star_allele_calling2

September 30, 2022

Workflow

Please see here: https://app.diagrams.net/#G1HzMMyRkVhr8Y_M1wMmXwmZagIEpak1Bb

```
[ ]: from Bio import SeqIO
     from sklearn.metrics import r2_score
     from scipy.signal import find_peaks, peak_prominences, peak_widths
     import numpy as np
     from matplotlib import pyplot as plt
     import pandas as pd
     import re
     import warnings
     warnings.filterwarnings('ignore')


     def loadFSA(aFSA):
         """Load FSA file
         Args:
             aFSA (str): A path to a FSA file.

         Returns:
             SeqIO: A data loaded from FSA file.
         """

         return(SeqIO.read(aFSA, 'abi'))



     def generateReferenceRange(reference):
         """Generate all range from reference data

         Args:
             reference (list, required): A list contain all bp locations of␣
      ↪refernces.
                                                        Defaults to [15, 20, 25, 35, 50, 62, 80,␣
      ↪110, 120].

         Returns:
```

```
        list:   The list contant all generated range of the references
                Example: [(15, 20), (20,25)]
    """

    referenceRange = []

    for i,_ in enumerate(reference):
        if i < len(reference)-1:
            referenceRange.append((reference[i], reference[i+1]))

    return referenceRange



def getRawData(FSA, chanel):
    """Extract raw intensity by chanel.

    Args:
        FSA (class): A class load FSA file.
        chanel (str): An interested intensity chanel.

    Returns:
        Tuple: A tuple about a chanel intensity data.
    """
    return FSA.rawdata[chanel]



class FSA:
    def __init__(self, aFSA) -> None:
        self.data = SeqIO.read(aFSA, 'abi')
        self.name = self.data.name
        self.id = self.data.id
        self.description = self.data.description
        self.rawdata = self.data.annotations['abif_raw']

    def panel(self):
        """Extract panel from fsa name. The first element of the name was␣
 ↪consider as the panel.

        Returns:
            str: name of the panel
        """
        return self.name.split('-')[0]

    @property
    def reference_intensity(self):
        """Extract reference intensity, in the fsa raw data format, DATA105 is␣
↪defined for reference intensity chanel.

        Returns:
            tupe: A tupe of all reference intensity data.
        """
        return self.rawdata['DATA105']

    @property
    def chanel_size(self):
        """Return chanel size, it will be used for generate number of points␣
↪and make basepairs prediction.

        Returns:
            int: Length of the intensity chanel.
        """
        return len(self.reference_intensity)

    def extract_intensity(self, chanel, range=None):
        """Extract the intensity by a specific chanel.
           In the FSA file, there 4 chanels (DATA1, DATA2, DATA3, DATA4)␣
↪following 4 nucleotides (ATCG)

        Args:
            chanel (str): chanel name for [DATA1, DATA2, DATA3, DATA4]
            range (tupe, optional): A tupe defines an intersted range for␣
↪extract. It was resulted in by `get_index_by_base_range` function. Defaults␣
↪to None.

        Returns:
            tupe: intensity of the selected chanel
        """
        if range:
            return self.rawdata[chanel][range[0]:range[1]]
        else:
            return self.rawdata[chanel]


    @property
    def sample_name(self):
        return extract_sample_name(self.name)

class Reference:
    def __init__(self, aRef) -> None:
        self.size = aRef
        self.range = generateReferenceRange(self.size)
        self.length = len(self.size)
```

```
def finding_peaks(intensity, min_width=None, min_height=500, min_prominence =␣
↪None):
    """Finding Peaks based on intensity data

    Args:
        intensity (tupe, required): intensity data from a chanel
        min_width (int, optional): threshold minimum width of the peaks.␣
↪Defaults to None. Reference value: 1
        min_height (int, optional): threshold minimum height of the peaks.␣
↪Defaults to 500.
        min_prominence (int, optional): threshold minimum depth of the peaks.␣
↪Defaults to None. Reference value 50

    Returns:
        peaks: a list of detected peaks
        heights: a list of corresponding detected peaks
    """

    peaks, heights = find_peaks(intensity, height=min_height)
    heights = heights['peak_heights']

    if min_prominence:
        prominence = peak_prominences(intensity, peaks)
        conditions =  np.where(prominence[0] >= min_prominence)
        peaks = peaks[conditions]
        heights = heights[conditions]

    if min_width:
        width = peak_widths(intensity, peaks)
        conditions = np.where(width[0] >= min_width)
        peaks = peaks[conditions]
        heights = heights[conditions]

    return peaks, heights


class LeastSquared:

    """Building a LeastSequared model to predict the size of DNA based on␣
↪intensity data points.
      Each fsa can be different each run, therefore, model will be build based␣
↪on specific data from FSA and reference.
```

```
        The method of model was refer from gene mapper reference. More␣
↪information can be found here:
         Sizing: https://www2.unbc.ca/sites/default/files/sections/genetics/
↪microsat.pdf
         Peak scanner:  https://apps.thermofisher.com/apps/peak-scanner/help/
↪GUID-CEE18138-6788-48FA-8E08-4CE939F4B9D8.html

    """



    def __init__(self, peak, reference_size, degree = 3):
        self.peak = peak
        self.reference_size = reference_size
        self.degree = degree
        self.popt = np.polyfit(self.peak, self.reference_size, self.degree)
        self.ref_predicted = np.polyval(self.popt, self.peak)
        # self.ref_predicted = np.round(self.ref_predicted, 2)


    @property
    def r2_score(self):
        return r2_score(self.reference_size, self.ref_predicted)

    def val_plot(self, title):
        """Plot a validation figure from reference and predicted result.

        Args:
            title (str): Title of the figure, it can be a fsa name.
        """
        _min = np.min(self.peak) - 100
        _max = np.max(self.peak) + 100
        xn = range(_min, _max +1)
        yn = np.polyval(self.popt, xn)

        fig, ax = plt.subplots(figsize=(5,3), dpi = 150)
        alpha = 0.8
        ax.plot(xn, yn, color = 'blue', label = 'Predicted', alpha = alpha)
        ax.plot(self.peak, self.reference_size, 'or', label = 'Reference',␣
↪alpha =alpha)

        for peak, size in zip(self.peak, self.reference_size):
            ax.text(peak, size + 4, f'{size}', fontsize =6, ha='center')

        ax.set_xlabel('Points')
        ax.set_ylabel('Size (bp)')
        ax.set_title(title, fontsize = 8)
        fig.text(0.2, 0.80, f'$R^{2} = {str(self.r2_score)}$')
        plt.legend(loc='lower right')
        plt.tight_layout()
        plt.show()

    def predict(self, x):
        """Predict basepairs based on range of points

        Args:
            x (list): A list of points want to be predicted by built model

        Returns:
            np.array: An array includes predicted basepairs.
        """
        return np.round(np.polyval(self.popt, x),2)

    def fit(self, x:list):
        """predicted base bairs

        Args:
            x (list): data points
        """
        self.predicted_base = self.predict(x)



class Allele:
    def __init__(self, base:str, basetype:str, is_forward:bool, min_bin:int,␣
 ↪max_bin:int, min_height:int = 500) -> None:
        """Create allele class

        Args:
            base (str): tested nucleotide [A,T,C,G]
            basetype (str): whether base is wildtype or mutant
            min_bin (int): min bin set for peak detection
            max_bin (int): max bin set for peak detection
            min_height (int, optional): min height for peak detection. Defaults␣
 ↪to 500.
        """
        self.base = base
        self.basetype = basetype
        self.min_bin = min_bin
        self.max_bin = max_bin
        self.min_height = min_height
        self.is_forward = is_forward

    @property
    def color(self):
        """Return color based on marker's direction and target base

        Returns:
            str: color
        """
        return forward_color.get(self.base) if self.is_forward else␣
↪reverse_color.get(self.base)

    @property
    def data_chanel(self):
        """Return data chanel based on marker's direction and target base

        Returns:
            _type_: _description_
        """
        return data_chanel_map.get(self.base) if self.is_forward else␣
↪data_chanel_map.get(reverse_map.get(self.base))



# define the color of forward
forward_color = {
    'Ref': 'orange',
    'A': 'green',
    'T': 'red',
    'C': 'black',
    'G': 'blue'
    }

# define the color of reverse
reverse_color = {
    'Ref': 'orange',
    'A': 'red',
    'T': 'green',
    'C': 'blue',
    'G': 'black'
}

# define data chanel
data_chanel_map = {
    'Ref': 'DATA105',
    'G':'DATA1',
    'A':'DATA2',
    'C':'DATA3',
    'T':'DATA4'
}
```

```python
# revese complementation of base.
reverse_map = {
    'A':'T',
    'T':'A',
    'G':'C',
    'C':'G'
}


class Marker:

    def __init__(self, gene:str, marker_name: str, marker_label:str, panel:str,␣
 ↪is_forward:bool, alleles = []) -> None:
        """Creat a marker class

        Args:
            gene (str): gene name
            marker_name (str): marker name
            marker_label (str): marker label
            panel (str): panel name, it should be identical with chanel␣
 ↪detected from FSA file
            is_forward (bool): direction of this marker, True mean forward,␣
 ↪False mean reverse.
            alleles (list, optional): Allele class information. Defaults to [].
        """
        self.gene = gene
        self.marker_name = marker_name
        self.marker_label = marker_label
        self.is_forward = is_forward
        self.alleles = alleles
        self.panel = panel
        self.direction = 'Forward' if self.is_forward else 'Reverse'
        # self.num_alleles = 0

    def add_allele(self, allele):

        """Update allele to the list of allele in this marker.
            It also update the direction of the marker to this allele.
        """
        allele.is_forward = self.is_forward
        self.alleles.append(allele)

    @property
    def num_alleles(self):
        return len(self.alleles)
    def update_allele_config(self, allele_number:int, min_bin:int=None, max_bin:
↪int=None, min_height:int=None):
        """update allele configuration to adjust the bin size and height to␣
↪call the peaks

        Args:
            allele_number (int): number of allele in the marker. It ussually␣
↪can be 0,1 since each marker often have 2 alleles, but somes may have more␣
↪than 2.
            min_bin (int, optional): Min bin size for this allele. Defaults to␣
↪None.
            max_bin (int, optional): Max bin sizx for this allele. Defaults to␣
↪None.
            min_height (int, optional): Min height for this allele. Defaults to␣
↪None.
        """
        allele = self.alleles[allele_number]

        if min_bin:
            allele.min_bin = min_bin

        if max_bin:
            allele.max_bin = max_bin

        if min_height:
            allele.min_height = min_height

        self.alleles[allele_number] = allele


def get_index_by_base_range(base_pred, base_ranges=(0,200)):
    """Get index of orginal intensity based on intested basepairs.


