

Engineering Welfare Traits in livestock

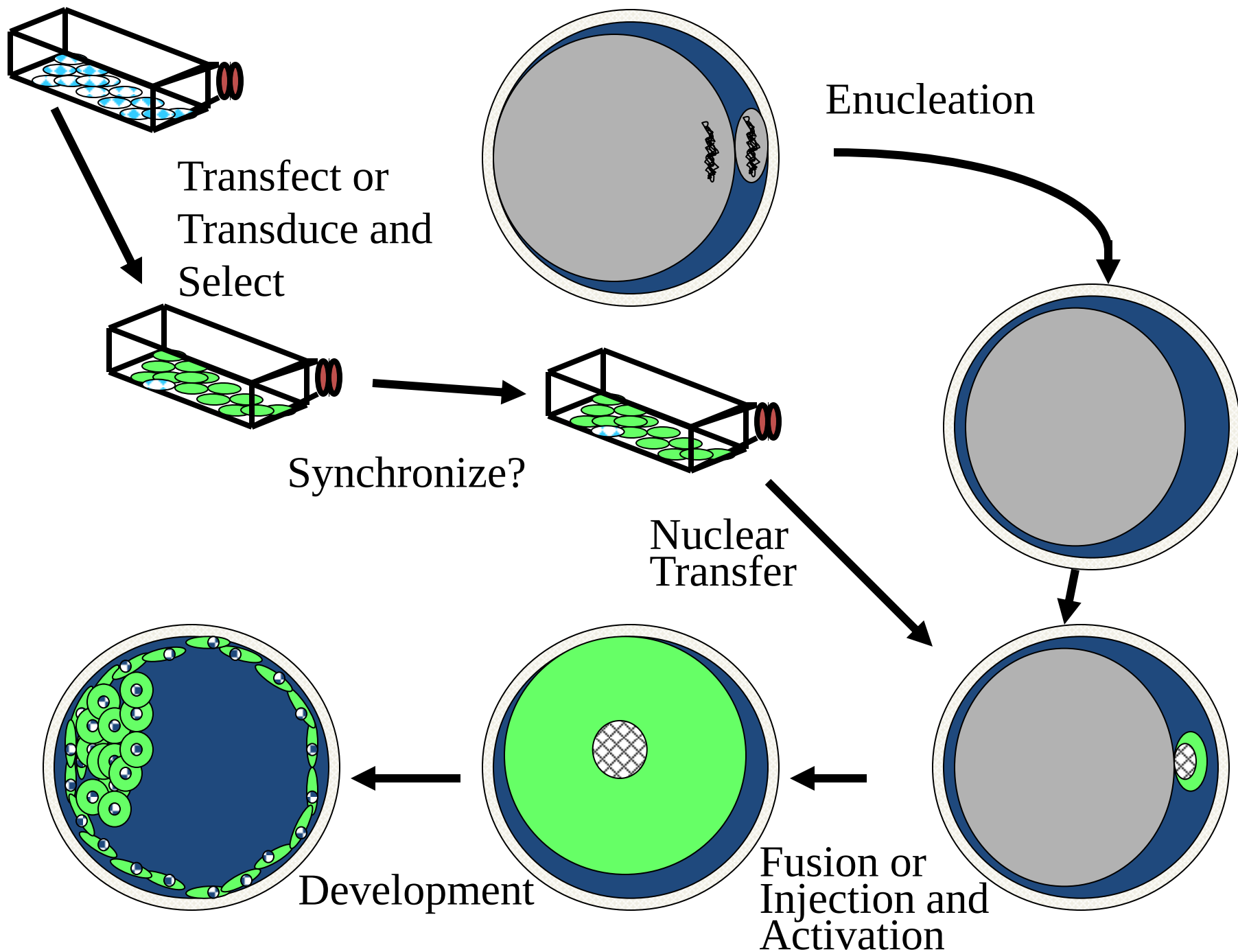
Bhanu Telugu

Canonical problem

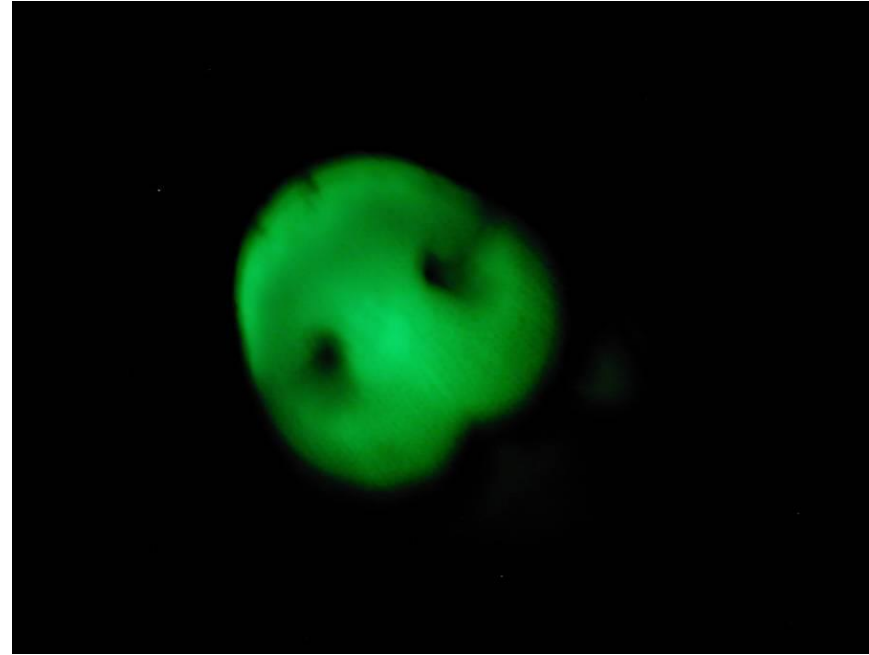
(length of pregnancy)

Species	Gestation Length
Mouse	21 days
Rabbit	1 mo
Bitch, Queen	63 days
Sow	3 mos, 3 wks, 3 days (3.8 mos; 114 days)
Ewe, Doe	5 mos (145 days)
Cow	9 mos
Mare	11 mos

