RNA-seq Quality Control in BIOS freeze 2

OPENING UP THE BBMRI GENOMICS INFRASTRUCTURE IN THE NETHERLANDS

AMSTERDAM, 21ST OF SEPTEMBER, 2016

ANNIQUE CLARINGBOULD

RNA-seq data in BIOS
Freeze 1

- Freeze 1: 2,116 unrelated samples
- Several current papers use freeze 1 data

Hypothesis-free identification of modulators of genetic risk factors

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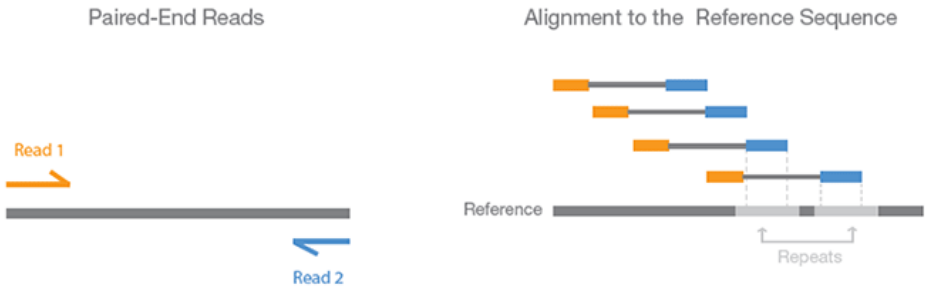
Disease variants alter transcription factor levels and methylation of their binding sites

Marc Jan Bonder[1,*], René Luijk[2,*], Daria V. Zhernakova[1,**], Matthijs Moed[2,**], Patrick Deelen[1,3,**], Martijn Vermaat[4,**], Maarten van Iterson[2], Freerk van Dijk[1,3], Michiel van Galen[3], Jan Bot[5], Roderick C. Slieker[2], P. Mila Jhamai[6], Michael Verbiest[3], H. Eka D. Suchiman[2], Marijn Verkerk[6], Ruud van der Breggen[2], Jeroen van Rooij[6], Nico Lakenberg[2], Wibowo Arindrarto[8], Szymon M. Kielbasa[7], Iris Jonkers[2], Peter van 't Hof[7], Irene Nooren[5], Marian Beekman[2], Joris Deelen[2], Diana van Heemst[9], Alexandra Zhernakova[1], Ettje F. Tigchelaar[1], Morris A. Swertz[1,3], Albert Hofman[10], André G. Uitterlinden[6], René Pool[11], Jenny van Dongen[11], Jouke J. Hottenga[11], Coen D.A. Stehouwer[12], Carla J.H. van der Kallen[12], Casper G. Schalkwijk[12], Leonard H. van den Berg[13], Erik. W van Zwet[8], Hailiang Mei[7], Yang Li[1], Mathieu Lemire[14], Thomas J. Hudson[14,15,16], the BIOS Consortium, P. Eline Slagboom[2], Cisca Wijmenga[1], Jan H. Veldink[13], Marleen M.J. van Greevenbroek[12], Cornelia M. van Duijn[17], Dorret I. Boomsma[11], Aaron Isaacs[17,##], Rick Jansen[18,##], Joyce B.J. van Meurs[6,##], Peter A.C. 't Hoen[4,#], Lude Franke[1,#], Bastiaan T. Heijmans[2,#]

Refined mapping of autoimmune disease associated genetic variants with gene expression suggests an important role for non-coding RNAs

Isis Ricaño-Ponce[a], Daria V. Zhernakova[a], Patrick Deelen[a, b], Oscar Luo[c], Xingwang Li[c], Aaron Isaacs[d], Juha Karjalainen[a], Jennifer Di Tommaso[a], Zuzanna Agnieszka Borek[a], Maria M. Zorro[a], Javier Gutierrez-Achury[a], Andre G. Uitterlinden[d], Albert Hofman[d], Joyce van Meurs[d], BIOS consortium[e],

