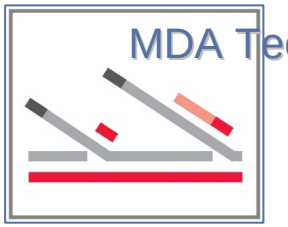
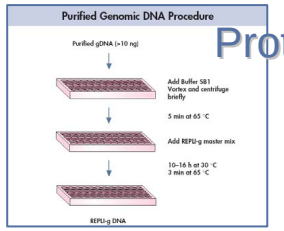
QIAGEN Whole Genome Amplification REPLI-g

Eliminating Sample Limitations, Potential Use for Reference Material

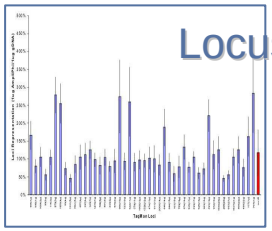
MDA Technology



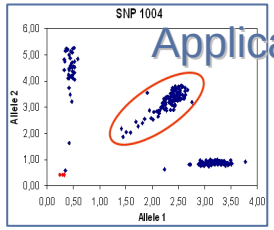
Protocols



Locus Bias



Applications



Kits & Service

Summary

QIAGEN REPLI-g WGA
DNA Shortage Solved

MDA Technology

10.000 fold amplification

Very small amounts of sample
small needle biopsies
Preimplantation genetic diagnosis (PGD)

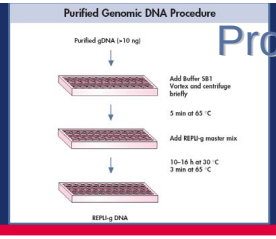
Limited amount of sample
Precious and irretrievable samples
Genome wide studies

WGA
"Immortalization"
Create more DNA in Sample

"Unlimited" Number of Analyses
Sequencing
SNP genotyping
Patient genotyping
....

REPLI-g Mini / Midi Kit –

Sample Types & Single-Tube Protocol



Protocols

Alkaline Denature/ Lysis

Neutralize

REPLI-g Mix

Incubate at 30°C

40 µg DNA (Midi)

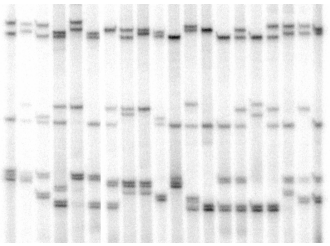
10 µg DNA (Mini)

Direct amplification

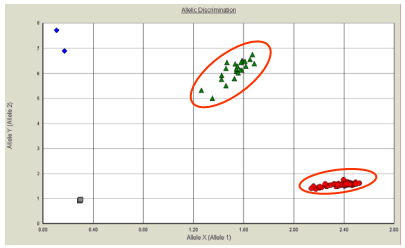
- 10 ng gDNA
- 0.5 µl Blood
- Cell Culture
- Biopsy

QIAamp first

- Blood spots
- Buccal swab
- Mouthwash
- Serum/Plasma



STR Genotyping

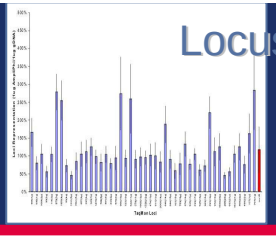


TaqMan SNP Assay

Published with validation in *Genome Research* **2003** (13) **954**

Amplification Bias ?

Experimental Design of Study



0.3 – 300 ng Genomic DNA

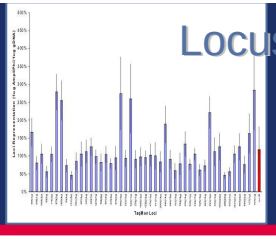
No amplification | **DOP** | **PEP** | **MDA (REPLI-g)**

