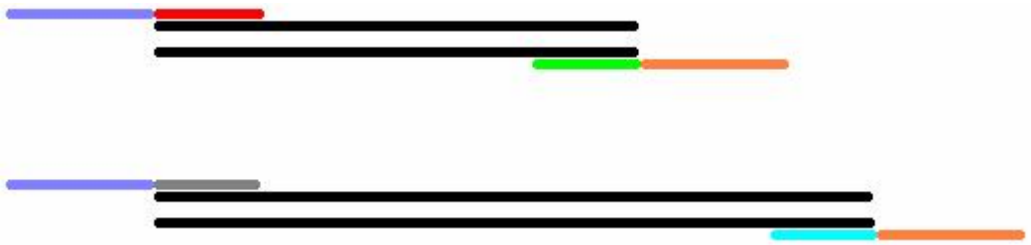
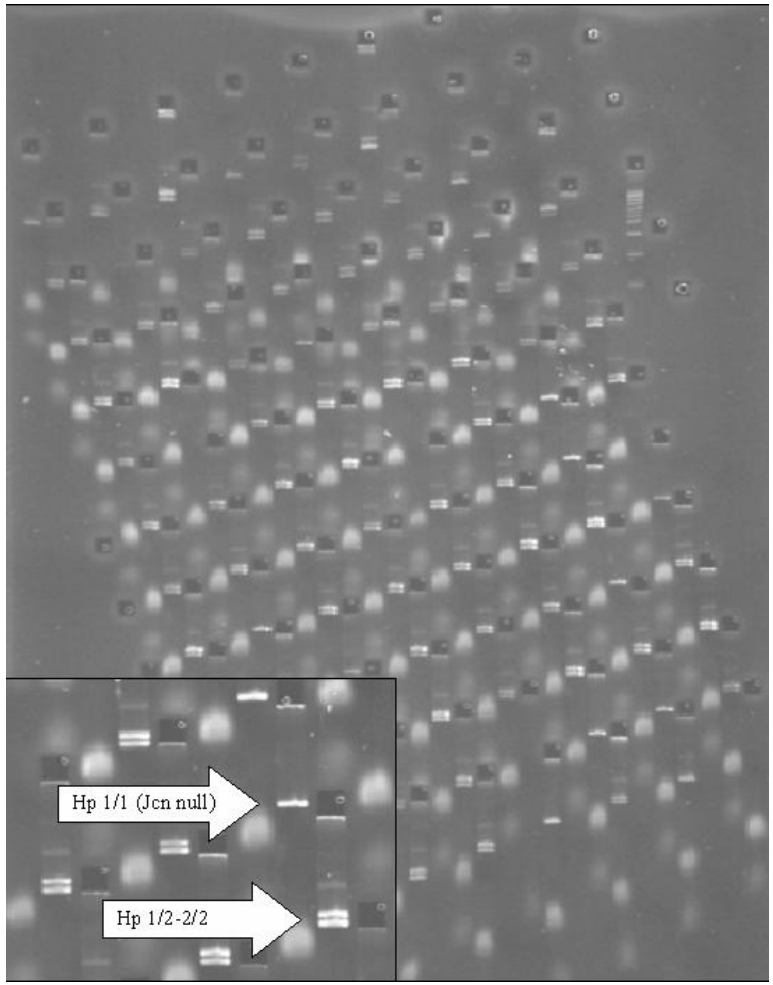


- 4 primers – 4 different properties
- – original template ratios??
- Numerous possible confounding factors –
  - DNA quantity, DNA quality
  - Different fragment sizes
  - Protein
  - EDTA
  - Mg
  - pH
  - log effect of different amp. efficiencies over e.g. 35 cycles



- Better
  - 1 forward universal primer
  - 1 reverse universal primer

MADGE gel assay for Haptoglobin junction null allele



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Roche LightTyper

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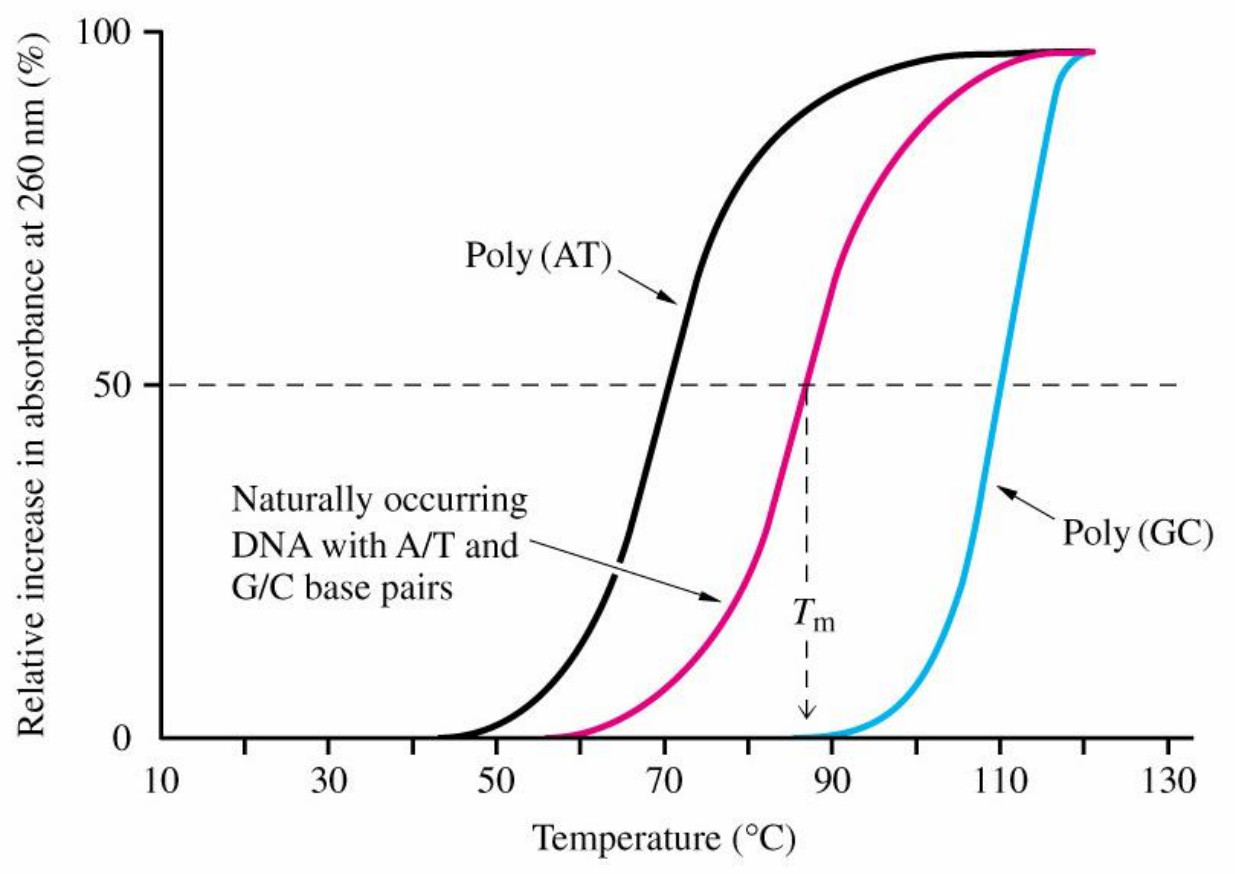
Differential melting of A/T- or G/C-rich fragments



Horton, Moran, Ochs, Rawn, Scrimgeour. Principles of Biochemistry. Prentice-Hall.