RNA-seq data in BIOS
Freeze 2



Freeze 2 initial numbers	Number of samples
BIOS + NTR extra	4543
BIOS only	3824

- Samples available from
  CODAM, LLDeep, LLS, NTR, PAN, RS
- Paired-end sequencing Illumina's Hiseq2000
- Intended number of reads: >15M
  - Re-sequenced several samples
- Many factors can influence the quality of the data
- Goal: usable set of unrelated samples with gene expression (and genotype) data of good quality

Picture source: Paired-End Sequencing web page Illumina,
http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing_assay.html

Main strategy

1. Match RNA to DNA for each individual based on data
   - Resolve mix ups
   - Merge topped up samples
2. Use quality metrics as cut-offs
3. Sample selection

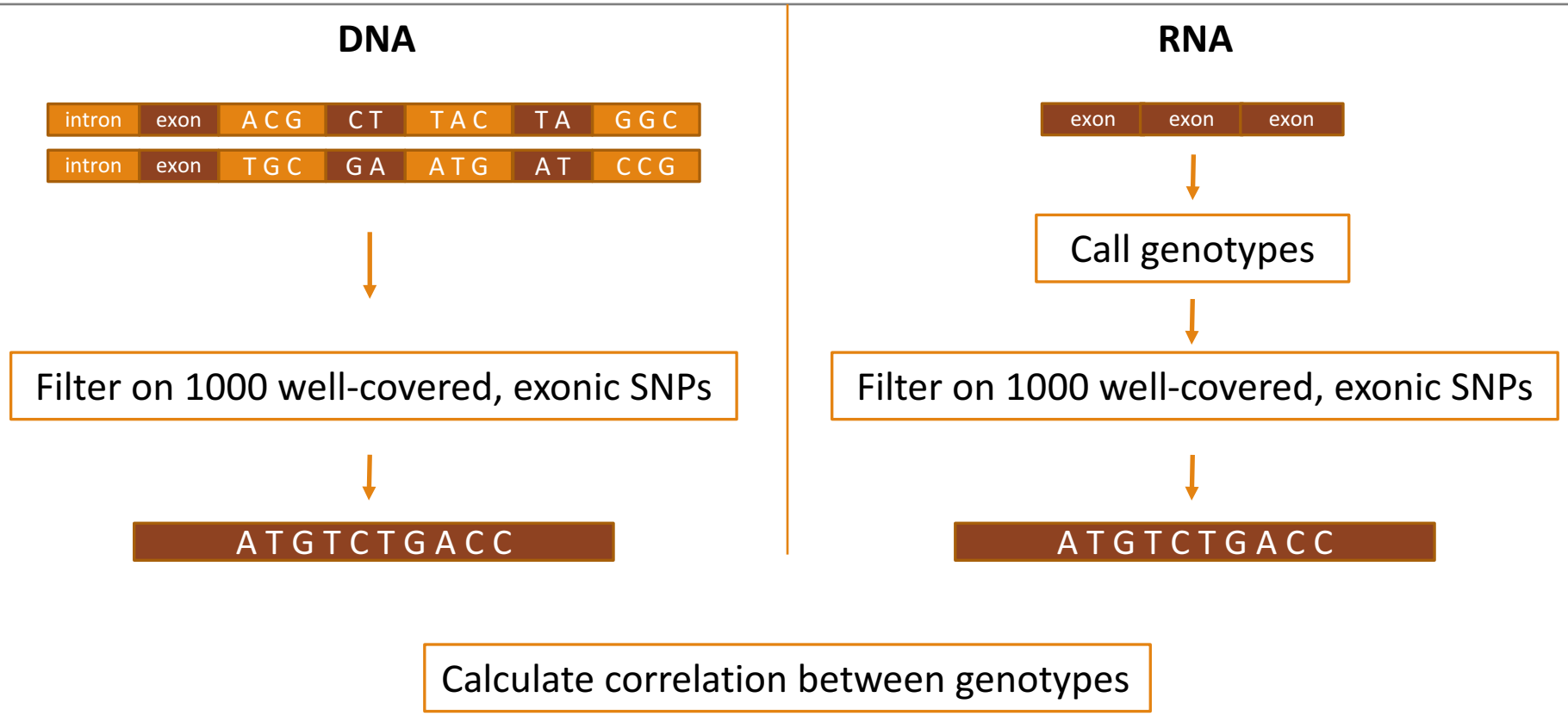
DNA-RNA matching
Method

- Extract genotypes from RNA-seq data
  - Alignment
  - Quantification
  - Genotype calling (Unified Genotyper)

