Table:

<table>
<tr><td>Expected product and size:</td><td colspan="2">pA15A, 2 kb</td></tr>
<tr><td>H_2O (reaction volume - reagents added =)</td><td colspan="2">34.5 µL</td></tr>
<tr><td>Buffer: Phusion HF Buffer (5X)</td><td colspan="2">10 µL</td></tr>
<tr><td>Primer 1: 15_01</td><td colspan="2">2 µL</td></tr>
<tr><td>Primer 2: 15_02</td><td colspan="2">2 µL</td></tr>
<tr><td>DNA Template: pA15A miniprep, 1ng/µL dilution</td><td colspan="2">1 µL</td></tr>
<tr><td>Polymerase: Phusion</td><td colspan="2">0.5 µL</td></tr>
<tr><td>Total reaction volume:</td><td colspan="2">50 µL</td></tr>
<tr><td rowspan="7">PCR Program:</td><td colspan="2">Program name: iGEM PHUSION</td></tr>
<tr><td>98°C</td><td>2 min</td></tr>
<tr><td>98°C</td><td>30 s</td></tr>
<tr><td>(anneal temp)°C</td><td>30 s</td></tr>
<tr><td>72°C</td><td>(extend time) min</td></tr>
<tr><td colspan="2">repeat steps 2-4: 30 times</td></tr>
<tr><td>72°C</td><td>4 min</td></tr>
<tr><td colspan="3">Additional info:
- (did you use GC buffer instead?)
- (did you prepare the tubes and have someone else put them into the machine when it was free or when their PCRs were also ready to run?)</td></tr>
</table>

Preparations:

- Fill ice bucket.
- Find and thaw primers and buffer.
- Make 1 ng/µL dilution of template if PCRing from a miniprep.

Procedure:

1) Acquire and label caps of PCR tubes; place on ice.
2) Pipette water, buffer, primers, and template. (Use an (n+1)X master mix if more than 2 tubes, where n is the number of tubes.)
3) Start PCR machine: Temperature Measurement Mode > Algorithmic, Sample Volume > 50 µL, Hot Start? > YES, Hot Start Temperature > 98°C. Machine will preheat to 98°C and pause.
4) Add 0.5 µL Phusion polymerase per tube.

5) Replace tubes in machine and hit resume button to start thermocycling.

Assembled 2015 by Olya Spassibojko. Adapted from 2012. Source: iGEM 2015.