# analyze_50_cycles_mage

March 15, 2017

## 1 Experiment 1 Repeat Analysis started 2016-07-07

In preparation for manuscript.

```
In [1]: import collections
        from collections import defaultdict
        import operator
        import os
        import re
        import sys

        from Bio import SeqIO
        import matplotlib.pyplot as plt
        import numpy as np
        import pandas as pd
        from scipy import stats

        print 'pandas version', pd.__version__

        %matplotlib inline

        sys.path.append('../')
        from common_code import data_util
        from common_code import model_fitting
```

```
pandas version 0.19.2
```

### 1.1 Constants

```
In [2]: NUM_SAMPLES = 96

        # NOTE: We later drop 6 samples suspecting cross-contamination,
        # thus we end up analyzing 90 samples, as described in manuscript.
```

### 1.2 Helper functions

Below, we'll find it useful to compare variants using a common primary key. For this purpose, we'll use tuples of the form (POSITION, REF). Let's make a helper function for generating the primary key.

```
In [3]: def get_variant_key_for_df_row(df_row):
            """Parses unique key for a variant from Dataframe row.

            Returns key as tuple of the form: (POSITION, REF).
            """
            return (df_row['POSITION'], df_row['REF'])
```

### 1.3 Data Import and Preparation

Import data. Similar to previous analyses.

```
In [4]: MG1655_GENBANK = 'data/mg1655_U00096.2.gb'
        mg1655_seq_record = SeqIO.read(MG1655_GENBANK, 'gb')
        mg1655_seq_str = str(mg1655_seq_record.seq)
```

SNP data (exported from Millstone).

```
In [5]: MELTED_VARIANT_DATA = (
                'data/fix_rec_variants_millstone_export_all_strains_2016_10_07.csv')
        melted_variant_data_snps_only_df = pd.read_csv(
                MELTED_VARIANT_DATA, low_memory=False)

        # Rename columns
        melted_variant_data_snps_only_df.rename(
                columns={'EXPERIMENT_SAMPLE_LABEL': 'BARCODE'}, inplace=True)

        # Get rid of rows that don't have sample in them (catch-all rows for VariantSets from Mi
        melted_variant_data_snps_only_df = melted_variant_data_snps_only_df[
                melted_variant_data_snps_only_df['BARCODE'].notnull()]

        # Drop samples named C321.*. These were included in the Millstone alignment
        # but we are only focusing on Experiment 1 analysis here.
        melted_variant_data_snps_only_df = melted_variant_data_snps_only_df[
                melted_variant_data_snps_only_df['BARCODE'].apply(
                        lambda b: not bool(re.match('C321.*', b)))]

        # Drop samples with LONG ref. We ignore SVs in this analysis.
        def _is_sv(row):
            if pd.isnull(row['REF']):
                assert bool(re.match('LONG.*', row['ALT']))
                return True
            return bool(re.match('LONG.*', row['REF']))
        melted_variant_data_snps_only_df = melted_variant_data_snps_only_df[
                melted_variant_data_snps_only_df.apply(lambda row: not _is_sv(row), axis=1)]
```

Import the designed UAG-to-UAA SNPs data and make sure all are accounted for.

```
In [6]: # NOTE: These were manually updated.
        amber_snps_df = pd.read_csv(
                'data/mg1655_uag_designs__freebayes_consolidated__2016_10_07.csv')
        amber_snps_df.rename(columns={'POS': 'POSITION'}, inplace=True)

        # Some UAGs are counted as a single SNP because they are adjacent.
        SINGLE_SNP_COMBO_AMBERS = [
            633969, # 633970
            745946, # 745949
        ]

        # Some are not called but I visually confirmed they are there in Millstone.
        UAG_NOT_CALLED_BUT_VISUALLY_VERIFIED = [
            1431010, # Poor mapping quality region
        ]
        amber_snps_df = amber_snps_df[
                amber_snps_df['POSITION'].apply(
                        lambda p: p not in UAG_NOT_CALLED_BUT_VISUALLY_VERIFIED)]

        NUM_AMBER_SNPS = 321 - len(SINGLE_SNP_COMBO_AMBERS) - len(UAG_NOT_CALLED_BUT_VISUALLY_VE

        assert NUM_AMBER_SNPS == len(amber_snps_df), len(amber_snps_df)

        # Create (pos, ref) tuple set.
        amber_pos_ref_tuple_set = set(amber_snps_df.apply(
                get_variant_key_for_df_row, axis=1))
        assert NUM_AMBER_SNPS == len(amber_pos_ref_tuple_set)
```

Add Experiment data, e.g. wells, barcodes, lineage/timepoint identities, etc. These were calculated in Analysis 1.

```
In [7]: METADATA = (
                'data/exp_1_sequencing_wells_barcodes_samples.csv')

        experiment_metadata_df = pd.read_csv(METADATA)
        experiment_metadata_df = experiment_metadata_df.rename(
                columns={'barcode': 'BARCODE'})
        experiment_metadata_df['pos'] = (
                experiment_metadata_df['pos'].apply(
                        data_util.normalize_well_name))

        # Add sequencing layout data.
        LAYOUT_TO_MAGE_CYCLE_MAPPING_DATA = (
                'data/exp_1_sequencing_layout_to_mage_cycle_mapping.csv')
        layout_to_mage_cycle_mapping_df = pd.read_csv(LAYOUT_TO_MAGE_CYCLE_MAPPING_DATA)
        layout_to_mage_cycle_mapping_df.rename(columns={'well': 'pos'}, inplace=True)
        experiment_metadata_df = pd.merge(
                experiment_metadata_df,
                layout_to_mage_cycle_mapping_df[['pos', 'actual_mage_cycle']],
                how='inner',
                on='pos'
        )

        C321_I4_DT = experiment_metadata_df[
                experiment_metadata_df['pos'].apply(
                        lambda p: p in ['H10', 'H11', 'H12'])]['doubling_time'].mean()
        print 'C321_I4_DT', C321_I4_DT

        # Wild-type doubling time was not measured on this plate, but approximated from other pl
        # using relative ratios.
        ECNR1_DT = 47
        print 'ECNR1_DT', ECNR1_DT

        experiment_metadata_df[-5:]
```

```
C321_I4_DT 87.0065433333
ECNR1_DT 47
```

```
Out[7]:     pos rc_barcode BARCODE   sample  doubling_time  lineage  time_point  \
        91  H08     GACAGA  TCTGTC    03-06      70.113961        3           6
        92  H09     GCGCTA  TAGCGC    05-06      60.102475        5           6
        93  H10     GAAGTC  GACTTC  C321_I4      85.300355        0           0
        94  H11     TTGATT  AATCAA  C321_I4      85.964804        0           0
        95  H12     CGACTC  GAGTCG  C321_I4      89.754471        0           0

            actual_mage_cycle
        91                 40
        92                 34
        93                  0
        94                  0
        95                  0
```

Merge with SNP data.

```
In [8]: len_before_merge = len(melted_variant_data_snps_only_df)
        melted_variant_data_unfiltered_df = pd.merge(
                melted_variant_data_snps_only_df,
                experiment_metadata_df,
                how='inner',
                on=['BARCODE'])
        assert len_before_merge == len(melted_variant_data_unfiltered_df), (
                len_before_merge, len(melted_variant_data_unfiltered_df))
```

Below, we'll find it useful to compare variants using a common primary key. For this purpose, we'll use tuples of the form (POSITION, REF). Let's make a helper function for generating the primary key.

Filter out mutations that we know are not real (e.g. adjacent to lambda prophage scar) or inconsequential (mobile insertion elements) , or that we have determined are difficult to confirm via sequencing (e.g. homopolymer runs).

```
In [9]: # First determine which keys to delete.
        # Maintain map so we can quickly query by key, but also
        # inspect by value.
        to_delete_variant_keys_to_data_row_map = {}

        MANUAL_IGNORE_SET = set([
            # lambda prophage locus
            (809582, 'A'),
            (806566, 'C'),

            # prfA deletion locus
            (1264224, 'C'),
            (1265309, 'C')
        ])

        for _, row in melted_variant_data_unfiltered_df.iterrows():
            variant_key = get_variant_key_for_df_row(row)
            if variant_key in to_delete_variant_keys_to_data_row_map:
                continue

            if variant_key in amber_pos_ref_tuple_set:
                continue

            if pd.isnull(row['ALT']):
                continue

            # Ignore homopolymer runs.
            if len(row['ALT']) > 1:
                # Grab 2nd from end in case full homopolymer contained in ALT.
                # e.g. (3758149, AGGGGGGC)
                if len(row['ALT']) > 2:
                    last_nt = row['ALT'][-2]
                else:
                    last_nt = row['ALT'][-1]
                homopolymer_start = row['POSITION'] + 1
                if mg1655_seq_str[homopolymer_start:homopolymer_start + 5] == last_nt * 5:
                    to_delete_variant_keys_to_data_row_map[variant_key] = row
                    continue

            if len(row['REF']) > 2:
                last_nt = row['REF'][-2]
                homopolymer_start = row['POSITION'] + 1
                if mg1655_seq_str[homopolymer_start:homopolymer_start + 5] == last_nt * 5:
                    to_delete_variant_keys_to_data_row_map[variant_key] = row
                    continue

            # Manually ignore some mutation (e.g. lambda prophage).
            if variant_key in MANUAL_IGNORE_SET:
                to_delete_variant_keys_to_data_row_map[variant_key] = row
                continue

            # Ignore insertion elements.
            if not pd.isnull(row['INFO_EFF_GENE']) and row['INFO_EFF_GENE'][:3] == 'ins':
                to_delete_variant_keys_to_data_row_map[variant_key] = row
                continue


        print 'Num variant keys to delete', len(to_delete_variant_keys_to_data_row_map)

        # Sanity checks for SNPs that we should be ignoring.
        TEST_DELETED_VARIANT_KEYS = [
            # false positive homopolymer run
            (3758149, 'AGGGGGGC'),
            (4473579, 'CGGGGGGC'),
            (3509760, 'CAAAAAAAAC'),

            # insertion element
            (1426076, 'T'),

            # prfA
            (1264224, 'C'),
        ] + list(MANUAL_IGNORE_SET)

        for test_deleted in TEST_DELETED_VARIANT_KEYS:
            assert test_deleted in to_delete_variant_keys_to_data_row_map, test_deleted
```

```
Num variant keys to delete 189
```

```
In [10]: # Filter out the variants to delete identified above.
         melted_variant_data_df = melted_variant_data_unfiltered_df[
                 melted_variant_data_unfiltered_df.apply(
                         lambda row: not get_variant_key_for_df_row(row) in
                                 to_delete_variant_keys_to_data_row_map,
                         axis=1)][:]
```

```
In [11]: # Add an assert to make sure data doesn't change without us knowing.
         NUM_SNPS_CALLED = 2250
         assert NUM_SNPS_CALLED == len(set(melted_variant_data_df['POSITION'])), (
                 len(set(melted_variant_data_df['POSITION']))

         NUM_SAMPLES = 96
         assert NUM_SAMPLES == len(set(melted_variant_data_df['BARCODE']))
```

**NOTE**: We can still have more than one row per Variant if there are multiple alts.

Update GT_TYPEs based on AF as Freebayes was probably too conservative with this and to account for where we possibly had contamination.

```
In [12]: # Save the original GT_TYPEs to another column.
         melted_variant_data_df['original_GT_TYPE'] = melted_variant_data_df['GT_TYPE']

         AF_ALT_THRESHOLD = 0.7

         AF_REF_THRESHOLD = 0.1

         def _update_gt_type(row):
             af = row['AF']
             if af >= AF_ALT_THRESHOLD:
                 return 2
             elif af <= AF_REF_THRESHOLD:
                 return 0
             else:
                 return row['GT_TYPE']

         melted_variant_data_df['GT_TYPE'] = melted_variant_data_df.apply(
                 _update_gt_type, axis=1)
```

### 1.3.1 Identify variants in the starting strain C321_I4 relative to MG1655

**NOTE**: Similar to Analysis 2.

We called variants relative to MG1655, so we need to determine variants in the starting strain as a reference point to identify reverted and new variants in descendant strains. Note that we ran freebayes in diploid mode, even though E. coli is haploid, so that we can more easily discern structural variations. Because of this, variants may be called as homozygous ref, heterozygous, or homozygous alt.

We'll leverage the fact that we sequenced 3 clones of the starting strains so that we can deal with data. We'll say that a variant is considered to be present in the starting strain if it was called at least heterozygous (GT_TYPE = 1 or GT_TYPE = 2) in all 3 clones, and called homozygous alt (GT_TYPE = 2) in at least one of the clones. We'll ignore the actual ALT value for now, digging deeper when necessary. We're okay with ignoring ALT for now because we are interested in observing the transition from some ALT back to REF.

Create structure containing all variants in starting set.