    Args:
        base_pred (_type_): _description_
        ranges (tuple, optional): _description_. Defaults to (0,200).

    Returns:
        _type_: _description_
    """
    min_index = np.where(base_pred >= base_ranges[0])[0][0]
    max_index = np.where(base_pred >= base_ranges[1])[0][0]

    return (min_index, max_index)
```

```
def plot_raw_intensity(fsa,min_index, max_index, select_base,␣
↪base_range=(20,80)):
    fig, ax = plt.subplots(1,1, figsize =(12,3), dpi = 200)
    ax.set_xlim(*base_range)
    for base in data_chanel_map:
        chanel = data_chanel_map.get(base)
        intensity = fsa.extract_intensity(chanel, (min_index, max_index))
        color = forward_color.get(base)
        alpha = 0.6 if base == 'Ref' else 1

        ax.plot(select_base, intensity, color = color, label = chanel, alpha =␣
↪alpha)
    plt.legend(loc='upper left')
    plt.tight_layout()
    plt.show()


def generate_markers(peak_table):

    maker_list = {}
    for _,row in peak_table.iterrows():
        m = Marker(row.gene, row.marker, row.marker_label, row.panel, row.
↪is_forward, [])

        m.define_colors = {row.w_base:row.w_color, row.m_base:row.m_color}

        a1 = Allele(row.w_base, 'wildtype', row.is_forward, row.w_min, row.
↪w_max)
        # a1.set_color(row.is_forward)
        a2 = Allele(row.m_base, 'mutant', row.is_forward, row.m_min, row.m_max)
        # a2.set_color(row.is_forward)
        m.add_allele(a1)
        m.add_allele(a2)
        maker_list[m.marker_name] = m

    return maker_list


def finding_peak_by_markers(markers:dict, fsa:FSA, panel:str, select_base:list,␣
↪index_range:tuple):
    """Finding peak by marker dictionary and update the called result to the␣
↪marker dictionary

    Args:
        markers (dict): A dictionary of markers that includes defined␣
↪information to call a peak such as bin size, height, data chanel and base
        fsa (FSA): FSA data loaded from fsa file
        panel (str): Panel definition, it should be match with panel defined in␣
↪the file name, need to be discussed with PGx to make consensus naming␣
↪convention.
        select_base (list): a list of predicted basepair from model, it was␣
↪used to match with bin size and data points.
        index_range (tuple): index data point to extract intensity data
    """

    for marker_name in markers:
        marker = markers.get(marker_name)
        marker.sample_name = fsa.sample_name
        if marker.panel == panel:
            for i,allele in enumerate(marker.alleles):
                intensity = fsa.extract_intensity(allele.data_chanel,␣
↪(index_range[0],index_range[1]))
                _min = np.where(select_base >= allele.min_bin)[0][0]
                _max = np.where(select_base <= allele.max_bin)[0][-1]
                i_base = select_base[_min:_max]
                i_intensity = intensity[_min:_max]
                peak,height = finding_peaks(i_intensity, min_height= allele.
↪min_height, min_width=0.7, min_prominence=30)

                if peak.size == 1:
                    allele.is_detected = True
                    allele.peak = peak
                    allele.size = i_base[peak]
                    allele.height = height
                    allele.status = 'ok'
                    allele.message = ''
                elif peak.size > 1:
                    allele.is_detected = True
                    allele.peak = peak
                    allele.size = i_base[peak]
                    allele.height = height
                    allele.status = 'warning'
                    allele.message = 'More than 1 peaks detected!'
                else:
                    allele.is_detected = False
                    allele.peak = ''
                    allele.size = ''
                    allele.height = ''
                    allele.status = ''
                    allele.message = 'Peak(s) could not be detected. Please␣
↪check peak ranges if required!'
```

```python
                # print('append allele', marker.marker_name)
                marker.alleles[i] = allele

            markers[marker_name] = marker

    # update genotype
    update_marker_genotype(markers, panel)


def update_marker_genotype(markers:dict, panel:str):
    """Update the genotype of each marker based on the peak calling process.

    Args:
        markers (dict): a dictionary of a called marker
        panel (str): panel
    """
    for marker_name in markers:
        marker = markers.get(marker_name)
        if marker.panel == panel:
            genotype = ''
            phenotype = ''
            is_called = False
            basetype = []
            called_base = []
            for j, allele in enumerate(marker.alleles):
                if allele.status == 'ok':
                    called_base.append(allele.base)
                    basetype.append(allele.basetype)

            if len(called_base) == 1:
                genotype = f'{called_base[0]}{called_base[0]}'
                is_called = True
            elif len(called_base) == 2:
                genotype = f'{called_base[0]}{called_base[1]}'
                is_called = True

            if len(set(basetype)) == 1:
                if basetype[0] == 'wildtype':
                    phenotype = 'wildtype'
                else:
                    phenotype = 'homozygous mutant'
            elif len(set(basetype)) == 2 and len(basetype) == 2:
                phenotype = 'heterozygous'

            marker.genotype = genotype
            marker.is_called = is_called
            marker.phenotype = phenotype

            markers[marker_name] = marker


# def print_marker(markers):
#     status = False
#     for marker_name in markers:
#         marker = markers.get(marker_name)
#         if hasattr(marker, 'is_called'):
#             status = marker.is_called
#             print(marker.marker_name, marker.panel, status, marker.genotype,␣
 ↪marker.phenotype)
#         else:
#             print(marker.marker_name, marker.panel)


# def load_snapshot_file(snapshot_file):
#     df = pd.read_csv(snapshot_file, sep='\t').fillna("")
#     df['Allele 2'] = np.where(df['Allele 2'] == "", df['Allele 1'],␣
 ↪df['Allele 2'])
#     df["Genotype"] = df['Allele 1'] + df["Allele 2"]
#     df['Gene'] = [x[0] for x in df['Marker'].str.split("_")]
#     df['ExtractedSample'] = [extract_sample_name(x) for x in df['Sample␣
 ↪Name']]
#     df['SampleStatus'] = [is_valid_sample_name(x) for x in df['Sample Name']]

#     df = df[['Sample File', 'Sample Name', 'ExtractedSample', 'SampleStatus',␣
 ↪'Panel',
#               'Gene', 'Marker', 'Genotype', 'Allele 1', 'Allele 2']]
#     return df


# def is_valid_sample_name(sample_name, p='_S[0-9]+'):
#     pattern = re.compile(p)
#     if pattern.search(sample_name):
#         return True
#     else:
#         return False


# def format_call_markers(markers):
#     df = pd.DataFrame()
#     for marker_name in markers:
#         marker = markers.get(marker_name)
#         for allele in marker.alleles:
#             tmp = pd.DataFrame(allele.__dict__, index=[0])
#               tmp['color'] = allele.color
#               tmp['sample'] = marker.sample_name
#               tmp['gene'] = marker.gene
#               tmp['marker'] = marker.marker_name
#               tmp['panel'] = marker.panel
#               tmp['direction'] = 'Forward' if marker.is_forward else 'Reverse'
#               tmp['genotype'] = marker.genotype
#               tmp['phenotype'] = marker.phenotype
#               df = pd.concat([df, tmp])

#       ## marker table
#       marker_table = (df[df.columns[-7:]].drop_duplicates()).
 ↪reset_index(drop=True)

#       # allele table
#       cols = df.columns[:-2]
#       cols = cols[-5:].tolist() + cols[:-5].tolist()
#       allele_table = df[cols].reset_index(drop=True)

#       return marker_table, allele_table


# def extract_sample_name(sample_name, p='_S[0-9]+'):
#       pattern = re.compile(p)
#       if pattern.search(sample_name):
#           return pattern.split(sample_name)[0]
#       else:
#           # print(f'{sample_name} is not valid! Keep as origin')
#           return sample_name


def extract_sample_name(fsa_name:str, pattern = '[0-9]+-[0-9]+_'):

    """Extract sample name from fsa file name. It can be applied for bin text␣
 ↪file as well.
       But we need to define the file name convention with PGx team to have␣
 ↪rule to extract the sample name.

    Returns:
        str: sample name extracted from file name
    """
    p = re.compile(pattern)
    s = p.search(fsa_name)

    return s.group(0).replace('_', '')
```

```
class GenotypeResult:

    def __init__(self) -> None:
        self.is_from_file = False

    def from_bin_file(self, bin_file:str, marker_info:dict):
        """Since our result can be obtained from calling FSA file or from bin␣
↪text file. This function is to process data from bin text file

        Args:
            bin_file (str): bin text file obtained from gene mappeer
            marker_info (dict): Information of the defined marker includes␣
↪color, direction, label and other infomration.
        """

        self.file = bin_file
        self.raw_data = pd.read_csv(bin_file, sep='\t').fillna('')
        self.is_from_file = True
        self.clean_data = self._clean_from_bin_data(marker_info)
        self.sample_list = self.clean_data['sample'].unique().tolist()
        # self.marker_table = self._marker_table

    def from_fsa_call(self, called_markers:dict):
        """Process result called from FSA file

        Args:
            called_markers (dict): marker includes the peaking calling␣
↪information
        """
        self.is_from_file = False
        self.raw_data = called_markers

        clean_data = pd.DataFrame()
        for marker_name in called_markers:
            marker = called_markers.get(marker_name)
            for allele in marker.alleles:
                tmp = pd.DataFrame(allele.__dict__, index=[0])
                tmp['color'] = allele.color
                tmp['sample'] = marker.sample_name
                tmp['gene'] = marker.gene
                tmp['marker'] = marker.marker_name
                tmp['label'] = marker.marker_label
                tmp['panel'] = marker.panel
                tmp['direction'] = 'Forward' if marker.is_forward else 'Reverse'
                tmp['genotype'] = marker.genotype
                tmp['phenotype'] = marker.phenotype
                clean_data = pd.concat([clean_data, tmp])

        self.clean_data = clean_data.reset_index(drop=True)
        self.sample_list = self.clean_data['sample'].unique().tolist()


    def _clean_from_bin_data(self, marker_info:dict):
        """Detail function to process data from bin text file

        Args:
            marker_info (dict): Information of the defined marker includes␣
↪color, direction, label and other infomration.

        Returns:
            DataFrame: an updated data frame
        """

        if self.is_from_file:
            cols = ['Sample File', 'Sample Name', 'Marker', 'Panel', 'Allele␣
↪1', 'Allele 2',
                    'Size 1', 'Size 2', 'Height 1', 'Height 2']
            m_cols = ['sample_file', 'sample_name', 'marker', 'panel', 'base1',␣
↪'base2',
                    'size1', 'size2', 'height1', 'height2']
            df = self.raw_data[cols]
            df.columns = m_cols

            # extract gene, panel and sample
            df['gene'] = df.marker.apply(lambda x: x.split('_')[0])
