

# Multi-Capillary IGT 5 Dye Matrix Standard ABI 3130 & 3500 Series Genetic Analyzers

## Introduction

Proper spectral calibration is critical to evaluate multicolor systems with the Applied Biosystems Genetic Analyzers. The IGT 5-Dye Matrix Standard consists of DNA fragments labeled with five different fluorescent dyes, FAM, JOE, TMR, ROX, and TGI-ORANGE. These matrix fragments are used on the Applied Biosystems 3130, 3130xL, 3500 or 3500xL Genetic Analyzer to perform a spectral calibration on a specified dye set. The spectral calibration should be performed on dye set G5. Once generated, this file is applied during sample detection to calculate the spectral overlap between the five different dyes and separate the raw fluorescent signals into individual dye signals. The IGT 5-Dye Matrix Standard was developed for use with the 5-dye InnoTyper<sup>®</sup> 21 System. A matrix should be generated for each individual instrument.

## Product Component

Product	Volume Provided	Catalog #
IGT 5 Dye Matrix Standard for 3500 Series Genetic Analyzers	80 µL	21109M-35
IGT 5 Dye Matrix Standard for 3130 Series Genetic Analyzers	80 µL	21109M-31

**Storage Conditions:** Upon receipt, store the kit at -20°C. After initial use, store the kit at +4°C. We recommend that the matrix standards be stored with post amplification reagents and used separately with different pipettes, tube racks, etc.

**IMPORTANT!** IGT 5 Dye Matrix Standard is composed of oligonucleotides attached with fluorescent dyes which are light-sensitive. Protect the matrix standard from light when not in use.

**Reagents and Equipment:** IGT 5 Dye Multi-Capillary Matrix Standard Kit, Hi-Di<sup>™</sup> Formamide, POP-4<sup>™</sup> Polymer, Genetic Analyzer Buffer (10X) with EDTA, 3130 or 3500 capillary array (36cm), 1.5 mL polypropylene tubes, sample tubes and septa, thermal cycler, crushed ice or ice-water bath.

## Preparing Matrix Standard

1. Thaw and mix the matrix standard by vortexing the tube for 5-10 seconds prior to use. Do not centrifuge the matrix standards.
2. Prepare the matrix standard in a 1.5 mL polypropylene tube by combining IGT 5 Dye Matrix Standard with Hi-Di<sup>™</sup> Formamide. One capillary requires 1 µL of IGT 5 Dye Matrix Standard and 9 µL Hi-Di<sup>™</sup> Formamide. The table below shows the amount needed for 3130 and 3500 series instruments.

Component	3130	3130xL	3500	3500xL
IGT 5 Dye Matrix Standard	5 µL	18 µL	9 µL	26 µL
Hi-Di <sup>™</sup> Formamide	45 µL	162 µL	81 µL	234 µL

**Note:** The quality of formamide is critical. Use Hi-Di™ formamide. Freeze formamide in aliquots at –20°C. Multiple freeze-thaw cycles or long-term storage at 4°C may cause breakdown of formamide. Poor-quality formamide may contain ions that compete with DNA during injection, which results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

**Note:** There may be instrument-to-instrument variation in the sensitivity of detection. The matrix standard volumes described here may need to be optimized in individual laboratories, depending on the sensitivity of each Applied Biosystems Genetic Analyzer.

3. Vortex the mix thoroughly. Then centrifuge briefly.
4. Pipette 10 µL into each reaction well.

Instrument	Number of Capillaries	Well Positions
3130	4	A1 – D1
3130xL	16	A1 – H2
3500	8	A1 – H1
3500xL	24	A1 – H3

5. Cover tubes with septa strip.
6. Heat the tubes in the thermal cycler at 95°C for 3 minutes to denature the DNA.
7. Immediately place the tubes on ice for 3 minutes.
8. Place the plate in the 3130 or 3500 series 96-well standard plate base and cover with the plate retainer. Place the plate assembly in Position B for 3130 series / Position A for 3500 series on the autosampler.