First a structure with all variants.

```
In [13]: all_variant_pos_ref_tuple_set = set(melted_variant_data_df.apply(
                 get_variant_key_for_df_row, axis=1))
         print len(all_variant_pos_ref_tuple_set)
         assert NUM_SNPS_CALLED == len(all_variant_pos_ref_tuple_set)
```

```
2250
```

```
In [14]: # What are the duplicates.
         pos_observed = {}
         for (pos, ref) in all_variant_pos_ref_tuple_set:
             if pos in pos_observed:
                 print pos, ref, pos_observed[pos]
             pos_observed[pos] = ref
```

Now we'll create structures that contain variants that occur in all 3 starting strains, as determined by these two criteria points mentioned above:

1. At least het (GT_TYPE = 1 or GT_TYPE = 2) in all 3 clones of C321_I4.
2. Called homozygous alt (GT_TYPE = 2) in at least 1 clone.

```
In [15]: starting_strain_variants_df = melted_variant_data_df[
                 melted_variant_data_df['sample'] == 'C321_I4']

         # Variants that are at least HET in the starting strain.
         variants_called_at_least_het_in_starting_strains_df = (
                 starting_strain_variants_df[
                         (starting_strain_variants_df.GT_TYPE == 2) |
                         (starting_strain_variants_df.GT_TYPE == 1)])
         print 'num at least het', len(variants_called_at_least_het_in_starting_strains_df.POSIT

         # There's probably a pandas way to do this elegantly, but I'm going to
         # do it iteratively for now.

         # First, build a map from position to list of GT_TYPES.
         position_to_gt_type_list_map = defaultdict(list)
         for idx, row in variants_called_at_least_het_in_starting_strains_df.iterrows():
             position_to_gt_type_list_map[row['POSITION']].append(row['GT_TYPE'])

         MANUALLY_VERIFIED_SNP_IN_STARTING_STRAIN = set([
             3726133, 3509760, 1757699, 4472155, 1632334])

         # Now keep only those positions that satisfy (1) and (2) above,
         # or are in the manual exception list.
         positions_to_keep = []
         for position, gt_type_list in position_to_gt_type_list_map.iteritems():
             if ((len(gt_type_list) >= 3 and 2 in gt_type_list) or
                     position in MANUALLY_VERIFIED_SNP_IN_STARTING_STRAIN):
                 positions_to_keep.append(position)

         # Filter down the DataFrame to the positions satisfying our constraints.
         starting_strain_variants_passing_filter = pd.merge(
                 variants_called_at_least_het_in_starting_strains_df,
                 pd.DataFrame({'POSITION': positions_to_keep}),
                 how='inner',
                 on='POSITION')

         # Get the (POSITION, REF) tuple set.
         variants_in_starting_strain_set = set(
                 starting_strain_variants_passing_filter.apply(
                         get_variant_key_for_df_row, axis=1))
         print 'num variants in starting strain', len(variants_in_starting_strain_set)
         assert 651 == len(variants_in_starting_strain_set) # break assert if unexpected code ch
```

```
num at least het 675
num variants in starting strain 651
```

### 1.3.2 Import Target Mutations Data

And check against called mutations from experiment.

#### UAG-to-UAA (Amber) Mutations

```
In [16]: # Check that all UAGs are accounted for.
         assert not amber_pos_ref_tuple_set - all_variant_pos_ref_tuple_set, (
                 amber_pos_ref_tuple_set - all_variant_pos_ref_tuple_set)

         # Check all UAGs accounted for in starting strains.
         assert not amber_pos_ref_tuple_set - variants_in_starting_strain_set
```

**Designed reversions** We import designed reversions. Note the commented code that follows where we create the designed set csv that adjusts for discrepancies between designs and how Freebayes calls them.

```
In [17]: # NOTE: Freebayes called some variants differently than they were designed
         # (e.g. REF/ALT of GCC/GC vs GC/G). We make a copy of the top designed SNPs
         # and consolidate them manually in the csv file.
         designed_set_df = pd.read_csv(
                 'data/snp_data_top_ranked_final__freebayes_consolidated__2016_10_07.csv')

         designed_set_df = designed_set_df.rename(
                 columns={'POS': 'POSITION', 'ref': 'REF'})

         # Create (pos, ref) tuple set.
         designed_variant_pos_ref_tuple_set = set(designed_set_df.apply(
                 get_variant_key_for_df_row, axis=1))
         assert 127 == len(designed_variant_pos_ref_tuple_set)

         # GK (2016-10-07): Visually confirmed that these are homopolymers and filtered out abov
         MANUALLY_CONFIRMED_IGNORE_DESIGN_SET = set([
                 (2212355, 'GCC'), (4036960, 'A'), (4472155, 'T'), (1683560, 'GC'),
                 (1622373, 'AT'), (1867040, 'GT'), (3707578, 'G'), (2198468, 'GA'),
                 (3726133, 'TCCCCCCCCG'), (3509760, 'CAAAAAAAAC')])

         # Not observed in Experiment 1 at all.
         missing_variant_set = (
                 designed_variant_pos_ref_tuple_set -
                 all_variant_pos_ref_tuple_set -
                 MANUALLY_CONFIRMED_IGNORE_DESIGN_SET)
         assert not missing_variant_set

         print 'Designs considered for reversion: ', (
                 len(designed_variant_pos_ref_tuple_set) -
                 len(designed_variant_pos_ref_tuple_set - all_variant_pos_ref_tuple_set))
```

```
Designs considered for reversion:  117
```

The code below, now commented out, programatically fixed the Freebayes output to match our designs.

```
In [18]: # updated_design_representation = []
         # for _, row in designed_set_df.iterrows():
         #     variant_key = get_variant_key_for_df_row(row)
         #     if variant_key in all_variant_pos_ref_tuple_set:
         #         updated_design_representation.append({
         #             'POSITION': row['POSITION'],
         #             'REF': row['REF'],
         #             'ALT': row['alt']
         #         })
         #     else:
         #         # Try to figure out the actual representation.
         #         pos = variant_key[0]

         #         # First try exact
         #         matches_df = melted_variant_data_df[
         #             (pos == melted_variant_data_df['POSITION']) &
         #             (melted_variant_data_df['GT_TYPE'] == 2)][:]
         #         if not len(matches_df):
         #             matches_df = melted_variant_data_df[
         #                 (pos - 10 <= melted_variant_data_df['POSITION']) &
         #                 (melted_variant_data_df['POSITION'] <= pos + 10) &
         #                 (melted_variant_data_df['GT_TYPE'] == 2)][:]
         #         matches_df.drop_duplicates('POSITION', inplace=True)
         #         if len(matches_df) == 1:
         #             updated_design_representation.append({
         #                 'POSITION': matches_df['POSITION'].values[0],
         #                 'REF': matches_df['REF'].values[0],
         #                 'ALT': matches_df['ALT'].values[0]
         #             })
         #         elif len(matches_df) > 1:
         #             assert False, (pos, len(matches_df))
         #         else:
         #             updated_design_representation.append({
         #                 'POSITION': row['POSITION'],
         #                 'REF': row['REF'],
         #                 'ALT': row['alt']
         #             })

         # updated_reversion_designs_df = pd.merge(
         #     designed_set_df[['PRIORITY_SET', 'PRIORITY_INDIVIDUAL', 'POSITION', 'EFF_GENE']],
         #     pd.DataFrame(updated_design_representation),
         #     on='POSITION')

         # updated_designs_pos_ref_tuple_set = set(updated_reversion_designs_df.apply(
         #         get_variant_key_for_df_row, axis=1))
         # assert 127 == len(updated_designs_pos_ref_tuple_set)

         # # GK (2016-10-07): Visually confirmed that these are homopolymers and filtered out ab
         # MANUALLY_CONFIRMED_IGNORE_DESIGN_SET = set([
         #         (2212355, 'GCC'), (4036960, 'A'), (4472155, 'T'), (1683560, 'GC'),
         #         (1622373, 'AT'), (1867040, 'GT'), (3707578, 'G'), (2198468, 'GA')])

         # # Not observed in Experiment 1 at all.
         # missing_variant_set = (
         #         updated_designs_pos_ref_tuple_set -
         #         all_variant_pos_ref_tuple_set -
         #         MANUALLY_CONFIRMED_IGNORE_DESIGN_SET)
         # print 'Not observed in Experiment 1 at all. Ignoring %d:' % len(missing_variant_set),

         # updated_reversion_designs_df.to_csv(
         #     'data/snp_data_top_ranked_final__freebayes_consolidated__2016_10_07.csv',
         #     index=False)

         # designed_variant_pos_ref_tuple_set = updated_designs_pos_ref_tuple_set
```

**Add columns to the data that indicate how positions relate to the starting strain.**
0 = same allele as starting C321_I4
1 = alt allele

```
In [19]: MUTATION_TYPE__AMBER = 'AMBER'

         MUTATION_TYPE__AMBER_REVERSION = 'AMBER_REVERSION'

         MUTATION_TYPE__FIXED = 'FIXED'

         MUTATION_TYPE__UNTARGETED_REVERSION = 'UNTARGETED_REVERSION'
```

```python
MUTATION_TYPE__DE_NOVO = 'DE_NOVO'

MUTATION_TYPE__NONE = 'NONE'

def determine_mutation_type(row):
    """Determines the type of mutation. Possibilities:
        (FIXED, DE_NOVO, UNTARGETED_REVERSION, NONE)

    NOTE: This used to be represented in 2 different columns
    IS_FIXED and IS_DE_NOVO but we realized we additionally had
    mutations of type UNTARGETED_REVERSION. Rather than adding
    more boolean columns, use this enum column.
    """
    variant_key = get_variant_key_for_df_row(row)

    if variant_key in amber_pos_ref_tuple_set:
        if row['GT_TYPE'] == 2:
            return MUTATION_TYPE__AMBER
        else:
            return MUTATION_TYPE__AMBER_REVERSION
    if variant_key in designed_variant_pos_ref_tuple_set:
        if row['GT_TYPE'] == 0:
            return MUTATION_TYPE__FIXED
    elif variant_key in variants_in_starting_strain_set:
        if row['GT_TYPE'] == 0:
            return MUTATION_TYPE__UNTARGETED_REVERSION
    elif row['GT_TYPE'] == 2:
        # Neither in designed set or mutation in starting strain.
        return MUTATION_TYPE__DE_NOVO

    # Default. Meets no interesting condition.
    return MUTATION_TYPE__NONE

melted_variant_data_df['MUTATION_TYPE'] = melted_variant_data_df.apply(
        determine_mutation_type, axis=1)

# Add column indicating signal relative to starting strain, either 0 or 1.
# These are either reversions, or de novo mutations.
# Excludes ambers, or weak evidence mutations.
melted_variant_data_df['signal_relative_to_C321'] = (
        melted_variant_data_df['MUTATION_TYPE'].apply(
                lambda mt: mt in [
                        MUTATION_TYPE__FIXED,
                        MUTATION_TYPE__DE_NOVO,
                        MUTATION_TYPE__UNTARGETED_REVERSION
                ])).astype(int)
```

Visually inspect the ones that claim to be amber reversions.

```
In [20]: melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] ==
                         MUTATION_TYPE__AMBER_REVERSION].drop_duplicates('POSITION')
```

```
Out[20]:              UID  POSITION CHROMOSOME REF  ALT BARCODE  \
         5806    a855d089   1632334   U00096.2   C  NaN  CCGTGA
         166581  f69c77fe   1286207   U00096.2   G    A  CCAACC

                                                        INFO_EFF   GT IS_HET  GQ  \
         5806    [u'splice_region_variant+stop_retained_variant...  NaN    NaN NaN
         166581  [u'intergenic_region(MODIFIER|||n.1286207G>A||...  0/1   True NaN

                           ...             pos rc_barcode sample  doubling_time  \
         5806              ...             B12     TCACGG  05-07      60.662941
         166581            ...             G02     GGTTGG  03-02      73.378095

                 lineage  time_point actual_mage_cycle  original_GT_TYPE  \
         5806          5           7                43               NaN
         166581        3           2                19               1.0

                   MUTATION_TYPE  signal_relative_to_C321
         5806    AMBER_REVERSION                        0
         166581  AMBER_REVERSION                        0

         [2 rows x 52 columns]
```

Visual inspection confirms these are really there, just weren't called correctly by Freebayes. Correct these manually.

```
In [21]: melted_variant_data_df['MUTATION_TYPE'] = (
                 melted_variant_data_df['MUTATION_TYPE'].apply(
                         lambda mt: mt if mt != MUTATION_TYPE__AMBER_REVERSION else MUTATION_TYP
```

