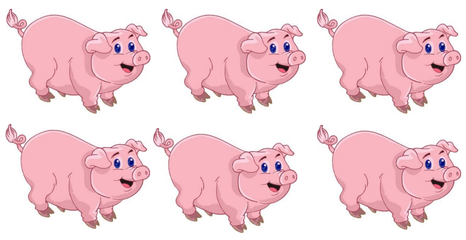
Detection of reciprocal translocations using short read sequencing

Aniek Bouwman, Marinus te Pas, Martijn Derks, Martien Groenen, Roel Veerkamp, Marleen Broekhuijse, Barbara Harlizius

Background

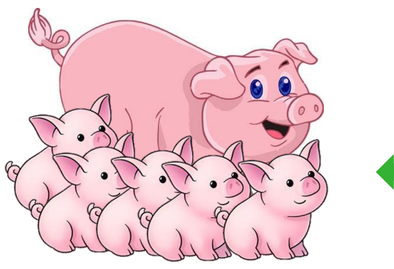
Breeding candidates

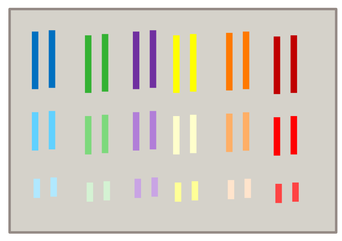


Pig Breeding Program

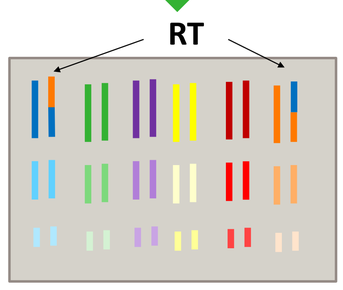
Karyotype screening

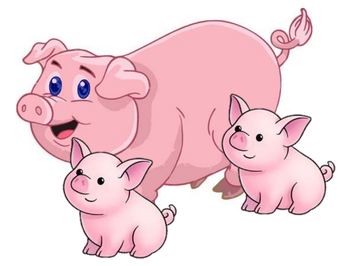
normal





0.4% abnormal karyotype
- Trisomy
- Mosaicism
- **Reciprocal translocations (RT)**





Subfertility

Material

Reference genome animal
23x coverage
paired end
short reads

Control animals (n=10)
20-30x coverage
paired end
short reads

RT cases (n=6)
30x coverage
paired end
short reads

Objective

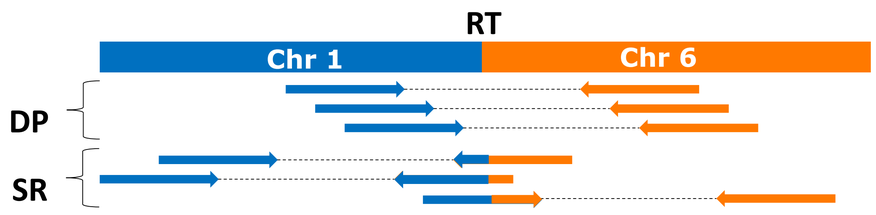
Identify reciprocal translocations detectable with karyotyping using short read paired end sequencing

To replace karyotype screening when sequencing breeding candidates becomes a (cost efficient) routine

Methods

Sequence alignment: Sus Scrofa build 11.1

Detection: discordant pairs (**DP**) & split reads (**SR**)



Software: Delly (detection), Jbrowse (visualization)

Basic filtering: contigs, low quality, imprecise

Final filtering: mapping quality, read support, reciprocal breakpoint present
Using reference & control animals: genome build problems, common issues (e.g. repeats)

Visual inspection: normal reads present; forward DP & reverse SR left; reverse DP & forward SR right

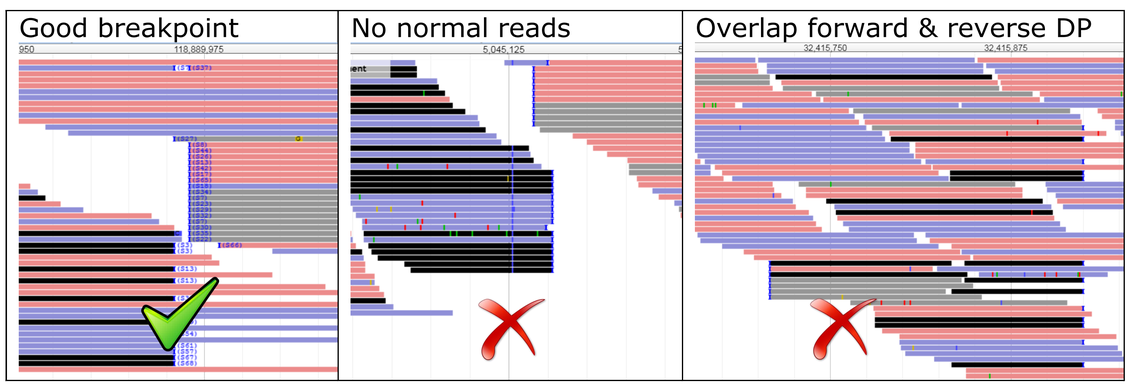


Figure 1. JBrowse visual of a reciprocal translocation and two false positive breakpoints. Black reads are forward discordant reads, grey are reverse discordant reads, blue are reverse (split) reads, red are forward (split) reads.

Conclusions

- Reciprocal translocations **detectable** using short read sequence
- Software outputs many **false positive** translocations – but they can be identified as false

Results

Pig 1, 2, 3, 5: reciprocal translocation **detected**

Pig4: visual inspection **debatable**

Pig 3 & 4 full sibs, and relative of Pig 1 - same RT

Pig 6: nothing passed visual inspection – **false neg**.

Table 1. Reciprocal translocation chromosomes (RT chr), number of detected inter-chromosomal translocations by Delly output, after filtering steps, and after visual inspection

	PIG1	PIG2	PIG3	PIG4	PIG5	PIG6
RT chr	2,4	6,8	2,4	2,4	7,14	1,16
Delly out	73,923	96,378	85,597	94,223	94,683	78,946
Basic filt	1,127	1,426	1,185	1,175	1,503	1,116
Final filt	30(15)	56(28)	34(17)	38(19)	68(34)	44(22)
Visual insp	**1(2,4)**	**1(6,8)**	**1(2,4)**	**1(2,4)**	**1(7,14)**	**FN**

To do: - Automate visual inspection
- Test specificity using (additional) controls
- Confirm breakpoints with PCR

Animal Breeding and Genomics
Wageningen University & Research
P.O. Box 338, 6700 AH Wageningen
Contact: aniek.bouwman@wur.nl
T + 31 (0)317 48 04 95

BREED4FOOD

WAGENINGEN UNIVERSITY & RESEARCH