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Target gene:- ~49% G/C

Reference gene:- ~58% G/C

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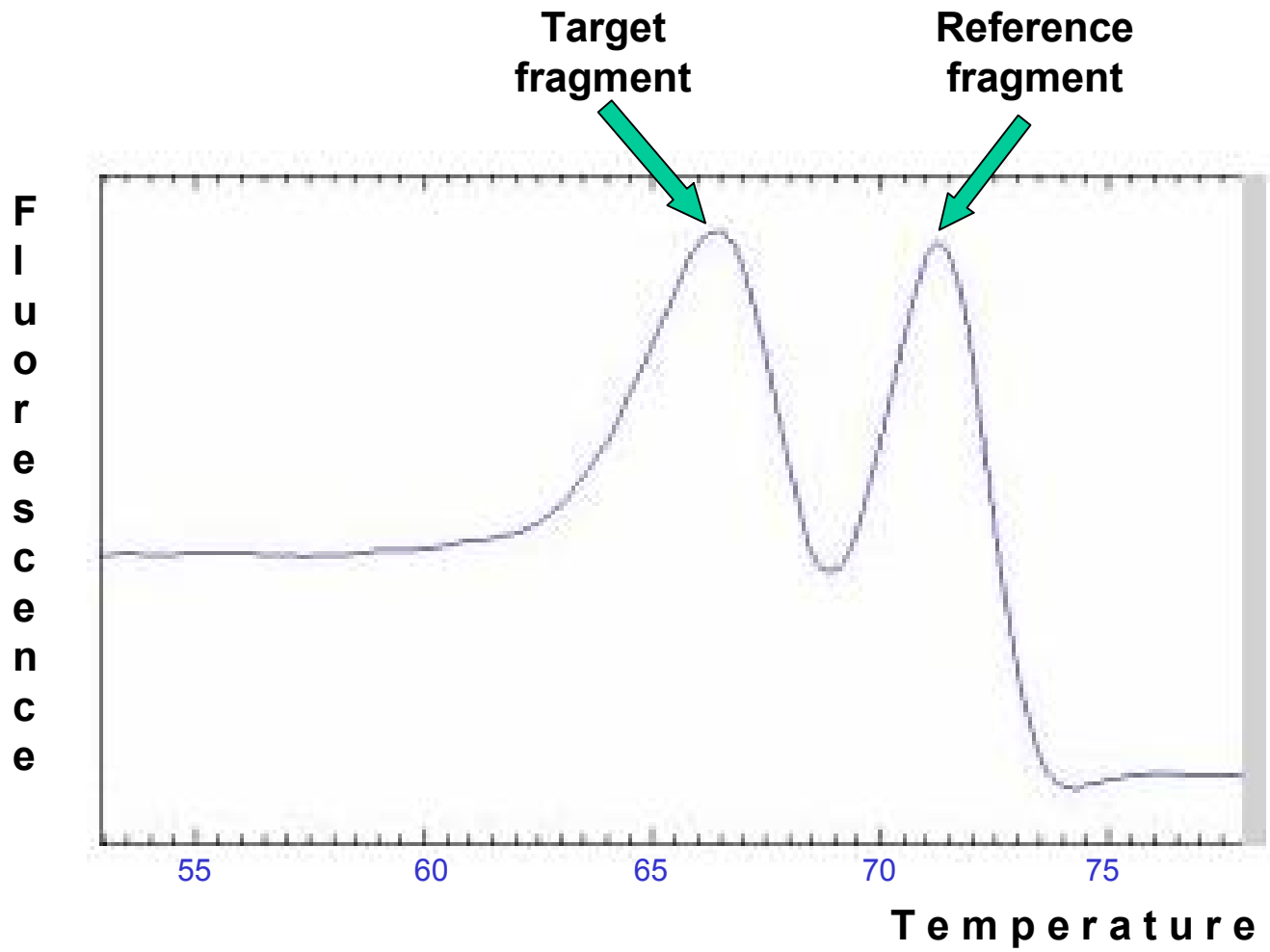
Trial of Target/Reference fragment differential melting



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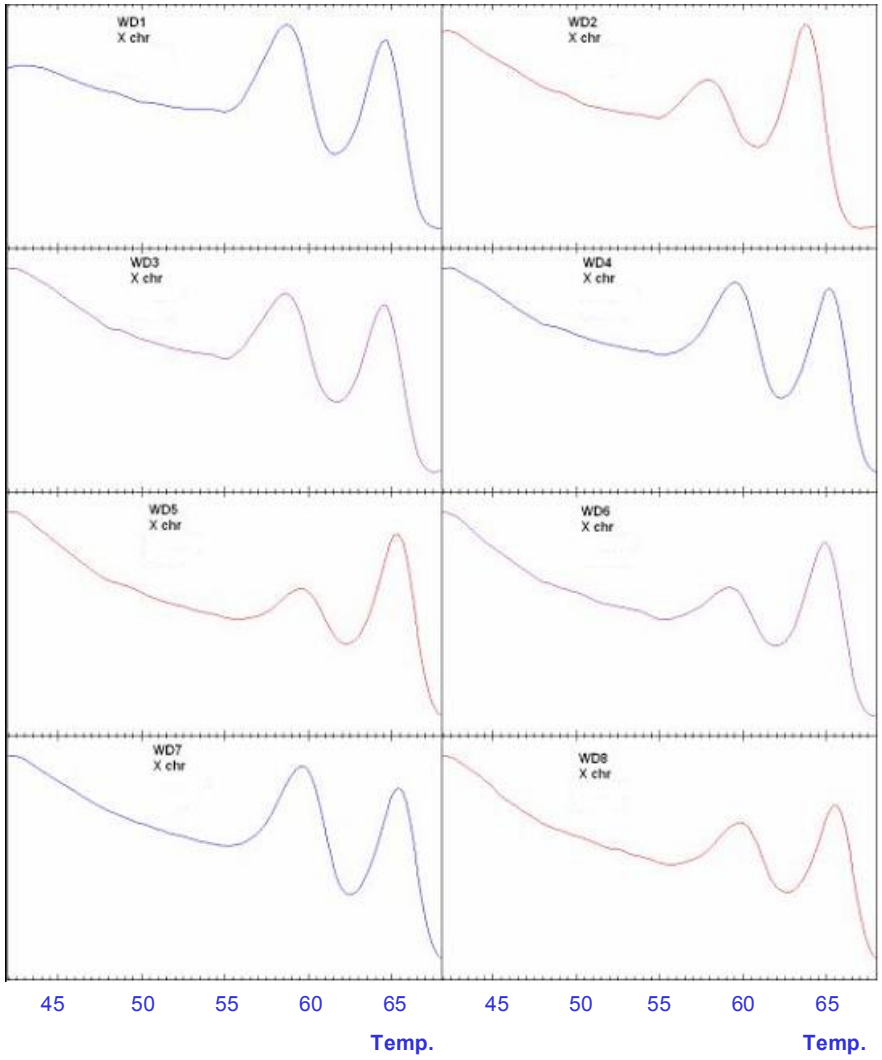
X chromosome complement, by melting profile



