

Provision of Genotyping Support Services (GSS): An IRRI perspective

The Generation Challenge Program (GCP) - Genotyping Support Services assists developing country institutes in applying molecular marker technology for crop improvement. GCP engaged IRRI as an expert genotyping services provider responsible for legal and phytosanitary requirements for shipment of rice DNA, for genotyping of samples, and for providing the client institution and GCP with the data generated from the services described in each work order. As host agent for GCP, CIMMYT provides information on the client institution and the marker data for use in genotyping. Two work orders from the China National Rice Research Institute (CNRRI) and the Crops Research Institute in Ghana, respectively, are currently in place to screen a breeding population for the presence of *Xa7* and *Xa21* bacterial blight resistance genes and *Rf3* and *Rf4* fertility restorer genes, and analyze the genetic diversity of African rice germplasm. In the pipeline is the genotyping of CBSN-derived *indica* x *indica* lines from India. With these initial work orders, we hope to provide GCP with feedback on the logistics needed to complete each work order efficiently and effectively – from getting the DNA to IRRI, and the data back to the client and GCP. Similarly, IRRI will learn what it takes to provide genotyping services to partners and collaborators.

Work Orders: scope, timing and payment

Two work orders (WO) are in progress: (1) screening a breeding population for bacterial blight resistance genes *Xa7* and *Xa21* and for fertility restorer genes *Rf3* and *Rf4*, based on a request by CNRRI and, (2) analyzing the genetic diversity of African rice germplasm, requested by the Crops Research Institute, Ghana (Table 1). In the pipeline is a project from India for genotyping CBSN-derived *indica* x *indica* lines.

Total compensation, has been obtained through competitive cost estimate, is based on the number of samples and markers analyzed for each WO.

Delivery of results is specified in each work order but may change after a written agreement from GCP.

Table 1. Summary of work orders in place and in the pipeline.

Work Order	Genetic Materials	Samples (no.)	Services required	Application
J. Wu – China	Parental and F_n progeny lines	625 DNA in 5 replicates	Genotyping for <i>Xa7</i> , <i>Xa21</i> , <i>Rf3</i> and <i>Rf4</i>	Marker-assisted selection
M. D. Asante – Africa	Rice germplasm collection of CRI, Kumasi, Ghana	122	Genotyping for genetic diversity using 40 SSR type markers	Genetic diversity analysis
S. Manonmani – India	CBSN derived <i>indica</i> x <i>indica</i> lines	500	Genotyping for <i>Rf3</i> and <i>Rf4</i> and Waxy genes (oligos Wx484 and Wx485)	Marker-assisted selection

Requirements: paper work, DNA quality, shipping DNA

Paper work – An Import Permit needs to be secured by IRRI from the Philippines' Bureau of Plant Industry Quarantine Office containing the required details of the samples to be imported. The client institution should provide a Phytosanitary Certificate issued by the authorized Plant Quarantine Officer of the country of origin which is enclosed with the DNA package during transport.

DNA quality - IRRI provides a protocol for the extraction of DNA suitable for Polymerase Chain Reaction (PCR) as well as a procedure for checking the quality of the DNA. This is to ensure that IRRI receives uniform concentrations of the DNA and is PCR-ready. This will prevent any delays in the genotyping analysis.

Shipping DNA - DNA samples should be dried prior to shipment to protect DNA from degradation during transport. Instructions on re-solution of the DNA should be provided by the client institution to IRRI.

Table 2. Requirements for pre-shipment, shipment, and post-shipment of DNA from client institute to IRRI

Requirement	IRRI	Client Institute	Remarks
Pre-shipment	Apply for a Philippine Import Permit (PIP)	Apply for a Phytosanitary Certificate (PC) issued by the authorized Plant Quarantine Officer	PIP application 2-3 weeks before expected arrival of samples.
	Send a copy of PIP to client, keep original	Give list of materials to IRRI	
DNA quality assurance	Send protocols for DNA extraction and DNA quality check to client	DNA extraction and quality check should be based on protocol sent by IRRI	
Shipment of DNA	Prepare Tax Exemption Certificate (TEC) for Customs clearance	PC, copy of PIP and list of materials should be included in shipment	Shipment may take 3-4 days
		Send DNA samples dried down to prevent degradation; instructions on re-solution of the DNA should be provided to IRRI.	Clearance processing time at customs varies; may take longer than expected
Upon receipt of DNA	DNA to pass through IRRI Seed Health Unit (SHU) for inspection by Philippine Quarantine Services officer; need original copy of PIP		Will take 2-3 days due to quarantine officer's fixed schedule of visit to IRRI
	Electronic DNA list uploaded in SHU shareportal and central database		
	Genotyping services as specified in Work Order commence		