**Somatic cell nuclear transfer (SCNT)/
cloning is the most popular approach**



SCNT as a means for generating GE pigs

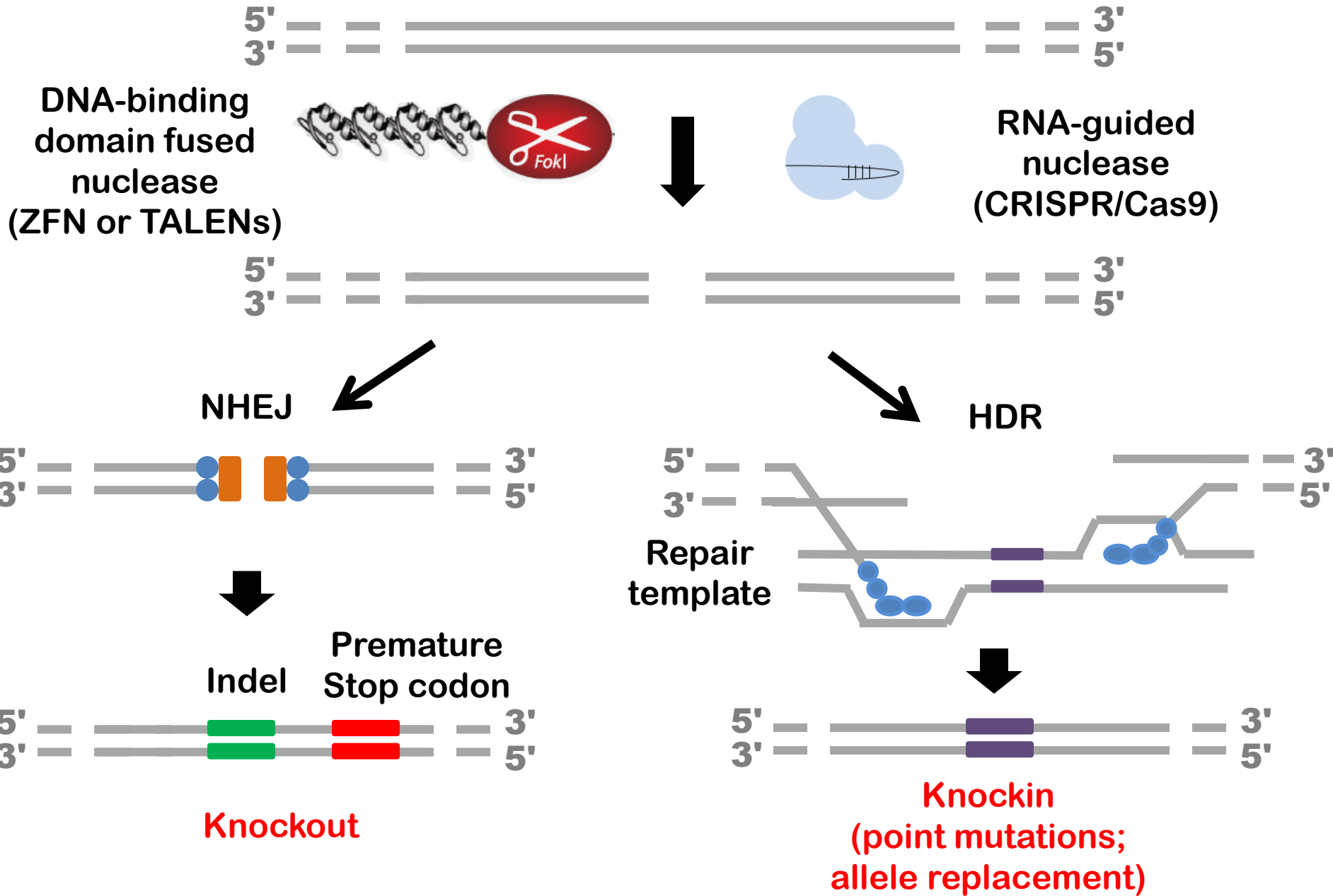


Courtesy: NSRRC, University of Missouri

Genome editing in the CRISPR era



CRISPR era



Introducing site specific modifications

Project: Eliminating boar taint



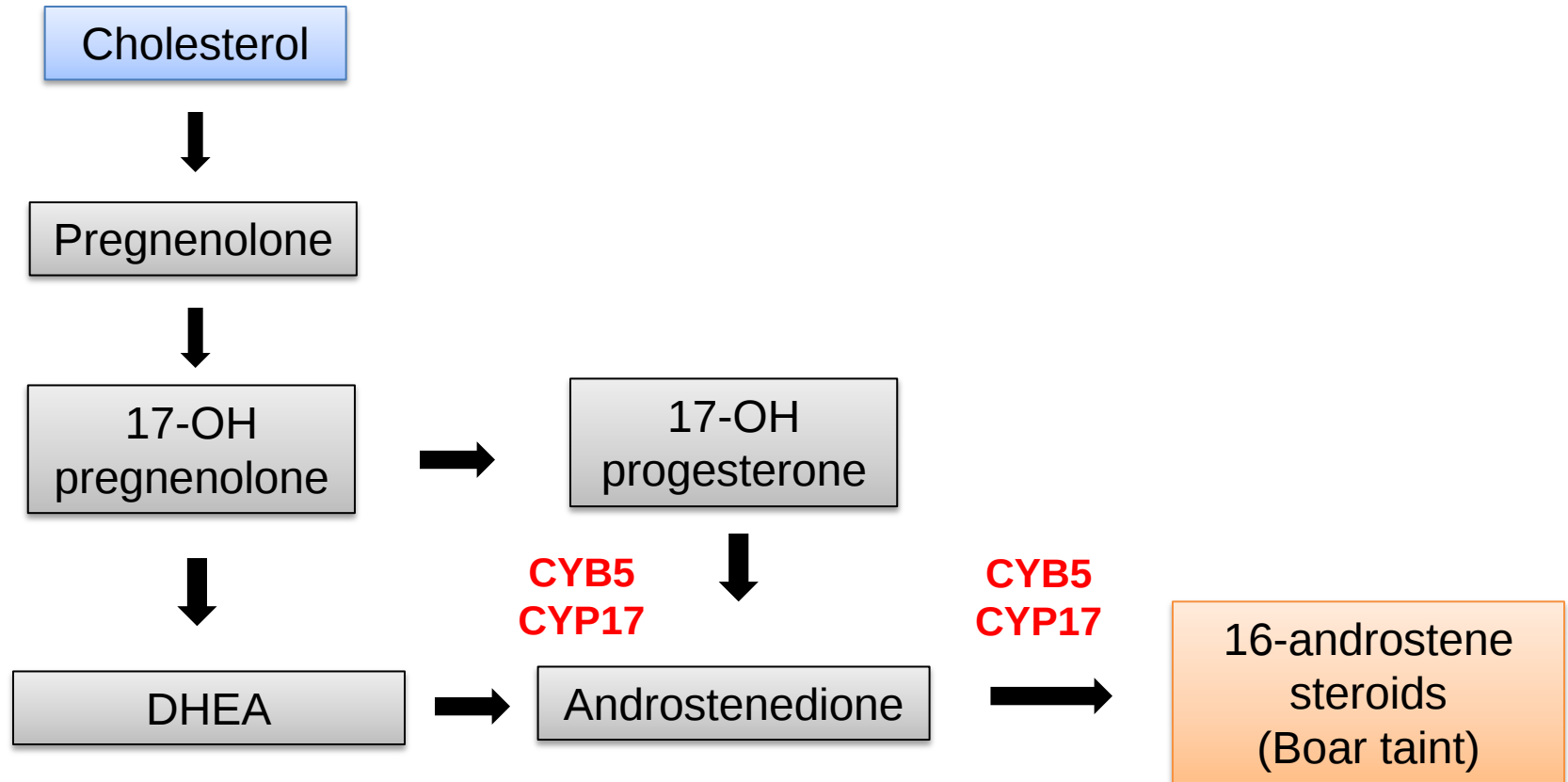
Boar taint

- Boar taint is an offensive off order and taste found in uncastrated male pigs.
- The major compounds responsible for boar tainted pork are **androstenone** and skatole, and both compounds are accumulated in fat.