            df['panel'] = df.panel.apply(lambda x: x.split('-')[1])
            df['sample'] = df['sample_name'].apply(lambda x: '-'.join(x.
↪split('-')[1:]))

            # generate genotype
            df['genotype'] = df.apply(lambda row: row['base1'] + row['base1']␣
↪if row['base2'] == '' else row['base1'] + row['base2'], axis=1)

            # update phenotype

            def _get_type_base(marker):
                wildtype_base = []
                mutant_base = []
                for allele in marker.alleles:
                    if allele.basetype == 'wildtype':
                        wildtype_base.append(allele.base)
                    else:
                        mutant_base.append(allele.base)
                return wildtype_base, mutant_base


            def _update_phenotype(marker_name, genotype):
                marker = marker_info.get(marker_name)
                wildtype, mutant = _get_type_base(marker)
                _genotype = pd.Series(list(set(genotype)))

                if len(_genotype) == 1:
                    if all(_genotype.isin(wildtype)):
                        return 'wildtype'
                    else:
                        return 'homozygous mutant'
                elif len(_genotype) ==  2:
                    if all(_genotype.isin(mutant)):
                        return 'homozygous mutant'
                    else:
                        return 'heterozygous mutant'
                elif len(_genotype) == 0:
                    return ''
                else:
                    raise ValueError('invaid genotype input!')

             # df['phenotype'] = df.genotype.apply(lambda x: 'homozygous' if x␣
↪in ['AA', "GG", "CC", "TT"] else 'heterozygous')
            df['phenotype'] = df.apply(lambda row:␣
↪_update_phenotype(row['marker'], row['genotype']), axis = 1)

            # df['direction'] = '' # we dont know marker inforamtion in this␣
↪file, need to be updated later

            df = pd.wide_to_long(df, stubnames=['base', 'size', 'height'],
                                 j='n_bases', i=['marker', 'sample'], sep='')
            df = df.reset_index()

            def _color(marker, base):
                return marker.define_colors.get(base)

            # def _direction(marker)

            df['color'] = df.apply(lambda row: _color(marker_info.get(row.
↪marker), row.base), axis = 1)
            df['direction'] = df.apply(lambda row: marker_info.get(row.marker).
↪direction, axis = 1)
            df['label'] = df.apply(lambda row: marker_info.get(row.marker).
↪marker_label, axis = 1)
```

```
        return df


    def marker_table(self, sample_name = None):
        """Return a marker data information table

        Args:
            sample_name (str, optional): Filter marker table by sample name if␣
↪required. Defaults to None.

        Returns:
            DataFrame: marker table
        """

        cols = ['sample', 'gene', 'marker', 'label', 'panel', 'direction',␣
↪'genotype', 'phenotype']

        df = self.clean_data[cols].drop_duplicates()

        if sample_name:
            df = df[df['sample'] == sample_name]

        return df



    def allele_table(self, sample_name = None, called_filter=True):

        """Return a allele data information table. It can be different from bin␣
↪file or fsa file.
           Because some information we could not get from bin text file result␣
↪and fsa file.

        Returns:
            _type_: _description_
        """

        if self.is_from_file:

            cols = ['sample', 'gene', 'marker', 'label', 'panel', 'direction',
                    'base', 'size', 'height', 'color']

        else:
            cols = ['sample', 'gene', 'marker', 'label', 'panel', 'direction',
                    'base', 'basetype',         'min_bin', 'max_bin',␣
↪'min_height',
                    'is_forward', 'is_detected', 'peak', 'size', 'height',
                    'status', 'message', 'color']


        df = self.clean_data[cols].drop_duplicates()

        if sample_name:
            df = df[df['sample'] == sample_name]

        if called_filter:
            if self.is_from_file:
                df = df[pd.notna(df.color)]
            else:
                df = df[df.is_detected]

        return df


    def plotting_qc(self, sample_name:str):
        """Plot QC chromatogram based on called genotype: peak size, peak␣
↪height.
            It will plot all panel of this sample.

        Args:
            sample_name (str): name of sample
        """

        allele_data = self.allele_table(sample_name)

        for panel in allele_data.panel.unique():
            plot_qc(allele_data[allele_data.panel == panel])


def generate_intensity(size, height):

    '''Generate intensity base on height of each allele'''

    # generate width based on height

    _range = 2.5 if height < 4000 else 2.7

    width = np.arange(-1*_range,_range,0.01)

    intensity = height*np.exp(-15*np.log(1+(width**2)/10))
    # set 1 first and last intensity = 0 to make sure all all k
    intensity[0] = 0
    intensity[-1] = 0

    return width, intensity


def plot_qc(data, xlim =(20,80),
            figsize =(15,4),
            dpi = 200,
            alpha = 0.8,
            marker_labels=True,
            ex_height = 0.2,
            ex_annotation = 0.05):

    data = data.sort_values('height')
    max_height = np.max(data.height)

    fig, ax = plt.subplots(figsize=figsize, dpi=dpi)

    ax.set_xlim(*xlim)
    ax.set_ylim(0, max_height +  max_height * ex_height)
    ax.grid(linestyle='--', alpha = 0.3, color ='gray', linewidth = 0.3)

    for idx, row in data.iterrows():

        width, y = generate_intensity(row['size'], row.height)

        x = width + row['size']
        # print(row.size)

        ax.fill(x, y, color=row.color, alpha=alpha)

    # process marker information
    props = dict(boxstyle='round', facecolor='#f8f9fa', alpha=0.5)
    if marker_labels:
        tmp = data.groupby(['label'], as_index=False).agg({'size':np.average,␣
 ↪'height':np.max})

        for _, row in tmp.iterrows():
            ax.text(row['size'],
                    row.height + max_height * ex_annotation,
                    row.label,
                    ha='center',
                    fontsize = 11,
                    bbox=props)
```

```python
    plt.tight_layout()
    plt.show()



def call_from_fsa(fsa_files:list, target_markers:dict, reference:list,␣
 ↪val_plot=True, intensity_plot = True):
    # load reference size
    ref = Reference(reference)

    markers = target_markers.copy()
    # print_marker(markers)

    for fsa_file in fsa_files:

        fsa = FSA(fsa_file)
        ref_intensity = fsa.reference_intensity
        ref_peaks, ref_peaks_height = finding_peaks(ref_intensity,␣
 ↪min_height=800)

        # build model
        model = LeastSquared(ref_peaks, ref.size, degree=3)

        if val_plot:
            model.val_plot(fsa.name)

        # predict base from model
        pred = model.predict(range(fsa.chanel_size))

        # extract interested range 0 to 200 bp

        min_index, max_index = get_index_by_base_range(pred,␣
 ↪base_ranges=(0,200))

        select_base = pred[min_index:max_index]

        # extract panel
        fsa_panel = fsa.name.split('-')[0]

        # find peak and update results to markers
        finding_peak_by_markers(markers,fsa, fsa_panel,select_base, (min_index,␣
 ↪max_index))

        if intensity_plot:
            plot_raw_intensity(fsa, min_index, max_index, select_base, (0,130))

    # print_marker(markers)
    return markers


class Definition:
    """This class is to load the tables data from definition file the already␣
 ↪processed before.
       There are 3 tables in this tables file and they are interconnecting each␣
 ↪other.
       This class will help us to quick extract the information from defintion␣
 ↪tables
    """
    def __init__(self, tables) -> None:
        self.tables = tables
        self.gene_table = self.tables['gene_table']
        # self.marker_table = self.tables['marker_table']
        self.genotype_marker_table = self.tables['genotype_marker_table']
        self.marker_table = self._add_gene_to_marker_table()


    def extract_geneid(self, gene):
        return self.gene_table[self.gene_table['gene'] == gene]['gene_id']


    def _add_gene_to_marker_table(self):
        return self.tables['marker_table'].merge(self.gene_table, how ='left',␣
 ↪on='gene_id')


    def extract_marker(self, gene=None):
        if gene:
            return self.marker_table[self.marker_table['gene'] == gene]
        else:
            return self.marker_table


    def get_target_marker(self, gene:str):

        df = self.genotype_marker_table
        if gene:
            df = self.genotype_marker_table[self.genotype_marker_table['gene']␣
 ↪== gene]

        genotype_id = df['genotype_id'].tolist()[0]
        marker_names = df[df['genotype_id'] == genotype_id]['marker']
        df = df[['gene', 'genotype', 'marker', 'value']].
 ↪pivot(index='genotype', columns='marker', values='value')
        df = df[marker_names]
        df['target'] = df[marker_names].apply(lambda row: '_'.join(row.values.
 ↪astype(str)), axis=1)

        return {'marker_name':marker_names, 'pattern':df}


# def check_if_marker_called(defined_markers, sample_markers):

#     df = defined_markers.merge(sample_markers, how='left', on='marker',␣
 ↪suffixes=('_defined', ''))

#     return df.marker[np.where(pd.isna(df.genotype))[0]]


def generate_sample_pattern(sample_markers, target_marker_names):
    """Generate comparison pattern of sample to compare with patterns from␣
 ↪definition tables.clear
       Example:
       Marker_01    AA
       Marker_02    CT

       Pattern: AA_(CT|TC)


    Args:
        sample_markers (DataFrame): A DataFrame contains all called genotypes
        target_marker_names (list): A list of the target marker name from␣
 ↪definition tables

    Returns:
        str: the generated pattern
    """
    df = sample_markers[sample_markers.marker.isin(target_marker_names)]
    cols = ['sample','gene', 'marker', 'genotype']
    df = df[cols]

    def _generate_pattern(genotype):

        if genotype not in ['AA', 'GG', 'CC', 'TT']:
            return f'({genotype}|{genotype[::-1]})'
        else:
            return genotype

    df['genotype'] = df['genotype'].apply(lambda x: _generate_pattern(x))
    df = df.pivot(index='sample', columns='marker', values='genotype')
    df = df[target_marker_names]
    df['exp_sample'] = df[target_marker_names].apply(lambda row: '_'.join(row.
 ↪values.astype(str)), axis=1)

    return df['exp_sample'][0]


def call_star_allele(sample_pattern, targets):
    """Matching pattern of sample and defintion table

    Args:
        sample_pattern (str): sample pattern
        targets (DataFrame): A data frame includes all generate pattern for all␣
 ↪combination star allele (diplotype) from definition table.

    Returns:
        (str):  All called diplotype (star allele combintations) that can match␣
 ↪with this sample.
                If more than one matched, the result will be joined and␣
 ↪separeted by "|". For example: *1/*2xN|*1xN/*2
    """
    matched = [bool(re.fullmatch(pattern=sample_pattern, string=target))
                        for target in targets['target']]
    genotype = '|'.join(targets.iloc[np.where(matched)].index.values.tolist())

    return genotype
```

