Sugar Research Australia

MICROSATELLIE MARKERS – CAN WE AGREE ON AN INTERNATIONAL SET FOR SUGARCANE VARIETY IDENTIFICATION?

G Piperidis, N Burrt, J Stephen and A D'Hont

ISSCT workshop

1-5 June 2015

Yes we can !

Outline

- Microsatellites – what are they used for?
- History of use at SRA (formerly BSES)
- Outsourcing: AGRF
- International tests
- Discussion session

SSRs – what are they used for?

- Variety identification
  - quality control of the selection program and new varieties prior to multiplication and distribution
  - quality control of the tissue culture propagation system to avoid propagation of incorrect variety
  - resolution of variety mix-ups
  - Identification of “unknown” varieties

SSRs – what are they used for?

- identification of duplicated accessions in the germplasm bank: germplasm management
- determination of selfing levels in seed samples
- identification of male progenitor of clones originated from polycrosses
- identification of hybrids/confirmation of parentage or identity

Maccheroni et al, ISSCT 2007

History of SSR use

- In the late 1990s, the International Sugarcane Microsatellite Consortium (SMC) funded by the International Consortium for Sugarcane Biotechnology (ICSB) developed about 270 sugarcane microsatellites (Cordeiro et al 2000)

- At SRA strong interest in developing/identifying and using SSR markers for variety identification (ISSCT, 2001)

SSR at SRA

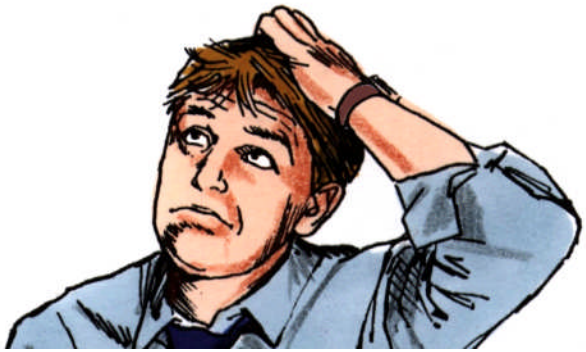
- Commenced 1999 – testing & screening 270 SSR primers for their usefulness in variety identification
- Using facilities at SRA
  - 33P & large polyacrylamide gels, manual scoring
- Primer pairs selected
  - Polymorphic
  - Clear profile, easy to score
  - Little or no stuttering
  - Repeatable, Reliable and Robust results

SSR at SRA

- 20 varieties sampled from two regions
- 19/20 consistent
  →Q96 ??

