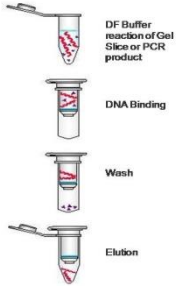
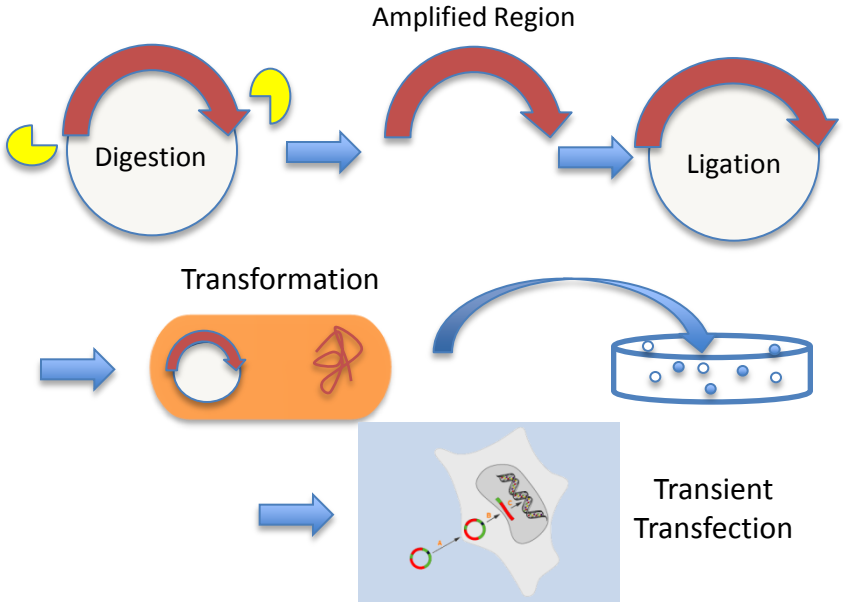
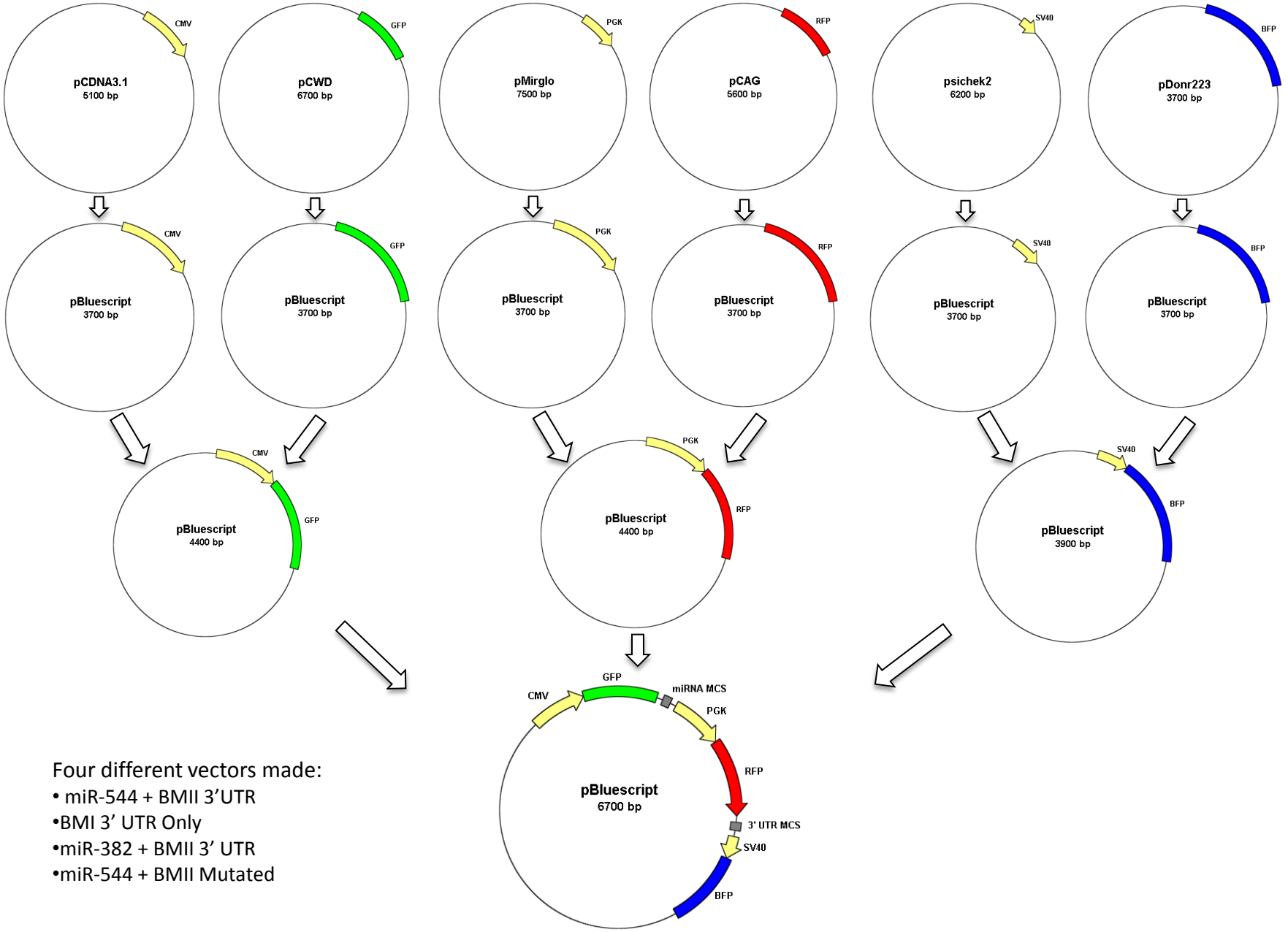
Cloning Protocol

- PCR amplification of genes
- Run through a .7% gel
- Cut correct size band out of gel
- Purify PCR product using a gel extraction kit
- Digest 1ug of pBlueScript with EcoRV
- Blunt end ligation of PCR products (CMV, GFP, PGK, etc.) into EcoRV cut site
- Transformation
  - 40ul competent cells + ligation
  - 7 minutes on Ice
  - 30 second heat shock (45C)
  - 2 minutes on ice
  - Add 250 ul LB no antibiotics
  - Incubate 45 minutes in 37C shaker
  - Plate on LB Amp plates with X-gal
- Blue/White selection- pick white colonies/ grow up over night
- Digestion of individual vectors
- Three point ligation into pBlueScript
- Transform/Plate with out X-gal
- Confirm by colony PCR
- Transfection of HEK-293 cells with expression construct
- Check for expression of fluorescence 24 hours post-transfection







Four different vectors made:

- miR-544 + BMII 3'UTR
- BMI 3' UTR Only
- miR-382 + BMII 3' UTR
- miR-544 + BMII Mutated