Genotyping activities

The genotyping analysis in each WO is defined by the client institution in consultation with IRRI and GCP. A GSS contract can be used for marker-assisted selection such as the work order from CNRRI, China (Fig. 1 - yellow highlight signifies genotyping step supported by the GSS – and Tables 1 & 3), or provide support for genetic diversity studies as in the case of the work order from the Crops Research Institute, Ghana.

The WO from CNRRI includes 625 lines in 5 replicates for a total of 3125. Upon arrival at IRRI, the DNA quality and concentration of each sample was checked. This showed that the DNA concentration of the samples was highly variable. Extracted using a kit, the 122 genetic materials from Ghana showed better quality and uniformity in concentration as expected. (Fig. 2)

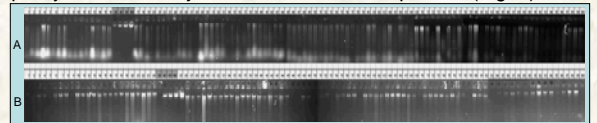


Fig. 2. Quality check of DNA using 1% agarose gels. A) WO from CNRRI and B) WO from Ghana

From the CNRRI WO, 2380 samples so far have been genotyped for *Xa7* and *Xa21* genes. Genotyping for *Rf3* and *Rf4* genes is underway. A preliminary run of a subset of the 122 lines from the WO from Ghana was also done using 10 SSR markers. Sample gels are shown in Figure 3.

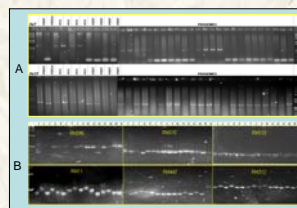


Fig 3. Samples of genotyping gels (A) WO from CNRRI (B) WO from Ghana.

Recommendations

Two major factors have been identified as key to process the work orders efficiently and effectively:

1. Comply with all the necessary requirements (i.e. permits, clearances, protocols) and consider the amount of time needed to process all of them in the overall timing/schedule of each work order (Table2).
2. Clients should provide DNA samples that are PCR ready - good quality, uniform in concentration, and in microplate format (for >200 samples).

Reference: Garris AJ, Tai TH, Coburn J, Kresovich S, and McCouch SR. 2005. Genetic structure and diversity in *Oryza sativa* L. *Genetics* 169:1631-1638

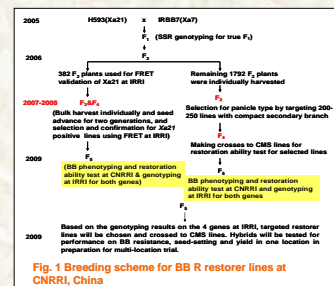


Fig. 1 Breeding scheme for BB R restorer lines at CNRRI, China

Table 3. Marker information used in the work order from CNRRI, China

Marker	Linked gene	Primer sequence	Approximate band size	Percent polyploidization (%)	Source and references
2027	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2028	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2029	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2030	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2031	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2032	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.

Table 4. Forty Rice SSR markers (GCP Project Number 2005-01g, Genotyping composite collections, Tier 1, rice) to be used in work order from Ghana

Marker	Linked gene	Primer sequence	Approximate band size	Percent polyploidization (%)	Source and references
2033	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2034	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2035	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2036	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2037	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2038	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2039	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2040	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2041	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2042	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2043	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2044	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2045	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2046	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2047	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2048	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2049	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2050	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2051	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2052	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2053	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2054	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2055	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2056	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2057	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2058	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2059	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2060	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2061	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2062	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2063	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2064	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2065	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2066	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2067	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2068	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2069	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2070	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2071	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2072	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2073	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2074	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2075	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2076	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2077	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2078	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2079	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2080	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2081	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2082	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2083	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2084	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2085	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2086	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2087	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2088	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2089	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2090	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2091	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2092	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2093	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2094	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2095	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2096	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2097	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2098	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.
2099	Xa7	TATGACAGCAATGATGCTGCTG	143 bp	0	Yang et al.
2100	Xa7	ATGATGATGATGATGATGATGATG	143 bp	0	Yang et al.