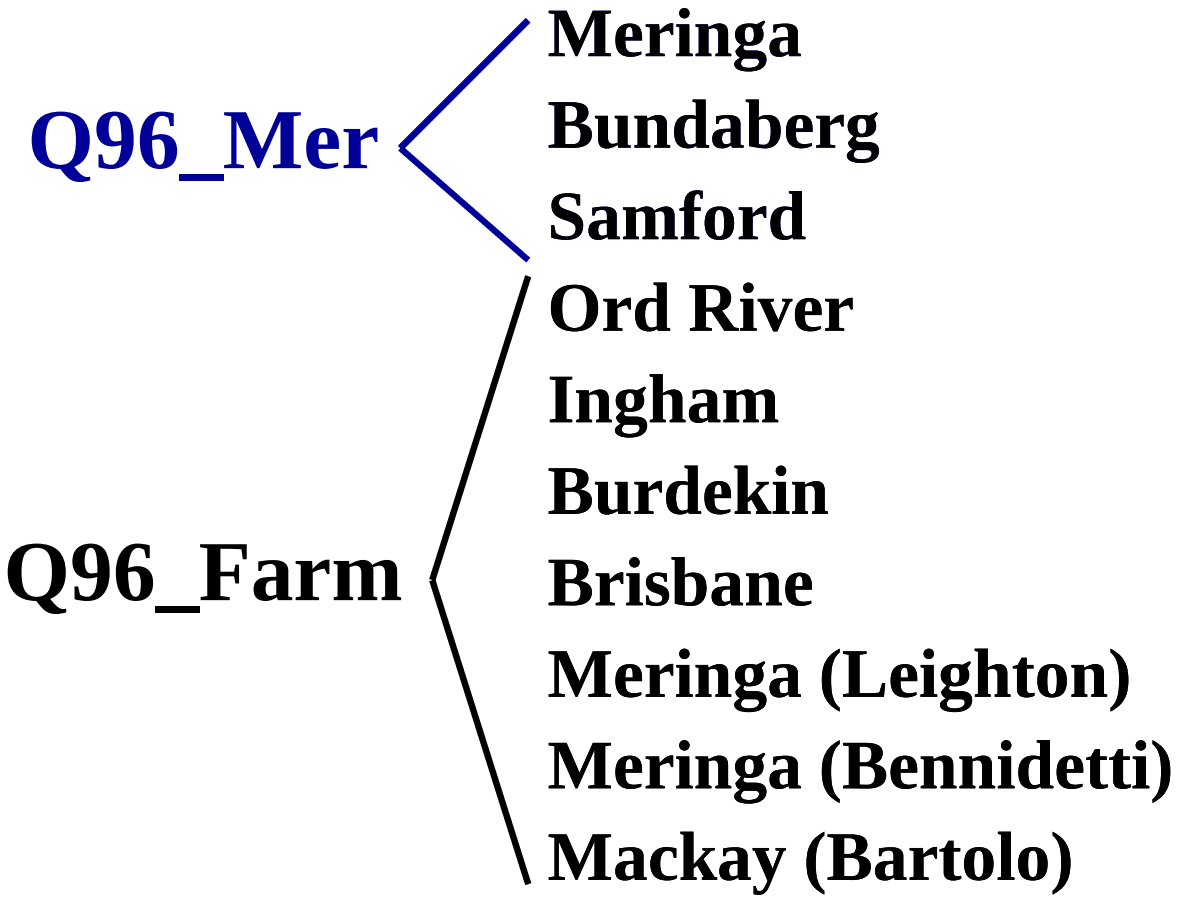


- Multiple Q96 samples tested





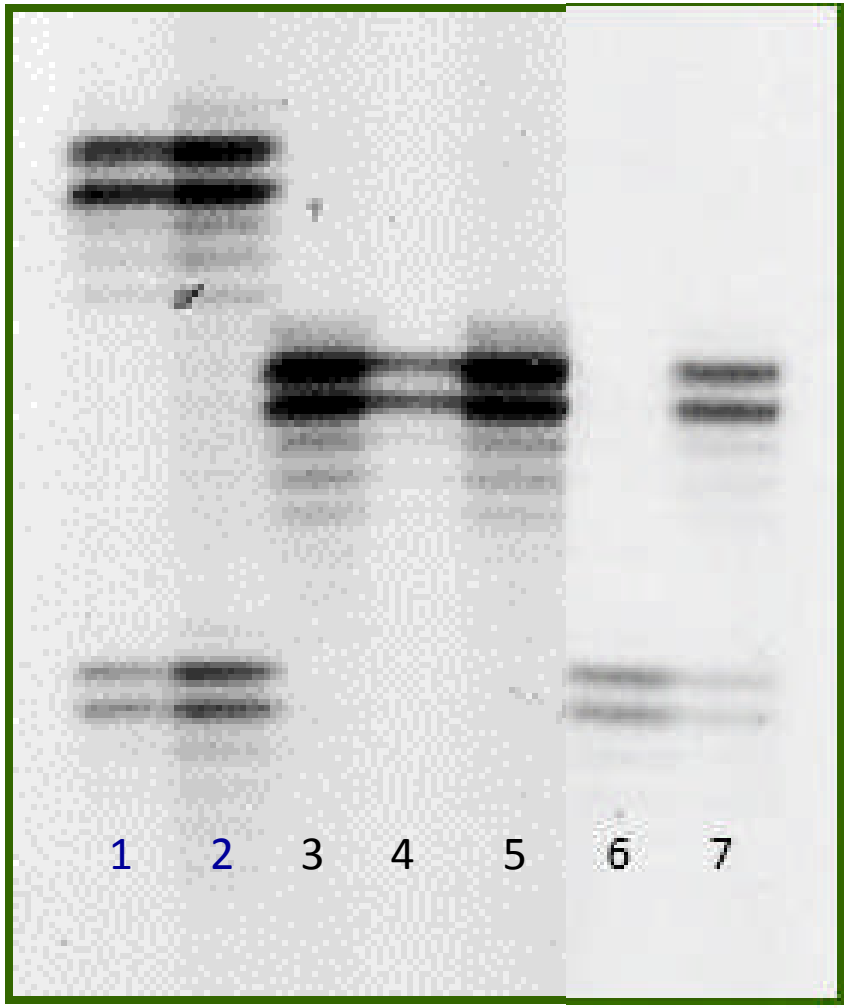
SSR and Q96


Q96_Mer
Meringa
Bundaberg
Samford
Q96_Farm
Ord River
Ingham
Burdekin
Brisbane
Meringa (Leighton)
Meringa (Bennidetti)
Mackay (Bartolo)

The real Q96 identified



6. Q63
7. Q68



SSR at SRA

- 2004: DNA fingerprinting should not be an SRA activity
  - Needed an external independent provider

- Australian Genome Research Facility (AGRF) contracted to provide the following:
  - Proof of concept
  - Secure searchable database of sugarcane DNA fingerprints
  - Deliver an identification verification service

SSR at AGRF

- Fresh leaf material collected by SRA and sent to AGRF