**Generate useful sets of (POSITION, REF) for fixed and de novo variants.**

```
In [22]: amber_variants_set = set(melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] == MUTATION_TYPE__AMBER].apply(
                         get_variant_key_for_df_row, axis=1))
         print len(amber_variants_set)
         assert NUM_AMBER_SNPS == len(amber_variants_set) # catch data changes

         amber_variants_df = melted_variant_data_df[
                 melted_variant_data_df.apply(
                         lambda row: get_variant_key_for_df_row(row) in
                                 amber_variants_set, axis=1)]
         print len(amber_variants_df)
```

```
318
30528
```

```
In [23]: # Identify fixed variants going relative to starting strain.
         variants_fixed_at_least_once = set(melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] == 'FIXED'].apply(
                         get_variant_key_for_df_row, axis=1))
         print len(variants_fixed_at_least_once)
         assert 99 == len(variants_fixed_at_least_once) # catch data changes

         variants_fixed_at_least_once_df = melted_variant_data_df[
                 melted_variant_data_df.apply(
                         lambda row: get_variant_key_for_df_row(row) in
                                 variants_fixed_at_least_once, axis=1)]
         print len(variants_fixed_at_least_once_df)
```

```
99
9527
```

Inspect the ones never observed.

```
In [24]: unobserved_design_versions_set = (
                 designed_variant_pos_ref_tuple_set -
                 MANUALLY_CONFIRMED_IGNORE_DESIGN_SET -
                 variants_fixed_at_least_once)
         designed_set_df[
                 designed_set_df.apply(
                         lambda row: get_variant_key_for_df_row(row) in unobserved_design_versio
                         axis=1)]
```

```
Out[24]:      PRIORITY_SET  PRIORITY_INDIVIDUAL  POSITION EFF_GENE   ALT      REF
         34              2                   35    162927     hrpB     A        G
         43              3                   44   1286375     rttR     C        T
         54              4                   55    280883     yagA    CG      CGG
         59              4                   60      7922     yaaJ  CAAA      CAA
         63              4                   64   1207756     tfaE    TA      TCA
         69              4                   70   1005635     ycbW    TG      TGG
         70              4                   71   4509169     fecE     T        C
         73              4                   74   1755775     ynhG    CT  CTCTGGT
         75              4                   76   3506472      php    GC      GCC
         85              4                   86   3181028     zupT     A        G
         88              4                   89   4322899     phnC     G        A
         92              5                   93   1072955     rutA     T        C
         98              5                   99   1448416     tynA     T        C
         103             5                  104   3338610     yrbG     C        T
         107             5                  108   2619329     purM     G        A
         108             5                  109   3306890     nlpI     A        G
```

```
121              5                  122   2272188     yejA     G        A
125              5                  126   2476114     dsdX     G        A
```

```
In [25]: untargeted_reversions_set = set(melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] == 'UNTARGETED_REVERSION'].apply(
                         get_variant_key_for_df_row, axis=1))
         print len(untargeted_reversions_set)

         untargeted_reversions_df = melted_variant_data_df[
                 melted_variant_data_df.apply(
                         lambda row: get_variant_key_for_df_row(row) in
                                 untargeted_reversions_set, axis=1)]
```

```
6
```

Inspected these by eye and most appear to be tough to call (e.g. homopolymers or paralogous regions). Only 4617936 is a solid call, but it happens in a single clone at lineage 5 timepoint 7 so it is difficult to infer anything meaningful.

```
In [26]: de_novo_variants = set(melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] == 'DE_NOVO'].apply(
                         get_variant_key_for_df_row, axis=1).values)
         print len(de_novo_variants)

         de_novo_variatns_df = melted_variant_data_df[
                 melted_variant_data_df.apply(
                         lambda row: get_variant_key_for_df_row(row) in
                                 de_novo_variants, axis=1)]
         print len(de_novo_variatns_df)
```

```
1336
128066
```

What about the remaining variants? These are neither in starting set, fixed, nor de novo.

```
In [27]: def is_variant_unaccounted_for(row):
             key = get_variant_key_for_df_row(row)
             return not (
                     key in de_novo_variants or
                     key in variants_in_starting_strain_set)
         unaccounted_for_variants_df = melted_variant_data_df[
                 melted_variant_data_df.apply(is_variant_unaccounted_for, axis=1)]

         unaccounted_for_variants_position_set = set(unaccounted_for_variants_df['POSITION'])
         print 'df size', len(unaccounted_for_variants_df)
         print 'unique variant positions', len(unaccounted_for_variants_position_set)
```

```
        print '# GT_TYPE=0', len(unaccounted_for_variants_df[unaccounted_for_variants_df['GT_TY
        print '# GT_TYPE=1', len(unaccounted_for_variants_df[unaccounted_for_variants_df['GT_TY
        print '# GT_TYPE=2', len(unaccounted_for_variants_df[unaccounted_for_variants_df['GT_TY
        assert len(unaccounted_for_variants_df[unaccounted_for_variants_df['GT_TYPE'] == 2]) ==
```

```
df size 25248
unique variant positions 263
# GT_TYPE=0 24105
# GT_TYPE=1 1143
# GT_TYPE=2 0
```

These are never GT_TYPE = 2 and mostly GT_TYPE = 0, so it appears that they are due to noise.

Thus it looks like that we've accounted for all variants. The remaining ones were all het or homo ref. A few sanity checks and useful data structures

```
In [28]: # Make sure all SNP positions are in some category.
         assert NUM_SNPS_CALLED == (len(de_novo_variants) +
                 len(variants_in_starting_strain_set) +
                 len(unaccounted_for_variants_position_set))

         SIGNAL_SNP_POSITIONS_SET = (
                 set([x[0] for x in de_novo_variants]) |
                 set([x[0] for x in variants_fixed_at_least_once]) |
                 set([x[0] for x in untargeted_reversions_set])
         )
         assert len(SIGNAL_SNP_POSITIONS_SET) == (
                 len(de_novo_variants) +
                 len(variants_fixed_at_least_once) +
                 len(untargeted_reversions_set))
         NUM_SIGNAL_SNPS = len(SIGNAL_SNP_POSITIONS_SET)

         # Sanity check: Another way of calculating that. Should match.
         assert NUM_SIGNAL_SNPS == len(
                 set(melted_variant_data_df[melted_variant_data_df['signal_relative_to_C321'] ==

         print 'de novo variants', len(de_novo_variants)
         print 'variants in starting strain', len(variants_in_starting_strain_set)
         print 'NUM_SIGNAL_SNPS', NUM_SIGNAL_SNPS
```

```
de novo variants 1336
variants in starting strain 651
NUM_SIGNAL_SNPS 1441
```

**Create a data structure that has annotation data only, useful for annotating model data.**

```
In [29]: METADATA_FIELDS = [
             'POSITION',
             'REF',
             'ALT',
             'MUTATION_TYPE',
             'INFO_EFF_GENE',
             'INFO_EFF_IMPACT',
             'INFO_EFF_AA',
             'GT_TYPE'
         ]

         variant_data_annotated_only_df = (
                 melted_variant_data_df[
                         (melted_variant_data_df['POSITION'].apply(
                                 lambda p: p in SIGNAL_SNP_POSITIONS_SET)) &
                         (melted_variant_data_df['GT_TYPE'].apply(lambda gt: gt in (1, 2)))])
         variant_data_annotated_only_df = (
                 variant_data_annotated_only_df.drop_duplicates(['POSITION', 'REF']))
         variant_data_annotated_only_df = variant_data_annotated_only_df[METADATA_FIELDS]

         # Add occurrence counts for these SNPS.
         positive_signal_df = melted_variant_data_df[
             melted_variant_data_df['signal_relative_to_C321'] == 1]
         counts_per_position_series = positive_signal_df['POSITION'].value_counts()
         counts_per_position_df = counts_per_position_series.to_frame(name='count')
         counts_per_position_df['POSITION'] = counts_per_position_df.index
         variant_data_annotated_only_df = pd.merge(
             variant_data_annotated_only_df,
             counts_per_position_df,
             how='left',
             on='POSITION')

         # Fix MUTTION_TYPE. The reason these may be broken is that
         # variant_data_annotated_only_df was made by
         # dropping duplicates by position and ref, which might not
         # have included a row of the appropriate GT_TYPE to have the
         # right annotation.
         def fix_mutation_type(row):
             variant_key = get_variant_key_for_df_row(row)
             if variant_key in variants_fixed_at_least_once:
                 return MUTATION_TYPE__FIXED
             elif variant_key in untargeted_reversions_set:
                 return MUTATION_TYPE__UNTARGETED_REVERSION
             elif variant_key in de_novo_variants:
                 return MUTATION_TYPE__DE_NOVO
             else:
                 return MUTATION_TYPE__NONE

         variant_data_annotated_only_df['MUTATION_TYPE'] = (
                 variant_data_annotated_only_df.apply(
                         fix_mutation_type, axis=1))

         # Assert all accounted for.
         assert not len(variant_data_annotated_only_df[
                 variant_data_annotated_only_df['MUTATION_TYPE'] == MUTATION_TYPE__NONE])

         assert len(variant_data_annotated_only_df) == len(
                 set(variant_data_annotated_only_df['POSITION']))
         # assert len(variant_data_annotated_only_df) == NUM_SIGNAL_SNPS
```

```
In [30]: print 'FIXED', len(
                variant_data_annotated_only_df[
                        variant_data_annotated_only_df['MUTATION_TYPE'] ==
                                MUTATION_TYPE__FIXED])

         print 'DE NOVO', len(
                variant_data_annotated_only_df[
                        variant_data_annotated_only_df['MUTATION_TYPE'] ==
                                MUTATION_TYPE__DE_NOVO])
```

```
FIXED 99
DE NOVO 1336
```

### 1.4 Analyze Mutation Dynamics

Look at dynamics of how mutations arise in the population.

First, for each sample, count the number of de novo and reversion mutations.

```
In [31]: per_clone_mutation_counts_df = experiment_metadata_df[[
                 'pos', 'BARCODE', 'sample', 'doubling_time', 'lineage', 'time_point', 'actual_m

         def get_mutation_counts(barcode):
             return melted_variant_data_df[
                 melted_variant_data_df['BARCODE'] == barcode]['MUTATION_TYPE'].value_counts()
         per_clone_mutation_counts_df = per_clone_mutation_counts_df.join(
                 per_clone_mutation_counts_df['BARCODE'].apply(get_mutation_counts))

         # Add HET counts.
         def get_orig_het_counts(barcode):
             return len(melted_variant_data_df[
                 (melted_variant_data_df['BARCODE'] == barcode) &
                 (melted_variant_data_df['original_GT_TYPE'] == 1)])
         per_clone_mutation_counts_df['ORIG_HET_COUNT'] = (
                 per_clone_mutation_counts_df['BARCODE'].apply(
                         get_orig_het_counts))

         def get_updated_het_counts(barcode):
             return len(melted_variant_data_df[
                 (melted_variant_data_df['BARCODE'] == barcode) &
                 (melted_variant_data_df['GT_TYPE'] == 1)])
         per_clone_mutation_counts_df['UPDATED_HET_COUNT'] = (
                 per_clone_mutation_counts_df['BARCODE'].apply(
                         get_updated_het_counts))

         # Add a column showing average rate of FIXED mutations.
         per_clone_mutation_counts_df['FIXED_per_MAGE_cycle'] = (
                 per_clone_mutation_counts_df[MUTATION_TYPE__FIXED] / per_clone_mutation_counts_

         # Add a column showing average rate of de novo mutations.
         per_clone_mutation_counts_df['DE_NOVO_per_MAGE_cycle'] = (
                 per_clone_mutation_counts_df[MUTATION_TYPE__DE_NOVO] / per_clone_mutation_count

         # Add a column showing ratio of de novo vs fixed.
         per_clone_mutation_counts_df['DE_NOVO_to_FIXED_ratio'] = (
                 per_clone_mutation_counts_df['DE_NOVO'] / per_clone_mutation_counts_df['FIXED']

         # Inspect one of these.
         per_clone_mutation_counts_df[per_clone_mutation_counts_df['sample'] == '01-01']
```

```
Out[31]:     pos BARCODE sample  doubling_time  lineage  time_point  actual_mage_cycle  \
        0    A01  AACCTG  01-01      78.331670        1           1                  5
        12   B01  CACCGT  01-01      77.343979        1           1                  5
        24   C01  AGTAAT  01-01      76.202194        1           1                  5
        36   D01  GCGCTA  01-01      71.441390        1           1                  5

             AMBER  DE_NOVO  FIXED    NONE  UNTARGETED_REVERSION  ORIG_HET_COUNT  \
        0    318.0      NaN    NaN  1930.0                   NaN              43
        12   318.0      6.0    1.0  1923.0                   NaN              21
        24   318.0      2.0    NaN  1928.0                   NaN              31
        36   318.0      3.0    1.0  1926.0                   NaN              34

             UPDATED_HET_COUNT  FIXED_per_MAGE_cycle  DE_NOVO_per_MAGE_cycle  \
        0                   30                   NaN                     NaN
        12                  11                   0.2                     1.2
        24                  17                   NaN                     0.4
        36                  25                   0.2                     0.6

             DE_NOVO_to_FIXED_ratio
        0                       NaN
        12                      6.0
        24                      NaN
        36                      3.0
```

