

Table of Contents

December 2020	2
CHAH plates with 10% sucrose	3
Arbitrary PCR	4
Arbitrary PCR reaction 2 agarose gel image.....	5
Thoughts.....	5
MG Tn mutagenesis plates from 11/18/20	6
Thoughts.....	6

December 2020**Tuesday, December 1, 2020****To Do:**

1. — Make EC LVS cells
2. — Electroporate pKL37 into LVS
3. — Pour plates

Results and Data:

Made new EC LVS

struck out plate yesterday, scraped up entire plate (somewhat older plate and plated rather late, so not terribly robust growth)

washed 3x in 10% sucrose

Resuspended in a bit too much 10% sucrose (200 uL)- wanted about 210 uL cells and obviously that was too much sucrose to add. Have about 300 ul cells and want to perform 4 electroporations, so use 75 uL per electroporation.

Number	Strain	Plasmid	Time constant
1	LVS	pKL37 pEXΔ <i>pmrA</i>	5.6
2	LVS	pKL37 pEXΔ <i>pmrA</i>	Arc
3	LVS	pKL37 pEXΔ <i>pmrA</i>	5.5
4	LVS	pKL37 pEXΔ <i>pmrA</i>	5.6

Used 75uL cells, 5 uL pKL37 (plasmid that JW prepped), recovered in 5 mL (should have been 4 mL!!) MHB at 12:00 PM shaking at 37°C.

Spin cells down at 6:15 PM (6.25 hours recovery) and plate on CHA-Kan plates (Michelle G. made Monday 11/23), 2 plates per EP.

Put at 37°C

Poured 300 mL CHA and 300 mL CHA-Kan plates

Wednesday, December 2, 2020**To Do:**

1. — Test CHA Kan plates

Results and Data:

The hemoglobin I used for the CHA-Kan plates had a lump of undissolved material that was undetected until I poured the hemoglobin into the CHA. Struck out some KanR cells to old CHA-Kan plates and these new ones to confirm that both can sustain single colonies and the hemoglobin deficit doesn't prevent growth.

Sunday, December 6, 2020**To Do:**

1. — Check CHA Kan plates
2. — Second arbitrary PCR reaction
3. — Look for 1° integrants from EP
 - a. — Patch out 1° integrants

- ~~b. Make sucrose plates~~
- ~~4. Clean up lab-ish~~
- ~~5. Check microscope objectives (40x, 25x, 10x)~~
- ~~6. Clean up office-ish~~
- ~~7. Pour plates?~~
- ~~8. Write RA1 job description~~
 - ~~a. Email DR for updated resume/CV~~
- 9. Grade papers
- 10. Order CHA
- 11. Read JC paper
- 12. Email SROs

Results and Data:

Media preparation:

CHA-Kan plates look good. Single colonies on both plates. Will probably still need more. Assess plate needs:

Dan:

CHA-Kan-X-gal - Make 1 flask, pour 2 x square plates, pour remaining large plates (~60 mL per plate)

Hannah:

CHA-Kan – we have enough to cover her initial electroporation. More wouldn't hurt

John:

Standard CHA. We have ~10 plates. More wouldn't hurt

Kathryn:

CHAH plates with 10% sucrose

In 500 mL flask, mix:

- 5 g Beef Heart Infusion
- 5 g Protease Peptone
- 5 g Glucose
- 0.5 g L-Cystine
- 7.5 g Agar

with 150 mL type I ddiH₂O. Stir on low heat until completely dissolved, about 10 minutes.

In 500 mL flask, dissolve 5 g hemoglobin in 250 mL ddiH₂O. Stir on low heat until completely dissolved, about 10 minutes.

Put both flasks in 10 qt InstantPot with ~400 mL distilled water. Pressure cook for 30 minutes.

