Increasing the power in paternity and relationship testing utilizing MPS for the analysis of a large SNP panel

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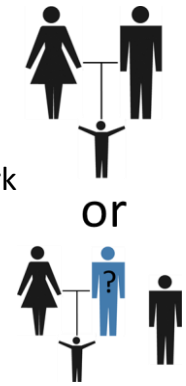
Our motivation for a NGS/MPS SNP panel

- Still a need for supplementary markers
  - Handle around 3000-5000 relationship cases each year
    - Inconclusive paternity/maternity cases.
    - Complex/distant relationship cases.
- Implement NGS/MPS technology for use in routine casework
  - ”Easy” to interpret.
  - Account for things not alwyss considered in a reseach project
    - QA aspects (over time).
    - Educate staff.



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Design & set up

140 SNP marker panel currently know as ” QIAseq Investigator SNP ID”

- 140 SNPs
  - 52 SNPforID (Sanchez et al., 2006)
  - 88 II SNPs (Pakstis et al., 2010)
- Each SNP covered by 2 forward and 2 reverse primers
  - 4 amplicons per locus

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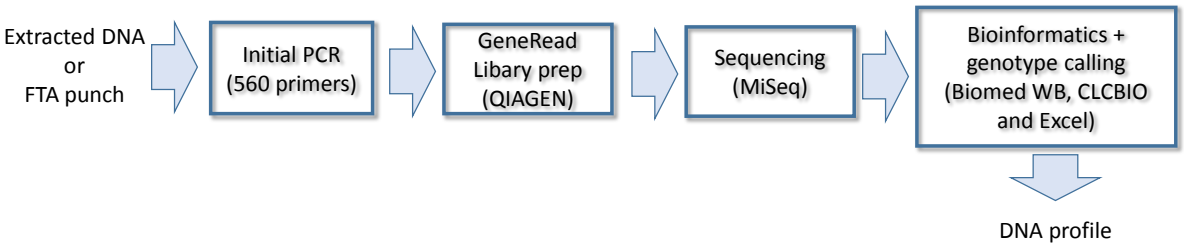
Lab workflow

(details i Grandell et al., 2016)



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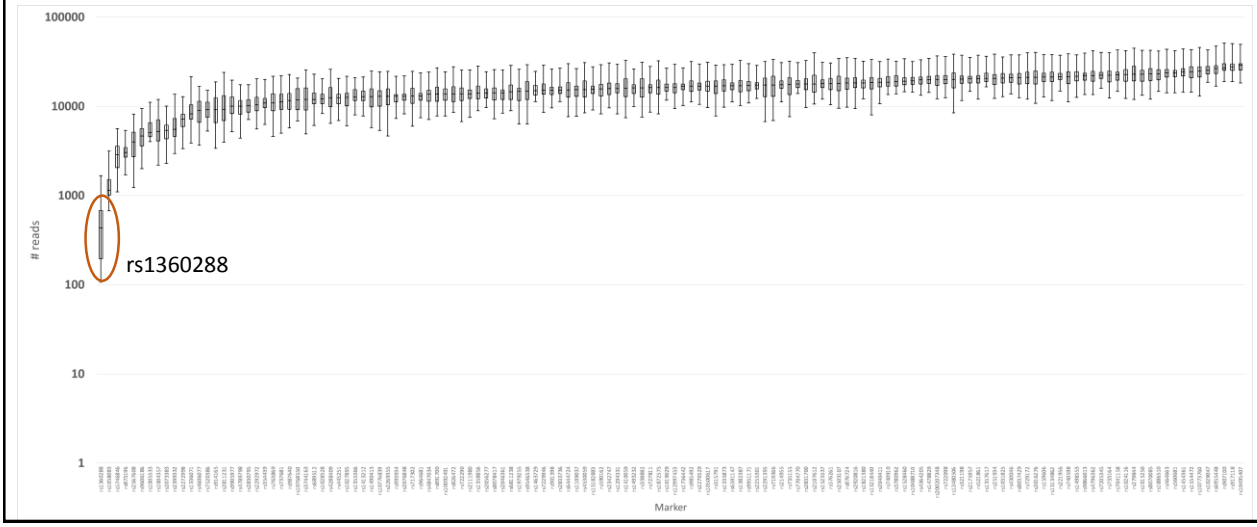
Result - Coverage