Drop samples with excessive HET counts. We found these HET counts to be significantly different than the rest of the wells. These likely suffered cross-contamination. This is why we describe 90 samples, not 96.

```
In [32]: high_het_barcodes_set = set(
                 per_clone_mutation_counts_df[
                         per_clone_mutation_counts_df['UPDATED_HET_COUNT'] > 30]['BARCODE'])
         print 'NUM HIGH HET BARCODES', len(high_het_barcodes_set)

         per_clone_mutation_counts_df = per_clone_mutation_counts_df[
                 per_clone_mutation_counts_df['BARCODE'].apply(
                         lambda b: b not in high_het_barcodes_set)]

         melted_variant_data_df = melted_variant_data_df[
                 melted_variant_data_df['BARCODE'].apply(
                         lambda b: b not in high_het_barcodes_set)]
```

```
NUM HIGH HET BARCODES 6
```

Aggregate by sample.

```
In [33]: aggregate_per_sample_mutation_counts_df = per_clone_mutation_counts_df.groupby('sample'
             'sample': {
                 'count': 'count'
             },
             'lineage': 'first',
             'time_point': 'first',
             'actual_mage_cycle': 'first',
             'doubling_time': {
                 'mean': 'mean',
                 'stdev': 'std'
             },
             'FIXED': {
                 'mean': 'mean',
                 'stdev': 'std'
             },
             'DE_NOVO': {
                 'mean': 'mean',
                 'stdev': 'std'
             },
             'UNTARGETED_REVERSION': {
                 'mean': 'mean',
                 'stdev': 'std'
             },
             'AMBER': {
                 'mean': 'mean',
                 'stdev': 'std'
             },
         })[['sample', 'lineage', 'time_point', 'actual_mage_cycle', 'doubling_time',
                 'FIXED', 'DE_NOVO', 'UNTARGETED_REVERSION', 'AMBER']]

         aggregate_per_sample_mutation_counts_df
```

```
Out[33]:        sample lineage time_point actual_mage_cycle doubling_time            \
                 count   first      first             first         stdev       mean
        sample
        01-01        4       1          1                 5      3.052262  75.829808
        01-02        3       1          2                14      2.871387  71.789395
        01-03        3       1          3                18      3.999653  73.915334
        01-04        4       1          4                20      4.001593  70.207219
        01-05        4       1          5                25      2.802409  65.267806
        01-06        4       1          6                31      3.068573  64.561708
        01-07        4       1          7                40      2.454961  62.669426
        03-01        4       3          1                 5      0.503411  82.107917
        03-02        4       3          2                19      2.479869  70.254887
        03-03        4       3          3                26      6.640756  66.323141
        03-04        4       3          4                28      6.990662  67.893865
        03-05        4       3          5                34      4.505766  62.819313
        03-06        4       3          6                40      2.586030  66.807928
        03-07        7       3          7                49      2.666364  58.299511
        05-01        4       5          1                11      4.179874  79.542669
        05-02        4       5          2                16      1.968539  72.614709
        05-03        4       5          3                21      4.830638  71.136283
        05-04        4       5          4                23      5.051717  73.612972
        05-05        3       5          5                28      1.892164  59.461666
        05-06        4       5          6                34      3.805866  62.951005
        05-07        7       5          7                43      1.877327  61.308703
        C321_I4      3       0          0                 0      2.402853  87.006543

                   FIXED               DE_NOVO            UNTARGETED_REVERSION       \
                   stdev       mean      stdev       mean                stdev mean
        sample
        01-01   0.000000   1.000000   2.081666   3.666667                  NaN  NaN
        01-02   0.577350   2.333333   2.516611   9.333333                  NaN  1.0
        01-03   3.055050   4.333333   1.154701  13.666667                  NaN  NaN
        01-04   3.403430   5.250000   2.500000  19.750000                  NaN  1.0
        01-05   1.732051   7.500000   3.403430  26.250000                  NaN  1.0
        01-06   0.957427   9.250000   3.095696  28.250000             0.000000  1.0
        01-07   2.160247  10.000000   5.123475  35.750000                  NaN  1.0
        03-01   0.000000   2.000000   2.753785   4.750000                  NaN  NaN
        03-02   2.943920   6.000000   5.560276  17.750000                  NaN  NaN
        03-03   4.573474   8.750000   1.707825  21.250000             0.000000  1.0
        03-04   4.924429   7.750000   1.414214  21.000000             0.000000  1.0
        03-05   4.509250   7.500000  12.311918  29.250000             0.577350  1.5
        03-06   2.943920   8.000000   8.883505  34.250000             0.000000  1.0
        03-07   2.225395   8.428571   7.967195  35.142857             0.894427  1.6
        05-01   1.892969   3.250000   2.645751   9.500000             0.000000  1.0
        05-02   1.825742  10.000000   4.434712  18.500000             0.707107  1.5
        05-03   1.707825   7.250000   2.828427  19.000000                  NaN  1.0
        05-04   1.258306   7.250000   4.898979  20.000000                  NaN  1.0
        05-05   2.000000   8.000000   1.732051  31.000000             0.000000  1.0
```

```
05-06    4.856267  11.750000   1.632993  33.000000              0.000000  1.0
05-07    4.041452  12.000000   8.202787  37.571429              1.732051  2.0
C321_I4       NaN        NaN   0.577350   2.333333                   NaN  NaN

        AMBER
        stdev   mean
sample
01-01     0.0  318.0
01-02     0.0  318.0
01-03     0.0  318.0
01-04     0.0  318.0
01-05     0.0  318.0
01-06     0.0  318.0
01-07     0.0  318.0
03-01     0.0  318.0
03-02     0.0  318.0
03-03     0.0  318.0
03-04     0.0  318.0
03-05     0.0  318.0
03-06     0.0  318.0
03-07     0.0  318.0
05-01     0.0  318.0
05-02     0.0  318.0
05-03     0.0  318.0
05-04     0.0  318.0
05-05     0.0  318.0
05-06     0.0  318.0
05-07     0.0  318.0
C321_I4   0.0  318.0
```