- The only effective method of eliminating boar taint is 1) by castration of male pigs, (2) pre-pubertal slaughter, and/or (3) administration of the boar taint vaccine Improvac (Pfizer).

QTL analysis

- A rare polymorphism in the porcine **CYB5** gene just upstream of the translational start site results in **decreased** production of CYB5 and decreased synthesis of androstenone (Peacock et al., 2008).
- A **CYB5** knockout mouse model has a dramatically low levels of testicular androgens (McLaughlin et al., 2010). Therefore, totaling eliminating the expression of CYB5 is not an option.

Boar taint etiology



Comparative genomics

Steroid binding pocket of CYB5A

	1	11	21	31	41
Rat CYB5	MAEQSDKDVK	YYTLEEIQKH	K DSKSTW V IL	HHKVYDLTKF	LEEHPGGEEV
human CYB5	MAEQSDEAVK	YYTLEEIQKH	N HSKSTW L IL	HHKVYDLTKF	LEEHPGGEEV
pig CYB5	MAEQSDKAVK	YYTLEEIQKH	N NSKSTW L IL	HHKVYDLTKF	LEEHPGGEEV

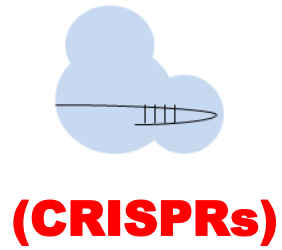
Steroid binding pocket of CYP17A1

	80	90	100	110	120
Human	QLAKEVLIKK	GKDFSGRPQM	A T L D IAS S NNR	K G I A F A D S GA	HWQL
Pig	QLAKEVLLKK	GKEFSGRPRV	M T L D IL S DNQ	K G I A F A D H GT	SWQL
Rat	QLAREVLIKK	GKEFSGRPQM	V T <u>Q</u> S <u>L</u> L S <u>D</u> <u>Q</u> G	K G <u>V</u> A F A D A GS	SW <u>H</u> L