(N=54, DNA extracted from blood samples)



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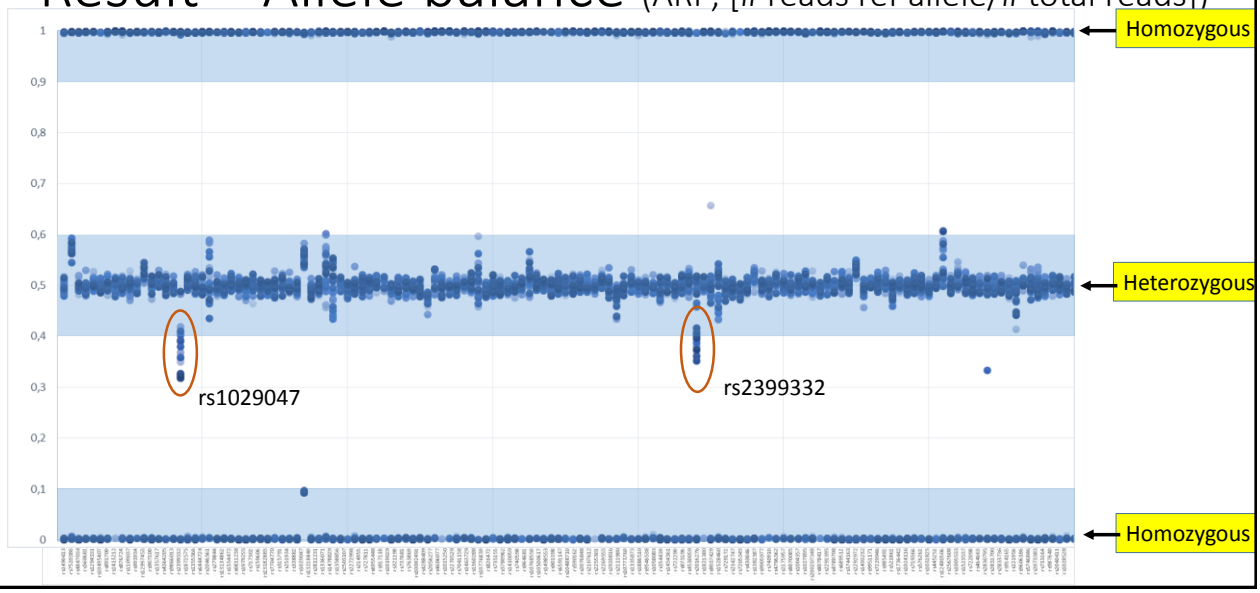
Result – Allele balance (ARF; [# reads ref allele/# total reads])



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Brief summary of the technical validation

(140 SNPs and 29 autosomal STRs)

- Removed 3 SNPs (rs1360288, rs2399332,rs4530059) due to technical reasons
- Accuracy, repeatability, sensitivity etc have been shown
  - details i *Grandell et al., 2016*
- Set thresholds criterias for genotyp calling:
  - Cov>200x,
  - ARF: 0.9-1 or 0-0.1 (homozygous)
  - ARF: 0.4-0.6 (heterozygous)
- FTA and high quality DNA extractions

