Canada and United States
Bilateral on Agricultural Biotechnology

Reviewers' Checklists

The Canadian Food Inspection Agency, Health Canada, and the United States Department of Agriculture's Animal and Plant Health Inspection Service, have prepared a series of checklists to be used by reviewers in the assessment process for the following six analytical techniques: Southern blot, Western blot, Northern blot, polymerase chain reaction, RNA dot blot, enzyme- linked immunosorbent assay (ELISA), and enzyme assays. The agencies are sharing these reviewers' checklists with potential applicants to provide guidance on the preparation of quality data.

Checklist for Northern Blot Data	**YES**	**NO**
Does the Northern blot have a figure number and title?		
Are lanes labeled on the blot?		
Does the figure legend describe each lane of the blot, including a description of the following for the RNA that was loaded: i. What type of material was loaded (eg. total purified RNA, poly-A RNA, crude prep, total plant extract)? ii. Source of the material loaded (eg. transformation event, tissue, developmental stage, any prior treatments to induce gene expression, etc.) iii. Quantity of material loaded in each lane?		
Does the text or figure legend describe how RNA was extracted prior to electrophoresis?		
Does the blot have appropriate positive and negative control lanes (positive control might demonstrate hybridization of the probe with itself; negative control might be the unmodified parental plant line or variety)?		
Is the gel system and Northern hybridization protocol described in the text or in a cited literature reference? Are any modifications of the cited protocols described in the petition text?		
Are the position of molecular size standards on the gel indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on the blot?		
Is there a description of the probe that was used for the hybridization? If so, is the description adequate (in the text or in a figure) to enable one to interpret the results?		
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments?		
Are any superfluous bands or background signals properly explained?		

Checklist for Southern Blot Data	**YES**	**NO**
Does the Southern blot have a figure number and title?		
Are lanes labeled on the blot?		
Does the figure legend describe each lane of the blot, including a description of the following for the DNA that was loaded on the gel: i. Type of DNA loaded (eg. entire plasmid, restriction fragment)? ii. Source of DNA loaded (eg. transformation event, tissue)? iii. Restriction digestions of DNA prior to loading gel? iv. Quantity of material loaded in each lane?		
Does the gel have appropriate positive and negative control lanes (positive control might demonstrate hybridization of the probe with itself; negative control might be the unmodified parental plant line or variety)?		
Is the gel system and Southern hybridization protocol described in the text or in a cited literature reference? Are any modifications of the cited protocols described in the petition text?		
Are the position of molecular size standards indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on the blot?		
Was an entire plasmid used as the probe for the hybridization? If so, is the plasmid described adequately in the text or in a figure to enable one to interpret the results?		
Was a restriction fragment used as the probe for the hybridization? If so, is the restriction fragment described adequately in the text or in a figure to enable one to interpret the results?		
Are any superfluous bands or background signals properly explained?		

Checklist for RNA Dot Blot Data	**YES**	**NO**
Does the dot blot have a figure number and title?		
Are lanes labeled on the blot?		
Does the figure legend describe each lane of the blot, including a description of the following for the RNA that was loaded: i. What type of material was loaded (eg. total purified RNA, poly-A RNA, crude prep, total plant extract) ii. Source of the material loaded (e.g., transformation event, tissue, developmental stage, any prior treatments to induce gene expression, etc.) iii. Quantity of material loaded in each lane?		
Does the text or figure legend describe how RNA was extracted prior to blotting onto the solid support?		
Does the blot have appropriate positive and negative control lanes (positive control might demonstrate hybridization of the probe with itself; negative control might be the unmodified parental plant line or variety)?		
Are the dot blot system and hybridization protocols described in the text or in a cited literature reference? Are any modifications of the cited protocols described in the submitted text?		
Is there a description of the probe that was used for the hybridization? If so, is the description adequate (in the text or in a figure) to enable one to interpret the results?		
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments?		