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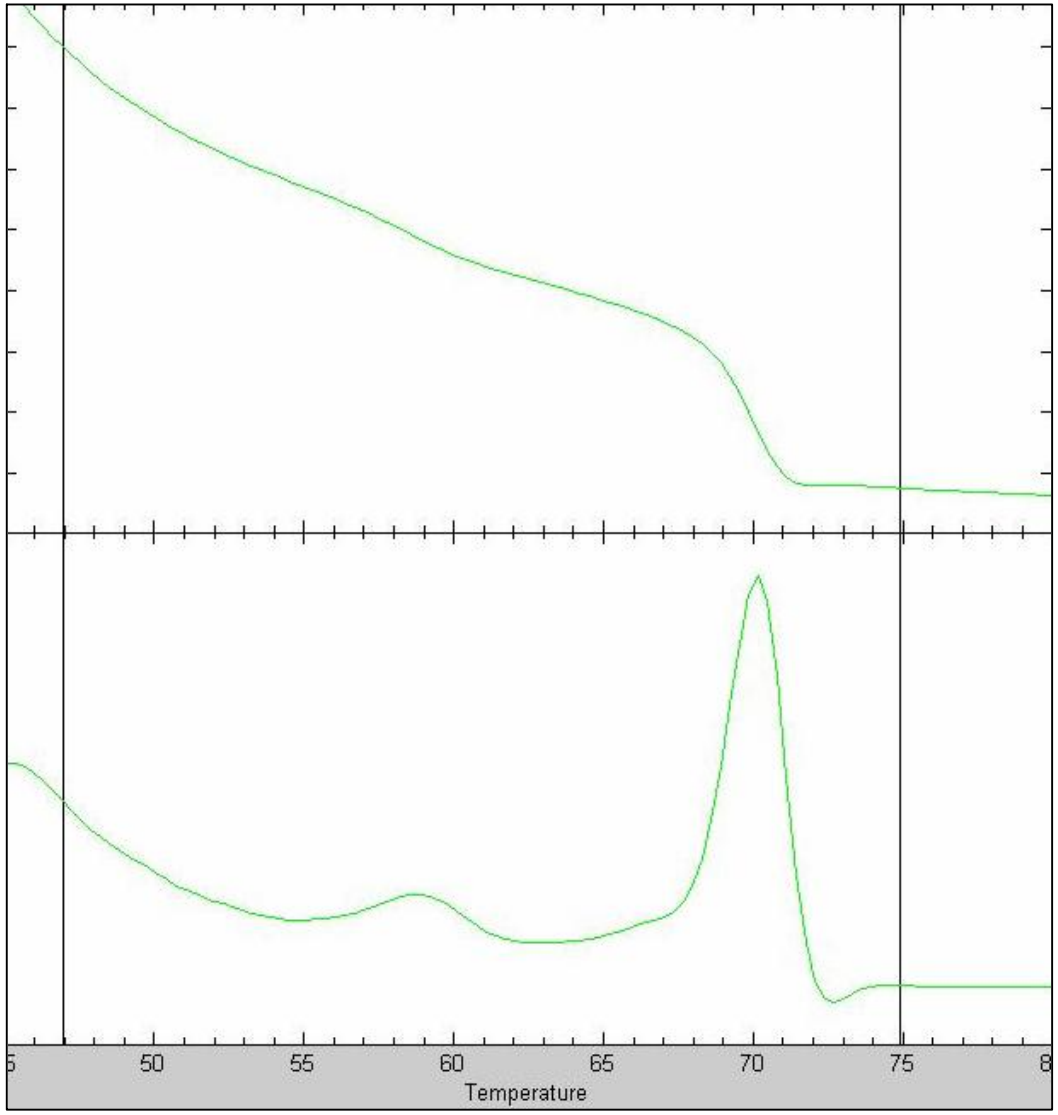
Haptoglobin duplication - null allele homozygous



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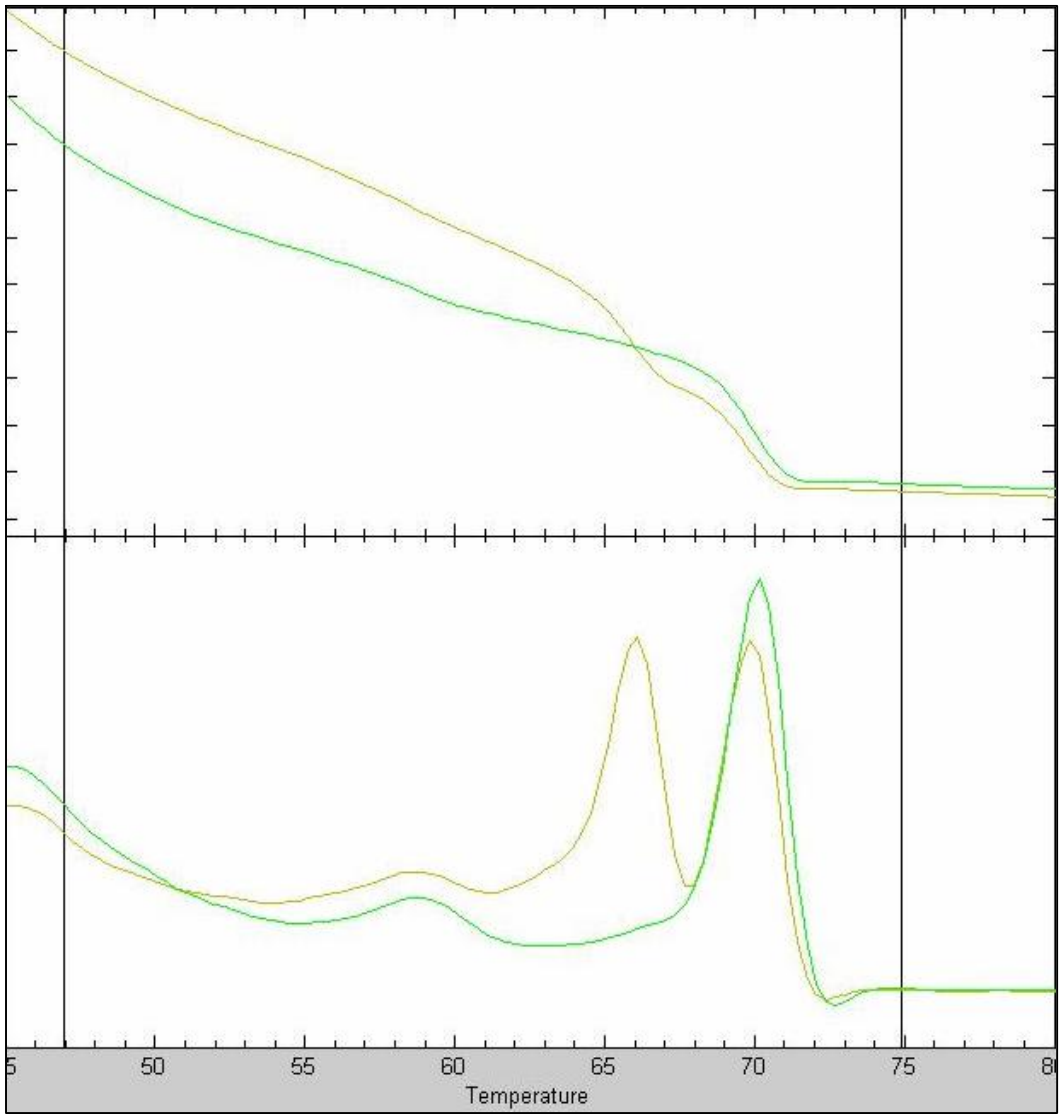
Haptoglobin duplication allele - heterozygous