- DNA-RNA matching
  - Check that correct samples are matched

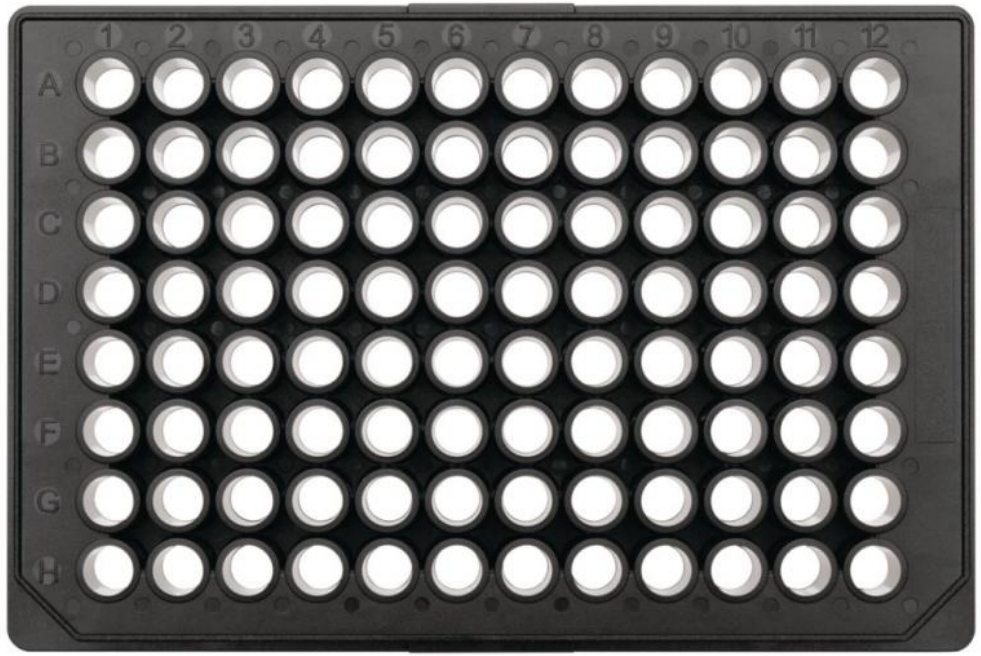
- **Remove** outliers
  - Ethnic
  - Heterozygosity

DNA-RNA matching
Method



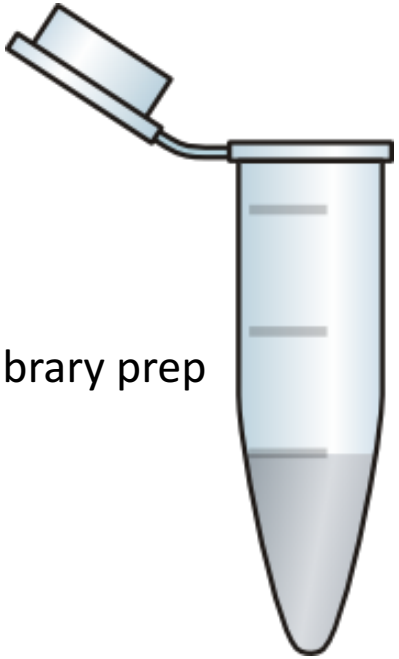
DNA-RNA matching
Resolving twin pairs and mix ups

- 96-well plate turned around
- Sample mix ups
- Identifying monozygotic & dizygotic twins
- **Remove** unresolved 39 mix ups



DNA-RNA matching
Merging RNA-seq runs

- Some low-read samples were re-sequenced
- Merge two RNA samples from one individual



One library prep

First round sequencing
(many reads)