- DNA extraction, PCR, fragment separation and scoring conducted at AGRF
  - AB3730 capillary electrophoresis for fragment separation
  - Scored using GeneMapper 3.7

SSR at AGRF

- Variety identification service commenced in 2006
- Cost is per sample sent (~$35)
- Results usually returned within 10 working days
- Integral part of SRA variety audit system
- ~ 6500 samples tested to date
- ~ 500 varieties fingerprinted

International tests

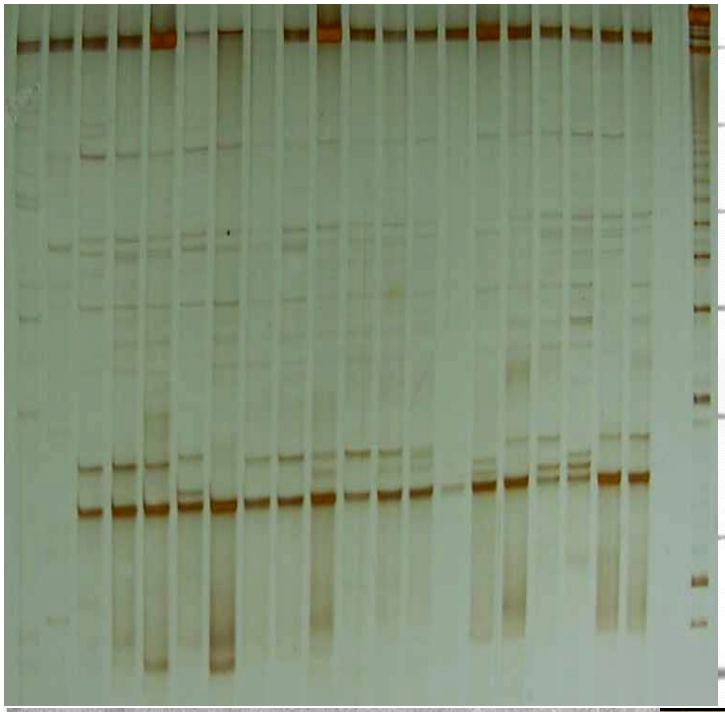
- 2002: international project to identify an agreed set of SSRs for variety identification purposes
  - First tests conducted with 6 labs, agreed set of 14 SSRs and 20 varieties (including spont off)