### Instrument Preparation / Spectral Calibration for ABI 3130 series with 36cm Capillary Using POP-4

1. Perform the spectral calibration as described in the ABI 3130 / 3130xL Genetic Analyzer User Manual with the following modifications.
2. To create a run module for IGT 5 Dye Matrix Standard, select “New” in the *Module Manager*.” Name the run module **Spect36\_POP4\_IGT\_G5**. Select “Spectral” in the Type drop-down list, and select “Spect36\_POP4” in the Template drop-down list.
3. To create an instrument protocol for IGT 5 Dye Matrix Standards, select “New” in the *Protocol Manager*.” Name the protocol **Spectral\_IGT\_G5** and make the following selections in the Protocol Editor then select “OK”:
  - “Spectral” in the Type drop-down list
  - “G5” in the Dye Set drop-down list
  - Note: Do NOT use “Any5Dye” as the dye set for this calibration, because the InnoTyper® 21 kit was developed with the G5 dye set filters. Using other dye sets will increase inter-color variation and may cause other spectral issues.

- “POP4” for the polymer
- “36” in the Array Length drop-down list
- “Matrix Standard” for the chemistry
- “Spect36\_POP4\_IGT\_G5” for Run Module

Select “Edit Parameters”, and make the following modifications:

- Change the lower condition bound to 4.0 and the upper condition bound to 20.0
  - Change the the Minimum Quality Score (Q value) to 0.95
4. In the *Plate Manager*, create a new plate record. In the dialog box that appears, select “Spectral Calibration” in the Application drop-down list, and select “96-well” as plate type. Add entries in the owner and operator windows. Name the plate and select “OK.”
  5. In the spectral calibration plate editor dialog box, place sample names in the appropriate cells. In the Instrument Protocol column, select **Spectral\_IGT\_G5**. Ensure that this information is present for each row that contains a sample name. Select “OK.”
  6. In the *Run Scheduler*, link the plate and select “Run.”
  7. Upon completion of the run, check the status of the spectral calibration in the Event Log window. A minimum of three of four capillaries for ABI 3130 / twelve of sixteen capillaries for ABI 3130xL is recommended to pass calibration. If fewer than the recommended numbers of capillaries pass, repeat the spectral calibration.

