DNA FINGERPRINTING BY PCR BASED MOLECULAR MARKERS - RAPD AND ISSR

By
Dr. Jaya Arora
Assistant Professor
Laboratory of Biomolecular Techhnology

Department of Botany, University college of Science
Mohanlal Sukhadia University, Udaipur

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INTRODUCTION

- Genetic markers indicate the genetic differences between different organs or species.
- There are three major types of genetic markers:
- (a) morphological markers (also called "classical“ or "visible" markers) which are phenotypic traits
- (b) biochemical markers, which are called isozymes, including allelic variants of enzymes

  (c) DNA markers (or molecular markers), which reveal sites of variation in DNA.
- By definition, **molecular markers** are **genetic** loci that can be easily tracked and quantified in a population and may be associated with a particular gene or trait of interest.
- Molecular markers are effective because they identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification.

List of Markers

List of Markers	Acronym
Restriction Fragment Length Polymorphism	RFLP
Random Amplified Polymorphic DNA	RAPD
Amplified Fragment Length Polymorphism	AFLP
Inter-simple sequence repeat	ISSR
Simple Sequence Repeats	SSR
Variable Number Tandem Repeat	VNTR
Oligonucleotide Polymorphism	OP
Single Nucleotide Polymorphism	SNP
Allele Specific Associated Primers	ASAP
Inverse Sequence-tagged Repeats	ISTR
Inter-retrotransposon Amplified Polymorphism	IRAP

RAPD AND ISSR MARKERS

- Besides detecting polymorphism, RAPDs and ISSRs are routinely used for testing the genetic fidelity of tissue culture plants.
- RAPD markers are decamer (10 nucleotides long) DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way. Such polymorphisms thus behave as dominant genetic markers**.**
- The technique was developed independently by two different laboratories (Williams et. al., 1990; Welsh and McClelland, 1990) and called as RAPD and AP-PCR (Arbitrary primed PCR) respectively.
- **ISSRs** are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. **ISSRs** are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18 bp).
- The PIC value for **ISSR markers** was higher (0.31) than RAPD **markers** (0.23). Hence, the **ISSR markers** were more discriminative than RAPD **markers.**

PREPARATION OF REACTION MIXTURE FOR RAPDS

- The volumes of reaction medium is 20 µl.

Reagent	Final Concentration in PCR	Required volume for reaction
Matrix DNA	200 ng	5 µl
Forward Primer	50 ng	1 µl
Reverse Primer	50 ng	1 µl
Master-mix	1X	10 µl
Autoclaved ddH_2O	-	3 µl
Total		20 µl

- Spin ⟶ Vortex ⟶ Spin

PCR Thermal Cycling Steps for RAPDs

- Program the PCR machine as fellow:

Steps	Temperature (˚C)	Time	Role
Initial denaturation	**94 °C**	**4 min**	For linearization
Denaturation	**94 °C**	**45 s**	45 Repeat Cycle
Annealing	**35°C**	**1 min**	
Extension (Ta)	**72 °C**	**1 min**	
Final Extension	**72°C**	**1 min**	
Hold	**8°C**	∞	

PREPARATION OF REACTION MIXTURE FOR ISSRS

- The volumes of reaction medium is 20 µl.

Reagent	Final Concentration in PCR	Required volume for reaction
Matrix DNA	200 ng	5 µl
Forward Primer	50 ng	1 µl
Reverse Primer	50 ng	1 µl
Master-mix	1X	10 µl
Autoclaved ddH_2O	-	3 µl
Total		20 µl