In vitro mutagenesis screen

CYB5 mutations with CYP17			16A/DHEA	
mutations	17OHP	DHEA	16A	ratio
R52M +L102Q	1.174	0.699	0.607	1.032
R52M +I112V	1.257	0.566	0.282	0.567
R52M +L102Q/I112V	1.500	1.162	0.750	0.282
R52M/D103S	1.282	0.761	0.457	0.600
R52M/S106A	1.484	0.529	0.616	1.167
R52M/NQ108QG	1.176	0.861	0.563	0.653
N62S + D103S	0.897	1.166	0.912	0.787
N62S + 104L	0.904	1.317	1.760	1.484
N62S + S106D	1.252	0.071	0.399	2.042
N62S +L102Q/I112V	1.032	0.963	0.748	0.765
R52M+N62S/D103S	1.195	0.827	0.534	0.645
R52M+N62S/S106A	1.437	0.546	0.799	1.462
R52M+N62S/NQ108QG	1.130	0.877	0.771	0.881
R52M+N62S + L102Q/D103S/I112V	1.130	0.839	0.333	0.426
R52M/G57R/N62S/T70S + L102Q/D103S/I104L/NQ108QG/I112V	1.536	0.257	0.503	1.979
G57R + D103S	0.950	1.085	0.905	0.836
G57R + NQ108QG	0.833	1.255	1.231	0.983
T70S + D103S	0.947	1.087	0.937	0.863
T70S + NQ108QG	0.855	1.221	1.201	1.180
N21K + D103S	1.132	0.835	0.490	0.585
L28V + D103S	1.068	0.924	0.643	0.693
N21K/L28V + D103S	1.110	0.867	0.588	0.677

A



(single stranded DNA)

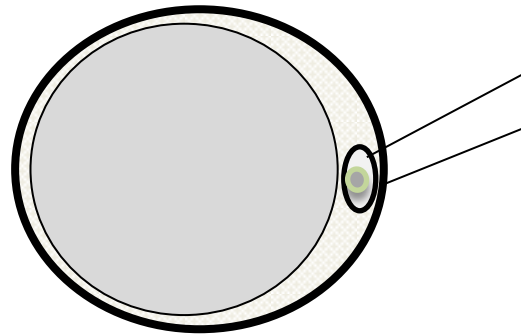
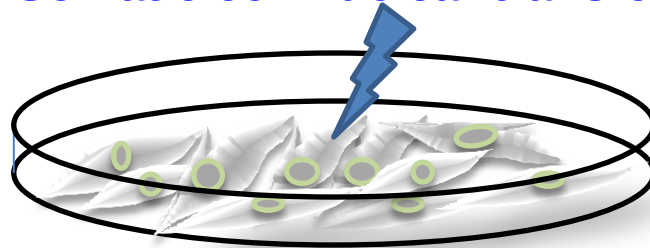
(OR)



(double stranded DNA)