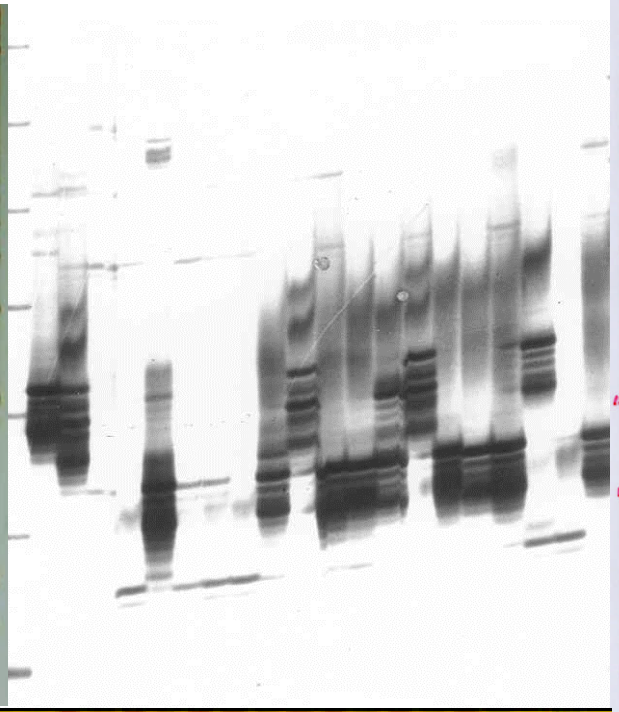
- Each lab used their own source of DNA and primers and conducted their own scoring of the markers generated