Plot mutations vs time for each lineage.

```
In [34]: LINEAGES = [1, 3, 5]

         TIME_POINTS = sorted(list(set(experiment_metadata_df['time_point'])))

         LINE_STYLES = ['bo-', 'go-', 'ro-']

         LINEAGE_NAMES = ['pool 1', 'pool 3', 'pool 5']


         def get_mutation_count_timeseries(lineage, mutation_type):
             mage_cycle_list = []
             mean_list = []
             std_list = []
             for time_point in TIME_POINTS:
                 if time_point == 0:
                     if mutation_type == 'doubling_time':
```

```
                mage_cycle_list.append(0)
                mean_list.append(C321_I4_DT)
                std_list.append(0)
            else:
                mage_cycle_list.append(0)
                mean_list.append(0)
                std_list.append(0)
            continue
        time_point_match = aggregate_per_sample_mutation_counts_df[
            (aggregate_per_sample_mutation_counts_df['time_point']['first'] == time_poi
            (aggregate_per_sample_mutation_counts_df['lineage']['first'] == lineage)
        ]
        mage_cycle_list.append(time_point_match['actual_mage_cycle'].values[0])
        mean_list.append(time_point_match[mutation_type]['mean'].values[0])
        std_list.append(time_point_match[mutation_type]['stdev'].values[0])
    return mage_cycle_list, mean_list, std_list

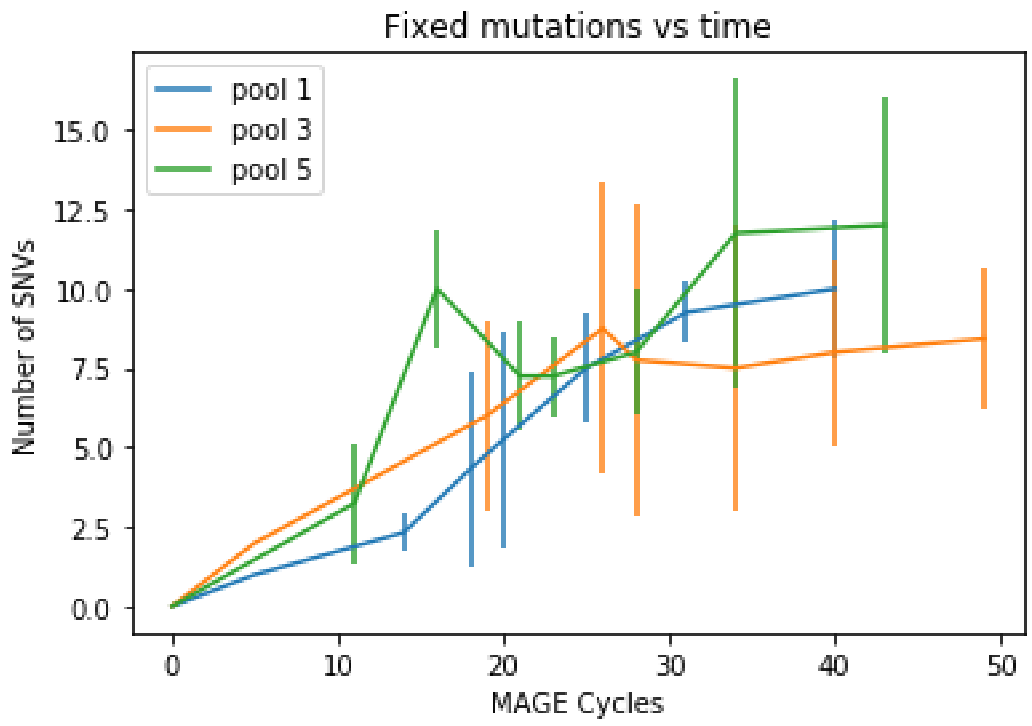

def plot_lineage(lineage, mutation_type, line_style):
    """Plot a single lineage.
    """
    mage_cycle_list, mean_list, std_list = get_mutation_count_timeseries(
            lineage, mutation_type)
    return plt.errorbar(
            mage_cycle_list, mean_list, yerr=std_list)

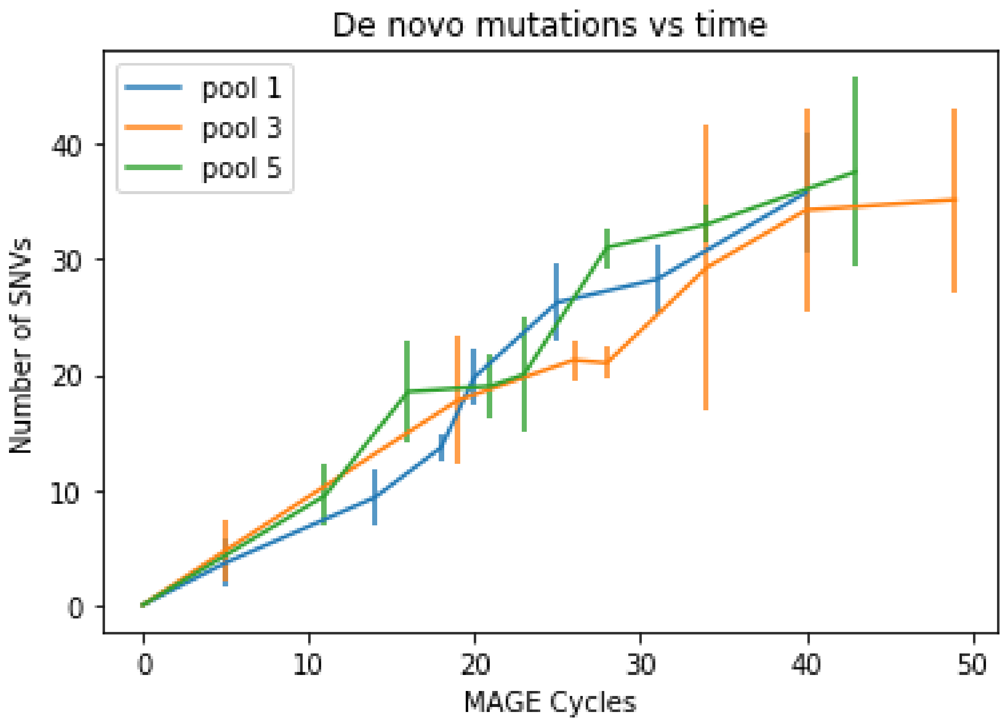

def plot_lineage_comparison(
        title, mutation_type, ylabel='Number of SNVs'):
    """Plots the 3 lineages together.
    """
    plt.figure()

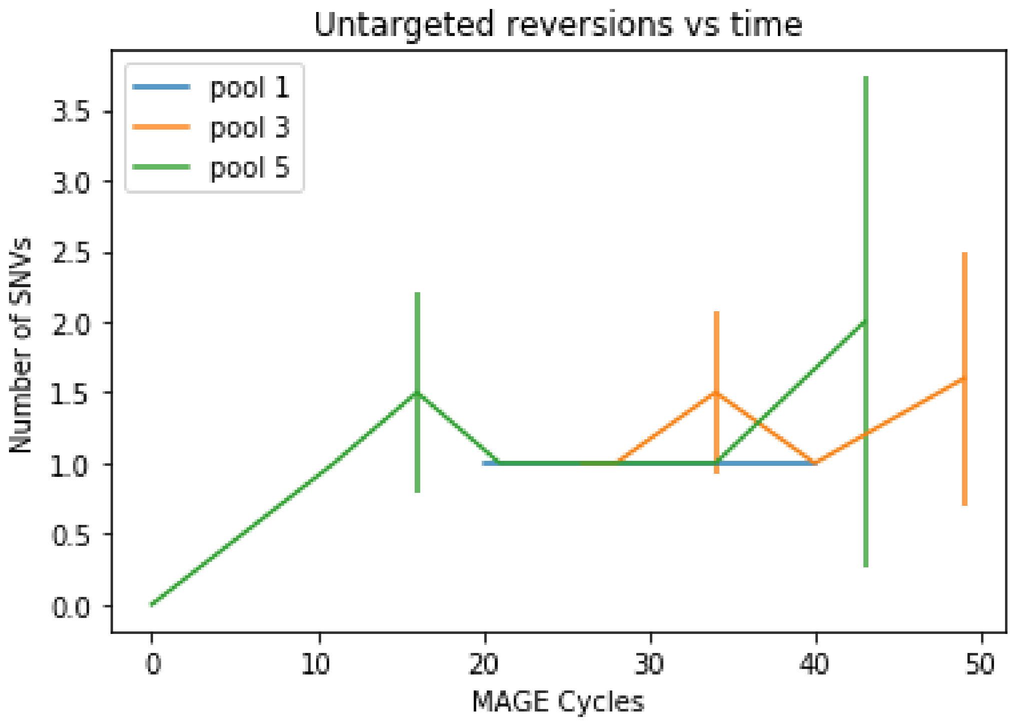
    lineage_plots = []
    for lineage, line_style in zip(LINEAGES, LINE_STYLES):
        lineage_plots.append(plot_lineage(lineage, mutation_type, line_style)[0])

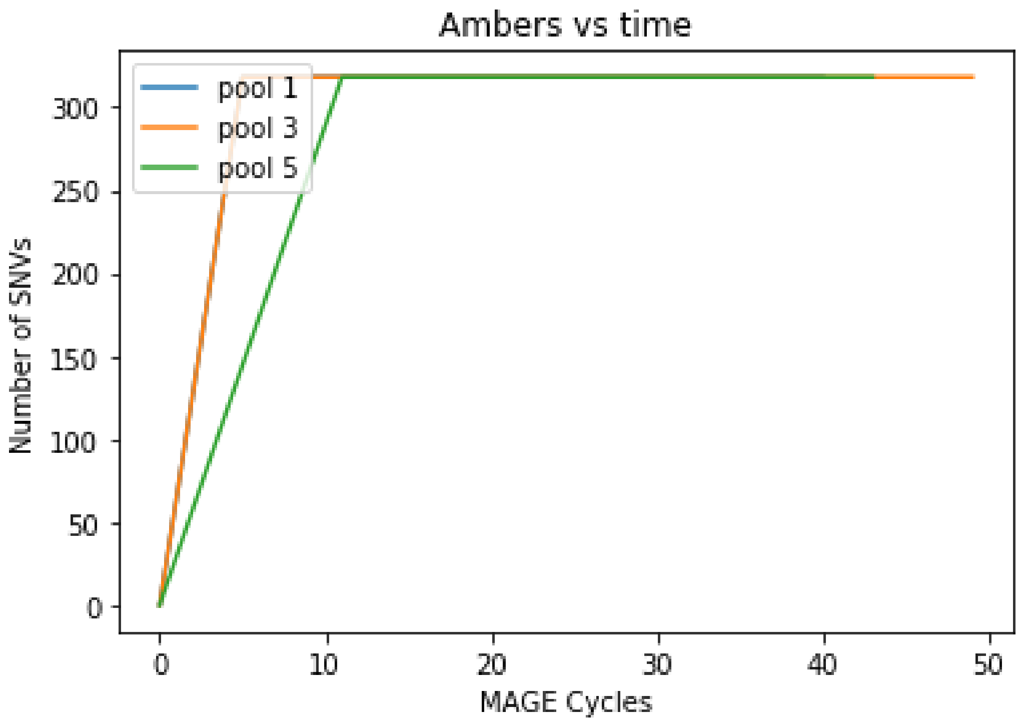
    plt.title(title)
    plt.xlabel('MAGE Cycles')
    plt.ylabel(ylabel)
    plt.legend(lineage_plots, LINEAGE_NAMES, numpoints=1, loc=2)

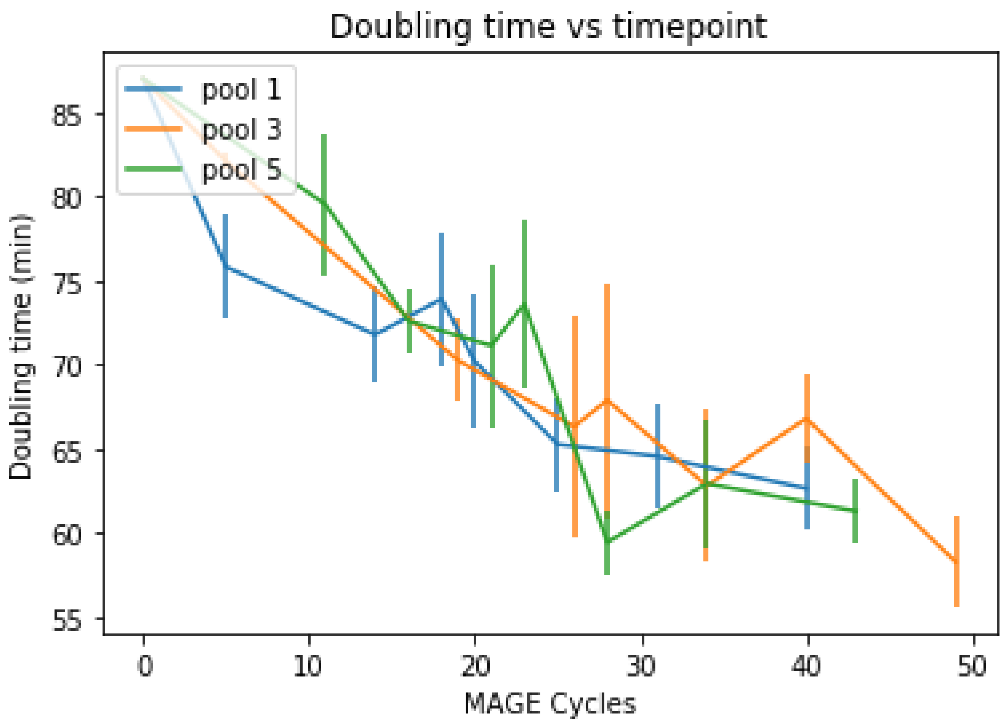
plot_lineage_comparison('Fixed mutations vs time', MUTATION_TYPE__FIXED)
plot_lineage_comparison('De novo mutations vs time', MUTATION_TYPE__DE_NOVO)
plot_lineage_comparison('Untargeted reversions vs time', MUTATION_TYPE__UNTARGETED_REVE
plot_lineage_comparison('Ambers vs time', MUTATION_TYPE__AMBER)
plot_lineage_comparison('Doubling time vs timepoint', 'doubling_time', ylabel='Doubling
```


Fixed mutations vs time
pool 1
pool 3
pool 5
Number of SNVs
0.0
2.5
5.0
7.5
10.0
12.5
15.0
0
10
20
30
40
50
MAGE Cycles


De novo mutations vs time
pool 1
pool 3
pool 5
Number of SNVs
0
10
20
30
40
0
10
20
30
40
50
MAGE Cycles


Untargeted reversions vs time
pool 1
pool 3
pool 5
Number of SNVs
MAGE Cycles
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
10
20
30
40
50


Ambers vs time
pool 1
pool 3
pool 5
Number of SNVs
MAGE Cycles
0
50
100
150
200
250
300
0
10
20
30
40
50



Plot individual counts, not averages.

```
In [35]: # We will still separate by lineage to give us
         # the option of coloring the points differently.

         def get_lineage_timeseries(lineage=None):
             mage_cycle_ordered_list = []
             doubling_time_ordered_list = []
             lineage_data_df = per_clone_mutation_counts_df[
                     per_clone_mutation_counts_df['lineage'] == lineage]
             return {
                 'mage_cycle_ordered_list': np.array(lineage_data_df['actual_mage_cycle']),
                 'doubling_time_ordered_list': np.array(lineage_data_df['doubling_time']),
                 'fixed_mutation_ordered_list': np.array(lineage_data_df[MUTATION_TYPE__FIXED]),
                 'de_novo_mutation_ordered_list': np.array(lineage_data_df[MUTATION_TYPE__DE_NOV
                 'de_novo_to_fixed_ratio': np.array(lineage_data_df['DE_NOVO_to_FIXED_ratio'])
             }

         lineage_0_data = get_lineage_timeseries(0)
         lineage_1_data = get_lineage_timeseries(1)
         lineage_3_data = get_lineage_timeseries(3)
         lineage_5_data = get_lineage_timeseries(5)
```

Plot doubling times.

```
In [36]: plt.figure()

         plt.plot(
                 lineage_1_data['mage_cycle_ordered_list'],
                 lineage_1_data['doubling_time_ordered_list'],
                 '.')

         plt.plot(
                 lineage_3_data['mage_cycle_ordered_list'],
                 lineage_3_data['doubling_time_ordered_list'],
                 'o')

         plt.plot(
                 lineage_5_data['mage_cycle_ordered_list'],
                 lineage_5_data['doubling_time_ordered_list'],
                 'x')
         plt.plot(
                 lineage_0_data['mage_cycle_ordered_list'],
                 lineage_0_data['doubling_time_ordered_list'],
                 '.')

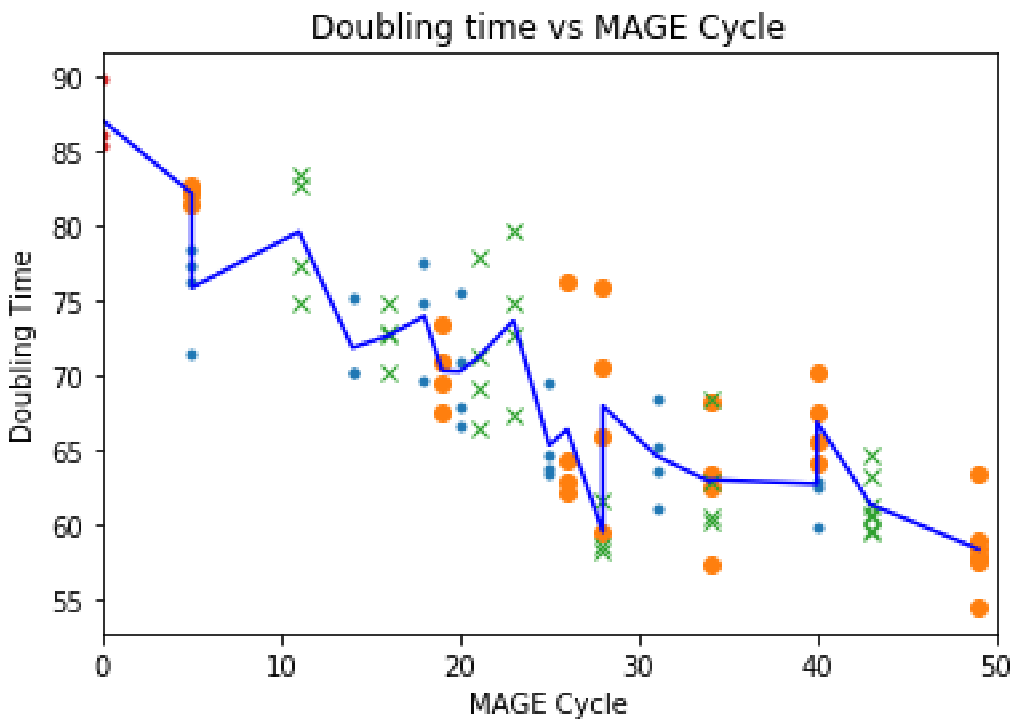
         # Plot mean.
         # Grab all mage cycles and means.
         # Lack of pandas skillz makes me do it the brute force way for now.
         data_obj_list = []
         for idx, row in aggregate_per_sample_mutation_counts_df.iterrows():
             x = row
             data_obj_list.append({
                 'lineage': x['lineage']['first'],
                 'actual_mage_cycle': x['actual_mage_cycle']['first'],
                 'doubling_time_mean': row['doubling_time']['mean'],
                 'doubling_time_stdev': row['doubling_time']['stdev']
             })
         mean_doubling_times_df = pd.DataFrame(data_obj_list)
         mean_doubling_times_df.sort_values('actual_mage_cycle', inplace=True)

         # Plot mean.
         plt.plot(
                 mean_doubling_times_df['actual_mage_cycle'],
                 mean_doubling_times_df['doubling_time_mean'], 'b')

         # # Plot stdev above and below.
         # stdev_above = (
         #         mean_doubling_times_df['doubling_time_mean'] +
         #         mean_doubling_times_df['doubling_time_stdev'])
         # plt.plot(
         #         mean_doubling_times_df['actual_mage_cycle'],
         #         stdev_above)
         # stdev_below = (
         #         mean_doubling_times_df['doubling_time_mean'] -
         #         mean_doubling_times_df['doubling_time_stdev'])
         # plt.plot(
         #         mean_doubling_times_df['actual_mage_cycle'],
         #         stdev_below)

         # plt.axhline(y=C321_FIX_DT)

         plt.title('Doubling time vs MAGE Cycle')
         plt.xlabel('MAGE Cycle')
         plt.ylabel('Doubling Time')
         plt.xlim([0, 50])
         plt.show()
```



Similarly, plot individual mutation counts rather than averages.