0.1 Load defined marker with colors

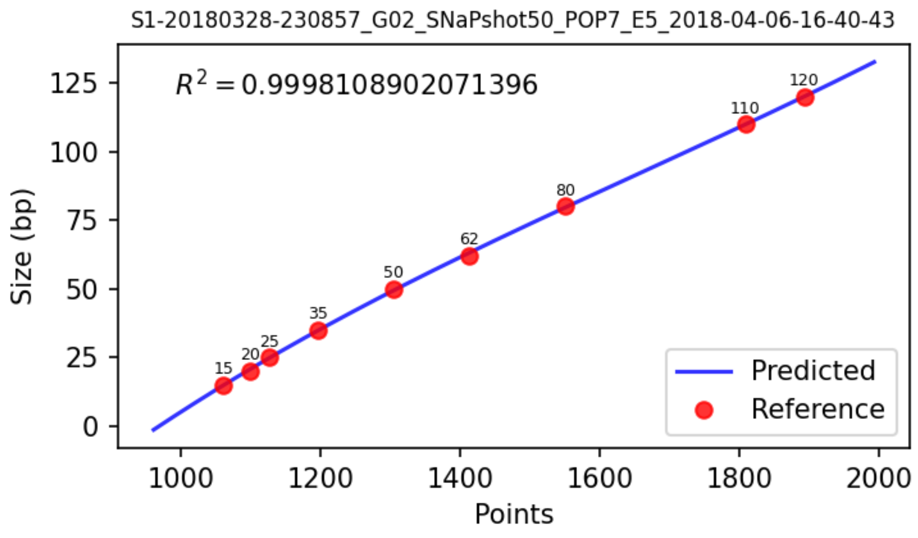
This form is test form and it was based on the format from PGx team.

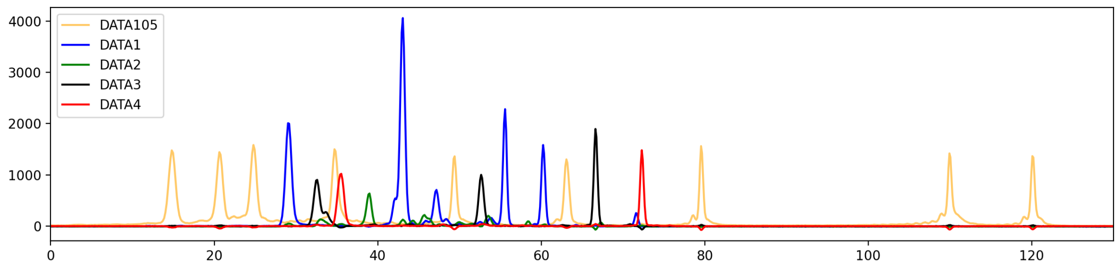
However this form have a disavantage if testing allele is not biallelic for example triallelic (A > C/T). and this requires a new format to overcome this issue.