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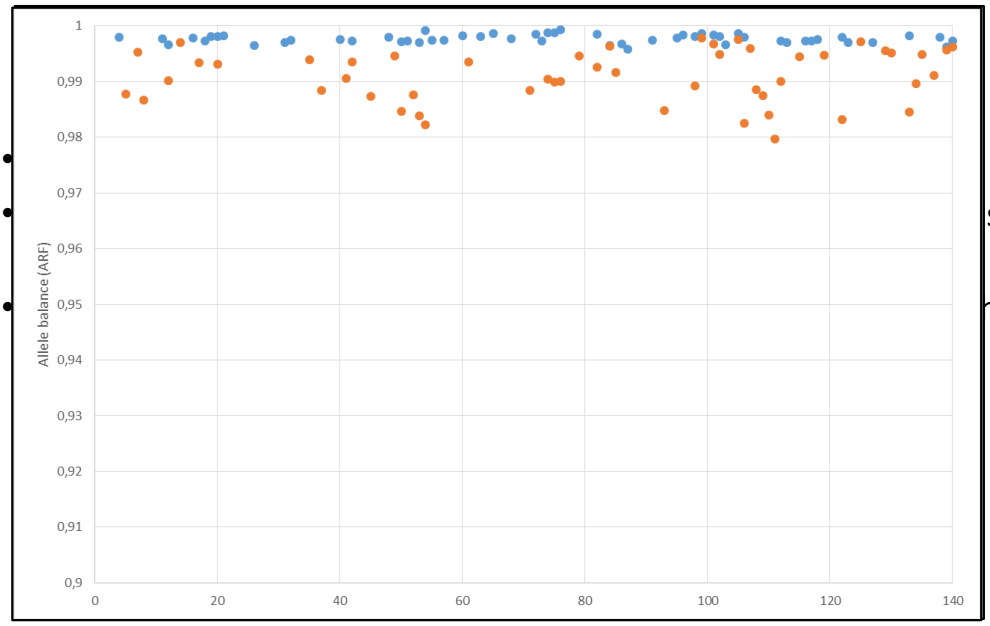

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Validation of the biostatistic features of the panel

(137 SNPs and 29 autosomal STRs)

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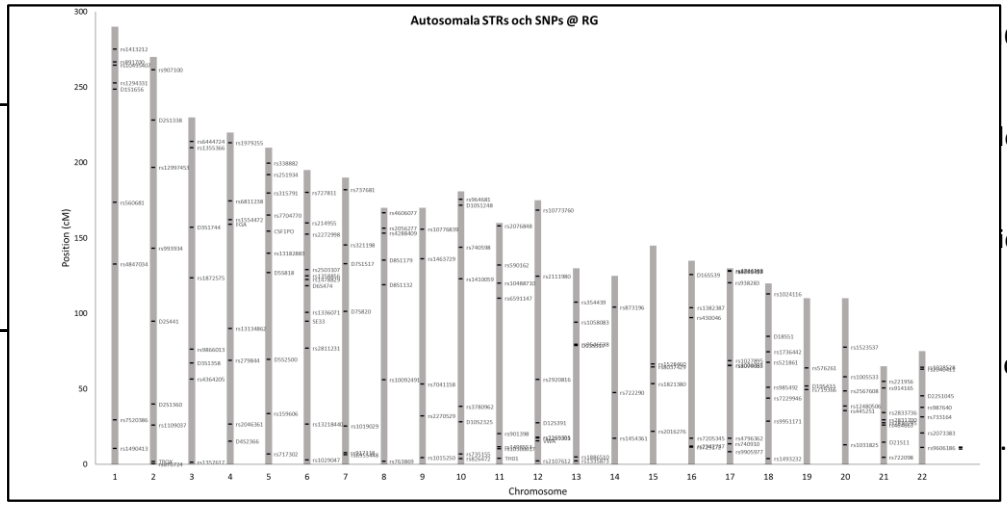

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Validation of the biostatistic features of the panel

(137 SNPs and 29 autosomal STRs)

- Genotype data/(haplotype data) from 49 Swedish individuals.
- Linkage analysis based on data from HapMap 3
  - Estimation of recombination rates.
- LD analysis based on 49 swedish individuals AND 1000 Genomes project
  - Exact test.
  - SNAP (http://www.broadinstitute.org/mpg/snap)
- ”Expected” LRs for different case scenarios
  - Simulations

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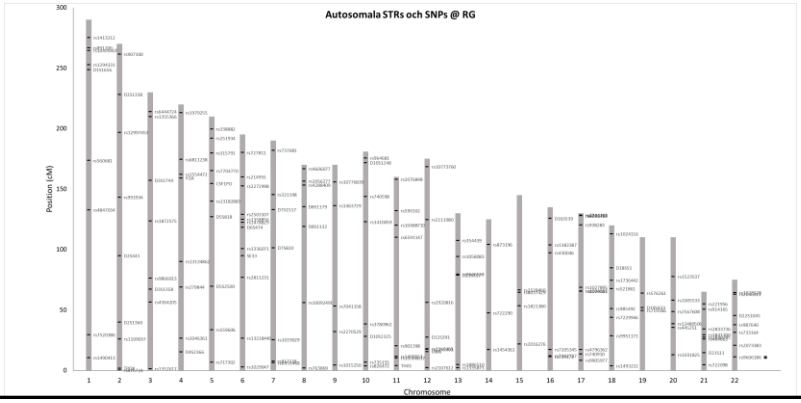
Validation of the biostatistic features of the panel
(131 SNPs and 29 autosomal STRs)