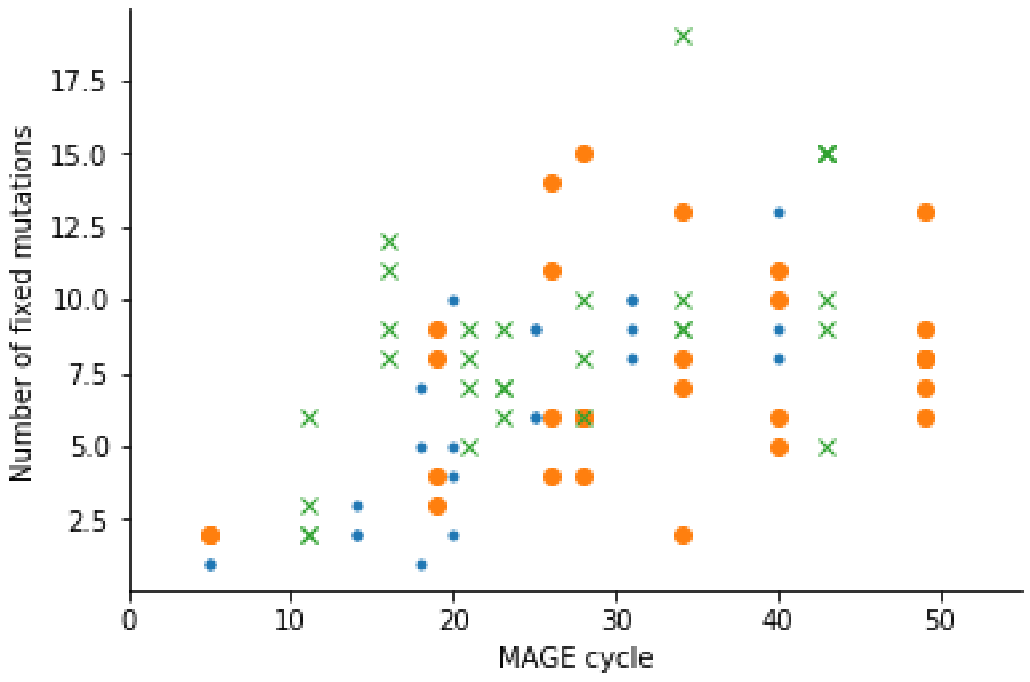
```
In [37]: def plot_mutation_list(lineage_data, mutation_key, style):
             plt.plot(
                     lineage_data['mage_cycle_ordered_list'],
                     lineage_data[mutation_key],
                     style)
```

Fixed mutations.

```
In [38]: plt.figure()
         plot_mutation_list(lineage_1_data, 'fixed_mutation_ordered_list', '.')
         plot_mutation_list(lineage_3_data, 'fixed_mutation_ordered_list', 'o')
         plot_mutation_list(lineage_5_data, 'fixed_mutation_ordered_list', 'x')
         # plot_mutation_list(lineage_0_data, 'fixed_mutation_ordered_list', '.')
         # plt.title('Fixed mutations vs MAGE Cycle')
         plt.xlabel('MAGE cycle')
         plt.ylabel('Number of fixed mutations')

         ax = plt.axes()
         ax.set_xlim([0, 55])
         ax.spines['top'].set_visible(False)
         ax.spines['right'].set_visible(False)
         ax.get_xaxis().tick_bottom()
         ax.get_yaxis().tick_left()

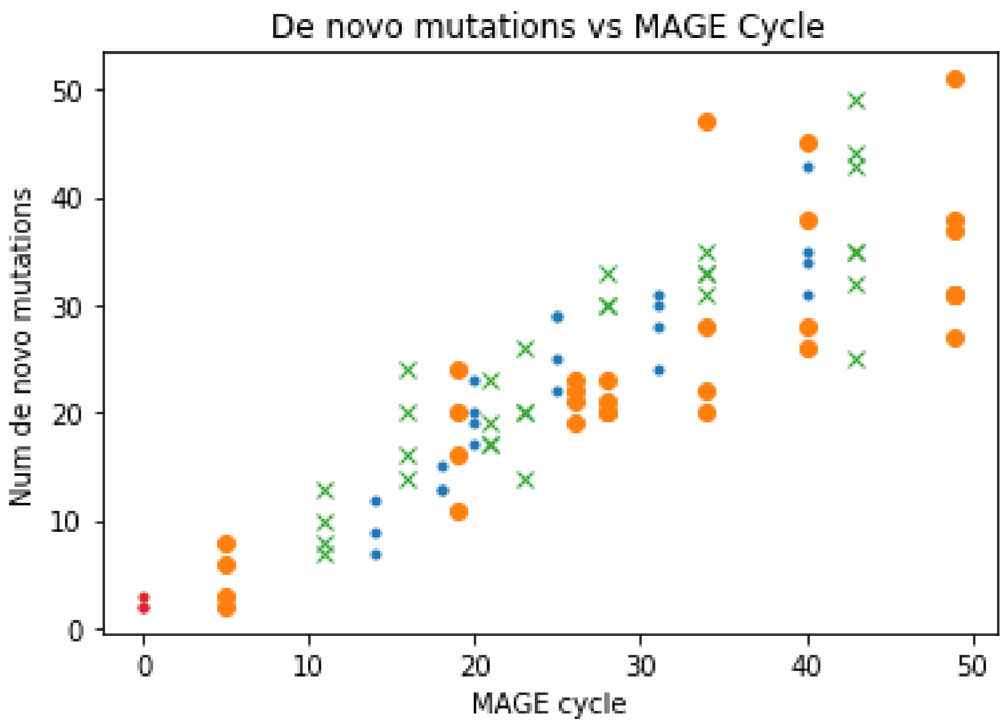
         plt.show()
```



De novo mutations.

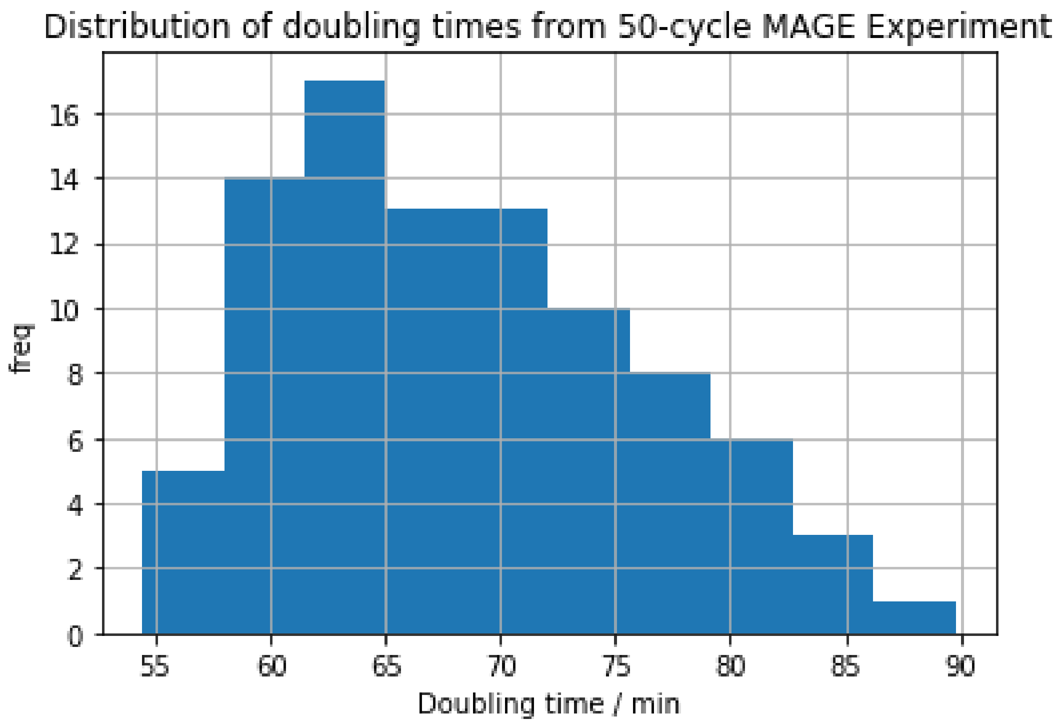
```
In [39]: plt.figure()
         plot_mutation_list(lineage_1_data, 'de_novo_mutation_ordered_list', '.')
         plot_mutation_list(lineage_3_data, 'de_novo_mutation_ordered_list', 'o')
         plot_mutation_list(lineage_5_data, 'de_novo_mutation_ordered_list', 'x')
         plot_mutation_list(lineage_0_data, 'de_novo_mutation_ordered_list', '.')
```

```
plt.title('De novo mutations vs MAGE Cycle')
plt.xlabel('MAGE cycle')
plt.ylabel('Num de novo mutations')
plt.show()
```

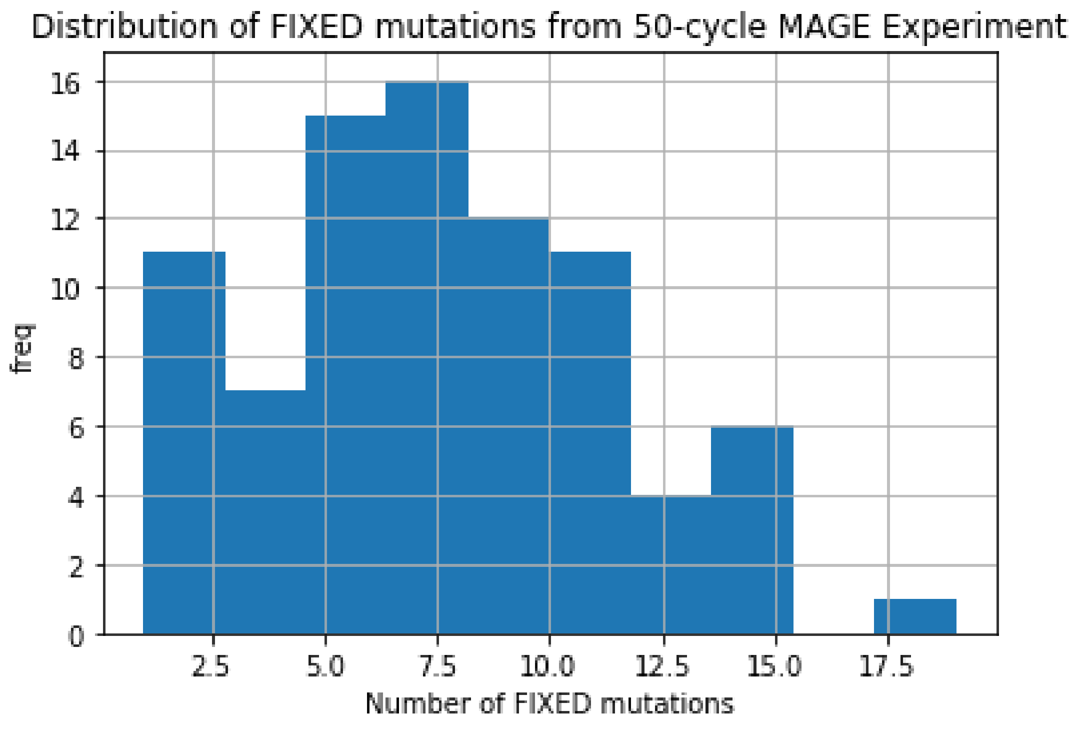


Curiously we see some clones with relatively few de novo mutations and others with many.