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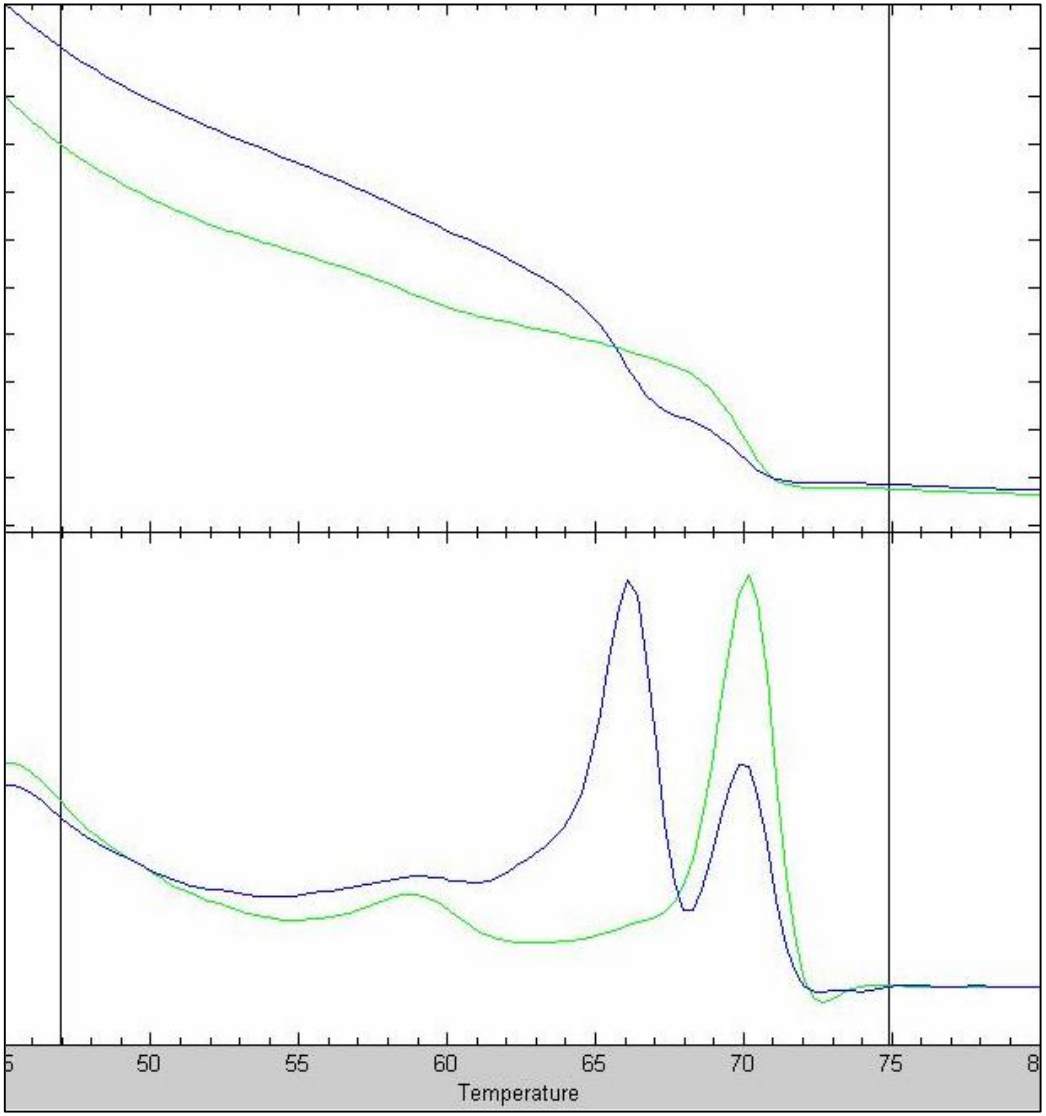
Haptoglobin duplication allele - homozygous



