**Selection of transgenic plants with antibiotics**
**Petri dish selection**
Kanamycin (pBI101)
Media
    1/2x MS, no sucrose, 0.8 % Sigma Agar (A1296)
    40µg/ml Kanaqmycin, 100µg/ml cefotaxim.

1. Sterilize 200µl (do  not exceed) seeds in 1.5 ml tube.
	* Wash 2x with 70% ethanol, remove as much debris as possible.
	* Wash 3 times with autoclaved water.
2. Resuspend in 0.1-0.2 % agar.  Make total volume of 1 ml.
3. Spread 250µl seed suspension/90 mm plate.
	* It is VERY important not to sow too many seeds/ plates.
	* Spread evenly (very important, too).
4. Cold-treat for 2 days.
5. Move to room tempearature.  It will take 4-5 days to distinguish green transgenic plants in yellow untransformed plants.

Hygromycin (pFGC1008)
1/4x MS, no sucrose, 0.8 % Sigma Agar (A1296)
    20-30µg/ml Hygromycin B, 100µg/ml cefotaxim.

1. Sterilize 200µl (do  not exceed) seeds in 1.5 ml tube.
	* Wash 2x with 70% ethanol, remove as much debris as possible.
	* Wash 3 times with autoclaved water.
2. Resuspend in 0.1-0.2 % agar.  Make total volume of 1 ml.
3. Spread 250µl-1 ml seed suspension/90 mm plate.
	* Spread evenly (very important).
4. Cold-treat for 2 days.
5. Move to room tempearature.  It will take 4-5 days to distinguish tall transgenic plants in short untransformed plants.

Hygromycin selection can be done in very high density (but drain the excess liquid from the media surface).  Resistance based on the root hair development can be observed under microscope after few days.
Hygromycin can loose titer over the time.  I used 20µg/ml for fresh purchase but increased to 35µg with 3 year-old stock.

Glyphosate (pMDC100-GAT)
1/4x MS, no sucrose, 0.8 % Sigma Agar (A1296)
    0.4 mM glyphosate (MW 169.07), 100µg/ml cefotaxim.

* Original glyphosate powder is 88% purity.

(I dissolved glyphosate in ethanol as 500x stock but will try to use methanol or DMSO to prepare higher concentration stock)

1. Sterilize 200µl (do  not exceed) seeds in 1.5 ml tube.
	* Wash 2x with 70% ethanol, remove as much debris as possible.
	* Wash 3 times with autoclaved water.
2. Resuspend in 0.1-0.2 % agar.  Make total volume of 1 ml.
3. Spread 250µl seed suspension/90 mm plate.
	* Spread evenly (very important).
4. Cold-treat for 2 days.
5. Move to room tempearature.  It will take 4-5 days to distinguish green transgenic plants with their root penetrating media in yellow untransformed plants.

Glean/chlorosulfuron (KIP011/pSOUP)
1/4x MS, no sucrose, 0.8 % Sigma Agar (A1296)
   0.167 µM  Glean (MW 357.77), 100µg/ml cefotaxim.

* Original Glean powder is 75% purity.

Glean stock solution (2.8 mM: 1.33 mg/ml of powder containing 75 % active ingredient in DW, filter sterilize)
Use 60 µl stock/L media

1. Sterilize 200µl (do  not exceed) seeds in 1.5 ml tube.
	* Wash 2x with 70% ethanol, remove as much debris as possible.
	* Wash 3 times with autoclaved water.
2. Resuspend in 0.1-0.2 % agar.  Make total volume of 1 ml.
3. Spread 250µl seed suspension/90 mm plate.
	* Spread evenly (very important).
4. Cold-treat for 2 days.
5. Move to room tempearature.  It will take 7 days to distinguish green transgenic plants with their root penetrating media in yellow untransformed plants.
* Glean stock solution (2.8 mM: 1.33 mg/ml of powders containing 75 % active  ingredient)  is not very stable.  Do not use after 2 weeks.

Basta (pSKI015)
1/4x MS, no sucrose, 0.8 % Sigma Agar (A1296)
  100µg/ml cefotaxim.