While autoclaving, warm sterilized 50% sucrose in water bath at 55°C

After all components are sterilized and equilibrated to 55°C, pour hemoglobin into CHA flask. Add 100 mL 50% sucrose to CHA-hemoglobin flask. Mix media, use sterile pipette, pour ~24 mL media per plate.

Prep more CHA, CHA-Kan, CHA-Kan-X-gal

Prepared 2 x 300 mL CHA in 1L flasks and brought to autoclave. Had to abort and re-start cycle about 20' in. Cycle completed, but flasks had boiled over (?!?!). Hannah has had many cycles fail before (common issue on first run of the day), but this is new. ☹



2x 300 mL CHA flasks, boiled over after a 30' cycle aborted and re-started.

Arbitrary PCR

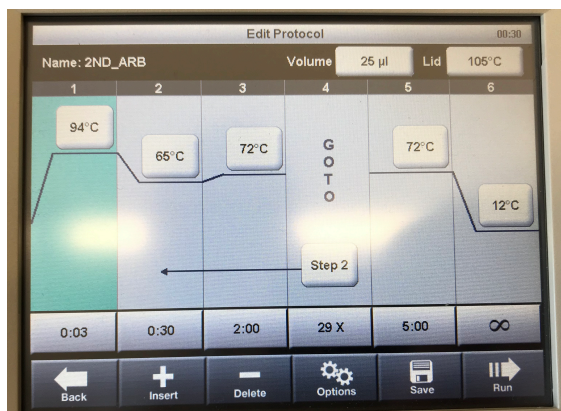
Following up for Michelle on her last arbitrary PCR reaction, now that we have more OneTaq. She had performed a 1° reaction with either Tn3 gDNA or LVS, using a gradient of temps for the annealing temp. See Michelle's notebook for details.

We're low on 10 mM dNTPs, so adjust formula to use 2.5 mM dNTPs. Also, have 16 reactions but err on the side of prepping more master mix.

Component	Stock Concentration	Final Concentration	Volume of each component (uL)	
			Per reaction	Master Mix
			# of reactions here: 17	
			# of reactions allowing for error: 18.3	
ddiH ₂ O			15.125	276.8
OneTaq Rxn Buffer	5X	1X	5	91.5
dNTPs	2.5 mM	0.2 mM	2	36.6
KROL88 Arb2	100 µM	4.0 µM	1	18.3
External Specific Primer: KROL92 Tn_Mar3	10 µM	0.4 µM	1	18.3
PCR 1 Amplicon, diluted 1:10	-	-	0.75	-
OneTaq Polymerase	5 units/uL	6.25 units	0.125	2.3
Total volume			25	457.5
Thermocycler program:	Step	Temp	Time	
	1	94°C	30"	
	2	65°C	30"	
	3	72°C	120"	
	4	Go to step 1, repeat 29x		
	5	72°C	5'	
	6	12°C	hold	

Made up reaction, of course forgot that the total volume is 25 uL instead of 20 uL so only put 19.2 uL in reaction tube and used 1 uL of 1:10 diluted PCR.

Went to put tubes in thermocycler and discovered that this was the saved program for the second arbitrary PCR reaction:

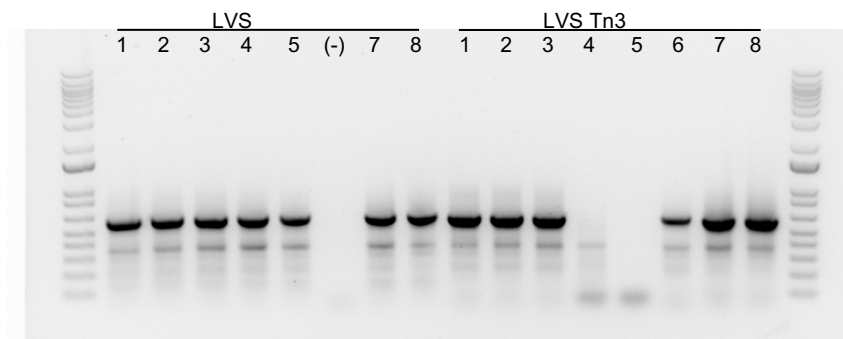


Looks like there was a programming mistake. 94°C for only 3 seconds and it never cycled back to 94°C, just between 65°C and 72°C.