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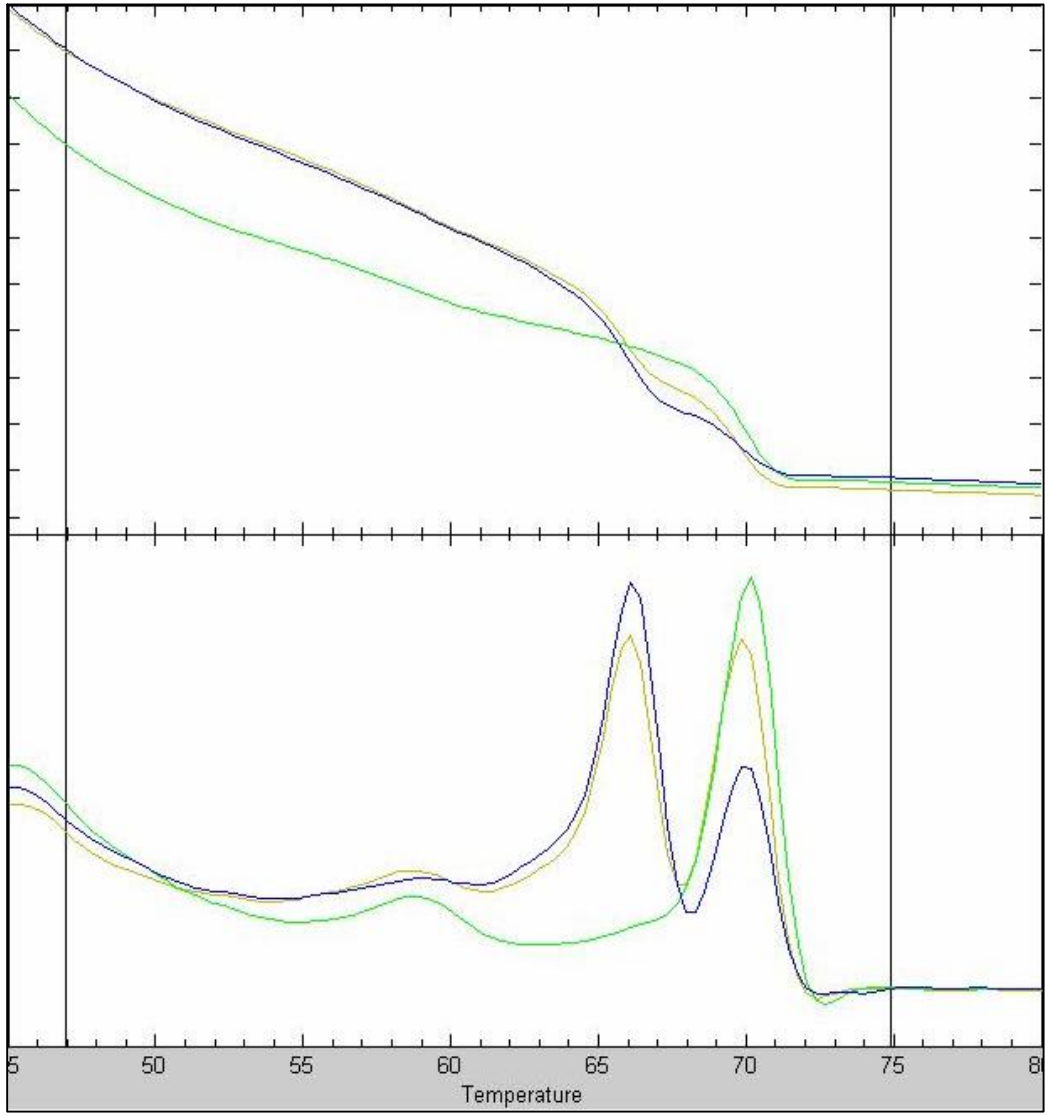
Haptoglobin duplication – composite profiles



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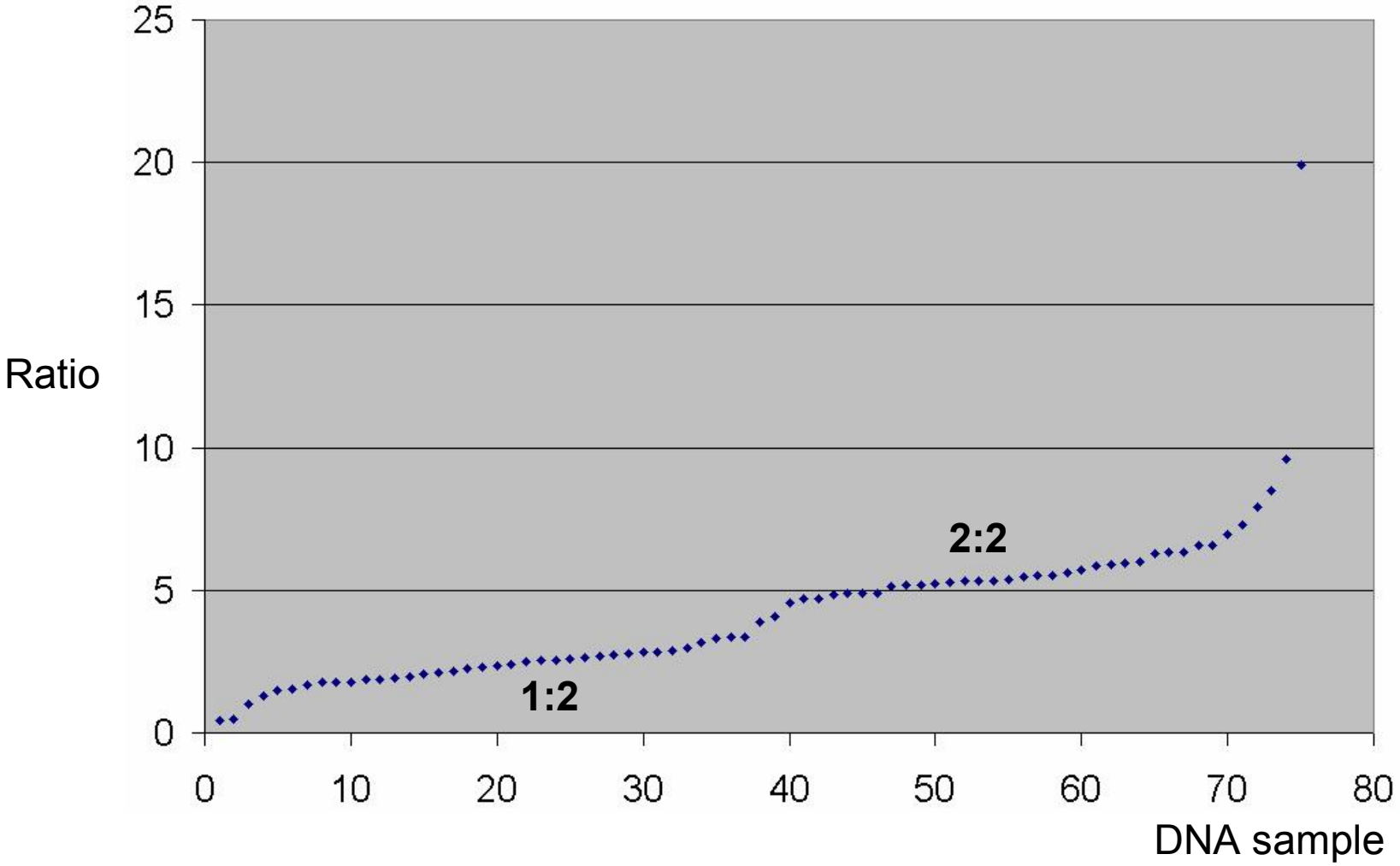
Hp Junction/Reference fragment – area under curve ratios