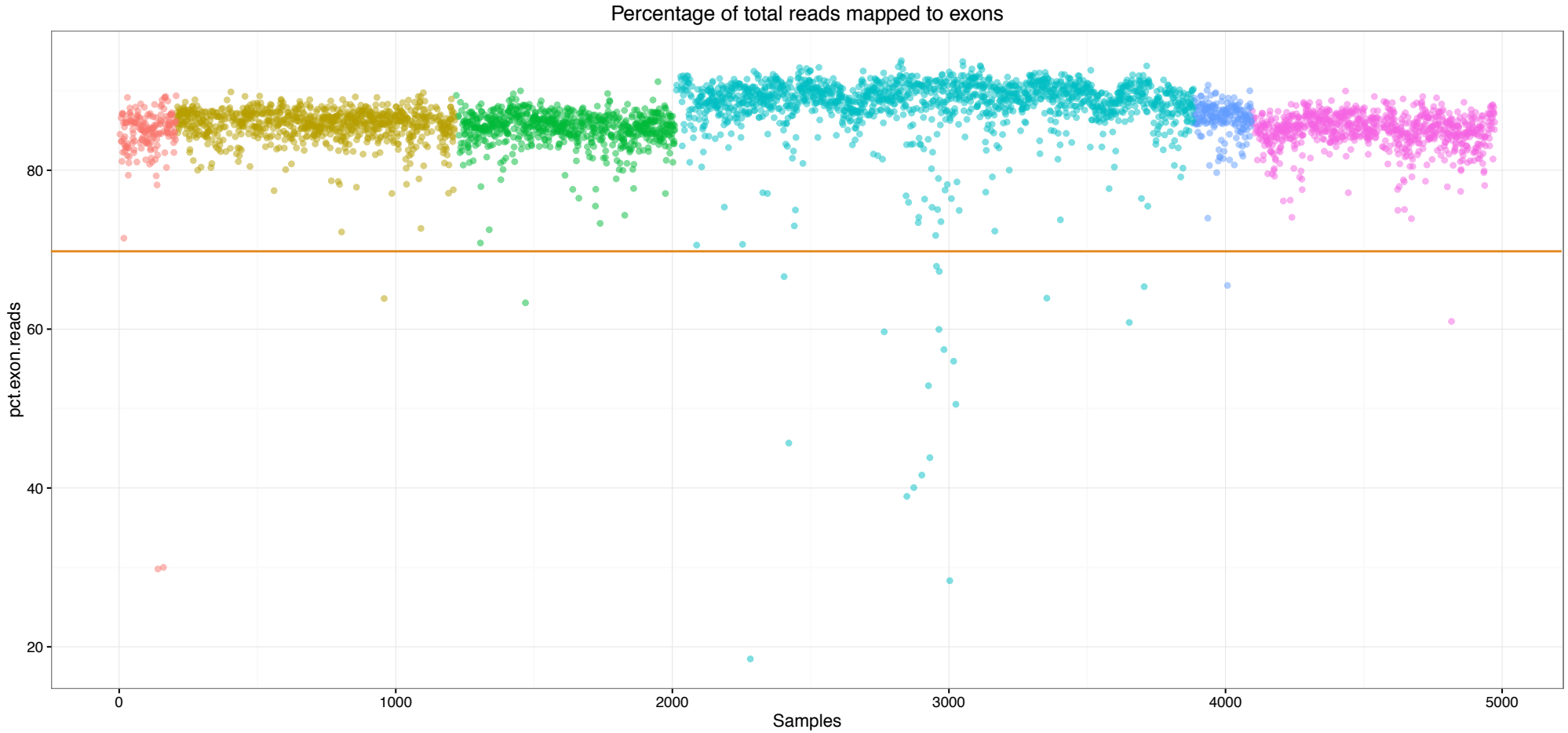
Second round sequencing
(top up reads)