1. Sterilize 200µl (do  not exceed) seeds in 1.5 ml tube.
	* Wash 2x with 70% ethanol, remove as much debris as possible.
	* Wash 3 times with autoclaved water.
2. Resuspend in 0.1-0.2 % agar.  Make total volume of 1 ml.
3. Spread 250µl seed suspension/90 mm plate.
	* Spread evenly (very important).
4. Cold-treat for 2 days.
5. Move to room tempearature.
6. After 7-10 days, in clean bench, spray plates with 30µg/ml Liberty herbicide (diluted in autoclaved water, used sprayer that have been treated with 70% ethanol and washed with autoclaved water)
	* Commercial Liberty herbicide is 11.33% (113.3g/L).  Add 26.4µl/100mL DW.

Asulam (pCB301-Sul)
1/4x MS, no sucrose, 0.8 % Sigma Agar (A1296)
    30 µg/ml Asulox herbicide, 100µg/ml cefotaxim.
Commercial Asulox herbicide is 355.89g/L.  Filter sterilize and add 84.2µl/L to the media.

1. Sterilize 200µl (do  not exceed) seeds in 1.5 ml tube.
	* Wash 2x with 70% ethanol, remove as much debris as possible.
	* Wash 3 times with autoclaved water.
2. Resuspend in 0.1-0.2 % agar.  Make total volume of 1 ml.
3. Spread 250µl seed suspension/90 mm plate.
	* Spread evenly (very important).
4. Cold-treat for 2 days.
5. Move to room tempearature.

**Soil selection**
Basta (pSKI015)

1. Prepare soil.  Make sure to include Banlot to prevent Pithium infection, and malathon to prevent insects.
2. Sow seeds with high density.
3. Mix seeds (1ml seeds/big tray) and sands (1:1) in salt shaker and shake onto the surface of soils.
4. Water from the bottom with Banlot containing water.  Make sure the surface became wet.  If needed, drain excess water.
5. Keep in cold room for 2-5 days.
	* Seeds on the soil are less efficient to germinate.  Longer cold treatment improves germination. 3-5 days are good starting points.
6. Move to growth chamber set to 21-24°C.
7. After 1 week, spray with 30µg/ml Liberty solution for 3 consecutive days.  After 5days interval, treat with 3 more sprays to eliminate escapes.
	* Liberty is a very light sensitive chemical.  Store the diluted solution in the dark.
8. Untransformed plant start to die after second spray of 3 sprays.

Glyphosate (pMDC100-GAT)

1. Prepare soil.  Make sure to include Banlot to prevent Pithium infection, and malathon to prevent insects.
2. Sow seeds with high density.
3. Mix seeds (1ml seeds/big tray) and sands (1:1) in salt shaker and shake onto the surface of soils.
4. Water from the bottom with Banlot containing water.  Make sure the surface became wet.  If needed, drain excess water.
5. Keep in cold room for 2-5 days.
	* Seeds on the soil are less efficient to germinate.  Longer cold treatment improves germination. 3-5 days are good starting points.
6. Move to growth chamber set to 21-24°C.
7. After 1 week, spray with 1-2 mM glyphosate (0.2-0.4g/L active ingredient) solution.  Transgenic plants will keep growing whereas non-transformed plants will slowly turn yellow.  After 5days, transgenic plants will be distinguishable.
* Generally, single spray will prevent emergence of escapes perhaps due to the toxicity of glyphosate absorbed in the soil.  If spraying adult plants, use 1 mM or less.
* pMDC100-GAT has 35S-NPTII marker as well.  It seems these markers may interfere in T2 generation.  We observed high frequency of Kan S and glyphosate S lines in T2 lines.

Glean/chlorosulfuron (KIP011/pSOUP)

1. Prepare soil.  Make sure to include Banlot to prevent Pithium infection, and malathon to prevent insects.
2. Sow seeds with high density.
3. Mix seeds (1ml seeds/big tray) and sands (1:1) in salt shaker and shake onto the surface of soils.
4. Water from the bottom with Banlot containing water.  Make sure the surface became wet.  If needed, drain excess water.
5. Keep in cold room for 2-5 days.
	* Seeds on the soil are less efficient to germinate.  Longer cold treatment improves germination. 3-5 days are good starting points.
6. Move to growth chamber set to 21-24°C.
7. After 1 week, spray with 1.73 µM Glean (620 µl stock/L spray solution) solution.  Transgenic plants will keep growing whereas non-transformed plants will slowly turn yellow.  After 7 days, transgenic plants will be distinguishable
* Glean stock solution (2.8 mM: 1.33 mg/ml of powders containing 75 % active  ingredient)  is not very stable.  Do not use after 2 weeks.