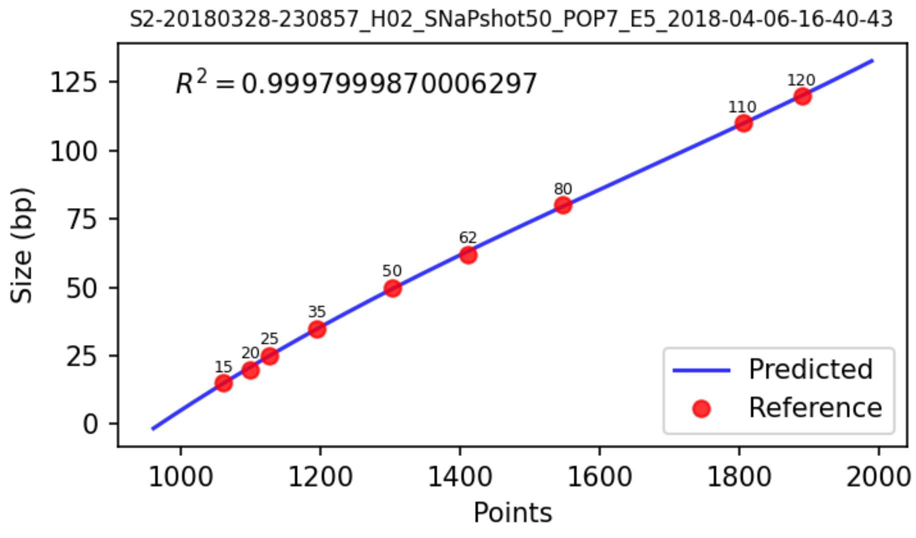
```
[ ]: import pandas as pd
     peak_table = pd.read_excel('./resource/test_bin_peak.xlsx')
     defined_markers = generate_markers(peak_table)

     liz120 = [15, 20, 25, 35, 50, 62, 80, 110, 120]
```

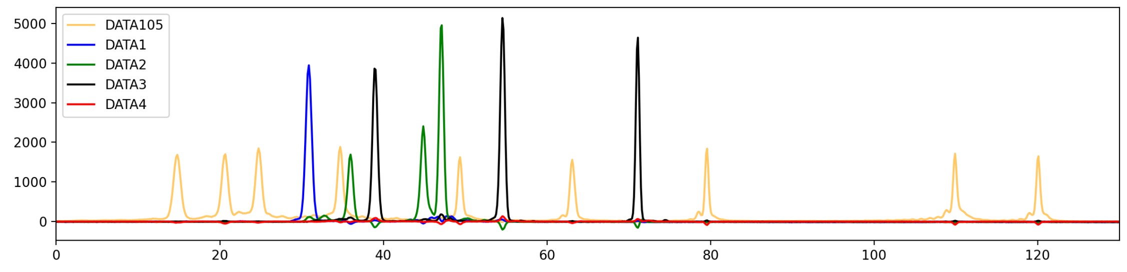
0.2 Example call from fsa file with CYP2D6 and 2 panels

```
[ ]: fsa_files = ['./raw_data/fsa/PA180404A/
       ↪S1-20180328-230857_G02_SNaPshot50_POP7_E5_2018-04-06-16-40-43.fsa',
                   './raw_data/fsa/PA180404A/
       ↪S2-20180328-230857_H02_SNaPshot50_POP7_E5_2018-04-06-16-40-43.fsa']
     liz120 = [15, 20, 25, 35, 50, 62, 80, 110, 120]

     import pickle
     with open('./resource/tables.pdata', 'rb') as t:
         tables = pickle.load(t)

     definition = Definition(tables)

     #load origin markers
     s1 = call_from_fsa(fsa_files, defined_markers, liz120)

     # call_from_fsa(fsa_files, defined_markers, liz120)
         # input:
             # 1. fsa_files
             # 2. defined_markers = generate_markers
                 # Input: (1): test_bin_peak.xlsx
             # 3. liz120: ref size: e.g.: [15, 20, 25, 35, 50, 62, 80, 110, 120]


     # print(s1)

     gene = 'CYP2D6'

     g = GenotypeResult()
     g.from_fsa_call(s1)

     for sample in g.sample_list:

         sample_data = g.marker_table(sample)

         targets = definition.get_target_marker(gene)
         sample_pattern = generate_sample_pattern(sample_data,␣
       ↪targets['marker_name'])

         result = call_star_allele(sample_pattern, targets['pattern'])
         print(f'{sample}:{result}')
```


S1-20180328-230857_G02_SNaPshot50_POP7_E5_2018-04-06-16-40-43
$R^2 = 0.9998108902071396$
Size (bp)
Points
Predicted
Reference
15
20
25
35
50
62
80
110
120

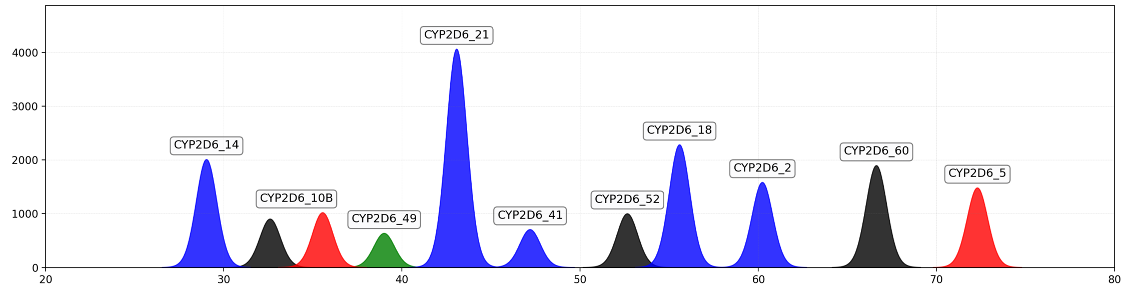

DATA105
DATA1
DATA2
DATA3
DATA4

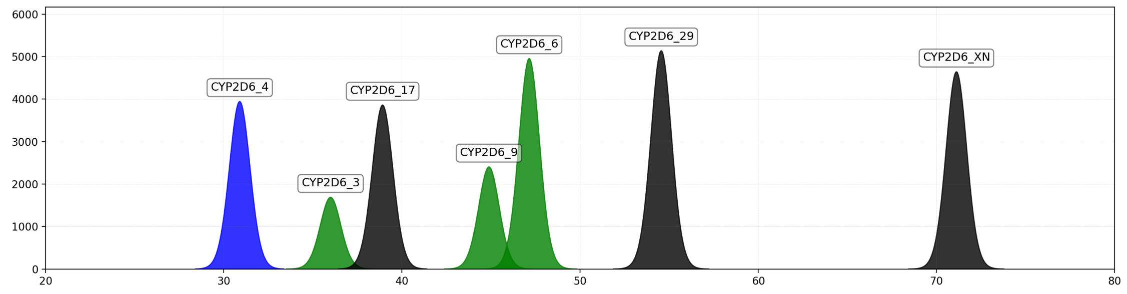

S2-20180328-230857_H02_SNaPshot50_POP7_E5_2018-04-06-16-40-43
$R^2 = 0.9997999870006297$
Size (bp)
Points
Predicted
Reference
15
20
25
35
50
62
80
110
120



20180328-230857:*1/*10B

```
[ ]: g.plotting_qc('20180328-230857')
```





0.3 Example call from bin file

```
[ ]: g2 = GenotypeResult()
     g2.from_bin_file('./raw_data/bin_text/PA180404A_CYP2D6_Example.txt',␣
      ↪defined_markers)

     gene = 'CYP2D6'

     for sample in g2.sample_list:
         sample_data = g2.marker_table(sample)
```

```
    # defined_markers = definition.extract_marker(gene)
    targets = definition.get_target_marker(gene)
    sample_pattern = generate_sample_pattern(sample_data,␣
↪targets['marker_name'])

    result = call_star_allele(sample_pattern, targets['pattern'])

    print(f'{sample}:{result}')
```

```
20180328-230857:*1/*10B
PTC:*1/*1
NTC:
```