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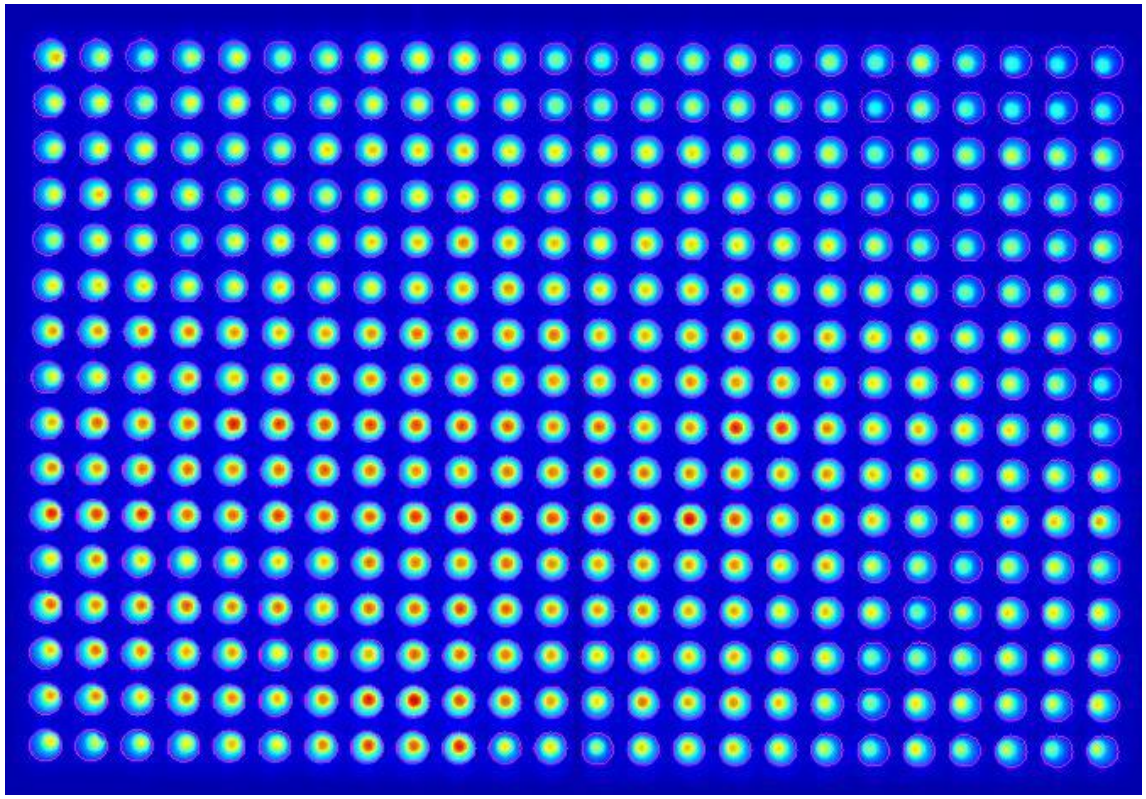
384-well microplate fluorescing in LightTyper



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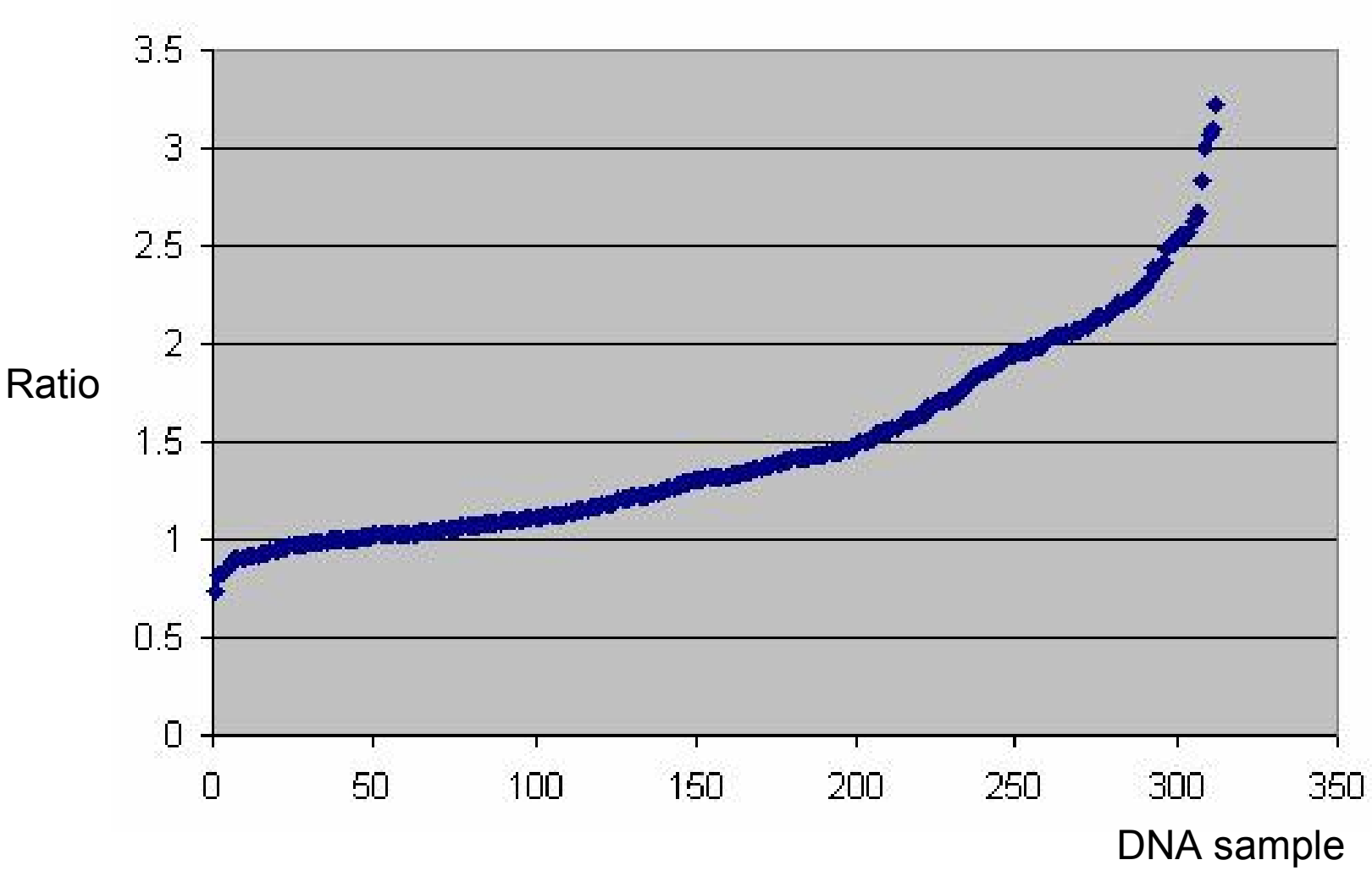
BWHHS 384-well microplate – Hp Jcn/Ref peak height ratios