- Spin ⟶ Vortex ⟶ Spin

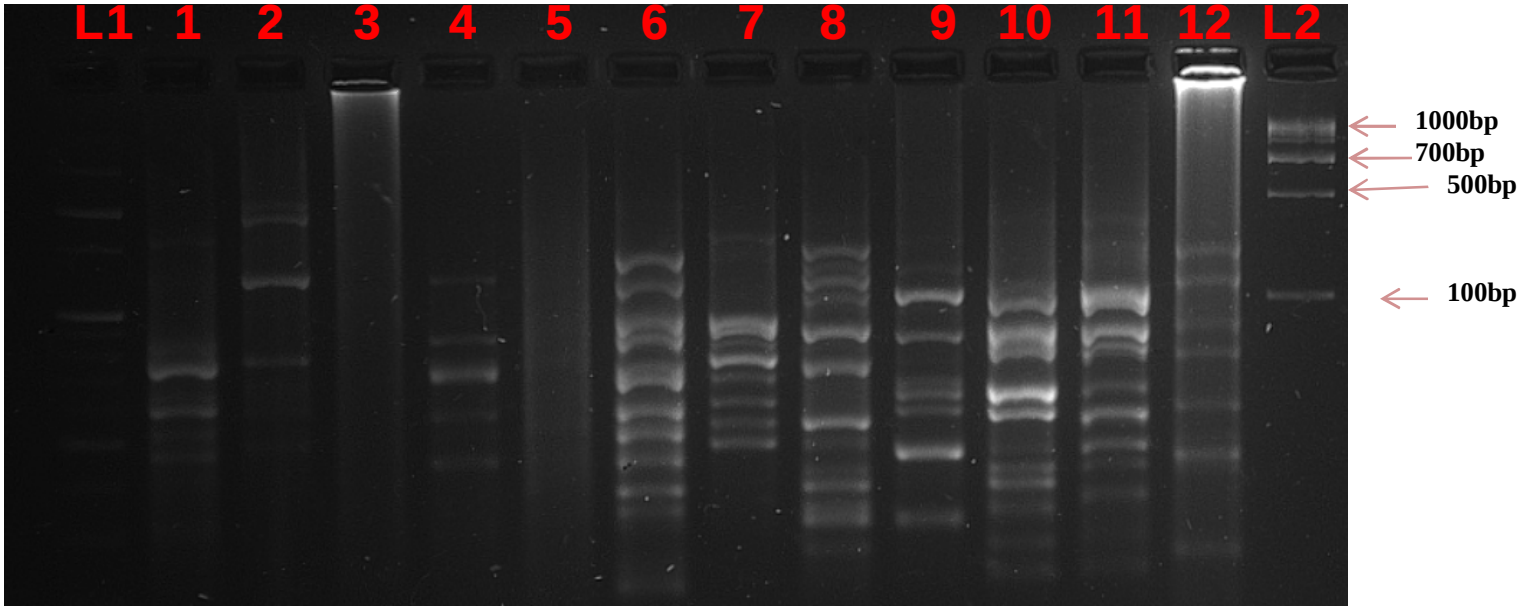
PCR Thermal Cycling Steps for ISSRs

- Program the PCR machine as fellow:

Steps	Temperature (˚C)	Time	Role
Initial denaturation	**94 °C**	**4 min**	For linearization
Denaturation	**94 °C**	**1 min**	36 Repeat Cycle
Annealing	**According to Tm of Primer**	**1 min**	
Extension (Ta)	**72 °C**	**1 min**	
Final Extension	**72°C**	**10 min**	
Hold	**4°C**	∞	

OBSERVATION

- After PCR amplification Amplified DNA Runs on 1.2 % gel electrophoresis.
- The number of fragments observed in the profile will reflect the number of amplified loci on templet DNA.



Gel electrophoresis of a RAPD and ISSR amplification profiles obtained with different primers (Lane L1: GeneRuler 100 bp, Lane L2: GeneRuler 1 Kb DNA Ladder (Bangalor Genei), 1-12 *in vitro* shoots of *P. tuberosa* (1–6 amplified with RAPD Primers and 7-12 amplified with ISSR Primers)) .

Table 1. Data on RAPD and ISSR primers used in the detection of genetic stability in in vitro raised plantlets of *P. tuberosa*.

Marker	Name of Primer	Primer sequence (5' -3')	Tm (ºC)	No. of amplicons	Size range of amplicons (bps)
RAPD	OPAT-01	CAGTGGTTCC	36	4	10-50
	OPP-08	ACATCGCCCA	36	5	50-500
	OPK-09	CCCTACCGAC	36	0	0
	OPN-02	ACCAGGGGCA	36	5	20-100
	OPN-05	ACTGAACGCC	36	0	0
	OPN-07	CAGCCCAGAG	36	11	20-250
ISSR	UBC-801	ATATATATATATATATT	42.5	7	80-100
	UBC-802	ATATATATATATATATG	46.8	10	20-250
	UBC-803	ATATATATATATATATC	42.9	7	10-150
	UBC-804	TATATATATATATATAA	43.3	8	10-200
	UBC-805	TATATATATATATATAC	45.0	11	10-150
	UBC-806	TATATATATATATATAG	52.1	7	10-250

Thank You