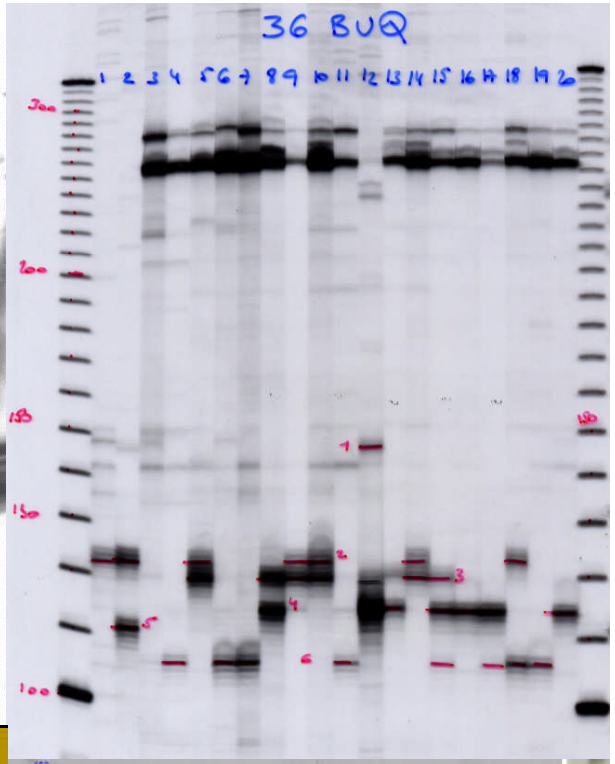
- Lot of difficulties/inconsistencies

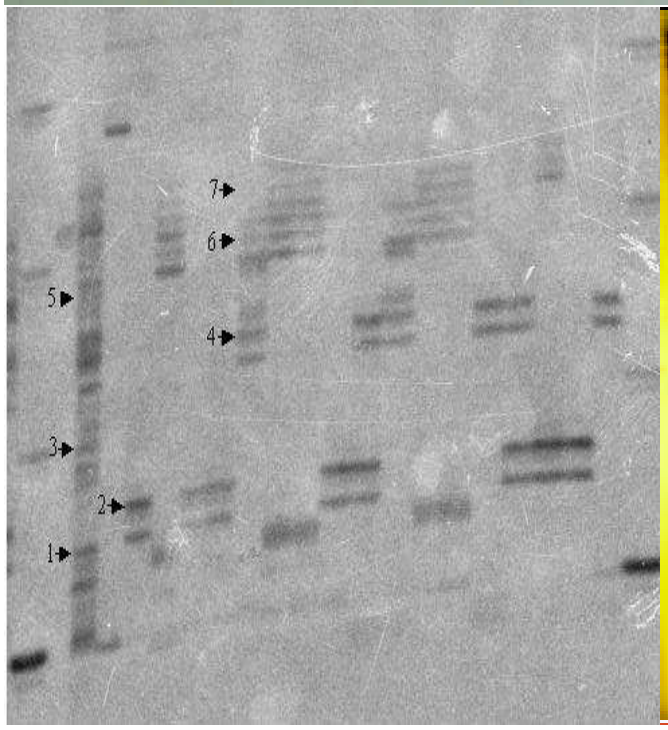
International tests

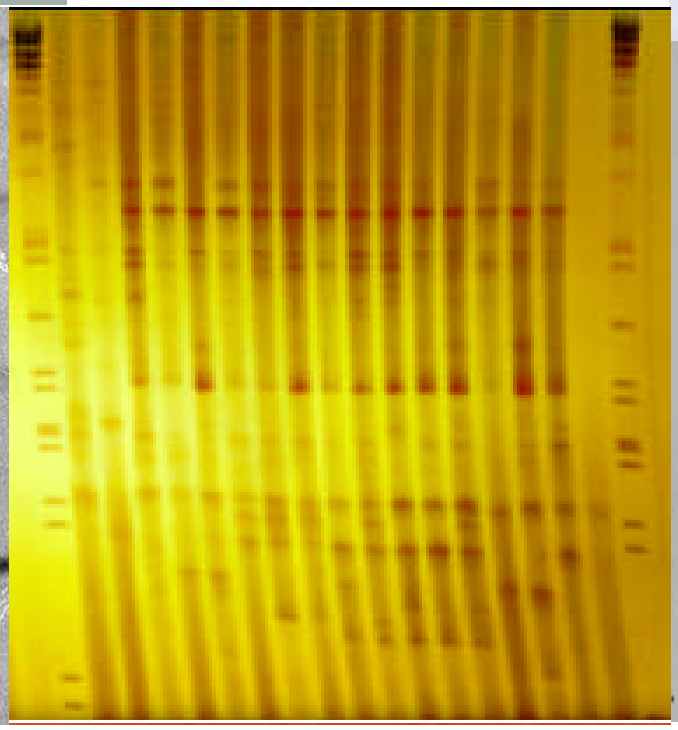
- Difficulties :
  - silver staining vs autoradiograph
  - length of gel run
  - scanned images
  - different size marker
  - incorrect scoring
  - scoring outside of expected size range
  - mislabelling of varieties