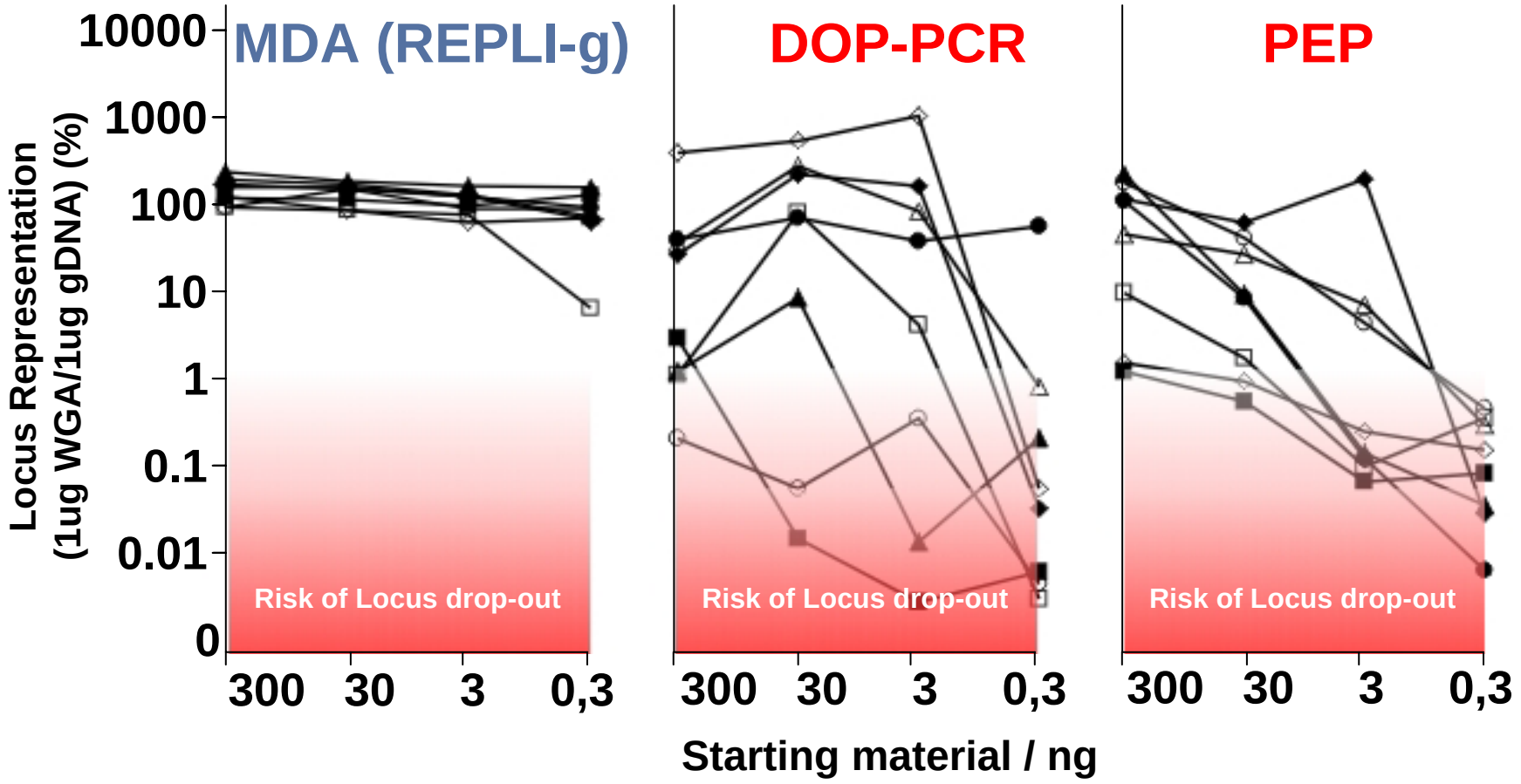
TaqMan analysis for copy number of 8 loci

DOP-PCR: Degenerate Oligonucleotide Priming
PEP-PCR: Primer Extension Pre-amplification
MDA: Multiple Displacement Amplification with REPLI-g