[ ]:
```
g2.marker_table()
```

[ ]:
```
              sample    gene      marker       label panel direction genotype  \
0    20180328-230857  CYP2D6  CYP2D6_001   CYP2D6_14    S1   Forward       GG
2    20180328-230857  CYP2D6  CYP2D6_002  CYP2D6_10B    S1   Forward       CT
4    20180328-230857  CYP2D6  CYP2D6_003   CYP2D6_49    S1   Reverse       TT
6    20180328-230857  CYP2D6  CYP2D6_004   CYP2D6_21    S1   Forward       GG
8    20180328-230857  CYP2D6  CYP2D6_005   CYP2D6_41    S1   Forward       GG
10   20180328-230857  CYP2D6  CYP2D6_006   CYP2D6_52    S1   Reverse       GG
12   20180328-230857  CYP2D6  CYP2D6_007   CYP2D6_18    S1   Forward       GG
14   20180328-230857  CYP2D6  CYP2D6_008    CYP2D6_2    S1   Reverse       CC
16   20180328-230857  CYP2D6  CYP2D6_009   CYP2D6_60    S1   Reverse       GG
18   20180328-230857  CYP2D6  CYP2D6_010    CYP2D6_5    S1   Reverse       AA
20   20180328-230857  CYP2D6  CYP2D6_011    CYP2D6_4    S2   Forward       GG
22   20180328-230857  CYP2D6  CYP2D6_013   CYP2D6_17    S2   Forward       CC
24   20180328-230857  CYP2D6  CYP2D6_012    CYP2D6_3    S2   Forward       AA
26   20180328-230857  CYP2D6  CYP2D6_015    CYP2D6_6    S2   Reverse       TT
28   20180328-230857  CYP2D6  CYP2D6_014    CYP2D6_9    S2   Forward       AA
30   20180328-230857  CYP2D6  CYP2D6_016   CYP2D6_29    S2   Reverse       GG
32   20180328-230857  CYP2D6  CYP2D6_017   CYP2D6_XN    S2   Reverse       GG
34               PTC  CYP2D6  CYP2D6_001   CYP2D6_14    S1   Forward       GG
36               PTC  CYP2D6  CYP2D6_002  CYP2D6_10B    S1   Forward       CC
38               PTC  CYP2D6  CYP2D6_003   CYP2D6_49    S1   Reverse       TT
40               PTC  CYP2D6  CYP2D6_004   CYP2D6_21    S1   Forward       GG
42               PTC  CYP2D6  CYP2D6_005   CYP2D6_41    S1   Forward       GG
44               PTC  CYP2D6  CYP2D6_006   CYP2D6_52    S1   Reverse       GG
46               PTC  CYP2D6  CYP2D6_007   CYP2D6_18    S1   Forward       GG
48               PTC  CYP2D6  CYP2D6_008    CYP2D6_2    S1   Reverse       CC
50               PTC  CYP2D6  CYP2D6_009   CYP2D6_60    S1   Reverse       GG
52               PTC  CYP2D6  CYP2D6_010    CYP2D6_5    S1   Reverse       AA
54               PTC  CYP2D6  CYP2D6_011    CYP2D6_4    S2   Forward       GG
56               PTC  CYP2D6  CYP2D6_013   CYP2D6_17    S2   Forward       CC
58               PTC  CYP2D6  CYP2D6_012    CYP2D6_3    S2   Forward       AA
```

```
60                PTC  CYP2D6  CYP2D6_015   CYP2D6_6    S2  Reverse       TT
62                PTC  CYP2D6  CYP2D6_014   CYP2D6_9    S2  Forward       AA
64                PTC  CYP2D6  CYP2D6_016  CYP2D6_29    S2  Reverse       GG
66                PTC  CYP2D6  CYP2D6_017  CYP2D6_XN    S2  Reverse       GG
68                NTC  CYP2D6  CYP2D6_001  CYP2D6_14    S1  Forward
70                NTC  CYP2D6  CYP2D6_002 CYP2D6_10B    S1  Forward
72                NTC  CYP2D6  CYP2D6_003  CYP2D6_49    S1  Reverse
74                NTC  CYP2D6  CYP2D6_004  CYP2D6_21    S1  Forward
76                NTC  CYP2D6  CYP2D6_005  CYP2D6_41    S1  Forward
78                NTC  CYP2D6  CYP2D6_006  CYP2D6_52    S1  Reverse
80                NTC  CYP2D6  CYP2D6_007  CYP2D6_18    S1  Forward
82                NTC  CYP2D6  CYP2D6_008   CYP2D6_2    S1  Reverse
84                NTC  CYP2D6  CYP2D6_009  CYP2D6_60    S1  Reverse
86                NTC  CYP2D6  CYP2D6_010   CYP2D6_5    S1  Reverse
88                NTC  CYP2D6  CYP2D6_011   CYP2D6_4    S2  Forward
90                NTC  CYP2D6  CYP2D6_013  CYP2D6_17    S2  Forward
92                NTC  CYP2D6  CYP2D6_012   CYP2D6_3    S2  Forward
94                NTC  CYP2D6  CYP2D6_015   CYP2D6_6    S2  Reverse
96                NTC  CYP2D6  CYP2D6_014   CYP2D6_9    S2  Forward
98                NTC  CYP2D6  CYP2D6_016  CYP2D6_29    S2  Reverse
100               NTC  CYP2D6  CYP2D6_017  CYP2D6_XN    S2  Reverse

              phenotype
0              wildtype
2    heterozygous mutant
4              wildtype
6              wildtype
8              wildtype
10             wildtype
12             wildtype
14             wildtype
16             wildtype
18             wildtype
20             wildtype
22             wildtype
24             wildtype
26             wildtype
28             wildtype
30             wildtype
32             wildtype
34             wildtype
36             wildtype
38             wildtype
40             wildtype
42             wildtype
44             wildtype
46             wildtype
```

```
48                wildtype
50                wildtype
52                wildtype
54                wildtype
56                wildtype
58                wildtype
60                wildtype
62                wildtype
64                wildtype
66                wildtype
68
70
72
74
76
78
80
82
84
86
88
90
92
94
96
98
100
```

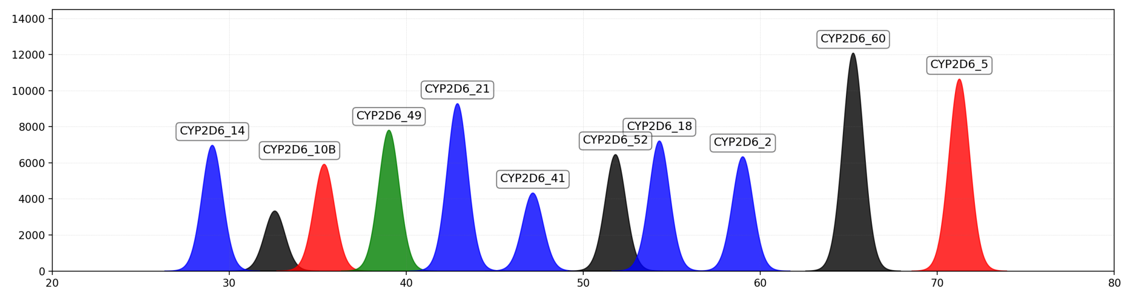
[ ]:
```
g2.marker_table().head(1)
```

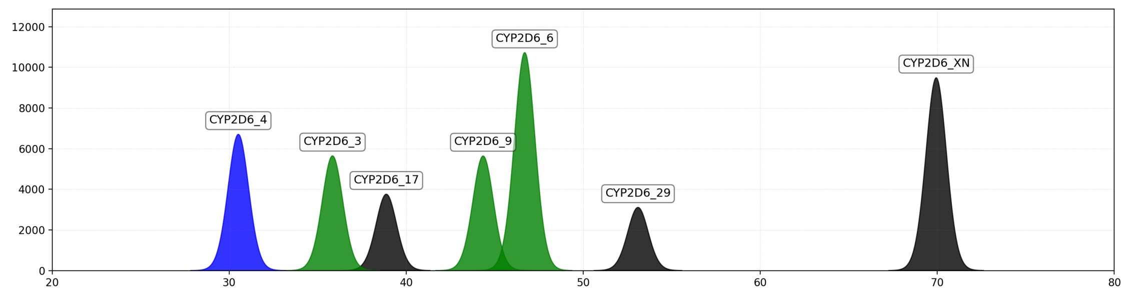
[ ]:
```
            sample    gene      marker      label panel direction genotype  \
0  20180328-230857  CYP2D6  CYP2D6_001  CYP2D6_14    S1   Forward       GG

  phenotype
0  wildtype
```

[ ]:
```
g2.allele_table(sample_name='20180328-230857', called_filter=True).head(1)
```

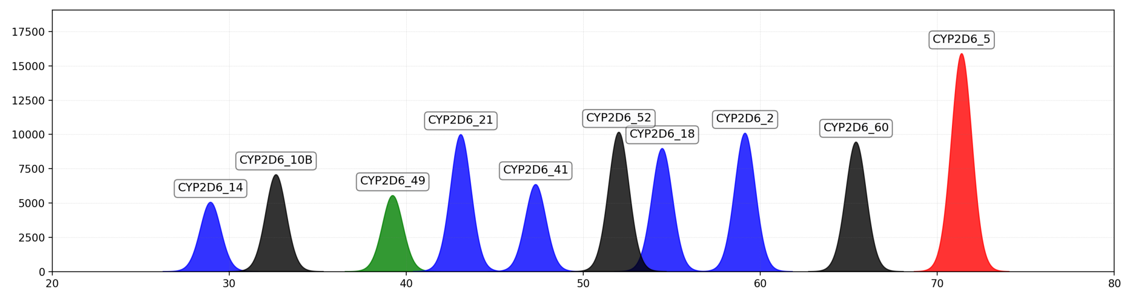
[ ]:
```
            sample    gene      marker      label panel direction base   size  \
0  20180328-230857  CYP2D6  CYP2D6_001  CYP2D6_14    S1   Forward    G  29.04

   height color
0  6967.0  blue
```

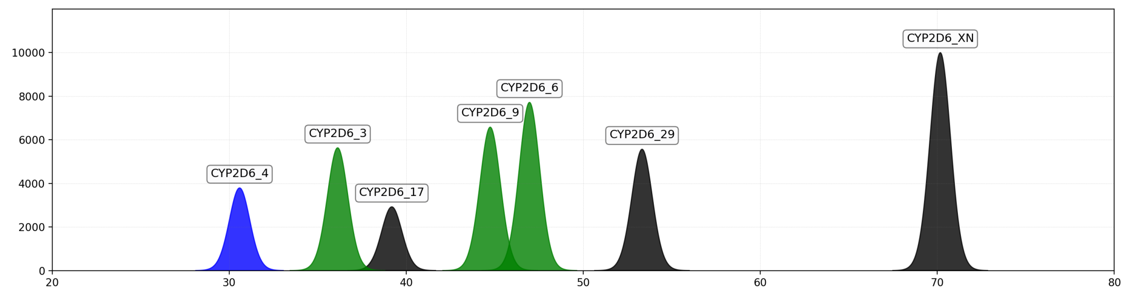
[ ]:
```
g2.plotting_qc('20180328-230857')
```





```
[ ]: g2.plotting_qc('PTC')
```





