Multiplexed RiCA V.4 Release note:

The IRRI's Rice Custom Amplicon assay SNP Panel V.4: Updated, revised and with more traits, now available through IRRI – AgriPlex Genomics collaboration.

Introduction:

The use of DNA markers and molecular breeding shortens the time to market of new crop lines and lowers the overall cost of breeding. Molecular markers also contribute to faster and cheaper seed quality assurance practices by substituting expensive and time-consuming grow-outs with early lab testing (for genetic purity, trait confirmation, and adventitious presence). Presently, the most efficient and commonly used molecular markers are Single Nucleotide Polymorphisms (SNPs).

SNPs are single nucleotide changes that are heritable, codominant, and abundant throughout eukaryotic genomes. The advent of next generation sequencing technologies and genotyping by targeted sequencing provides an attractive method for mid-density SNP genotyping; Agriplex Genomics' PlexSeq is such a platform that enables effective mid-density SNP panel by high level multiplexing.

The International Rice Research Institute (IRRI) designed the first version of the 1K-Rice Custom Amplicon (1k-RiCA) panel. The 955 SNP markers were multiplexed in-to a PlexSeq™ panel as the 1k-RiCA v2. We describe here the next generation of the panel: RiCA v4, the way it evolved and list some of its new features as a molecular breeding and seed QA tool.

Characterizing the version 4 of the 1k Rice Custom Amplicon (RiCA) panel

Version 4 of the IRRI Rice Custom Amplicon SNP panel is made of 1,040 markers and maintains the basic structure of the original panel:

- Genomic screen: 797 SNPs originating from the Cornell 6KArray Infinium Rice chip [2] and the 3,000 Rice Genomes Project [5, 6, 7]. The markers are evenly distributed throughout the genome and were selected based on call rate higher than 95% and high minor allele frequencies (MAF≥0.4).
- Trait-related markers: 205 SNPs associated with different important trait genes/QTLs.
- Purity Markers: 22 SNPs chosen for their discriminatory ability amongst elite indica material, used in determination of genetic purity, identity, and hybridity.

The revision of the panel included performance analysis of RiCA version 2: the results of 5 projects adding up to 5,187 individuals were analyzed. Markers were scored on their success rate and robustness. Based on these criteria 48 lowest performing SNPs were removed from the panel.

In addition, the panel was augmented with more trait associated markers (see Table 1). Altogether 133 new SNPs were added to the revised panel, which now targets over 90 genes and QTLs of interest.

Table 1. List of traits and genes represented on the AgriPlex RiCA panel.

Trait category	Trait	Gene targets
Disease	Blast	Pi9 (3 alleles), Pik (11 alleles), Pii, Pita, Ptr, pi21, Pi35, Pi54, qPi33
	Bacterial blight	qXa4, xa5, qXa7, xa13, Xa21, Xa23, qXa26, Sweet14
	Brown planthopper	Bph9, Bph17, Bph32
	Other insects	qGm4
	Virus	TSV1, TBV1, rymv1-2, rymv1-4, rymv1-5, rymv2, RYMV3, STV11
Grain quality	Amylose	Waxy (6 different alleles distinguished)
	Chalkiness	PGC8.2, GW5
	Aroma	fgr-1
	Gelatinisation temp.	Alk (3b and 4)
	Other grain quality	NAS3 (grain zinc), LOX3 (spoilage), SBE-I, SBE-IIb, SBE-III, GPT1
	Grain size/shape	GS3, TGW6, SLG7
Abiotic stress	Drought	qDTY1.1, qDTY2.2, qDTY3.1, qDTY3.2, qDTY 4.1, qDTY12.1, Dro1
	Cold	Cold1, qSCT1, qCST10, qPSST6
	Heat	TT1
	Salinity	qSIS1.2, Saltol, qSOR1
	Anaerobic germination	qAG1, qAG3
	Submergence	Sub1
	Other abiotic stresses	BET1 (boron toxicity)
Yield components	Heading date	Ghd7, DTH8, Hd2/PRR37, Hd1, Hd6a, RFT1, Hd3a, ehd1
	Miscellaneous	DEP1, NAL1, WFP1, HIS1, GNP1, GFR1, FZP1, NGR5, SCM2
Hybrid		Rf3, Rf4, ms-IR36, WA-CMS, tms5

PlexSeq™: The mid-density multiplexed SNP genotyping solution

The revised SNP collection was again multiplexed and validated as a PlexSeq panel. The most important attribute of PlexSeq is its simplicity. PlexSeq™ workflow consists of:

- Primer design and multiplex prediction: A proprietary algorithm that uses artificial intelligence to identify the optimal sets of compatible primers that can be mixed in one PCR amplification.
- Crude DNA isolation.
- Thermocycling: highly multiplexed Primary PCR followed by secondary barcoding PCR.
- Pooling and Sequencing: barcoded amplicons are combined, purified, quantitated, and uploaded on an NGS sequencers.

This workflow is amenable to automation; all steps are carried out on liquid handlers and high-capacity thermocyclers. The efficiency and usefulness of the panel is further supported by AgriPlex' s large collection of barcode combinations. These allow simultaneous sequencing of up to 55,000 individuals; thus, effectively, the number of individuals tested simultaneously is limited only by the sequencer's capacity.

In addition, the panel is a collection of PCR primers not tethered to a surface (e.g.: chips). This provides the flexibility to dynamically customize and alter the composition of the markers in the panel so it best fits the germplasm or the application. Once the sequencing is complete, a proprietary genotype calling analysis software, provides an automated sequencer to data report workflow.

The 1k RiCA V4 is available as a service from AgriPlex Genomics. The panel and software are also available as a kit to be used by in-house genotyping laboratories.

References:

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