Amplification Bias

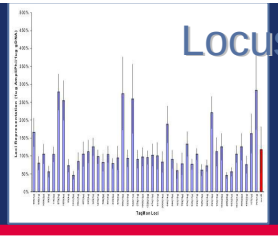
Experimental Result with 8 TaqMan Loci

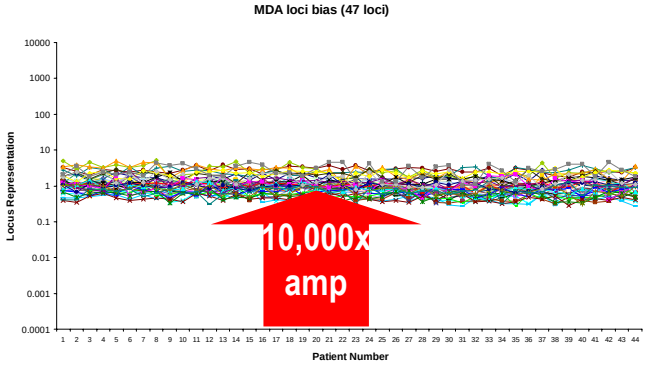


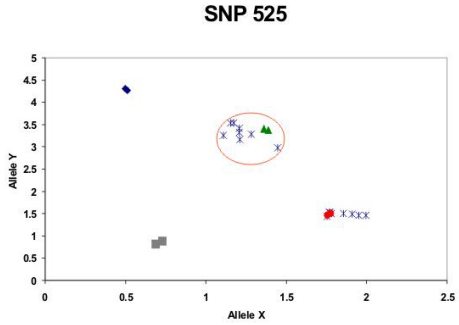


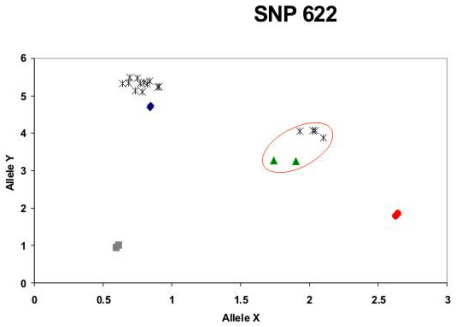
Risk of Locus drop-out → **Data compromised** → **Incorrect data interpretation**