QC metrics
Pipeline output

- Output from QC pipeline includes many variables
- All of these were plotted to check which thresholds are informative
- exon mapped / genome total = percentage of total reads mapping to exons

avg_deletion_length	avg_mapped_length
start_mapping_time	avg_input_length
pct_unique_mapped	end_time
num_unique_mapped	pct_unmapped_mismatch
num_splice_annotated	genome_duplicates
num_splice_noncanonical	exon_duplicates
pct_unmapped_other	exon_mapped
num_splice_total	genome_total
num_splice_atac	genome_mapped
num_splice_gcag	exon_total
num_input	genome_insert_std
rate_insertion_per_base	genome_insert_mean
pct_mapped_multiple	R2_raw_GC_mean
rate_mismatch_per_base	R2_raw_GC_std
start_job_time	R1_raw_GC_mean
pct_unmapped_short	R1_raw_GC_std
mapping_speed	R1_clean_GC_mean
avg_insertion_length	R2_clean_GC_mean
pct_mapped_many	R2_clean_GC_std
rate_deletion_per_base	R1_clean_GC_std
num_splice_gtag	MEDIAN_5PRIME_TO_3PRIME_BIAS
num_mapped_many	MEDIAN_3PRIME_BIAS
num_mapped_multiple	MEDIAN_5PRIME_BIAS