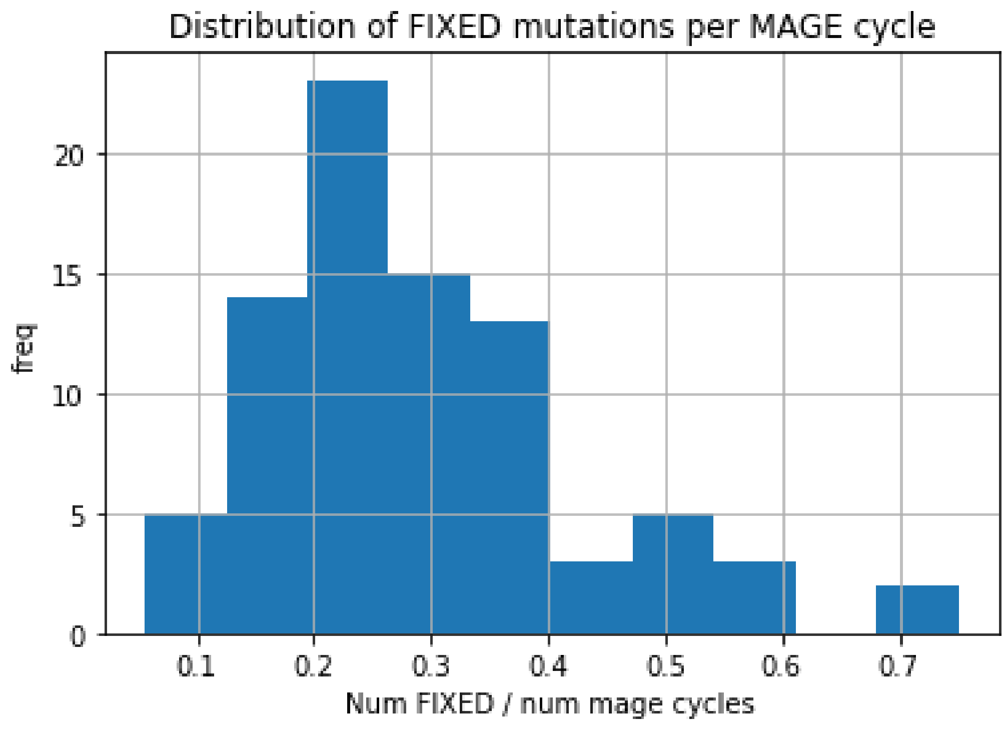
```
In [40]: plt.figure()
         per_clone_mutation_counts_df['doubling_time'].hist()
         plt.title('Distribution of doubling times from 50-cycle MAGE Experiment')
         plt.xlabel('Doubling time / min')
         plt.ylabel('freq')
         plt.show()
```



```
In [41]: plt.figure()
         per_clone_mutation_counts_df[MUTATION_TYPE__FIXED].hist()
         plt.title('Distribution of FIXED mutations from 50-cycle MAGE Experiment')
         plt.xlabel('Number of FIXED mutations')
         plt.ylabel('freq')
         plt.show()
```



```
In [42]: plt.figure()
         per_clone_mutation_counts_df[
                 per_clone_mutation_counts_df['actual_mage_cycle'] > 0][
                         'FIXED_per_MAGE_cycle'].hist()
         plt.title('Distribution of FIXED mutations per MAGE cycle')
         plt.xlabel('Num FIXED / num mage cycles')
         plt.ylabel('freq')
         plt.show()
```



What are doubling times of clones with most FIXED mutations?

```
In [43]: per_clone_mutation_counts_df.sort_values(MUTATION_TYPE__FIXED, ascending=False)[:5]
```

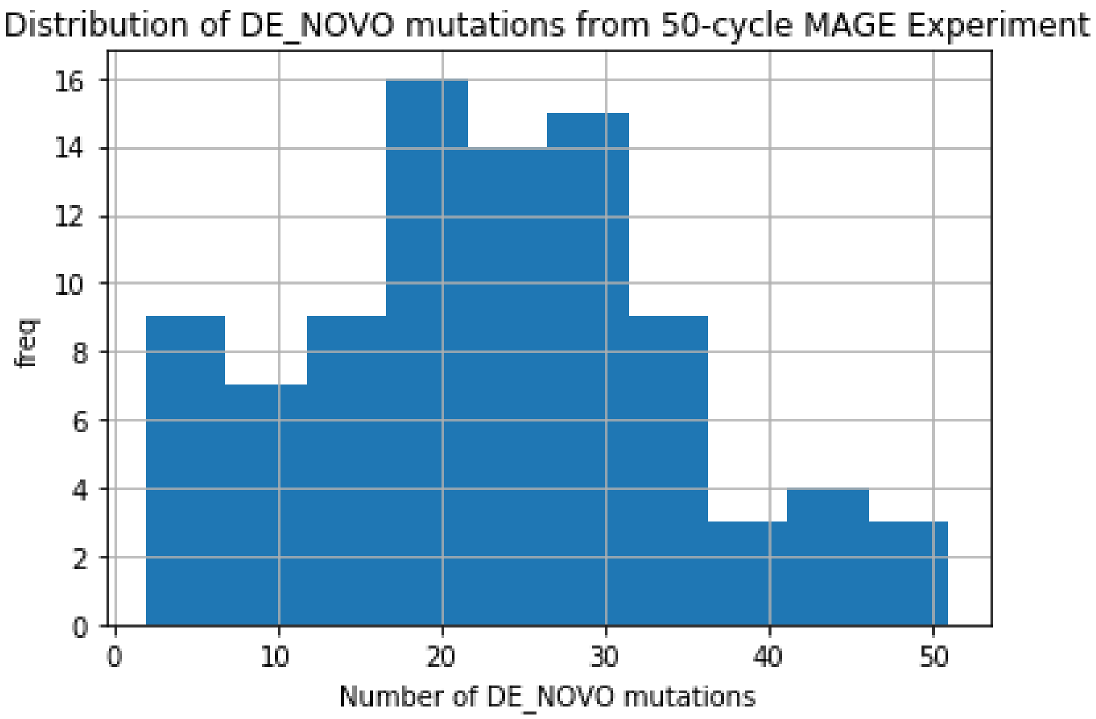
```
Out[43]:     pos BARCODE sample  doubling_time  lineage  time_point  actual_mage_cycle  \
         80  G09  TGAACA  05-06      68.370715        5           6                 34
         83  G12  AGGATA  05-07      63.150454        5           7                 43
         76  G05  TACTCA  03-04      65.921636        3           4                 28
         23  B12  CCGTGA  05-07      60.662941        5           7                 43
         47  D12  GAATGC  05-07      64.540241        5           7                 43

             AMBER  DE_NOVO  FIXED    NONE  UNTARGETED_REVERSION  ORIG_HET_COUNT  \
         80  318.0     33.0   19.0  1878.0                   NaN              20
         83  318.0     25.0   15.0  1891.0                   NaN              22
         76  318.0     21.0   15.0  1893.0                   1.0              12
         23  318.0     44.0   15.0  1867.0                   4.0              23
         47  318.0     43.0   15.0  1871.0                   1.0              23

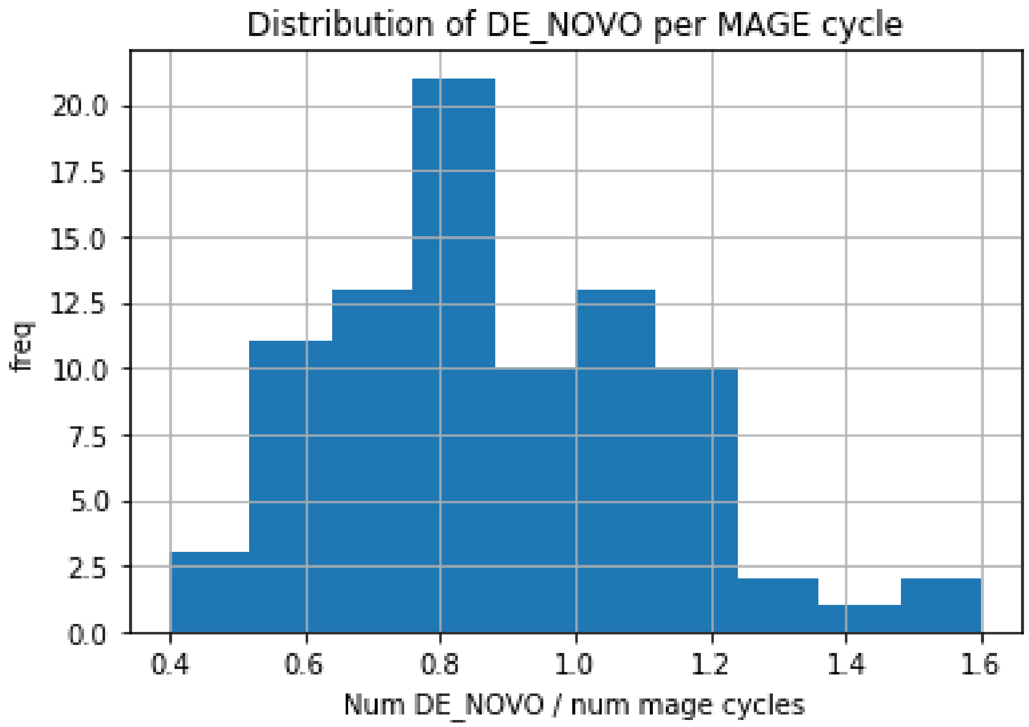
             UPDATED_HET_COUNT  FIXED_per_MAGE_cycle  DE_NOVO_per_MAGE_cycle  \
         80                 14              0.558824                0.970588
         83                 12              0.348837                0.581395
         76                 10              0.535714                0.750000
         23                 14              0.348837                1.023256
         47                 11              0.348837                1.000000
```

```
    DE_NOVO_to_FIXED_ratio
80                1.736842
83                1.666667
76                1.400000
23                2.933333
47                2.866667
```

```
In [44]: plt.figure()
         per_clone_mutation_counts_df[MUTATION_TYPE__DE_NOVO].hist()
         plt.title('Distribution of DE_NOVO mutations from 50-cycle MAGE Experiment')
         plt.xlabel('Number of DE_NOVO mutations')
         plt.ylabel('freq')
         plt.show()
```

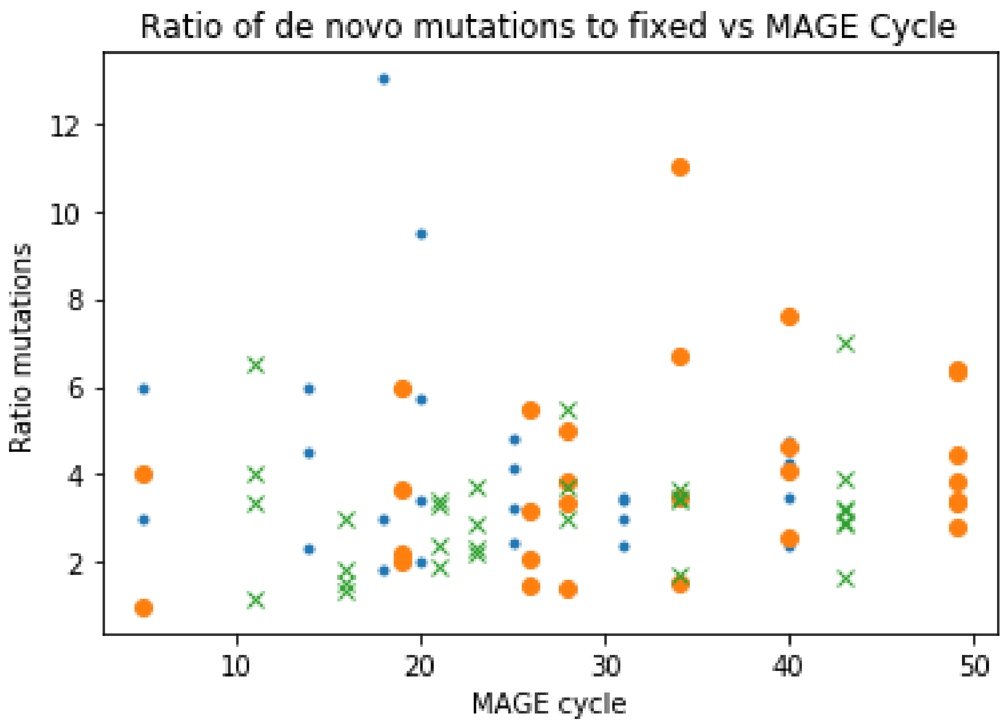


```
In [45]: plt.figure()
         per_clone_mutation_counts_df[
                 per_clone_mutation_counts_df['actual_mage_cycle'] > 0][
                         'DE_NOVO_per_MAGE_cycle'].hist()
         plt.title('Distribution of DE_NOVO per MAGE cycle')
         plt.xlabel('Num DE_NOVO / num mage cycles')
         plt.ylabel('freq')
         plt.show()
```



```
In [46]: plt.figure()
         plot_mutation_list(lineage_1_data, 'de_novo_to_fixed_ratio', '.')
         plot_mutation_list(lineage_3_data, 'de_novo_to_fixed_ratio', 'o')
         plot_mutation_list(lineage_5_data, 'de_novo_to_fixed_ratio', 'x')
         plot_mutation_list(lineage_0_data, 'de_novo_to_fixed_ratio', '.')

         plt.title('Ratio of de novo mutations to fixed vs MAGE Cycle')
         plt.xlabel('MAGE cycle')
         plt.ylabel('Ratio mutations')
         plt.show()
```



```
In [47]: print 'ECNR1_DT (min)', ECNR1_DT
         print 'C321_I4_DT (min)', C321_I4_DT

         original_defect = float(C321_I4_DT - ECNR1_DT) / ECNR1_DT

         def get_mean_timepoint_improvement(t):
             return aggregate_per_sample_mutation_counts_df['doubling_time']['mean'][
                     aggregate_per_sample_mutation_counts_df['time_point']['first'] == t].mean()

         print 'mean final clone improvement', (C321_I4_DT - get_mean_timepoint_improvement(7))
         final_clone_doubling_times = (
                 experiment_metadata_df[
                         experiment_metadata_df['time_point'] == 7]['doubling_time'])

         print 'original_defect', original_defect
         print 'Min improvement fraction', (C321_I4_DT - max(final_clone_doubling_times)) / floa
         print 'Max improvement fraction', (C321_I4_DT - min(final_clone_doubling_times)) / floa
```

```
ECNR1_DT (min) 47
C321_I4_DT (min) 87.0065433333
mean final clone improvement 0.656075928103
original_defect 0.851203049645
Min improvement fraction 0.532223845383
```

```
Max improvement fraction 0.813944135638
```