Low Amplification Bias
in Many Loci, Applications and Samples



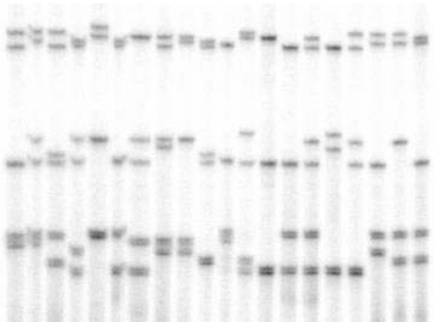




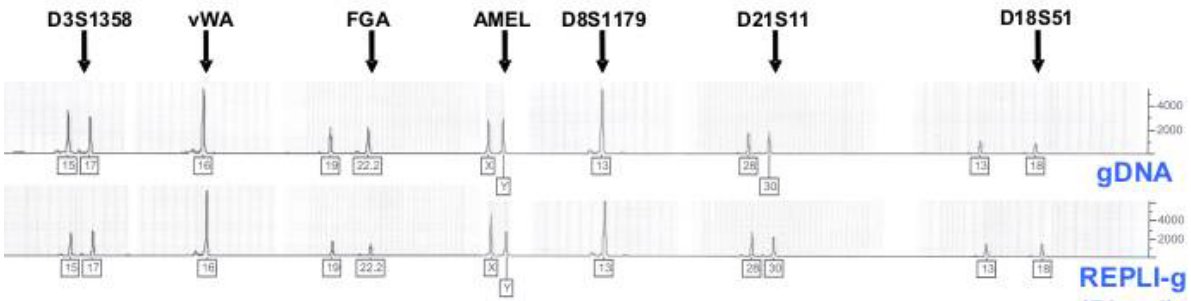


many SNPs and mutations across genome analysed by TaqMan

gDNA, whole blood, cells, DBS on S&S 903, IsoCode, FTA

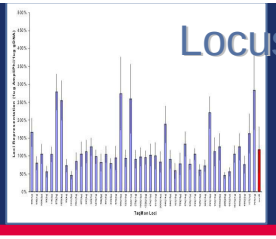


STR loci in Multiplex PCR

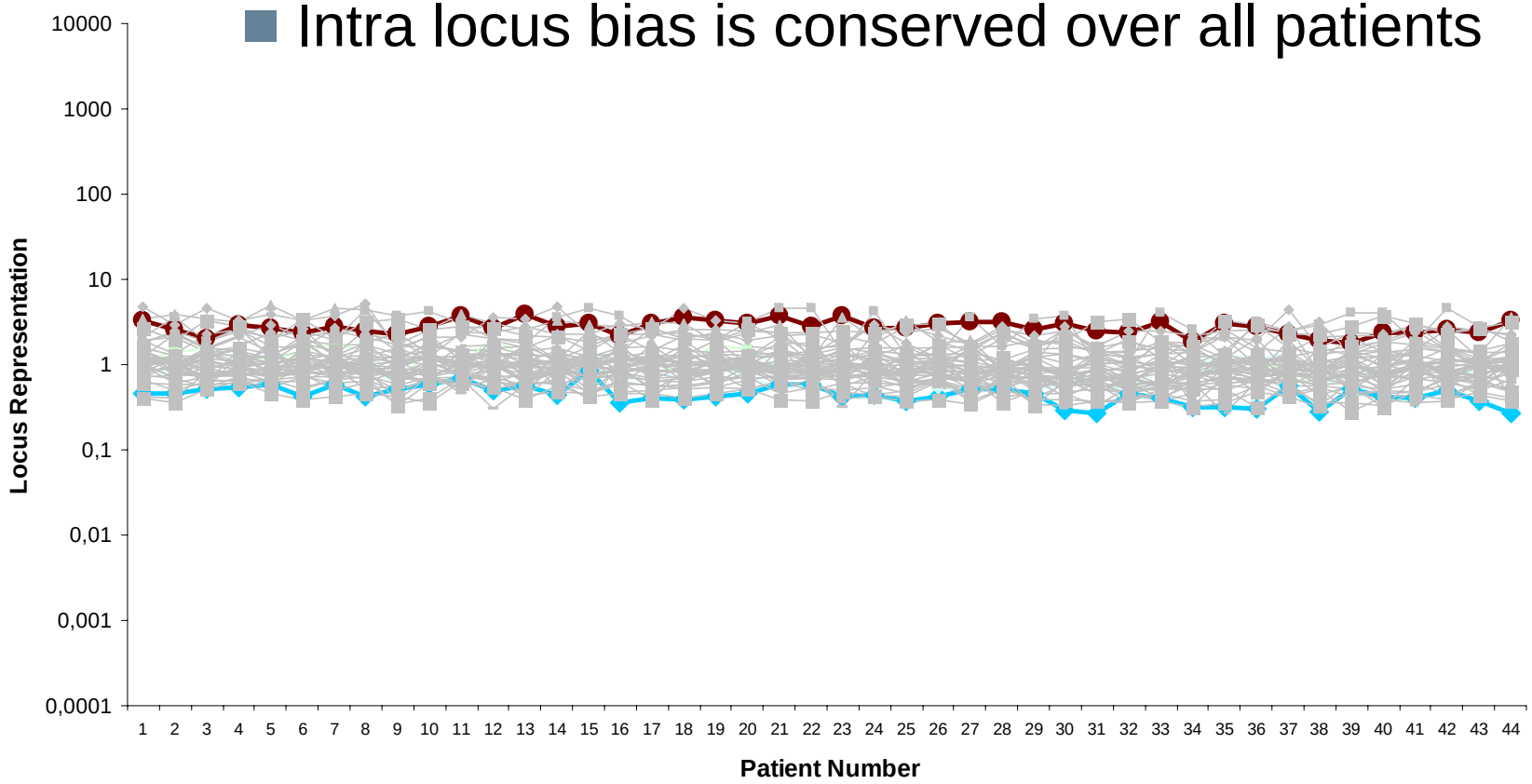


STRs analysed by GeneScan

Low Amplification Bias

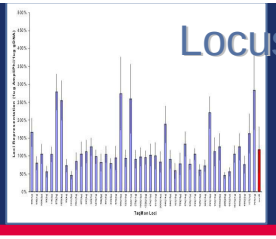


- Low bias bemonstrated for 47 arbitrary loci on gDNA samples from 44 different patients
  - Intra locus bias is conserved over all patients