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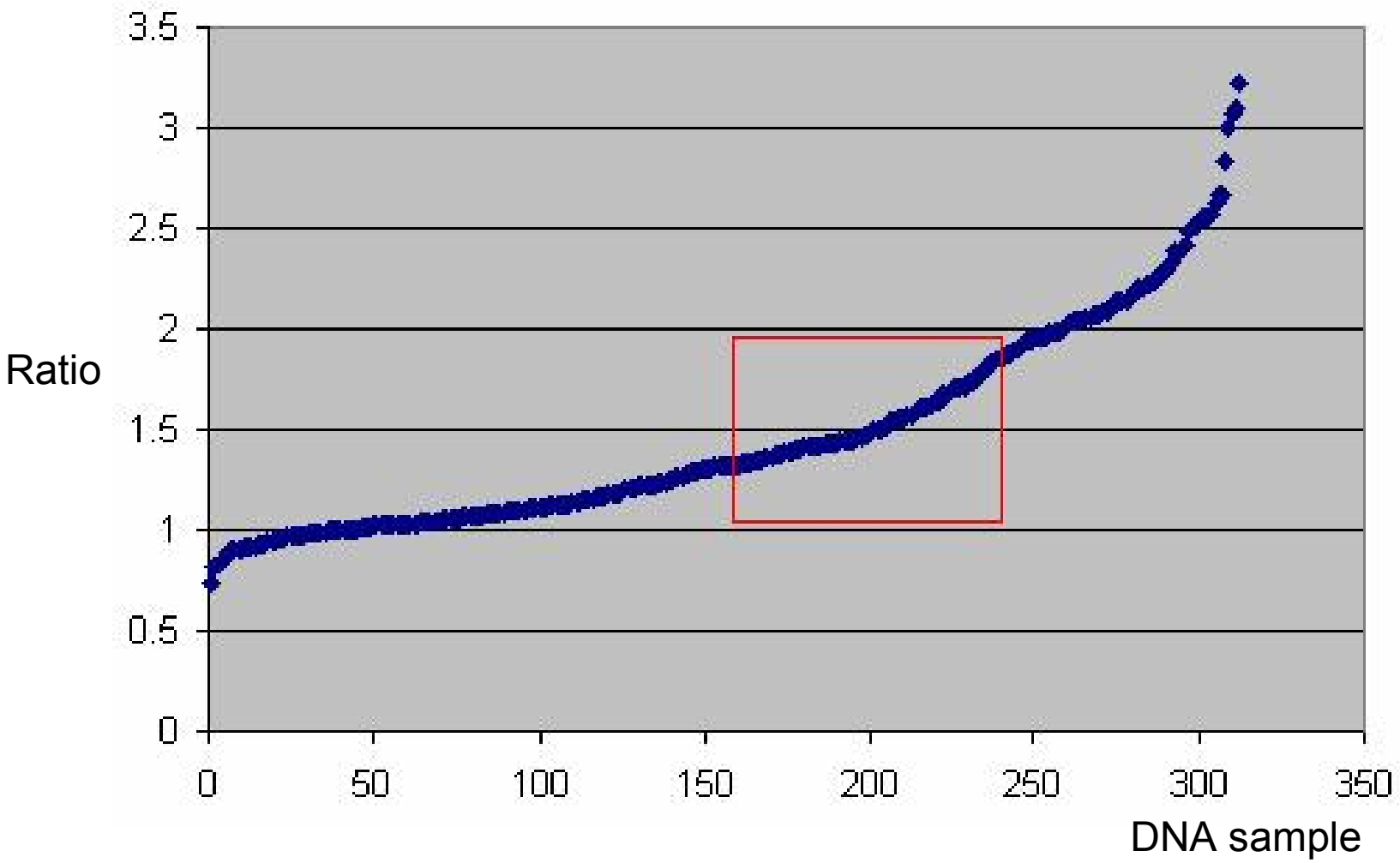

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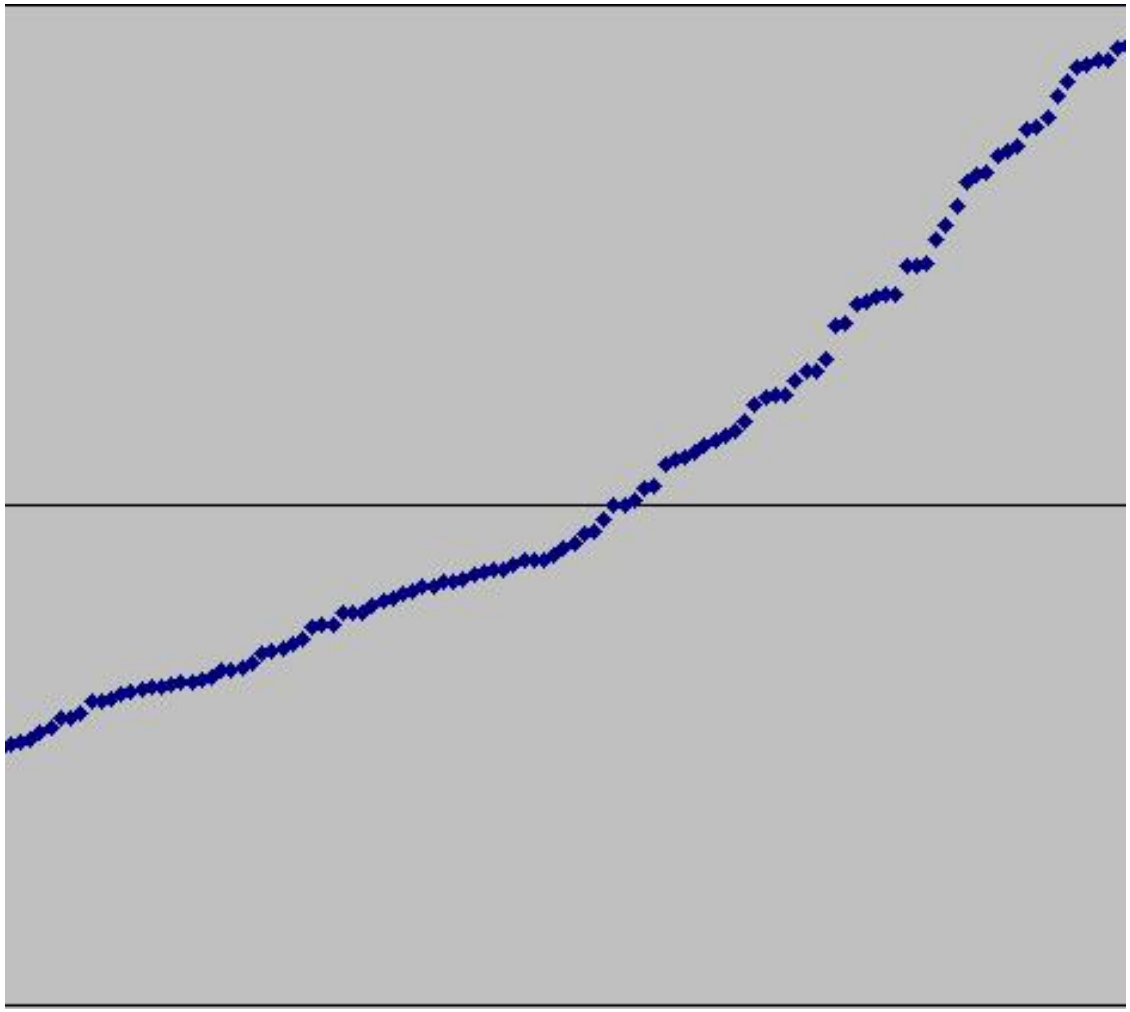
Hp Jcn/Ref ratios closeup