Checklist for Western Blot Data	**YES**	**NO**
Does the blot have a figure number and title?		
Are the lanes clearly labeled?		
Does the figure legend describe each lane of the blot, including a description of the following for the protein that was loaded: i. Type of material loaded (eg. crude, pure, total extract)? ii. Source of material loaded (eg. Transformation event, tissue type)? iii. Quantity of material loaded?		
Is the protein extraction method adequately described in either the text or the figure?		
Is the antibody or antiserum preparation protocol adequately described in the text, including an adequate description of the antigen and its purity? Has the specificity of antibody or antiserum been determined and described in the text or in a cited literature reference?		
Is the gel system and blotting protocol adequately described in the text or in a cited literature reference?		
Are the position of molecular weight standards indicated, and do they cover the appropriate range for the proteins expected to be detected on the blot?		
Does the blot include appropriate positive and negative controls?		
Was a normal serum control conducted?		
Are any superfluous bands or background signal properly explained?		
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments?		

Checklist for PCR Data	**YES**	**NO**
Does the PCR gel have a figure number and title?		
Are lanes labeled on the gel?		
Does the figure legend describe each lane of the gel, including a description of the following for the DNA that was loaded: i. What type of material was loaded (eg. plasmid fragment, amplified DNA)? ii. Source of material used in each reaction loaded (eg. transformation event, tissue)? iii. Quantity of material loaded?		
Are the position of molecular weight standards indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on gel?		
Does the text or figure legend describe how PCR amplification was performed prior to electrophoresis?		
Is there a description of the primers used for amplification in the text or in the figure sufficient to enable one to interpret the results?		
Does the gel have appropriate positive and negative control lanes (positive control might demonstrate specificity of the primers and the ability to amplify the appropriate size band; negative controls might include amplification with DNA from the unmodified parental plant line or variety, and amplification in absence of DNA template)?		
Was an entire plasmid or a restriction fragment used as the positive control template and is it adequately described in the text or in the figure legend for interpretation of the PCR results?		
Is the gel system and PCR protocol described in the text or in a cited literature reference? Are modifications of a cited protocol described in the text?		

Checklist for ELISA Data	**YES**	**NO**
Does the table have a number and a title?		
Are all entries clearly identified in the table and described in the text or table legend?		
Is the sample preparation method described?		
Is the antibody or antiserum preparation protocol adequately described in the text, including a description of the antigen and its purity? Has the specificity of antibody or antiserum been demonstrated and described in the text or in a cited literature reference?		
Is the ELISA protocol used described in the text or cited in the scientific literature? Any modifications to a cited protocol must be described.		
Were appropriate positive controls (e.g. purified protein) and negative controls (e.g. normal or preimmune serum, non-transformed plant material) used?		
When ELISA is being used to quantify protein expression in transformed plant tissues: i. Was a method for the determination of protein concentration in tissue samples presented in the text or in a cited literature reference? ii. Was a standard curve prepared and the limit of detection indicated? iii. Have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments? Was statistical analysis performed?		

Checklist for Enzyme Assays	**YES**	**NO**
Does the figure (or table) have a number and title?		
For graphical representations or tables, are the axis or columns labeled and the units indicated?		
Does the scale of the figure accurately represent and allow interpretation of the data?		
Does the legend or text describe: i. The substrate and amount used for the reaction? ii. The quantity and origin of the enzyme? iii. The temperature and pH?		
Does the text or legend describe the extraction and purification of the enzyme and the degree of purification achieved?		
If the enzyme used in the assay has not been isolated from the transformed plant but is derived from an expression system, has adequate data been presented to demonstrate its substantial equivalence to the plant expressed enzyme?		
Have the assay method and relevant information concerning the enzyme been provided in the text or in a cited literature reference?		
Are appropriate controls included in the assay?		
Has the stability of the enzyme and possible presence of enzyme inhibitors in different tissue extracts been taken into account in the design of the assay or the interpretation of the data?		
When relevant to the safety assessment, have the kinetics of the enzyme been calculated and where possible compared to published data?		
When quantitative analysis is performed, have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments? Was statistical analysis performed?		