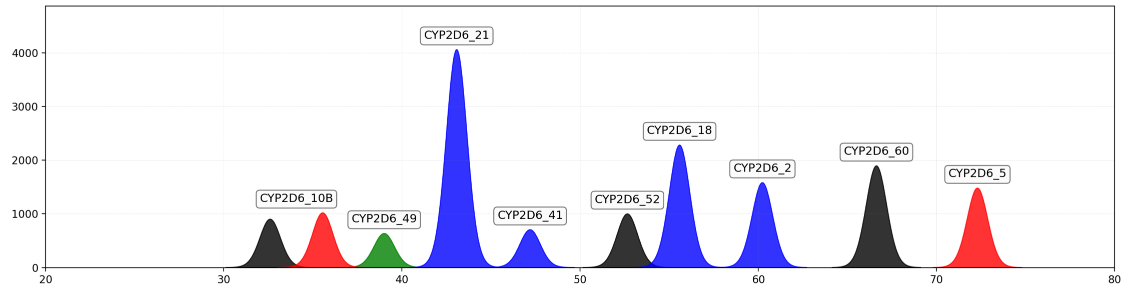
1 Update bin range to call genotype example

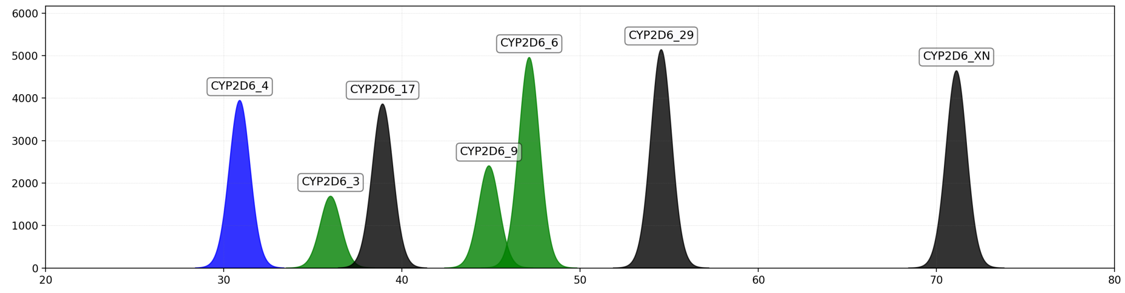
```
[ ]: fsa_files = ['./raw_data/fsa/PA180404A/
      ↪S1-20180328-230857_G02_SNaPshot50_POP7_E5_2018-04-06-16-40-43.fsa',
                   './raw_data/fsa/PA180404A/
      ↪S2-20180328-230857_H02_SNaPshot50_POP7_E5_2018-04-06-16-40-43.fsa']

     liz120 = [15, 20, 25, 35, 50, 62, 80, 110, 120]

     import pickle
     with open('./resource/tables.pdata', 'rb') as t:
         tables = pickle.load(t)

     definition = Definition(tables)

     s3_markers = generate_markers(peak_table)

     s3_markers['CYP2D6_001'].update_allele_config(allele_number = 0, min_bin = 20,␣
      ↪max_bin = 25)

     s3 = call_from_fsa(fsa_files, s3_markers, liz120, val_plot=False,␣
      ↪intensity_plot=False)

     g3 = GenotypeResult()
     g3.from_fsa_call(s3)

     g3.allele_table(called_filter=False).head(4)
```

```
[ ]:             sample    gene      marker       label panel direction base  \
     0  20180328-230857  CYP2D6  CYP2D6_001  CYP2D6_14    S1   Forward    G
     1  20180328-230857  CYP2D6  CYP2D6_001  CYP2D6_14    S1   Forward    A
     2  20180328-230857  CYP2D6  CYP2D6_002  CYP2D6_10B    S1   Forward    C
     3  20180328-230857  CYP2D6  CYP2D6_002  CYP2D6_10B    S1   Forward    T

        basetype  min_bin  max_bin  min_height  is_forward  is_detected peak  \
     0  wildtype       20       25         500           1        False
     1    mutant       27       36         500           1        False
     2  wildtype       28       38         500           1         True   32
     3    mutant       31       37         500           1         True   32

         size  height status                                      message  \
     0                       Peak(s) could not be detected. Please check pe…
     1                       Peak(s) could not be detected. Please check pe…
     2   32.6   901.0     ok
     3  35.55  1019.0     ok

        color
```

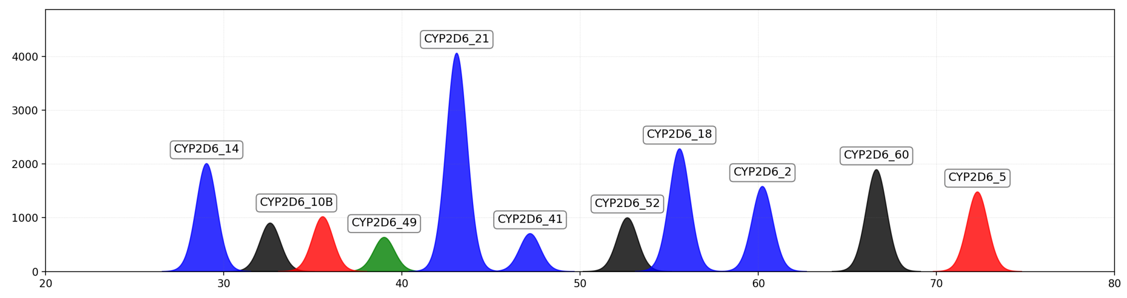
```
0    blue
1   green
2   black
3     red
```

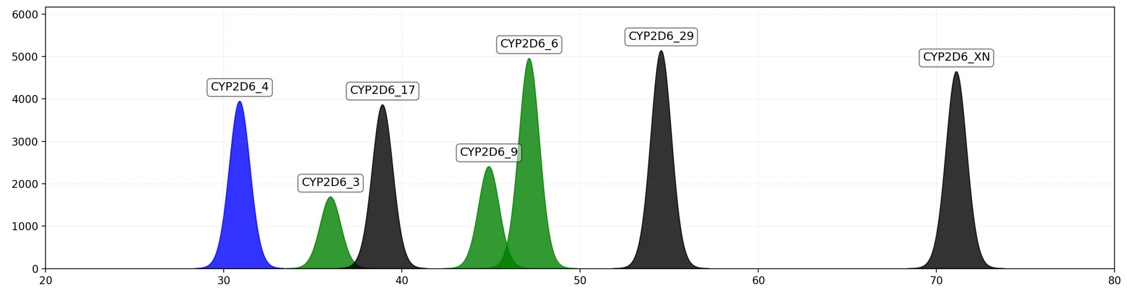
[ ]:
```
# qc plot
g3.plotting_qc('20180328-230857')
```





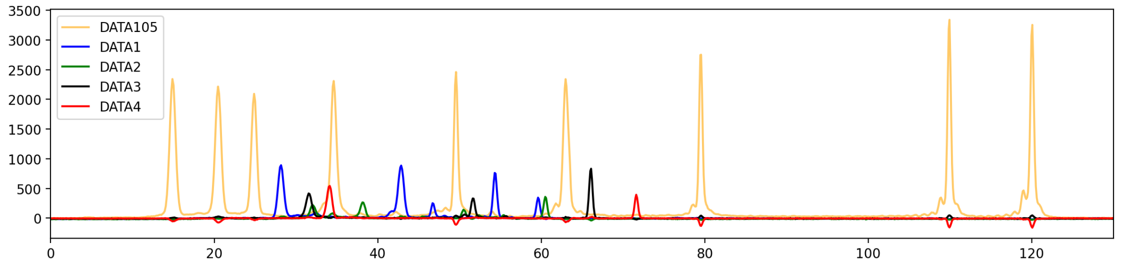
[ ]:
```
s3_markers['CYP2D6_001'].update_allele_config(allele_number = 0, min_bin = 27,␣
 ↪max_bin = 36)

s3 = call_from_fsa(fsa_files, s3_markers, liz120, val_plot=False,␣
 ↪intensity_plot=False)

g3_a = GenotypeResult()
g3_a.from_fsa_call(s3)

g3_a.plotting_qc('20180328-230857')
```