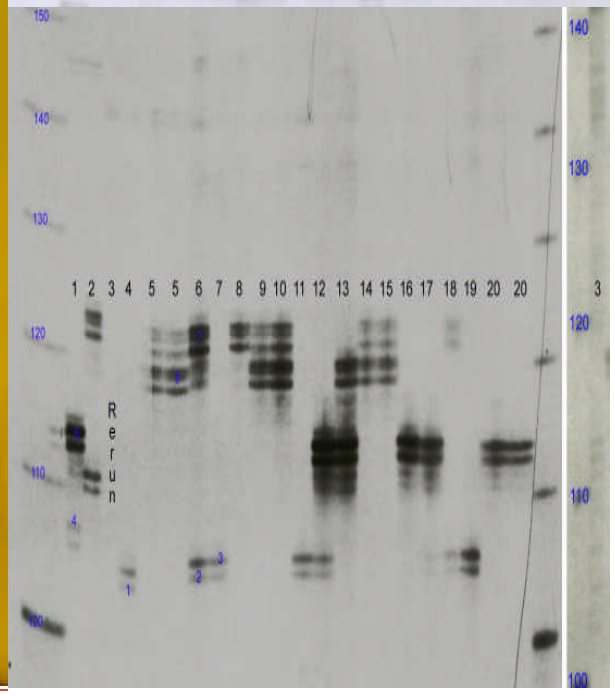





36 BUQ


7
6
5
4
3
2
1



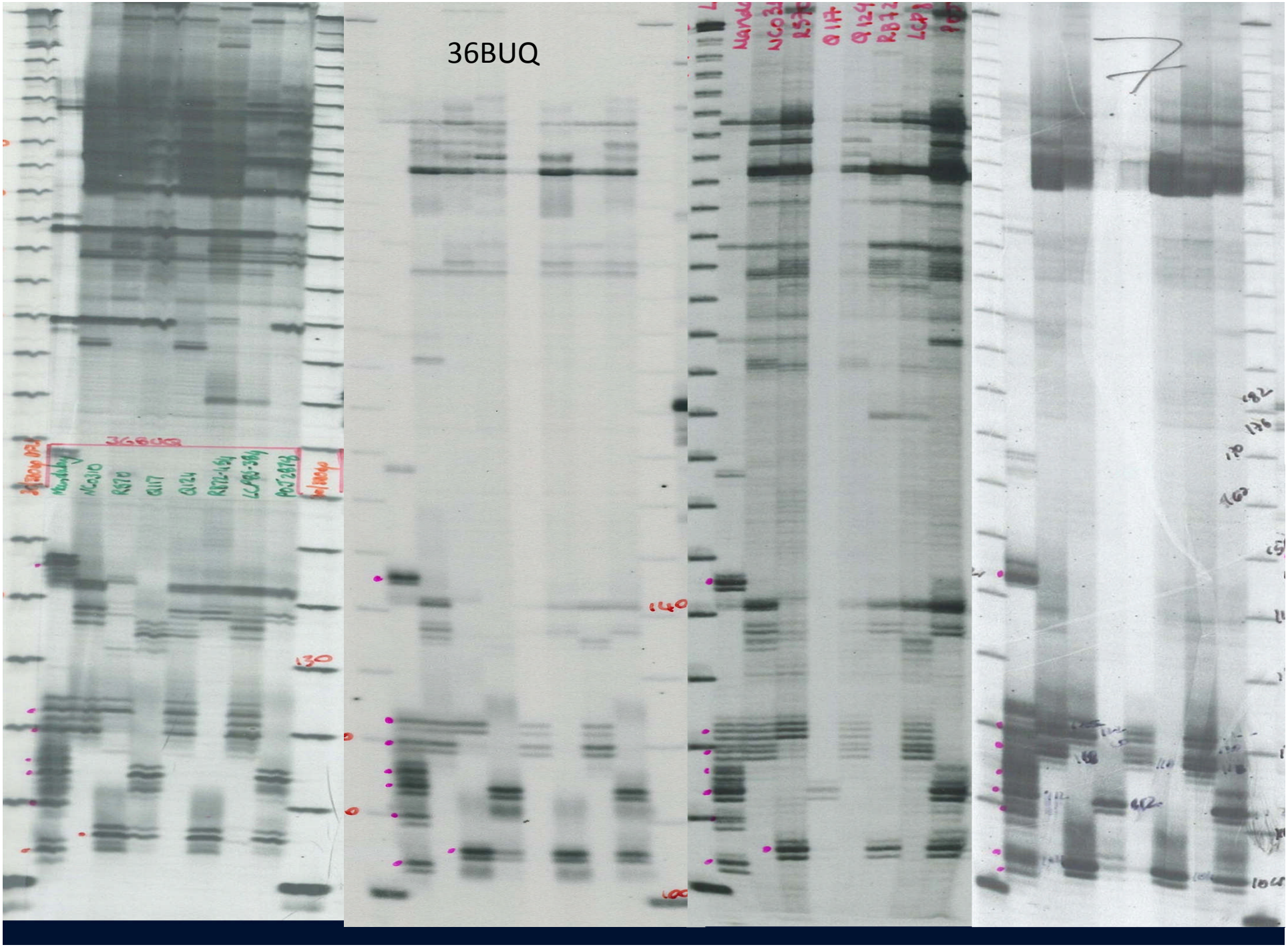

1 2 3 4 5 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 20
Rerun

International tests

SSR	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
334BS	8	13	11	9/8	8	5
336BS	8	24	16	8/9	20	3
749BS	7	9	15	6/6	18	5
36BUQ	7	6	8	8/7	6	5
278CS	9	21	16	7/8	8	2
286CS	6	21	9	8/9	10	10
119CG	14	11	11	9/9	14	5
mSSCIR10	11	22	18	6/6	8	7
mSSCIR12	13	24	13	15/10	14	4
mSSCIR19	9	21	17	8/9	16	8
mSSCIR25	11	9	10	5/5	7	5
mSSCIR55	5	10	9	5/7	11	3
mSSCIR56	9	26	16	11/10	12	10
mSSCIR60	10	33	13	6/7	22	6
Total	127	250	182	111/110	174	78

International tests – stage 2

- 2003 – 2004 2nd tests
  - 8 varieties x 10 SSRs x 6 labs
- DNA and primers from a single source distributed to all labs
- Specific PCR and gel conditions for each primer
- Results were much more consistent
  - Silver staining still an issue


36BUQ

SMC286CS

	Mandalay	NCo310	R570	Q117	Q124	RB72-454	LCP85-384	POJ2878
Marker 1	129							
Marker 2	130		130	130		130		130
Marker 3	133			133	133		133	
Marker 4		135		135	135	135	135	
Marker 5	137	137			137	137		
Marker 6	139					139	139	
Marker 7						140	140	
Marker 8		152			152			152
Marker 9	165							

International tests – stage 3

- Proposal:
  - Send freeze-dried ground material to all labs