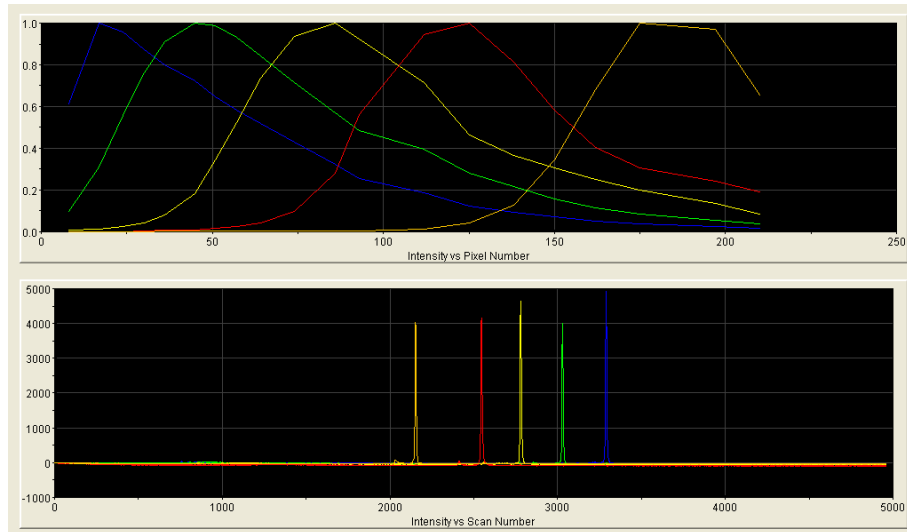
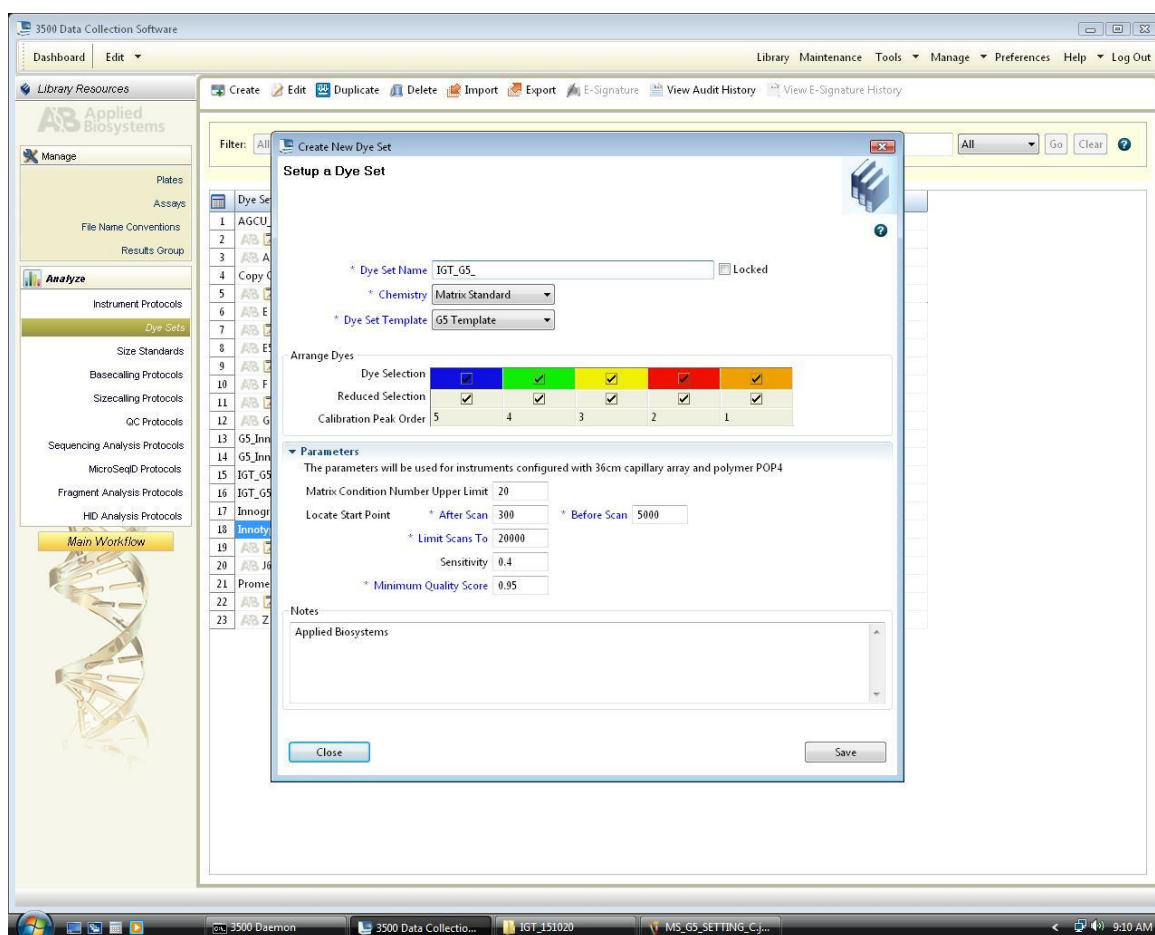


Figure 1: Representative Spectral Calibration Run on ABI 3130 Genetic Analyzer

**Note:** Peak heights in the range of 1,000–5,000RFU are ideal. Peak heights above 750RFU are required. If spectral peak heights are low, re-inject samples with increased injection time of up to 5 seconds. If peak heights are too high (reaching saturation), re-inject samples with decreased injection time of up to 5 seconds.

## Instrument Preparation / Spectral Calibration for ABI 3500 Series with 36cm Capillary Using POP-4

1. Perform the spectral calibration as described in the ABI 3500 / 3500xL Genetic Analyzer User Manual.
2. Set the oven temperature to 60°C, and then select the Start Pre-Heat icon at least 30 minutes prior to the first injection to preheat the oven.
3. To perform a spectral calibration with the IGT 5-dye chemistry, create a new dye set:
  - Navigate to the “Library”
  - Highlight “Dye Sets”
  - Select “Create”
4. The “Create a New Dye Set” tab will appear.
  - Name the Dye Set as “IGT\_G5”
  - Select “Matrix Standard” for the Chemistry
  - Select “G5 Template” for the Dye Set Template.
  - Note: Do NOT use “Any5Dye” as the dye set for this calibration, because the InnoTyper® 21 kit was developed with the G5 dye set filters. Using other dye sets will increase inter-color variation and may cause other spectral issues.
  - Change the parameters as follows and as noted on the screen shot below:
    - a. Matrix Condition Number Upper Limit: 20
    - b. Locate Start Point, After Scan: 300 Before Scan: 5000
    - c. Limit Scans To: 20000
    - d. Sensitivity: 0.4
    - e. Minimum Quality Score: 0.95



