

Characterize Tgenic Events in Breeding Stacks

- Speed-up Regulatory Approval
- Independent Event Confirmation
- Corn & Soybean & Cotton & Wheat etc.
- GLP Compatible Procedures/Protocols

Helping w/ Government Approval of Breeding Stacks

Technical Details of Molecular Characterization: USING BAC CLONES TO CONFIRM TGENIC EVENTS

Step 1 Reduce the Complexity of a Plant Stack Genome

• By digesting HMW gDNA and using propietary size selection we can reduce the size of the Stack genome by up to 90% depending on the location of the Event insertion.

Step 2 Make Custom BAC Library from the Plant Stack

• Amplicon Express is world renowned for its excellence in BAC library construction and have made over 2,500 BAC libraries in +21 years of business.

- AEX protocols are optimized for constructing high quality BAC libraries with fast turnaround times.
- This means your Breeding Stacks can be screened & Tgenic Events sequence-confirmed within a few weeks!

Step 3 Screen BAC library for Events of Interest

- AEX optimizes probes and builds screening tools at the same time your Breeding Stack BAC library is being made.
- Using DNA/DNA hybridization, AEX scientists identify long gDNA BAC clones containing your Event(s) of Interest.

Step 4 Confirm Each Tgenic Event with Two Independent BAC Clones

- AEX confirms the location of clone ends and sequences the event directly to validate the Tgenic locus.
- Most Regulatory Agencies require two independent confirmations of a Tgenic Event.
- AEX can screen for multiple Events simultaneously & our lab is compatible with GLP requirements for regulatory approval.

ADVANTAGES OF UTILIZING BAC LIBRARIES:

- Some Tgene targets can be difficult or impossible to amplify by PCR (AEX strategy does not rely on PCR for Event confirmation).
- BAC clones can simplify highly repetitive complex genomes down to only ~100Kb to 200Kb fragments.
- BAC clones allow you to determine haplotype & phase of each chromosome/allele.
- AEX has optimized Genome Reduction protocols to minimize # of clones screened.
- AEX can also use Fosmid cloning systems when needed (due to project constraints or HMW gDNA availability).
- Multiple Events can be screened simultaneously in a fast and cost effective manner.
- BAC clones serve as a permenant repository of your Tgenic Events (ideal for government agency approval).





BAC CLONES ARE NON-RECOMBINANT AND RELIABLE AND WELL ESTABLISHED For Technical Discussion & Pricing Contact: Europe: Jon Wittendorp <jon@ampliconexpress.com> +31-26-7519538 USA & other locations: Robert Bogden <bogden@ampliconexpress.com> 1-509-715-9251



Genome Reduction Strategy for Verification of Tgenic Events in Plant Breeding Stacks



*Several Tgenic Events can be found and verified simultaneously