- 6 SNPs removed due to sign of LD
- A genetic map was created



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”Expected” LRs

	H_1 true		H_2 true	
Case scenario (H_1 vs H_2)	**Median LR (29 STR)**	**Median LR (29 STR+ 131 SNP)**	**Median LR (29 STR)**	**Median LR (29 STR+ 131 SNP)**
Paternity (trio) vs Unrelated	7.0e+016	1.8e+032	0	0
Paternity/Maternity (duo) vs Unrelated	3.7e+011	2.6e+021	0	0
Full siblings vs Unrelated	4.2e+009	2.3e+018	4.0e-008	2.0e-017
Full siblings vs Half siblings	9.4e+002	1.2e+006	0.004637	3.4e-006
Paternity vs Uncle	6.9e+003	5.9e+007	0	0

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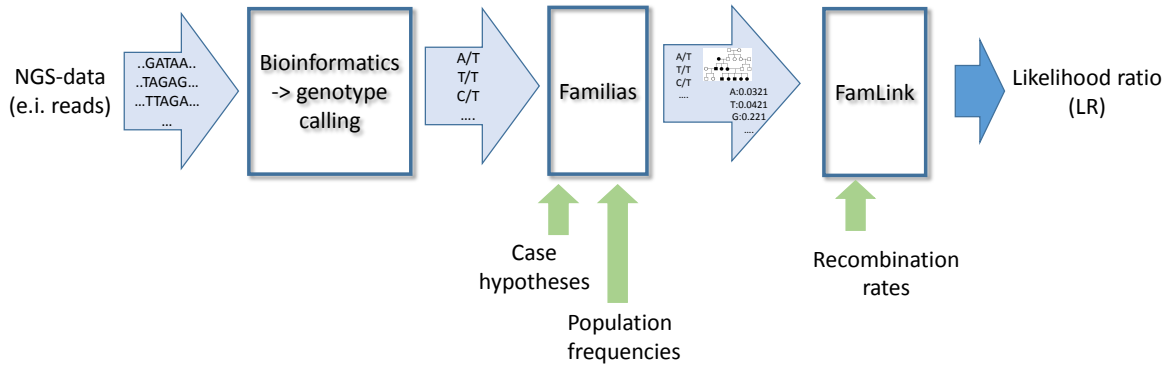
Workflow, case-by-case basis



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Results – Cases tested so far

(Apart from 40 parent-child tested during validation)

Outcome (posterior prob)	**#**
Conclusive (>99%)	13
Still inconclusive (5%-95%)	2

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Results – Real cases

Case 1

Woman + Child

Question: Is the woman the mother, aunt, full sibling or unrelated to the child?

Posterior probability (equal priors)

	Mother	**Aunt**	**Full sibling**	**Unrelated**
29 A-STR	93%	0.02%	7%	<0.001%
29 A-STR + 131 SNP	99.999%	<0.001%	0.001%	<0.001%

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Results – Real cases

Case 2

Mother + Child + Man

Question: Is the man a half-uncle or unrelated to the child?

Posterior probability (equal priors)

	Half uncle	**Unrelated**
29 A-STR	87%	13%
29 A-STR + 131 SNP	99.2%	0.8%

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Results – Real cases

Case 3

Woman + woman

Question: Are they half siblings?

Posterior probability (equal priors)

	Half siblings	**Unrelated**
29 A-STR	50%	50%
29 A-STR + 131 SNP	0.6%	99.4%

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Summary

- Validation
  - NGS typing methology
  - Biostatistical workflow
- Implemented in may 2016.
- Have solved earlier ”unsolved” cases.
- Aiming for accreditation (17025) next year.