- Select “Save”.
5. To perform the spectral calibration with the IGT 5-dye chemistry:
    - Go to the “Maintenance” tab
    - Select “Spectral”
  6. Under the Calibration Run tab,
    - Choose “Matrix Standard” from the “Chemistry Standard” drop-down menu
    - Choose “IGT\_G5” from the “Dye Set” drop-down menu
    - Check the “Allow Borrowing” box. Note: this setting may be changed at a later time; however, it is recommended that the “Allow Borrowing” function is enabled the first time a spectral calibration is run for this particular dye set to reduce the possibility of seeing “pull down” (or “mirror image”) peaks. (See note below.) Once the initial spectral calibration is accepted, it is possible to re-run the calibration to assess any failed capillaries.

**Note:** As noted in the 3500/3500xL Genetic Analyzer User Guide, the first time a spectral calibration is performed for each dye set, “pull-down” (or “mirror image”) peaks may be noticed. These pull-down peaks will eventually correct themselves once the run completes and the “Accept” button is selected. The pull-down peaks may still be visible in the “History View” but should no longer be visible after the spectral calibration is repeated.

7. Select “Start Run”.
8. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display, and choose either “Accept” or “Reject.” Refer to the *3500 Series Data Collection Software Version User Manual* for the acceptance criteria recommended by Applied Biosystems.
9. If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times

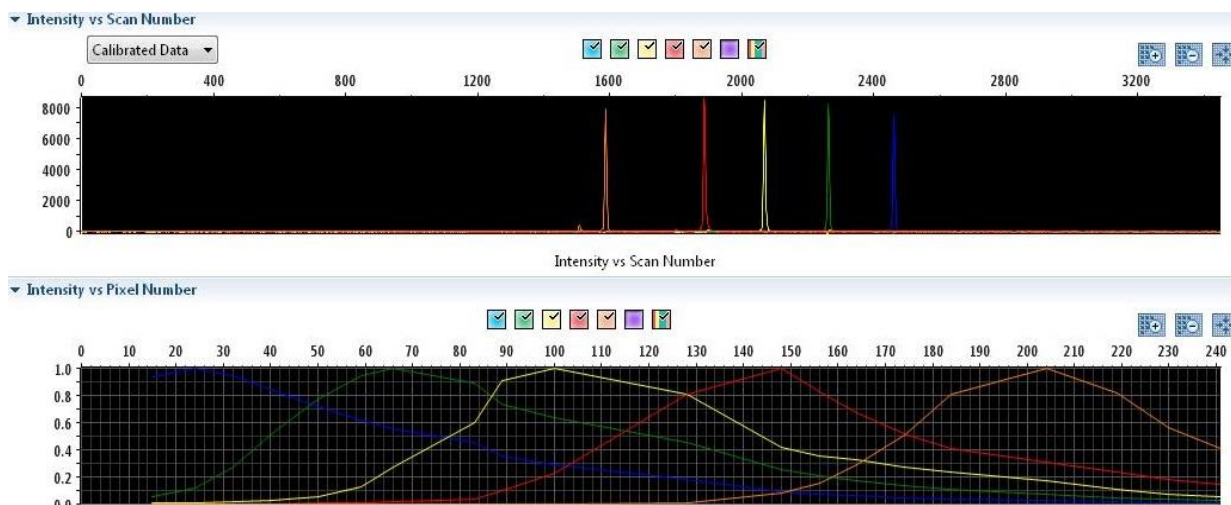


Figure 2: Representative Spectral Calibration Run on ABI 3500xL Genetic Analyzer

**Note:** Peak heights in the range of 4,000–10,000RFU are ideal. Peak heights above 750 RFU are required. If spectral peak heights are too low, re-inject samples with increased matrix standard amount (i.e. 1.5  $\mu$ L of matrix standard and 8.5  $\mu$ L HiDi formamide per reaction). If peak heights are too high (reaching saturation), re-inject samples with decreased matrix standard amount (i.e. 0.75  $\mu$ L matrix standard and 9.25  $\mu$ L HiDi formamide) per reaction.