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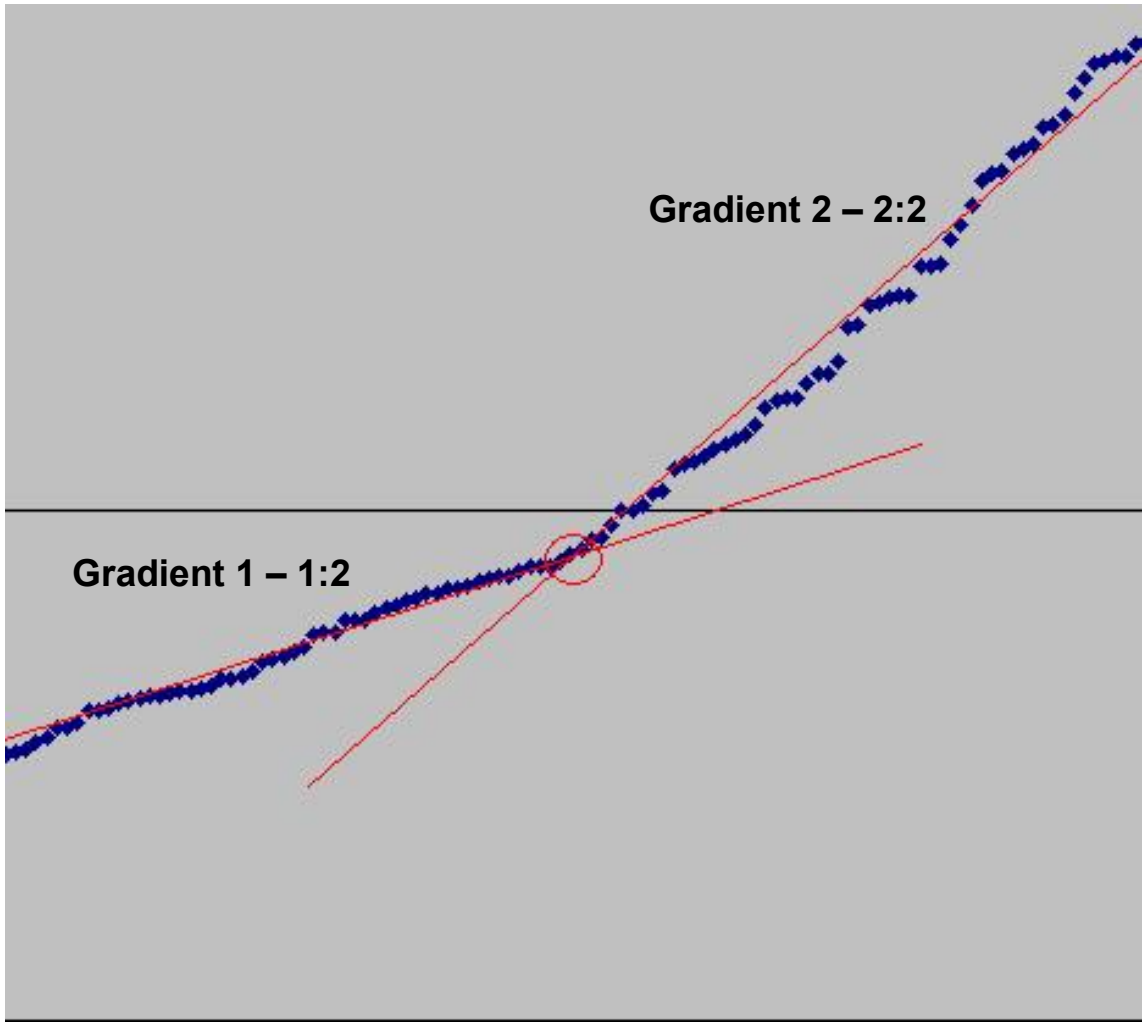
Hp Jcn/Ref ratio gradients



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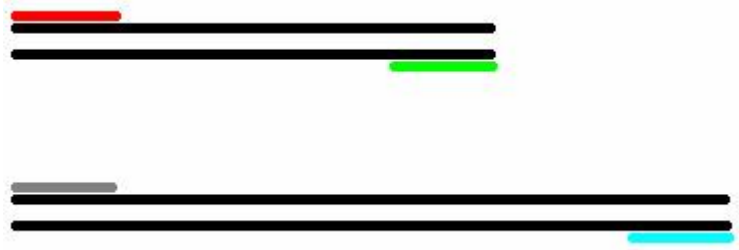

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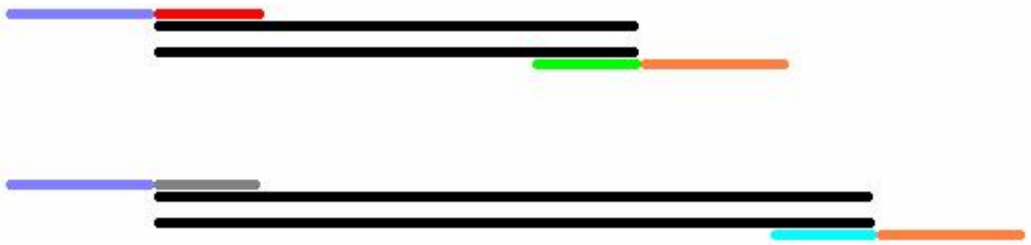

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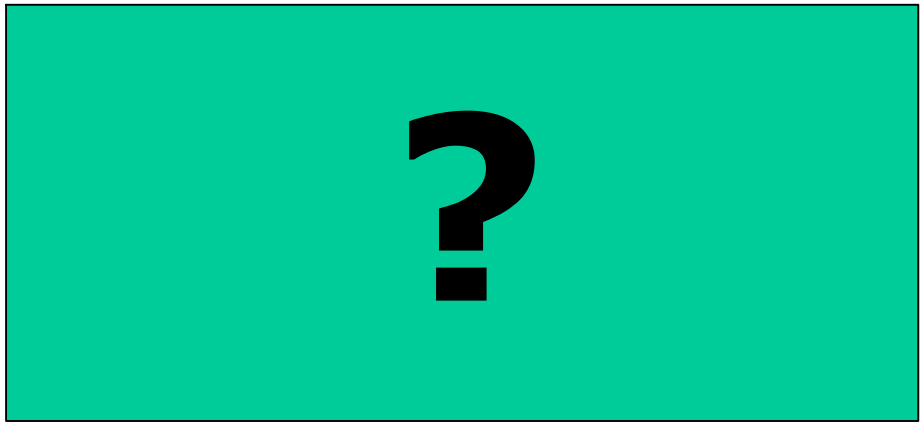

- Best
  - developments currently being patented
  - sorry, can’t disclose yet

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