QC metrics
Reads mapping to exons



Remove samples with < 70% exon mapping

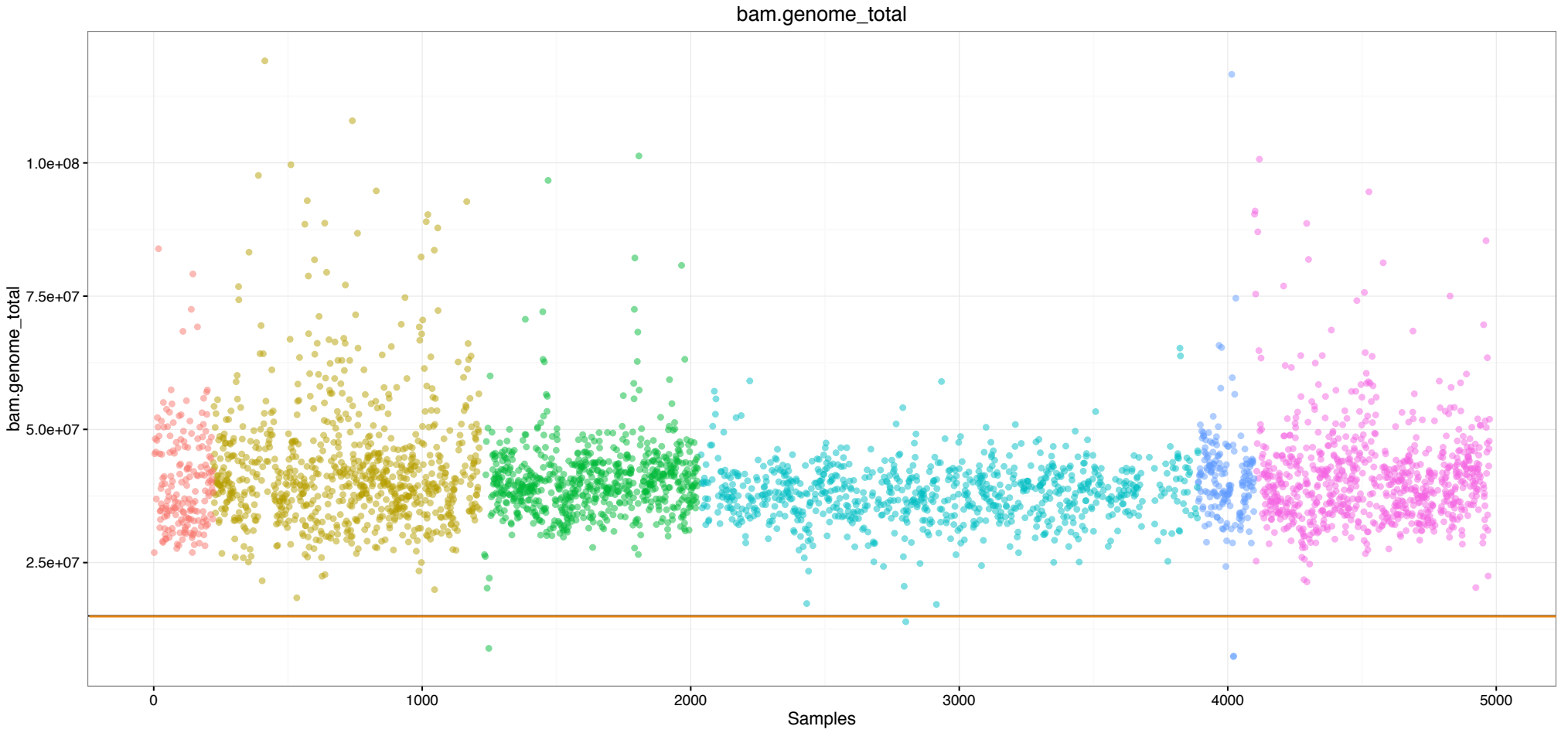
Sample selection
Family members

- **Remove** samples with first degree relationship
- Twins: take the RNAseq sample with higher % mapping to exons
- GoNL: include parents (more samples)

QC metrics
Mixupmapper

- Matching RNA to DNA again with a different method (MixupMapper)
- **Keep** samples with no match MixupMapper, but good match in earlier DNA-RNA mapping
- **Remove** samples with no match between RNA and DNA in both analyses
  - These samples cannot be recognized as a match
  - No match is an indication of RNA-seq quality: used as a QC metric

QC metrics
Total number of reads



Remove samples with total number of reads < 15M

Freeze 2 RNA-seq dataset

Step	Removed	Samples left
RNA sequencing		4543
Outliers	- 96	4447
Unsolved mix ups	- 39	4408
<70% mapped to exon	- 23	4385
No genotypes available	- 109	4276
No DNA – RNA match	- 109	4167
Related samples	- 684	3483
<15M reads	- 4	**3479**

Cohort	Freeze 2 DNA + RNA unrelated samples	Freeze 2 RNA only unrelated samples
CODAM	186	186
PAN	175	175
RS	777	777
LLS	697	697
LLDeep	825	825
NTR	819	928
Total	**3479**	3588

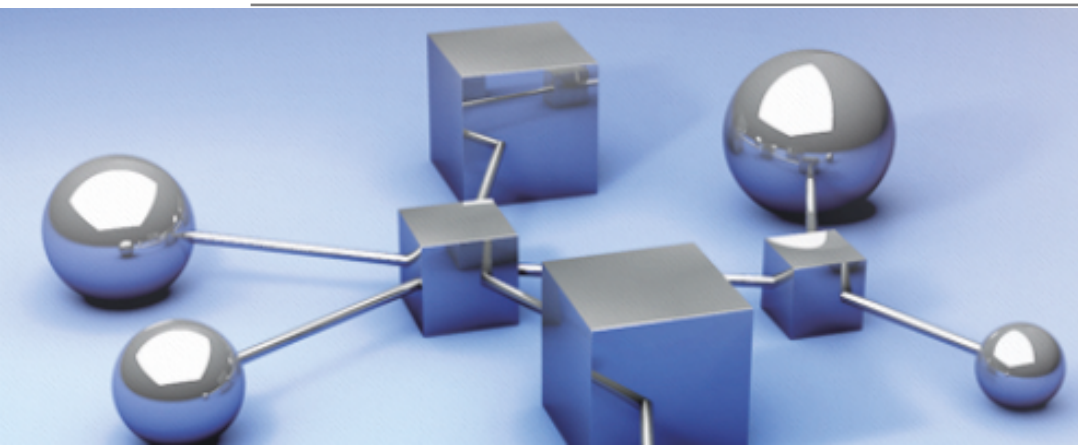
Concluding remarks

- Freeze 2 RNA-seq data is **ready for use**

- Keep in mind
  - Fairly lenient QC parameters
  - Cohort effects → adjust with covariate
  - Unrelated samples

- Quality control is a necessary **foundation for good research**

Acknowledgments



BBMRI.nl
Biobanking and
BioMolecular resources
Research Infrastructure
The Netherlands

Team effort by among others

Maarten van Iterson
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