Experiment Date: Tuesday, January 13, 2009

1. Original 10X Buffer Composition

Ingredient	Concentration in Original Buffer (M)	Concentration Provided (M)	Concentration Wanted in Tube 3	Concentration Wanted in Tube 4
Tris pH 8.3	0.2	2	Unchaged	Unchanged
MgCl2	0.015	0.2	Unchanged	Unchanged
KCl	0.25	2.5	0.125	1
Tween 20	0.5	10%	Unchanged	Unchanged

2. 10X Buffer Compositions ⟶

Tube #	Vol 2M Tris (µL)	Vol. 0.2M MgCl2 (µL)	Vol. 2.5M KCl (µL)	Vol. 10% Tween 20 (µL)	Vol. dH2O (µL)	Total Vol (µL)
#1: Original	10	7.5	10	5	67.5	100
#2: No KCl	10	7.5	0	5	77.5	100
#3: Half [KCl]	10	7.5	5	5	72.5	100
#4: 4x [KCl]	10	7.5	40	5	37.5	100

3. Master Mix Requirements

Ingredient	1 Rxn Vol (µL)
10X Buffer	5
dNTP	8
50% glycerol	10
Formamide	2.5
Total	25.5

4. PCR Reaction Volumes

Ingredient	Tube 1	Tube 2	Tube 3	Tube 4
Corresponding Master Mix	25.5	25.5	25.5	25.5
Primer Set #1	5	5	5	5
S. rimosis DNA	5	5	5	5
dH2O (sterile)	15	15	15	15
Taq pol.	0.5	0.5	0.5	0.5

1. Vortex and put PCR rxn tube into larger µ-fuge tube with a Kimwipe plug, and then spin for 10s.
2. Put all tubes into PCR Cycler