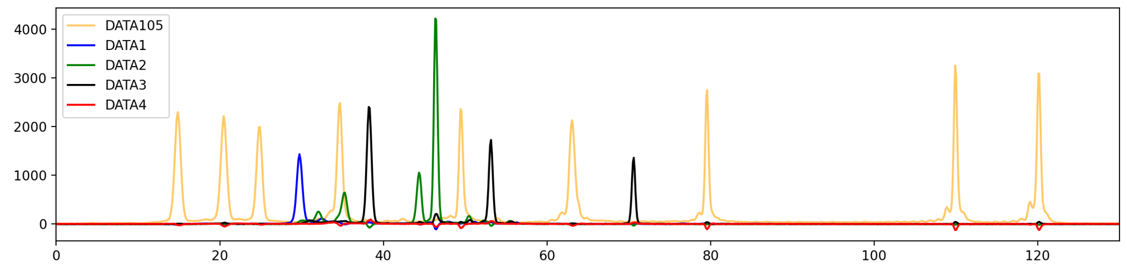




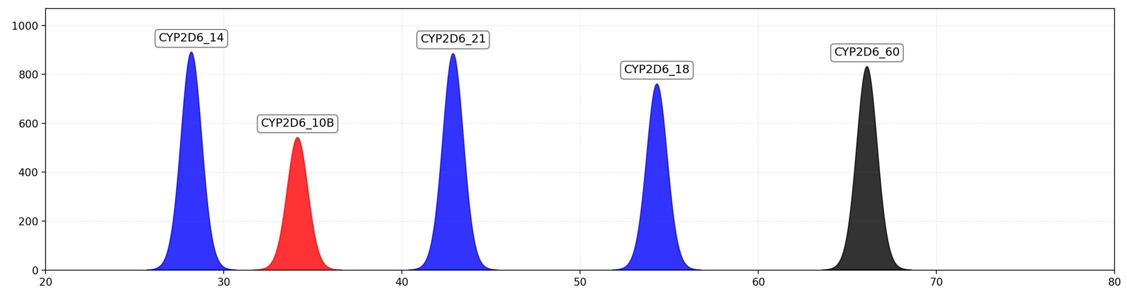
2 Example of adjusting height

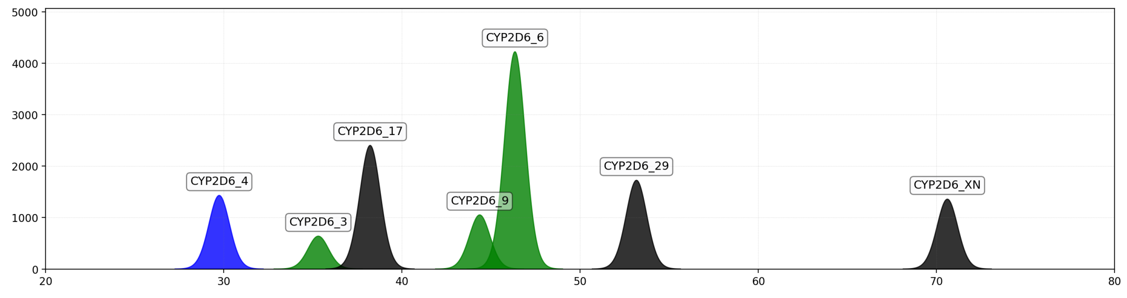
```
[ ]: # update height
fsa_files = ['./raw_data/fsa/PA181114A/
  ↪S1-20181106-730829_A10_SNaPshot50_POP7_E5_2018-11-23-16-40-43.fsa',
             './raw_data/fsa/PA181114A/
  ↪S2-20181106-730829_B10_SNaPshot50_POP7_E5_2018-11-23-16-40-43.fsa']

liz120 = [15, 20, 25, 35, 50, 62, 80, 110, 120]



s4_markers = generate_markers(peak_table)

s4 = call_from_fsa(fsa_files, s4_markers, liz120, val_plot=False,␣
  ↪intensity_plot=True)
```





[ ]:
```
g4 = GenotypeResult()
g4.from_fsa_call(s4)

g4.plotting_qc('20181106-730829')
```



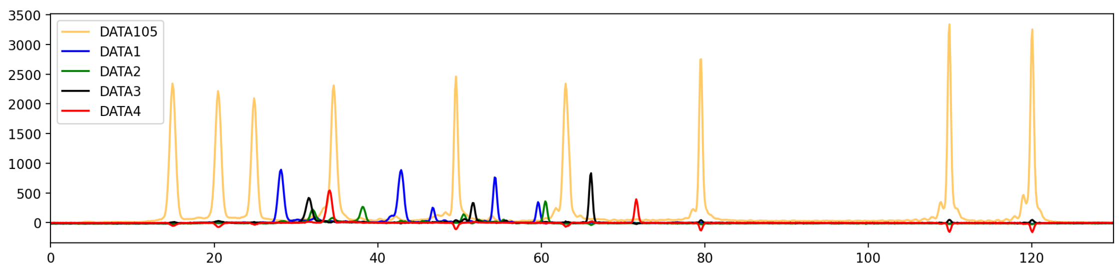


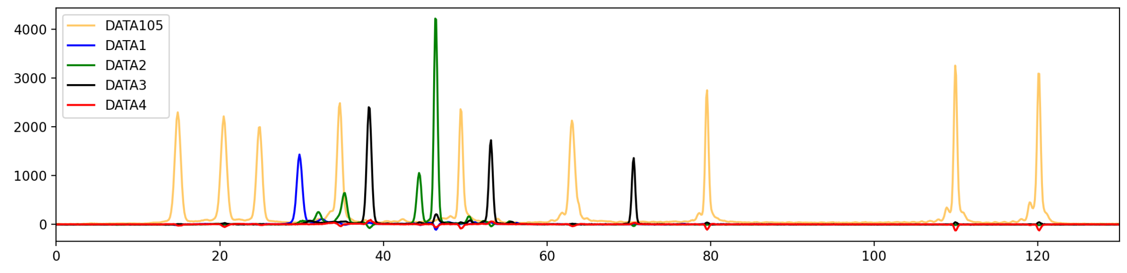
[ ]:
```
g4.allele_table(called_filter=False).head(4)
```

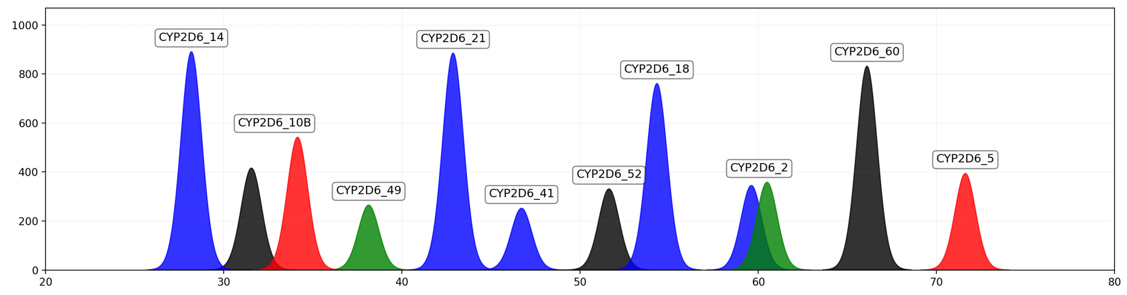
[ ]:
```
            sample    gene      marker       label panel direction base  \
0  20181106-730829  CYP2D6  CYP2D6_001   CYP2D6_14    S1   Forward    G
1  20181106-730829  CYP2D6  CYP2D6_001   CYP2D6_14    S1   Forward    A
2  20181106-730829  CYP2D6  CYP2D6_002  CYP2D6_10B    S1   Forward    C
3  20181106-730829  CYP2D6  CYP2D6_002  CYP2D6_10B    S1   Forward    T
```

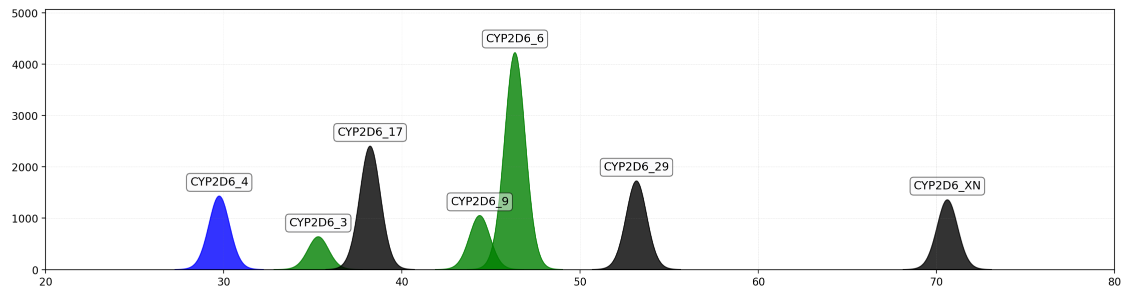
```
   basetype  min_bin  max_bin  min_height  is_forward  is_detected peak  \
0  wildtype       25       35         500           1         True   25
1    mutant       27       36         500           1        False
2  wildtype       28       38         500           1        False
3    mutant       31       37         500           1         True   25

    size height status                                          message  \
0  28.18  891.0     ok
1                       Peak(s) could not be detected. Please check pe…
2                       Peak(s) could not be detected. Please check pe…
3  34.14  542.0     ok

   color
0   blue
1  green
2  black
3    red
```

```
[ ]: for marker_name in s4_markers:
         marker = s4_markers.get(marker_name)

         for num, _ in enumerate(marker.alleles):
             marker.update_allele_config(allele_number= num, min_height =250)


     s4 = call_from_fsa(fsa_files, s4_markers, liz120, val_plot=False,␣
      ↪intensity_plot=True)
     g4 = GenotypeResult()
     g4.from_fsa_call(s4)

     g4.plotting_qc('20181106-730829')
```




DATA105
DATA1
DATA2
DATA3
DATA4


CYP2D6_14
CYP2D6_10B
CYP2D6_49
CYP2D6_21
CYP2D6_41
CYP2D6_52
CYP2D6_18
CYP2D6_2
CYP2D6_60
CYP2D6_5


CYP2D6_6
CYP2D6_17
CYP2D6_29
CYP2D6_4
CYP2D6_XN
CYP2D6_9
CYP2D6_3