  - Conduct a blind test
  - DNA extraction and PCR at each lab
  - Variety identification

- Primers:
  - SMC36BUQ, SMC278CS, SMC286CS, SMC119CG, SMC334BS, mSSCIR19, mSSCIR12, 068G08, 077C02, 175A08

Can we ?

- What's different now ?
  - Still a strong international interest
  - We are all more experienced
  - More labs using SSRs
  - Many more SSRs available – ESTs
  - New improved platforms for revealing SSR markers
  - Advanced software for allele identification

International tests: Future

- Email requesting information on SSR usage:
  - Primers
  - Detection system
- 11 respondents
  - 7 countries

International tests: Future

- Primers:
  - 5 labs using gSSRs (SMC and CIR), total 46 primers
  - 5 labs using EST derived SSRs: CV, SUCEST total 10 primers
    - CV29, CV37, CV38: 3 labs (5 in total)
  - 1 lab using EST from public database, 6 primers
- Detection system:
  - Polyacrylamide + silver staining x2
  - Polyacrylamide gel/4300 DNA Analyzer (Li-cor) including fluorescent dCTP x 4
  - ABI Sequencer x 5

Questions ?

Sugar Research Australia

DISCUSSION SESSION : CAN WE AGREE ON AN INTERNATIONAL SET FOR SUGARCANE VARIETY IDENTIFICATION?

ISSCT workshop

1-5 June 2015

Yes we can !

Most common primers

Primer	Labs
CV29	5
CV37	5
CV38	5
mSSCIR19	3
SMC119CG	2
SMC36BUQ	2
SMC278CS	2
SMC222CG	2
mSSCIR14	2