Somatic cell nuclear transfer

B



Genome edited animal



Rationale for Genome editing

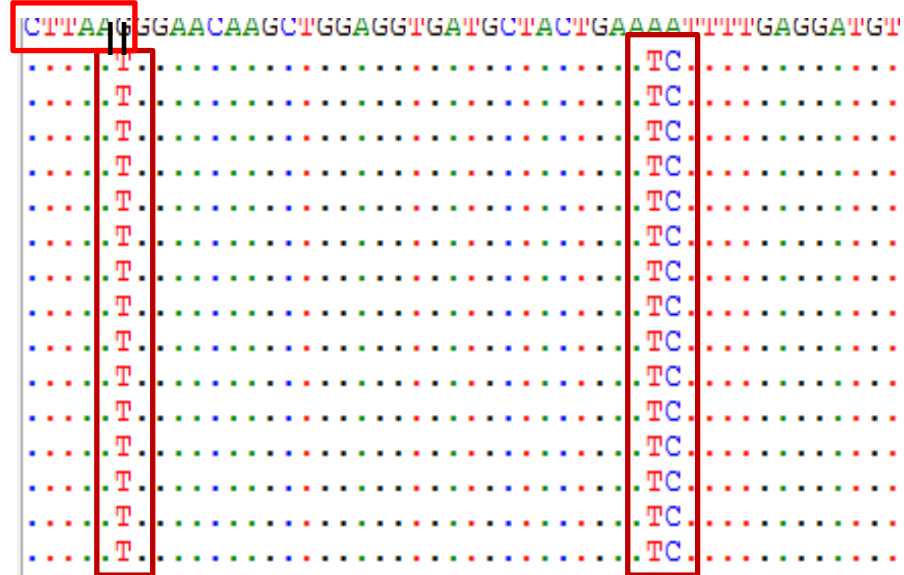
2) Gene modification- altering SNPs/QTNs

Targeting results in the loss
of AflII site

Query 481 CCAACAGCATCCTGGTGGGGAAGAAGTCTTAATGGAACAAGCTGGAGGTGATGCTACTGA 540
|||||
Sbjct 165928001 CCAACAGCATCCTGGTGGGGAAGAAGTCTTAAGGGAACAAGCTGGAGGTGATGCTACTGA 165928060
AflII

Query 541 ATCTTTTGAGGATGTTGGACACTCCACAGATGCTCGAGAGTTGTCCAAAACGTTTCATCAT 600
|
Sbjct 165928061 AAATTTTGAGGATGTTGGACACTCCACAGATGCTCGAGAGTTGTCCAAAACGTTTCATCAT 165928120

Afl



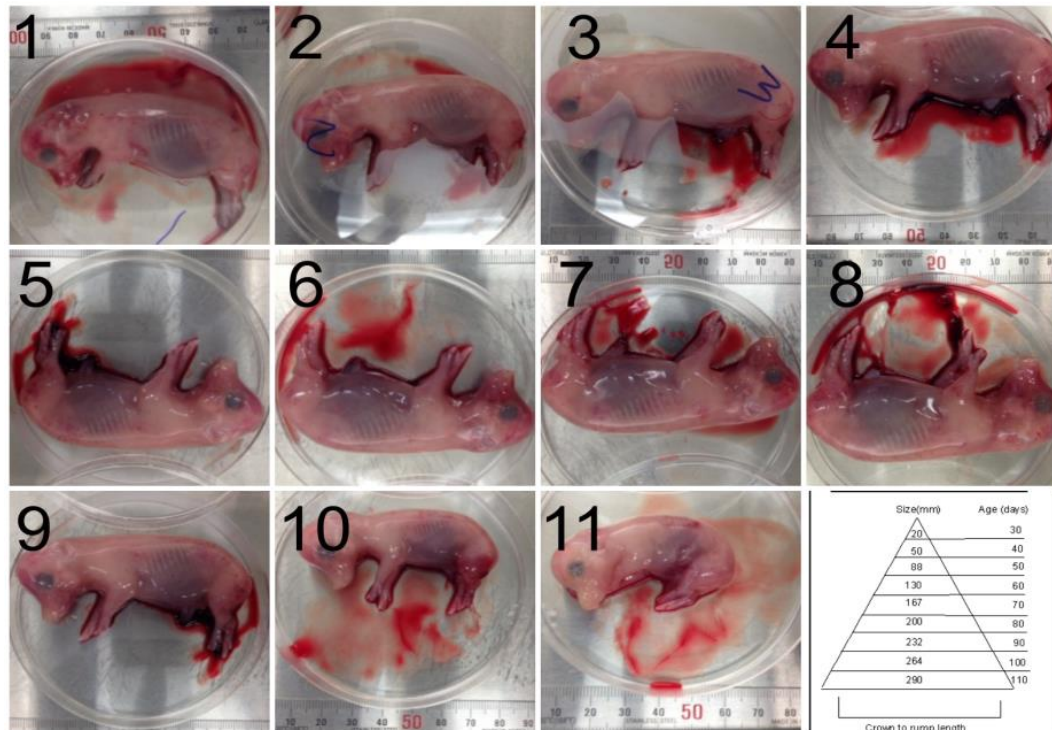
WT 1 2 3 4 5 6 7 8



Efficiency of generating genetically engineered pigs by SCNT and CRISPR/Cas9 system