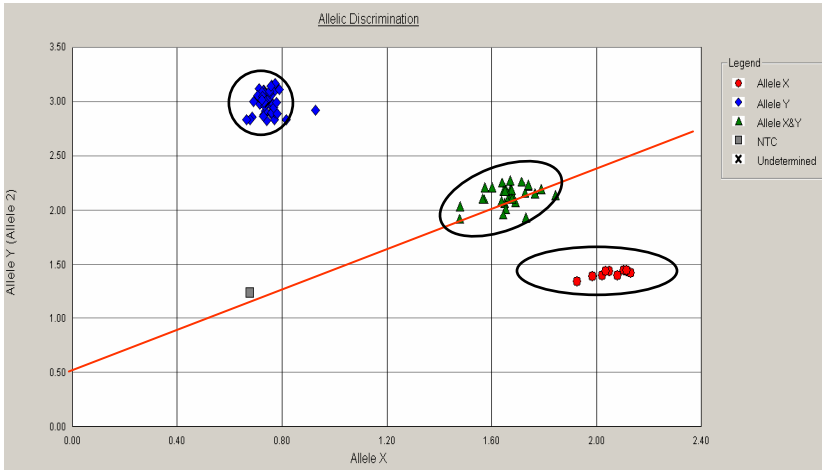


Detection and Elimination of Bias

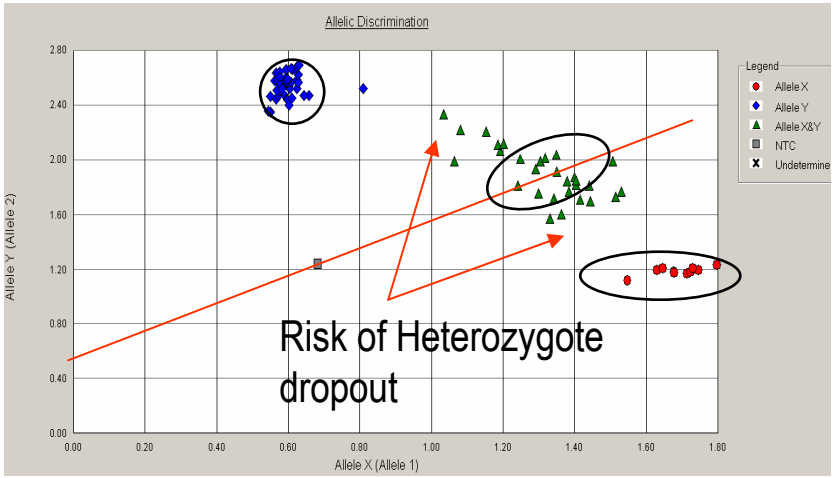
TaqMan SNP Genotyping



Ideal: Tight Clusters of Alleles



Sub-optimal: Allele Scatter



If allele bias is introduced in the whole genome amplification

- Heterozygotes are not clustered
- Heterozygotes may be difficult to score as it deviate from the diagonal
- Risk of Heterozygote dropout

=> repeat with more and pure gDNA, extend amplification and check again

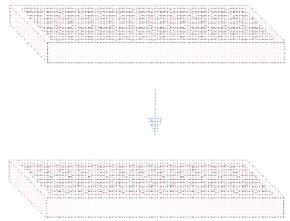
REPLI-g Kit and Service Offering

REPLI-g Mini / Midi Kits

- For genetic analysis and for archiving
- Including all buffers, primers, Polymerase.
- Now also includes denaturation reagent

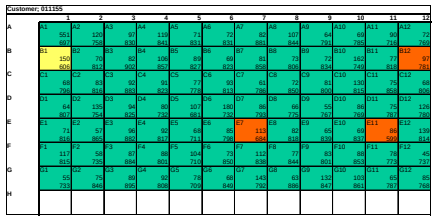
REPLI-g Screening Kit

- For rapid screening for mutation analysis
- Offers high-throughput format and procedure



REPLI-g WGA-Service

- Various output scales available
- Directly from gDNA and from biological samples
- Quantification and Quality Assessment included



REPLI-g WGA – Summary

- Consistent amplification from a variety of different starting materials
- Highly uniform amplification
  - across the whole genome with minimal, but detectable sequence bias
- Uniform and standardized DNA yield
  - for direct use in downstream applications without quantification
- Significantly more DNA from limited samples
  - have broader access to the genetic content
- List of published references
  - http://www.qiagen.com/RefDB/search_adv.asp
- Potential use for generation of standardized and immortal reference material where certified and approved reference material is not available