Correct the program to reflect the parameters indicated above and run the reactions.

Arbitrary PCR reaction 2 agarose gel image

Run ~ entire reaction on gel:



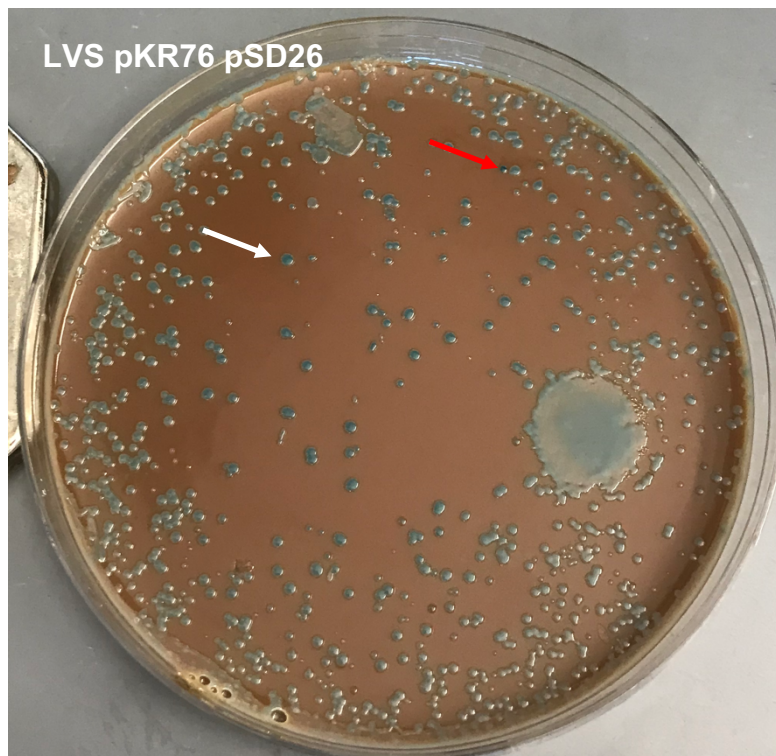
Thoughts

In 1st PCR, annealing temp for reaction 1 was 45°C, ranged up to 65°C for reaction 8.

All are producing non-specific product. Did I miss pipetting into LVS Tn3 reaction 5?

Regardless, the product created is not specific to the transposon insertion. it might be worth using only a single primer in reaction 1 and re-checking the annealing temps.

MG Tn mutagenesis plates from 11/18/20



Thoughts

Was about to throw old transformation plates away (Michelle's from 11/18/20) but it looked like there might be a single colony from one of the $\Delta pmrA \Delta priM$ pKR76 pSD26 X-gal Kan Nat plates. Streak it out onto CHA-Kan and see if it grows- really small and old, so may be too late

Also looked at the mutagenesis plates for LVS pKR76 plates. Noticed one colony looks pretty dark blue (red arrow)! Still have a few Kan X-gal plates- might be too old, but try streaking that colony plus a more normal colony (white arrow) onto Kan X-gal.

Monday, December 7, 2020

To Do:

1. Check on 1° integrants
 - a. Freeze down
 - b. Patch onto CHA
2. Check on patches from old transposon mutagenesis
3. Clean up lab, office
4. Write RA1 job description
5. Grade papers
6. Read JC paper
7. Email SROs

Wednesday, December 16, 2020

To Do:

1. Check on 1° integrants
 - a. Freeze down
 - b. Patch onto CHA

Results and Data:

Plan:

Reaction	Locus	Primers	Expected size (WT)	Expected size (Δ)
A	<i>pmrA</i>	KROL61, KROL62	1204	535