Cell sources	No. recipients	No. pregnancy	No. delivered	No. piglets (fetuses)
KI-CYP17a1	1	1/1 (100)	-	(11)

* Cloning efficiency that was obtained by total no. fetus / total no. transferred embryos



Screening of CYP17A1 targeted fetuses by BstZ17I digestion

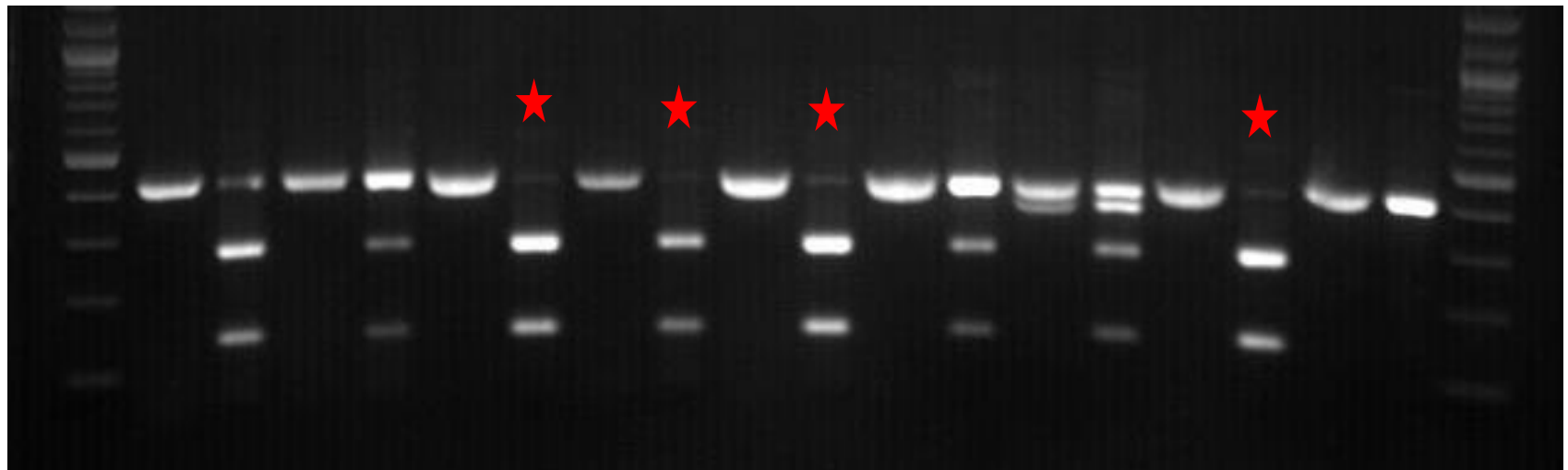
GAGTCTGATG GCTGTGTCCT TTCCTGCTTT CCAAAGATGA CTCTAagtAT D103S

10951 aCTGTCAGAC AACCAAAGG GGATTGCCTT CGCCGACCAT GGTACCTCCT

11001 GGCAGCTGCA TCGGAAGCTG GCACTGAGCA CCTTTTCCTT GTTCAAGGGT

11051 GGCAACCTGA AGCTGGAGAA CATCAGTGAG TGCCCAGCCG GCCCTGGGCT

New BstZ171 site



	P	D	P	D	P	D	P	D	P	D	P	D	P	D
Fetus	#1	#2	#3	#4	#5	#6	#7	#8	WT					

P: PCR product
D: Digest with BstZ17I

Boar taint project

Summary

- Edit *CYB5* locus on *CYP17*^{mut} background.
- Perform NT with the *CYB5* and *CYP17* double mutants and Wild type controls
- Screen for steroid profile at weaning and at puberty

Questions?