2-34 Spot Assay

FRIDAY, 2020-02-14

Goals:

- 1. Find out the prevalence of petite mutations in the dominance lines. When we previously did a spot assay on lines 35, 46, 35x35, and 35x46, none of the lines grew on YPG.
- 2. Ensure that line order is correct.

Spot on YPAD, YPG, and YPAD-G418 plates. Use dominance samples from fridge stock. Use 10 μL of undiluted sample.

MONDAY, 2020-02-17

Matthew took plates out of incubator and marked the plates and uploaded the results to the knockout records in dropbox.

Some evidence of contamination on at least 1 plate, maybe more. Some plates have spots with strange phenotypes - colonies within the spots, very red, etc.

- line 28: has small yellow colonies within the grown spot on ypg and ypad.
- line 115: only 2 colonies on ypad+g418
- line 123: very high growth compared to other spots on ypad and ypg, yellowish and glossy potential contamination.
- line 134: contamination large and hairy. It grew into 129 but you can still see 129 growth. Can't tell if 134 grew at all though.
- line 6x96: small yellow colonies present in the spot on ypg.
- Line 66x66 71x96 plate: lots of little satellite colonies.
- line 67x93: lots of little colonies on ypad+g418 instead of a spot.
- line 73x73: very pink appearance on ypad and ypg.
- line 115x20: did not grow, but I don't think it was spotted in the first place.
- line 77 control has a single small colony on ypad+g418. Growth or no?

Some plates have 'satellites', but I think they may just be from droplets.

Spot lines 115x20, 67x93, 134, and 123 again on ypad, ypg, and ypad+g418. And 123 again on ypad, ypg, and ypad+g418. In the standard stand

Linnea analyses the results

RDH status: only two abberrant cases. Line 115 and line 67x93 are both supposed to be wildtype at the RDH locus, but had small spots on YPAD + g418.

Can Matthew Stasiuk respot lines 77, 115, and 67x193 from freezer cultures? Else Linnea can do tomorrow:)

WEDNESDAY, 2020-02-19

Spot assay from monday needs extra day of growth for ypg and g418.

THURSDAY, 2020-02-20

Check spot assay.