The doubling time graph appears to asymptote around 20 cycles of MAGE. Count how many of the clones have doubling time below some threshold by 20 cycles of MAGE.

Count how many FIXED mutations observed in each pool.

```
In [48]: print 'All pools', len(set(melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] == MUTATION_TYPE__FIXED]['POSITION']))

         print 'Pool 1', len(set(melted_variant_data_df[
                 (melted_variant_data_df['lineage'] == 1) &
                 (melted_variant_data_df['MUTATION_TYPE'] == MUTATION_TYPE__FIXED)]['POSITION'])

         print 'Pool 3', len(set(melted_variant_data_df[
                 (melted_variant_data_df['lineage'] == 3) &
                 (melted_variant_data_df['MUTATION_TYPE'] == MUTATION_TYPE__FIXED)]['POSITION'])

         print 'Pool 5', len(set(melted_variant_data_df[
                 (melted_variant_data_df['lineage'] == 5) &
                 (melted_variant_data_df['MUTATION_TYPE'] == MUTATION_TYPE__FIXED)]['POSITION'])
```

```
All pools 99
Pool 1 24
Pool 3 58
Pool 5 86
```

Count DE NOVO mutations.

```
In [49]: print 'All pools', len(set(melted_variant_data_df[
                 melted_variant_data_df['MUTATION_TYPE'] == MUTATION_TYPE__DE_NOVO]['POSITION'])
```

```
All pools 1329
```

Export data for making figures in R.

```
In [50]: melted_variant_data_df[[
             'POSITION',
             'REF',
             'ALT',
             'BARCODE',
             'GT_TYPE',
             'sample',
             'doubling_time',
             'lineage',
             'time_point',
             'actual_mage_cycle',
             'MUTATION_TYPE',
             'signal_relative_to_C321'
         ]].to_csv('outputs/exp_1_data_export_minimal_columns.csv', index=False)
```

## 1.5 Model Fitting

First we define the procedure.

```
In [51]: def add_annotation_metadata(model_df):
             """Adds annotations.
             """
             model_with_metadata_df = pd.merge(
                     model_df,
                     variant_data_annotated_only_df,
                     how='inner', # why are some not found?
                     on=['POSITION']
             )
             assert len(model_df) == len(model_with_metadata_df)
             return model_with_metadata_df


         def run_linear_modeling(
                 filtered_variant_data_df,
                 doubling_time_source_df,
                 repeats=10,
                 test_size=20,
                 l1_ratio_list=[.1, .3, .5, .7, .9, .95, .99, 1],
                 min_coef_abs_threshold=None,
                 max_rank_threshold=None,
                 prune_and_repeat_cycles=1,
                 doubling_time_key='log_doubling_time'):
             """Function that runs our linear model technique.
             """
             # At most one of these for now. Could theoretically support both.
             assert min_coef_abs_threshold is None or max_rank_threshold is None

             # Return all runs of linear modeling for quantification purposes.
             lm_results_list = []

             sample_to_snp_occurrence_matrix_gt_type = (
                     model_fitting.generate_sample_to_signal_pivot_table(
                             filtered_variant_data_df))

             # Verify that they are sorted by barcode.
             index_list = list(sample_to_snp_occurrence_matrix_gt_type.index)
             assert index_list == sorted(index_list)

             # Get doubling times, only for included barcodes.
             doubling_times = model_fitting.get_doubling_times_array(
                     doubling_time_source_df,
                     barcode_filter=sample_to_snp_occurrence_matrix_gt_type.index,
                     doubling_time_key=doubling_time_key)
```

```
# We apply cross-validated ElasticNet in order to fit the parameters alpha and
# l1_ratio, while also identifying the most impactful SNPs. We repeat
# multiple times to get different fits depending on cross/train split.
current_elastic_cv_result = model_fitting.multiple_apply_elastic_net_cv(
        sample_to_snp_occurrence_matrix_gt_type,
        doubling_times,
        add_annotation_metadata,
        repeats=repeats,
        test_size=test_size,
        l1_ratio_list=l1_ratio_list)
lm_results_list.append(current_elastic_cv_result)

# Repeat using only the coefficients that pass some threshold defined by args.

# Determine what to use as tresholding.
if min_coef_abs_threshold is not None:
    thresholding = 'COEF'
elif max_rank_threshold is not None:
    thresholding = 'RANK'
else:
    thresholding = 'COEF'
    min_coef_abs_threshold = 0
assert thresholding in ['COEF', 'RANK']

for it in range(prune_and_repeat_cycles):

    # Prune SNPs that don't meet threshold.
    keep_snp_features = []
    if thresholding == 'COEF':
        # Calculate mean model coefficient for each observed SNP.
        snp_to_average_coef_dict = {}

        # Weighted by score.
        normalized_score_list = (
                np.array(current_elastic_cv_result['score_list']) /
                sum(current_elastic_cv_result['score_list']))

        for snp, coef_list in current_elastic_cv_result['snp_to_coef_list_dict'].it
            snp_to_average_coef_dict[snp] = sum(np.array(coef_list) * normalized_sc

        # Figure out which ones to keep based on threshold.
        for snp, coef in snp_to_average_coef_dict.iteritems():
            if coef <= 0 and abs(coef) > min_coef_abs_threshold:
                keep_snp_features.append(snp)
    elif thresholding == 'RANK':
        # Calculate mean ranking for each observed SNP.
        snp_to_average_rank_dict = {}
        for snp, rank_list in current_elastic_cv_result['snp_to_ranking_list_dict']
```

```
            not_None_rank_list = [x for x in rank_list if x is not None]
            snp_to_average_rank_dict[snp] = np.mean(not_None_rank_list)

        # Figure out which ones to keep based on threshold.
        for snp, rank in snp_to_average_rank_dict.iteritems():
            if abs(rank) <= max_rank_threshold:
                keep_snp_features.append(snp)
    else:
        raise AssertionError('Invalid thresholding: %s' % thresholding)

    sample_to_snp_occurrence_matrix_top_snps_only_df = (
            sample_to_snp_occurrence_matrix_gt_type[keep_snp_features])
    current_elastic_cv_result = model_fitting.multiple_apply_elastic_net_cv(
            sample_to_snp_occurrence_matrix_top_snps_only_df,
            doubling_times,
            add_annotation_metadata,
            repeats=repeats,
            test_size=test_size,
            l1_ratio_list=l1_ratio_list)
    lm_results_list.append(current_elastic_cv_result)

top_snps_repeated_elastic_cv_result = current_elastic_cv_result

# Calculate mean model coefficient for each observed SNP.
snp_to_average_coef_dict = {}

# Weighted by score.
normalized_score_list = (
        np.array(top_snps_repeated_elastic_cv_result['score_list']) /
        sum(top_snps_repeated_elastic_cv_result['score_list']))
for snp, coef_list in top_snps_repeated_elastic_cv_result['snp_to_coef_list_dict'].
    snp_to_average_coef_dict[snp] = sum(np.array(coef_list) * normalized_score_list

sorted_avg_coef_list = sorted(
        snp_to_average_coef_dict.items(), key=operator.itemgetter(1))
print 'Num SNPs with > 0 average coefficient:', len(sorted_avg_coef_list)

# Prepare report.
data_obj_list = []
for pos, coef in sorted_avg_coef_list:
    data_obj_list.append({
        'POSITION': pos,
        'model_coef': coef
    })

report_df = pd.merge(
    pd.DataFrame(data_obj_list),
    variant_data_annotated_only_df,
    on='POSITION')

return report_df, lm_results_list
```

Now in prep for running modeling, we filter variants to those that are fixed and any de novo that occur at least 2 times.

```
In [52]: melted_variant_data_df['log_doubling_time'] = np.log(melted_variant_data_df['doubling_t

         reverted_position_set = set(variant_data_annotated_only_df[
                 (variant_data_annotated_only_df['MUTATION_TYPE'] == MUTATION_TYPE__FIXED)
         ]['POSITION'])

         de_novo_position_set = set(variant_data_annotated_only_df[
                 ((variant_data_annotated_only_df['MUTATION_TYPE'] == MUTATION_TYPE__DE_NOVO) &
                 (variant_data_annotated_only_df['count'] >= 2))
         ]['POSITION'])

         keep_position_set = reverted_position_set | de_novo_position_set

         f_variant_data_df = melted_variant_data_df[
                 (melted_variant_data_df['POSITION'].apply(
                         lambda p: p in keep_position_set))
         ]

         total_num_snps_considered = len(f_variant_data_df['POSITION'].unique())
         print 'Num SNP features considered:', total_num_snps_considered
         print '...Reverted: ', len(reverted_position_set)
         print '...De Novo: ', len(de_novo_position_set)
         print 'Num samples considered:', len(f_variant_data_df['BARCODE'].unique())
```

```
Num SNP features considered: 234
...Reverted:  99
...De Novo:  135
Num samples considered: 90
```

Now run modeling.

NOTE: Modeling result / model_coef values hard-coded to match Fig. 3. The modeling result is stochastic depending on train-test split with respect to alleles with weaker effect. See **Methods**.

```
In [53]: LM_RESULT__ALL_DF, LM_RESULT__ALL__RESULTS_LIST = run_linear_modeling(
                 f_variant_data_df,
                 melted_variant_data_df,
                 repeats=100,
                 prune_and_repeat_cycles=1,
                 test_size=15,
                 doubling_time_key='log_doubling_time')

         LM_RESULT__ALL_DF
```

```
Num SNPs with > 0 average coefficient: 8
```

```
Out[53]:    POSITION  model_coef REF ALT MUTATION_TYPE INFO_EFF_GENE INFO_EFF_IMPACT  \
         0   4102449   -0.119654   A   G         FIXED          cpxA        MODERATE
         1   3990077   -0.046998   C   T       DE_NOVO          cyaA        MODERATE
         2   1263523   -0.044777   T   C         FIXED          hemA        MODERATE
         3   3092256   -0.035126   T   C       DE_NOVO          yggR        MODERATE
         4   1511492   -0.030327   T   C       DE_NOVO          ydcT        MODERATE
         5    200214   -0.011817   C   T         FIXED          bamA        MODERATE
         6    672170   -0.006232   C   A         FIXED          leuS        MODERATE
         7    322579   -0.001609   C   T       DE_NOVO          ykgF        MODERATE

                     INFO_EFF_AA  GT_TYPE  count
         0   p.Trp184Arg/c.550T>C      2.0     60
         1   p.Pro301Leu/c.902C>T      2.0     31
         2   p.Leu196Pro/c.587T>C      2.0     78
         3   p.Thr283Ala/c.847A>G      2.0     46
         4   p.Ser218Pro/c.652T>C      2.0     17
         5  p.Pro763Ser/c.2287C>T      2.0     10
         6  p.Val613Phe/c.1837G>T      2.0     18
         7  p.Pro340Ser/c.1018C>T      2.0     69
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